Pharmaceutical development of diacetylmorphine preparations for prescription to opioid dependent patients

Pharmaceutical development of diacetylmorphine preparations for prescription to opioid dependent patients

Farmaceutische ontwikkeling van toedieningsvormen voor diacetylmorfine ten behoeve van heroïne-afhankelijke personen

(met een samenvatting in het Nederlands)

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Voor mijn ouders
& voor René

Contents

Preface

Heroin (3,6-diacetylmorphine) is a di-ester of morphine that was introduced into medicine by Bayer in 1898 as a cough suppressant [1]. Its analgesic potency was only recognised decades later, when it had been banned from prescription in many countries due to its addictive properties [1]. Heroin is now one of the best known drugs of abuse, that is included in the United Nations list of Narcotic Drugs under International Control [2]. Nowadays, addiction has been accepted as a chronic, relapsing, psychiatric disorder and several pharmacological treatments have been developed to achieve three main goals: crisis intervention, detoxification and stabilisation or harm reduction [3]. Opioid antagonists (naloxone, naltrexone) and agonists (methadone, buprenorphine) have proven to be useful in the treatment of heroin addiction, but curing the disorder and achieving complete abstinence remains problematic for many addicts. Therefore, many treatment programs nowadays focus on stabilisation of drug use, improvement of well-being and reduction of drug-related harm [3]. In this context, there is considerable interest in heroin-assisted treatment: (co-)prescription of heroin to chronic, treatment-refractory opioid dependent patients. Several European Countries (Belgium, France, Germany, Spain, Switzerland, The Netherlands, United Kingdom), as well as Australia and Canada have planned or ongoing clinical trials on this subject [4]. These trials (and the routine heroin-assisted treatment programs that might result) will need pharmaceutical heroin (diacetylmorphine) to prescribe to the patients.

Research into the development of pharmaceutical forms of heroin for prescription to addicts can profit from the extensive knowledge that already exists on this substance. Chemically, heroin (3,6-diacetylmorphine) is a di-ester of morphine that is lipophilic in the base form, while its hydrochloride salt (pK_a 7.6) shows excellent solubility in water. This results in rapid absorption of the substance after administration and in rapid distribution into the tissues. Diacetylmorphine has a very short half-life in the circulation, due to rapid conversion to 6-acetylmorphine and morphine by esterase enzymes that are present in blood. Both main metabolites can be considered active metabolites, as binding to μ-receptors requires a free phenolic (3-OH) group in the morphinan structure, that diacetylmorphine lacks. Rapid peak plasma concentrations of diacetylmorphine and 6-acetylmorphine are thought to be related to the rewarding 'flash' effect, while sustained euphoria is associated with high plasma concentrations of morphine (-6-glucuronide).

Pharmaceutical dosage forms of heroin for prescription to addicts should comply with the usual requirements of safety, efficacy and quality of pharmaceutical products, but in addition, acceptability to the patients is important. Especially since heroin-assisted treatment is usually only an option for treatment-resistant addicts who have to be encouraged to participate in a treatment program. With regard to the acceptability to clients, rapid delivery of diacetylmorphine and 6-acetylmorphine into the circulation seems to be an important pharmacokinetic requirement for the pharmaceutical products to be developed. Obviously, this can be achieved by injection of a

diacetylmorphine hydrochloride solution, which is the most popular route of administration, clinically (in the UK) as well as on the street [5]. The second most popular method is heroin smoking via 'chasing the dragon', which has spread from South East Asia to several countries in Europe (The Netherlands in the 1970s, UK in the 1980s and Spain and Switzerland in the 1990s [6]) and is still gaining popularity [5]; in 2001, about 45% of the European addicts in treatment predominantly smoked heroin this way. Therefore, this thesis describes the development of pharmaceutical heroin for injection (diacetylmorphine for injection) as well as the development of pharmaceutical smokable pharmaceutical heroin: diacetylmorphine for inhalation.

The term heroin 'smoking' is somewhat misleading, as no burning is involved in 'chasing the dragon': addicts heat heroin powder on a piece of aluminium foil using a cigarette lighter, until it melts and evaporates, so they can inhale the vapours through a tube or drinking straw. Pharmaceutically, this process can be interpreted as administration of a systemically acting drug via inhalation after volatilisation. Its popularity can be explained by the efficiency of absorption of the lipophilic diacetylmorphine molecule across the large tissue area that is available in the airways. Furthermore, the blood flow from the lungs is directed straight to the brain, which makes a fast onset of action possible for the centrally acting drug. These assumptions were confirmed in a pharmacokinetic study that showed rapid absorption of diacetylmorphine after 'chasing the dragon' and a bioavailability of 52% compared to intravenously administered diacetylmorphine [7].

Street heroin has a variable composition and the additives present can influence the volatilisation process. These additives can be manufacturing impurities (active substances originating from the opium or morphine used in heroin synthesis and intermediates from the acetylation process: e.g., morphine, noscapine, acetylcodeine), diluents (mainly sugars, used as inert bulking agents), or adulterants (active drugs, with similar taste or effects to heroin: acetaminophen, caffeine, phenobarbitone, methaqualone, procaine, strychnine, quinine). The presence of some of these additives has been shown to affect the volatilisation process: caffeine, methaqualone [8], and barbital [8,9] were found to increase the recovery of diacetylmorphine in the vapours after volatilisation. Only caffeine was considered as an excipient for pharmaceutical heroin for inhalation after volatilisation, because it was least likely to show interfering pharmacologic effects. Studies of the thermal characteristics and volatilisation behaviour of powder mixtures of diacetylmorphine base and caffeine are described in this thesis, as well as the manufacturing process that was developed for dosing and packaging of the selected diacetylmorphine/caffeine powder mixture.

Practical application of diacetylmorphine for inhalation after volatilisation in heroin-assisted treatment programs posed new questions and challenges. For example, the procedure of 'chasing the dragon' has been developed by the users on the street and the efficiency of this mode of administration of pharmaceutical heroin might benefit from a more scientific approach. This thesis therefore also describes a comparison of

'chasing the dragon' with a standardised way of volatilising diacetylmorphine for inhalation, using a heating device and a specially designed sample holder.
Another question arising from the heating aspect of using diacetylmorphine for inhalation is the extent to which pyrolysis occurs. Several authors have reported the formation of degradation products of diacetylmorphine upon heating [8,10,11]. These substances, present in the vapours, might be associated with spongiform leukoencephalopathy [12] or impaired lung function [13] which have been reported as adverse effects of smoking street heroin. Even though pharmaceutical heroin has a higher purity than street heroin and the temperatures involved in 'chasing the dragon' are relatively low (compared to smoking tobacco, for example), the formation of toxic degradation products during volatilisation cannot be ruled out. This thesis therefore also describes the identification of the volatilised degradation products present in plastic straws, used by addicts for inhalation of pharmaceutical heroin after volatilisation.
Another important practical issue in heroin-assisted treatment is patient compliance, since the success of such programs depends on complete substitution of the patient's use of illicit heroin. Stable isotopically labelled heroin, added to pharmaceutical heroin for inhalation could be used as a urinary marker for detection of co-use of illicit heroin.

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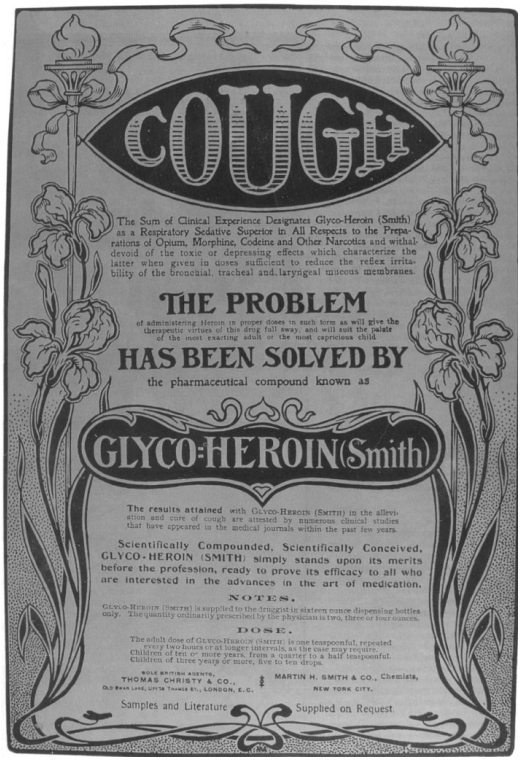
1 Introduction



Chapter 1.1

Pharmaceutical heroin for medical co-prescription to opioid dependent patients in methadone maintenance treatment

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Submitted for publication

Abstract

Presently, there is a considerable interest in heroin-assisted treatment: (co-)prescription of heroin to certain subgroups of chronic, treatment-resistant, opioid-dependent patients. In 2002, nine countries had planned (Australia, Belgium, Canada, France, Spain) or ongoing (Germany, The Netherlands, Switzerland, United Kingdom) clinical trials on this subject. These trials (and the routine heroin-assisted treatment programs that might result) will need pharmaceutical heroin (diacetylmorphine) to prescribe to the patients. Research into the development of pharmaceutical forms of heroin for prescription to addicts can profit from the large amount of knowledge that already exists regarding this substance. Therefore, in this paper we review the physicochemical and pharmaceutical properties of diacetylmorphine and the clinically investigated routes of administration. Routes of administration utilised on the street and the properties of street heroin are also discussed. Pharmaceutical heroin has to comply with the usual requirements of efficacy, safety, and quality of pharmaceutical products, but acceptability to patients is also an important requirement. Especially since heroin-assisted treatment is aimed at treatment-resistant addicts, who often have to be encouraged to participate (or to maintain participation) in a treatment program. This means that the most suitable products would have pharmacokinetic profiles mimicking that of diacetylmorphine for injection, with rapid peak concentrations of diacetylmorphine and 6-acetylmorphine, ensuring the 'flash effect' and the sustained presence of morphine(-6-glucuronide) creating the prolonged euphoria. Diacetylmorphine for inhalation after volatilisation (via 'chasing the dragon') seems to be a suitable candidate, while intranasal and oral diacetylmorphine are currently thought to be unsuitable. However, oral and intranasal delivery systems might be improved and become suitable for use by heroin dependent patients.

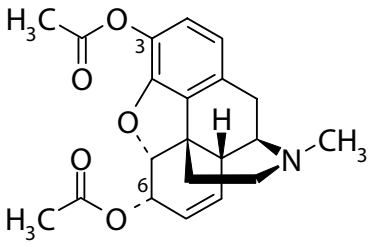
Introduction

Heroin (3,6-diacetylmorphine, acetomorphine, diamorphine) is a di-ester of morphine that was introduced into medicine by Bayer in 1898, as a cough suppressant to assist breathing in patients with severe lung disease [1]. It was known to be twice as potent a cough suppressant as morphine, but its analgesic potency (2-3 times that of morphine [2]) was only recognised decades later, when it had been banned from prescription in many countries due to its addictive properties [1]. Heroin is now one of the best known drugs of abuse, that it is included in the United Nations list of Narcotic drugs under international control [3]. However, heroin was not banned from medical practice completely, the drug and its preparations were still included in the national pharmacopoeias of 18 countries in 1953: Argentina, Austria, Belgium, Brazil, Finland, France, Germany, Greece, Italy, The Netherlands, Paraguay, Portugal, Romania, Switzerland, Turkey, Union of Soviet Socialist Republics, and the United Kingdom [4] and it is still present in the British Pharmacopoeia today [5].

Nowadays, addiction has been accepted as a psychiatric disorder and several pharmacological treatments have been developed to treat addiction to opioids. In the last decade, attention has also turned to heroin-assisted treatment: (co-)prescription of heroin to certain subgroups of chronic, treatment-resistant, opioid-dependent patients. In 2002, nine countries had planned (Australia, Belgium, Canada, France, Spain) or ongoing (Germany, The Netherlands, Switzerland, United Kingdom) clinical trials [6]. Most heroin-assisted treatment programs involve methadone with co-prescribed injectable heroin, although heroin tablets (UK, Spain) and cigarettes (UK, Switzerland) are also used. Street heroin is most commonly injected, snorted or smoked. The first route of administration poses little problems in heroin-assisted treatment programs, because parenteral use of diacetylmorphine is well-established in the UK, diacetylmorphine is administered intravenously, intramuscularly, subcutaneously or epidurally for chronic cancer pain, pain relief after myocardial infarction, (post)operative analgesia or in patient-controlled analgesia. However, it has proven more difficult to provide addicts that are used to snorting or smoking their street heroin with a suitable pharmaceutical alternative. In addition, alternative dosage forms could prove useful, even in countries where these routes of administration are unpopular compared to injecting, because they could be used by patients who wish to change their route of administration in order to avoid the risks associated with injecting or because of damaged veins. For the same reasons, non-injectable pharmaceutical dosage forms of diacetylmorphine could be stimulated in heroin-assisted treatment programs.

Research into the development of pharmaceutical forms of diacetylmorphine for prescription to addicts can profit from the large amount of knowledge that already exists regarding this substance. Therefore, in this paper we review the physicochemical and pharmaceutical properties of diacetylmorphine and the clinically investigated routes of administration. Routes of administration utilised on the street and the properties of street heroin are also discussed, because they have inspired the presented publications on pharmaceutical heroin for prescription to addicts.

Figure 1: Molecular structure of diacetylmorphine



Properties of diacetylmorphine

Physicochemical properties

Diacetylmorphine is a morphine ester, its synthesis involves replacement of the two hydroxyl groups at the 3 and 6 position of the morphine molecule by acetyl groups, for example via a simple procedure involving heating morphine with an excess acetic anhydride at 120°C for 5 min [7] (Figure 1). Diacetylmorphine synthesis using a combination of acetic anhydride with pyridine, benzene or sodium acetate has also been reported, as well as the use of a catalyst [8]. 3-Acetylmorphine was reported to be an intermediate in the acetylation reaction [8,9]. A base form exists, but the hydrochloride monohydrate salt is much more common in pharmaceutical dosage forms. Diacetylmorphine is a lipophilic substance with a partition coefficient (log P(octanol/water) = 52) between that of morphine (log P = 6) and fentanyl (log P = 955). As the pK_a of diacetylmorphine (7.6 [2]) is close to physiological pH, a large proportion is present in the lipophilic non-ionised form, favouring absorption, while the drug also profits from the excellent water solubility of the ionised form. Diacetylmorphine base is soluble in chloroform, alcohol and ether, but its solubility in water is only 1 gram/1700 mL, while diacetylmorphine hydrochloride is soluble in 2 parts water [10]. The melting point of diacetylmorphine base (173°C [10]) is lower than that of the hydrochloride salt (243-4°C [10], 229-233°C [2,7,11]), favouring its use in smoking or 'chasing the dragon' (see *Smoking heroin*). Two polymorphic forms have been reported for diacetylmorphine: form I, shaped like rods, oblique plates and needles with a melting point of 172-173°C and II, consisting of sperulites, melting at 168°C. Form II is readily converted to form I [12].

Stability

Hydrolysis is the main mechanism of degradation for diacetylmorphine. In aqueous solutions, the rate of hydrolysis depends on temperature and pH [13-15]. Partial hydrolysis results in 6-acetylmorphine, complete hydrolysis yields morphine. Maximal stability of diacetylmorphine in aqueous solution was found to be at pH 4-4.5 [13].

Diacetylmorphine base is known to turn pink and emit an acetic odour on prolonged exposure to air [10]. The discolouration might be due to oxidation, as it is a morphine derivative and morphine is known to be an oxygen sensitive drug [16]. Degradation of morphine in aqueous solutions to pseudomorphine and morphine-N-oxide is known to depend on the presence of oxygen and pH and such solutions show a yellow to brown discolouration. The extent of degradation of morphine into pseudomorphine has been associated with the degree of discolouration, but as this is a colourless substance it cannot be the cause. Pseudomorphine (a dimer) cannot be formed from heroin, because it lacks the free phenolic hydroxyl group [16].

Pharmacokinetics and pharmacodynamics

As mentioned above, the lipophilic nature of diacetylmorphine combined with its near-physiological pK_a results in rapid absorption into the systemic circulation after administration and in rapid distribution into the tissues. These topics (absorption and distribution) will be discussed in more detail in the sections on the different routes of administration.

Diacetylmorphine has a very short half-life in the circulation, due to rapid conversion to 6-acetylmorphine and morphine by esterase enzymes that are present in the blood (plasma and erythrocytes), the liver, and the brain [17-19]. Both substances are conjugated in the liver into 6-acetylmorphine-3-glucuronide and morphine-3- and -6-glucuronide, respectively. These hydrophilic compounds are subsequently excreted in urine [20-22]. Minor metabolites found in urine after diacetylmorphine intake include: normorphine-glucuronide, 6-acetylmorphine-3-glucuronide, normorphine [20], morphine-3,6-diglucuronide, and morphine-3-ethersulphate [23]. Results of pharmacokinetic studies will be discussed with each route of administration.

Diacetylmorphine is assumed to pass the blood-brain-barrier rapidly due to its lipophilicity, resulting in an almost instant effect. However, as binding to μ-receptors requires a free phenolic hydroxyl (3-OH) group in the morphinan structure, it is likely that diacetylmorphine does not bind these receptors and actually acts as a pro-drug for 6-acetylmorphine [24-26]. 6-Acetylmorphine and morphine should therefore be considered active metabolites of diacetylmorphine [24-28]. Even though the mechanism by which opioids produce euphoria is not entirely clear, μ-receptors in the brain seem to be involved, as well as dopaminergic neurons [26,29]. Stimulation of the μ-receptors in the ventral tegmental area of the brain is thought to lead to inhibition of GABA-ergic neurons, which in turn leads to cessation of the tonic inhibition of dopamine production in the nucleus accumbens and the mesocortical structures, resulting in the rewarding 'flash' effect [29]. 6-Acetylmorphine was found to be more potent at the μ-receptor than morphine [24,25]. It is therefore not surprising that the maximum concentrations of diacetylmorphine and 6-acetylmorphine in plasma seem to be related to the almost instant 'flash' or 'high' effect that occurs when addicts inject or inhale heroin. However, the exact relationship between plasma concentrations and effect remains a difficult issue to investigate. Peak plasma concentrations (C_{max}) of diacetylmorphine are difficult to determine exactly, because

of its rapid absorption and very short half-life in plasma. The C_{max} that is reported in studies on diacetylmorphine should therefore be considered an apparent C_{max} that is determined largely by the sampling schedule used and that is subject to considerable variation due to patient characteristics and sample timing with regard to dosing. Exposures to 6-acetylmorphine and morphine (glucuronides) are likely to be less variable and are preferred for the study of pharmacokinetic-pharmacodynamic relationships.

The estimated lethal dose of diacetylmorphine is 200 mg, but addicts may be able to tolerate up to 10 times as much. Fatalities have occurred after doses of 10 mg [2]. Doses of 5-10 mg (i.m./s.c.) every 4 hours are commonly used for analgesia [30]. Adverse effects of diacetylmorphine are similar to those of other opioid analgesics, with a larger potential for abuse, and with nausea, allergic reactions and hypotension occurring less commonly than with morphine. Pulmonary oedema can occur in addicts after an overdose. Most other adverse effects reported involve its abuse in an illicitly obtained and adulterated form [30].

Routes of administration

Clinical use

Clinically, diacetylmorphine is mostly used parenterally: in the UK, diacetylmorphine hydrochloride is licensed for use in the treatment of moderate to severe pain associated with acute myocardial infarction, surgical procedures and terminal illness and for the relief of dyspnoea in acute pulmonary oedema [31]. According to the market authorisation, it may be administered by intravenous, intramuscular, or subcutaneous route [31], but epidural administration of diacetylmorphine for post-operative or cancer pain has also been described extensively [32-36]. Diacetylmorphine hydrochloride is preferred over morphine for its superior solubility, its potency (2-4 times morphine) and a faster onset of action [26,37]. Furthermore, lipophilicity is considered preferable for opioids administered extradurally and intrathecally [32].

Tablets containing diacetylmorphine hydrochloride have market authorisation in the UK (Aurum Pharmaceuticals, no 12064/0001) for the relief of severe pain, particularly in terminal care, myocardial infarction, left ventricular pain and pulmonary oedema. Diacetylmorphine is also a component of the so-called Brompton mixture (aqueous mixture of variable composition, containing diacetylmorphine or morphine, cocaine and alcohol), that is in use with patients suffering from chronic severe pain in the UK and Canada [38]. Oral diacetylmorphine is considered to be 1.5 times more potent than morphine sulphate, which was suggested to be due to better absorption in the gastrointestinal tract [27,39,40]. It is however not surprising that its pharmacological effects are no different from morphine (when the potency difference was accounted for)[37], since first-pass metabolism was found to completely convert diacetyl-morphine into morphine after absorption [27].

Lipophilic opioids were also found to be absorbed better in the mouth in a study on sublingual absorption of opioids, but absorption of diacetylmorphine was not better than that of morphine, with a bioavailability of only 9% compared to intramuscular administration [41]. A comparison of inhaled (nebulised) morphine and diacetylmorphine solutions showed improved bioavailability due to its higher lipophilicity, which the authors believed could be promising results for inhalation as a route of administration of opioids to shocked patients [42]. Intranasal administration of diacetylmorphine (as nasal spray or drops) has been found safe, effective en acceptable for use in paediatric analgesia [43,44] and it has been adopted for this purpose in 16 of the larger emergency departments of hospitals in the UK since [31]. Furthermore, intranasal diamorphine in a special spray device for patient controlled analgesia was tested for postoperative pain and was found to be effective and well tolerated [45], but less effective compared to intravenous administration [46]. The lipophilic nature of diacetylmorphine could also explain its effect in suppressing pressure ulcer pain after dermal application as a gel [47]. Local application of diacetylmorphine as an analgesic has also been described for relief of bladder spasm (due to bladder carcinoma): intravesical administration of diacetylmorphine was found effective [48].

Injecting heroin

Intravenous injection is the most widely used mode of administration of heroin as a drug of abuse: In many EU countries, 60-80% of the heroin users in treatment predominantly injected the drug (data 1990-2001,[49]). However, the proportion of injectors varies considerably between countries and has changed over time, with levels of injection falling in almost all countries during the 1990s, although there is some evidence of more recent increases. Intravenous use of heroin is uncommon in Portugal and The Netherlands (10-15%) and has shown a large decrease (±60 to ±25%) in Spain. In 2002, about half of the heroin users in the EU predominantly injected [49]. The popularity of intravenous use of heroin was reflected in the prescription patterns of doctors in the UK: 92% of the (few) prescribing doctors prescribed diacetylmorphine to addicts as ampoules of freeze-dried powder for injection [50].

The pharmacokinetics of injected heroin probably account for much of its popularity: intravenous injection of diacetylmorphine rapidly results in peak plasma concentrations of diacetylmorphine (1.1-2.8 min) and 6-acetylmorphine (0.7-2.7 min) [51-53], that are often associated with the 'flash' or 'rush' effect. Both substances are hydrolysed rapidly, resulting in short half-lives, 1.3-3.8 min for diacetylmorphine [51-54] and 9.3-49 min for 6-acetylmorphine [52-54]. Morphine peak concentrations generally occur after 3.6-7.8 min and it is detectable in plasma for much longer (T½ 109-287 min) [51-53]. The same is true for the active conjugate, morphine-6-glucuronide (T_{max} 1 hour, T½ 4 hours) [53].

Intravenous drug use is considered the most harmful route of administration, for its many possible complications. Most of these result from the bad quality of the product

(impurities, adulterants, diluents, contamination with micro-organisms) and problems with the administration paraphernalia (contaminated needles, syringes, or acid solution). Problems of infection are the most common, for example: abscesses, collapsed veins, necrosis, sepsis, and endocarditis. However, many of these complications could be prevented, if pharmaceutical quality diacetylmorphine for intravenous administration would be used. This leaves the increased risk of overdose that is associated with intravenous drug use, as the most important disadvantage.

Smoking heroin

The term 'heroin smoking' is often used, but its exact meaning is not always clear, as two major types of heroin smoking can be distinguished: 'chasing the dragon' or smoking cigarettes containing heroin ('ack ack' for example). For reasons of clarity, in this paper the first will be termed 'inhalation after volatilisation', since smoking implies the use of cigarettes or burning, while 'chasing the dragon' (performed correctly) involves only volatilisation and inhalation of the vapours. The first description of heroin inhalation after volatilisation (Shanghai, 1920s) involved heating heroin pills in porcelain jars and inhaling the fumes through a bamboo tube [55]. This procedure was refined into what is now known as 'chasing the dragon': heating heroin on aluminium foil using a cigarette lighter and inhaling the fumes by mouth through a straw or tube. Movement of the melted substance over the surface of the foil and careful application of heat are attempts to obtain optimal control over the volatilisation process and to minimise charring. Over the years, 'chasing the dragon' has spread from South East Asia to several countries in Europe (The Netherlands in the 1970s, UK in the 1980s and Spain and Switzerland in the 1990s) [55] and it is still gaining in popularity [49]; in 2001, about 45% of the European addicts in treatment predominantly smoked heroin in this way [49].

Inhalation of diacetylmorphine has several advantages over intravenous administration. Inhalation of a given dose takes more time, which leads to increased control and less risk of overdose compared to injection of a bolus dose. In addition, the onset of intoxication will lead to respiratory depression which in turn automatically leads to a reduction of the heroin intake and the prevention of a serious overdose. It is a non-invasive route of administration with a much lower risk of infection and better social acceptability in some cultures. Furthermore, toxicity due to systemic exposure to impurities and adulterants present in street heroin is less likely, since many will not be inhalable and therefore not be available for absorption in the airways. On the other hand, heating may cause degradation of diacetylmorphine (hydrochloride) and the additives present in street heroin may also be susceptible to degradation and/or pyrolysis, which could lead to formation of volatile, toxic substances [56]. Such substances have been suggested as a cause for the occurrence of spongiform leuko-encephalopathy, a serious and rare, but recurrent toxicity that has been attributed to inhalation of heroin vapour, even though some reports of this toxicity involved injected heroin overdose [57] and snorted heroin [58]. It was first recognised in The Netherlands, where 47 cases were reported in 1981 [59], and since

then, reports from other parts of the world (Europe [60-62], the US/Canada [63-65]) have been published. The estimated mortality rate of 25% associated with spongiform leukoencephalopathy [59] has attracted much attention to this complication, that should however be considered very rare: less than 100 cases were reported in 18 years [65], while inhalation of heroin vapours was already quite common among addicts during that period, especially in Asia, where it was reported only once [66]. The cause for this condition was thought to be a toxin present in street heroin, but it was not identified, nor was the condition reproducible in animals exposed to heroin pyrolysate from suspect street heroin samples [59].
Heroin smoking was reported to have negative consequences for the pulmonary function of patients: chronic heroin smoking was related to an impaired lung function and a higher prevalence of dyspnoea [67]. However, almost all patients in this study also had a history of smoking tobacco, which caused part of the impairment of the lung function. Therefore, the authors concluded that further research is needed to quantify the separate effects of heroin smoking and tobacco smoking [67].

Snorting heroin

Intranasal use of opioids was the most common route of administration in the United States before 1930 (when intravenous use of heroin became popular) and snorting heroin made a comeback in the US around 1990 [68]. It is not very common in Europe, about 4% of the addicts in treatment use their heroin intranasally [49]. Sniffing heroin is thought to be a phase of involvement with heroin, in which the habit is developed and after which a transition to other modes of administration is often made [69]. The pharmacokinetic profile of intranasal administration is similar to the intramuscular route, with a relative potency of 50%. Even though lower blood concentrations and a slower onset of action are achieved compared to the intravenous route, adequate efficiency combined with reduced fear of infection and a non-invasive nature make intranasal administration an attractive alternative for injection of heroin [70].

Street heroin

A wide variety of street heroin types exist, differing in appearance (powder or course granules, different colours: white, brown, pink, red) and chemical composition. Studies suggest that different types are either more suitable for injection (‘white heroin’) or more suitable for smoking (‘brown heroin’) [71]. However, both brown and white heroin are used for injection. White heroin usually comprises heroin in hydrochloride form, which has good solubility, supporting its reputation of being particularly suitable for injection. Brown heroin, however, may be in the form of the hydrochloride or the base, which explains the need for acid in the preparation of an injection, because this results in conversion from diacetylmorphine base into the more soluble salt. The ‘cook-up procedure’ of injectable heroin concerns dissolving it in water using heat, and addition of acid (citric or ascorbic acid) to brown heroin. Heating the mixture reduces the likelihood of viral transmission, but the addition of

acidifiers, especially less common alternatives like vinegar and lemon juice, can cause additional problems of infection (disseminated candidiasis) due to contamination with molds or yeasts [71,72]. Reports of infection due to contaminated heroin have led to studies into the microflora of street heroin samples [73,74]. Several species of micro-organism were identified: *Aspergillus* spp. [74], *Bacillus* spp. [74,75], *Clostridium* spp., and *Staphylococcus* spp. [73]. *Bacillus cereus* found in a sample of street heroin was identified as the same strain that had caused crepitant cellulitis in the intravenous drug user that had used it [76].

The chemical composition of street heroin not only varies with diacetylmorphine being present as the salt or the base, but also in the amounts and identities of diluents and adulterants added. Many studies on the purity of street heroin have been published, and generally about 35-45% of a sample of brown heroin is identified as diacetylmorphine (hydrochloride) [77-81]. White heroin is usually much purer, with diacetylmorphine hydrochloride contents up to 85-95% [81,82]. The exact qualitative and quantitative composition of the rest of the samples is less well documented. Substances present in street heroin besides diacetylmorphine (hydrochloride) can be divided into manufacturing impurities, diluents and adulterants. The first category consists of morphine, codeine, papaverine, noscapine, acetylmorphine, acetylcodeine, etc.: active substances originating from the opium or morphine that was used in the synthesis of heroin and intermediates from the acetylation process. The second category comprises mainly sugars (glucose, lactose, sucrose, mannitol) that are used as inert bulking agents. Adulterants can be active drugs (paracetamol, caffeine, phenobarbitone, methaqualone, procaine, strychnine, quinine, piracetam), which, like heroin, have a bitter taste or that may mimic some of its effects [77-81,83]. The presence of some of the diluents and adulterants has been shown to affect the volatilisation process (while 'chasing the dragon'): caffeine, methaqualone, [56] and barbital [21,56] were found to increase the recovery of diacetylmorphine in the vapours after volatilisation. Furthermore, it is likely that the toxicity of street heroin used via 'chasing the dragon' is influenced by the presence of impurities, diluents and adulterants. For example, cotarnine could be formed on heating a sample containing noscapine hydrochloride, which is reported to give highly toxic fumes [56].

Pharmaceutical heroin for prescription to addicts

A growing number of (European) countries are developing programs for (the study of) heroin-assisted treatment for addicts (Germany, The Netherlands, Spain, Switzerland). Main goals are usually related to harm reduction by providing addicts with pure medication, hygienic circumstances, medical supervision and (compulsory) psycho-education and psychosocial support [6,49]. Suitable forms of pharmaceutical heroin are obviously needed for such programs, but surprisingly little has been published on this subject. Injectable and smokable forms of diacetylmorphine were expected to be required most frequently in heroin-assisted treatment programs,

considering the patterns in the routes of administration of heroin. In the EU, most of the addicts in treatment inject (±45%) or smoke (±45%) heroin and ±10% uses the oral route [49]. In the UK, licensed doctors prescribe diacetylmorphine to addicts in ampoules (92%), tablets (32%), reefers (marijuana cigarettes, 16%), powder (11%) or as a solution (5%), which were dispensed for unsupervised consumption at home, usually daily [50]. In a pilot study in Switzerland, prescription of intravenous, oral, or smoked diacetylmorphine was possible; 77% of the patients preferred injection [84].

An important requirement for pharmaceutical heroin would be its acceptability to clients, since heroin-assisted treatment is usually only an option for treatment-resistant addicts that have to be encouraged to participate in a treatment program. Furthermore, pharmaceutical heroin would have to comply with the usual requirements of efficacy, safety, and quality of pharmaceutical products. With regard to acceptability to clients, rapid delivery of unchanged diacetylmorphine and/or 6-acetylmorphine to the circulation seems to be an important pharmacokinetic requirement for diacetylmorphine for prescription to addicts. Addicts dissatisfied with using methadone or morphine replacement therapy report missing the 'flash' or 'rush' effect that is generally associated with the rate of achieving high diacetylmorphine or 6-acetylmorphine peak concentrations. Fast and sufficient delivery of diacetylmorphine and/or 6-acetylmorphine to the circulation is a prerequisite to ensure rapid absorption into the brain, where 6-acetylmorphine activity is superior to that of morphine [24,25].

Diacetylmorphine for injection

The safety and efficacy of diacetylmorphine for injection are not questioned, as marketed forms of this product already exist that have proven to be safe and effective. Ampoules containing 5, 10, 30, 100, and 500 mg of lyophilised diacetylmorphine hydrochloride have a market authorisation in the UK for use as an analgesic (manufacturers: Aurum, Berk, CP, Evans, Hillcross); especially the larger doses would be suitable for prescription of injectable heroin to addicts. The contents of these ampoules can be reconstituted with water, a 5% dextrose solution or a sodium chloride 0.9% solution [85]. Pharmaceutical quality of a newly developed form of diacetylmorphine for injection can be ensured by validation of the production process and following the quality control guidelines in the British Pharmacopoeia, for the bulk substance and the final product [86]. The stability of lyophilised formulations for diacetylmorphine was studied extensively by Poochikian et al. [14].

Heroin-assisted treatment programs for addicts in Switzerland use specially manufactured multi-dose 10 g ampoules of lyophilised diacetylmorphine hydrochloride for administration of average dosages of 500-700 mg per day [90,91]. The Dutch Heroin trial also uses multi-dose vials containing 3 grams of lyophilised diacetylmorphine hydrochloride to be reconstituted with 18 mL of Water for Injection [92]. The resulting 150 mg/mL solution is used for aseptic preparation of patient specific dosages up to 400 mg per gift and up to 1000 mg per day. Details on the lyophilisation process, the stability of the formulation solution and the final product

Table 1: Comparison of DAM inhalation techniques.

Study	**Rook et al. [53]**	**Rook et al. [53]**	**Mo et al. [21]**	**Mo et al. [21]**	**Stalder [87]**	**Jenkins et al. [54]**	**Speich [88]**	**Hendriks et al. [89]**	**Speich [88]**
Method	Chasing	Chasing	Chasing	Cigarette[1]	Cigarette[2]	Device[3]	Device[4]	Device[5]	Nebulisation
Number of patients	9	74	35	14	2	2	2	5	1
Dose	200-300	66-450	150-450	225-600	100 (*500)	10.5	100-300	50	536
Heroin type	A1	A1	D1	D2	B	B	A2	A3	C
Temperature				>500°C	>500°C	200°C	275°C	300°C	Amb.
In vitro recovery			68%	19%	2%	89%	53%	41%	45%
Bioavailability	52%	53%	38%	21%		12-324%	37%	38-45%	58%
Diacetylmorphine									
C_{max} *(µmol/L)*	1.85				1.27	0.551	0.52		0.91
T_{max} *(min)*					2.2	1-5	9.5		5.6/30.7
AUC *(hr·µmol/L)*	0.47				0.08	0.041	0.14		0.73
T½ *(min)*	3.2	7.6			2.5	3.3	4.3		
Morphine AUC *(hr·µmol/L)*	3.65				1.59*		1.06		2.17

[1] tobacco; [2] woodruff; [3] computer controlled heating device with nichrome wire coil; [4] TAS-oven heating device; [5] laboratory heating device with brass/aluminium sample holder;
Heroin types: A = diacetylmorphine base / caffeine anhydrate (A1 = 3:1 powder; A2 = 2:1 tablets; A3 = 1:2 tablets); B = diacetylmorphine base; C = diacetylmorphine hydrochloride, 173 mg/mL in aqua bidest; D = street heroin (D1 = 64% pure diacetylmorphine base; D2 = 92% pure diacetylmorphine hydrochloride)

and on the stability and antimicrobial properties of the reconstituted product have been published [93].

Diacetylmorphine for inhalation

Inhalation is gaining popularity as a route of administration for systemically acting drugs, which is mainly due to the large tissue area that is available for absorption of drugs in the airways. This provides a quick and non-invasive way of delivering drugs to the general circulation. Furthermore, blood flow from the lungs is directed straight to the brain, which makes a fast onset of action possible for centrally acting drugs. Nicotine uptake via cigarettes is the best-known practical example, but there are also reports of inhaled opioids. Morphine has been studied after administration as an aqueous aerosol from a nebuliser [42,94-96] or from a unit dose aerosol delivery system [97,98]. Bioavailability of nebulised morphine was found to be 5.5% [99] and 17% [94], while almost instantaneous absorption of significant amounts of morphine was reported for morphine dose aerosols with bioavailabilities of 59% [98] and 100% [97]. Administration of nebulised morphine-6-glucuronide resulted in only 6% bioavailability and maximum concentrations were observed after as long as 1.2 hours. Some of these differences in bioavailability can be attributed to the lipophilicity of the compounds; a more lipophilic substance is likely to show better absorption via the lungs. This assumption is supported by the rapid absorption ($T_{max} = 2$ min) of fentanyl, the most lipophilic opioid, after inhalation of an aerosol [100]. As diacetylmorphine is more lipophilic than morphine, its bioavailability per inhalation can be expected to be similar or better than found for morphine.

When different methods for inhalation of diacetylmorphine are compared, it is important to remember what goals should be achieved. Obviously, maximum amounts of unchanged diacetylmorphine from the dosage form should be made available for inhalation (recovery in vapour). Furthermore, for addicts, an efficient method for inhalation should achieve two goals: quick appearance of large enough peak concentrations of diacetylmorphine and 6-acetylmorphine for the 'flash' effect and sufficient exposures to the other metabolites for prolonged euphoria [52]. The results of several in vitro and in vivo studies into the efficiency of using heroin via inhalation are summarised in Table 1 and Table 2; they will be discussed in detail in the following three paragraphs.

Smoking

Development of pharmaceutical heroin for smoking has an inherent safety problem, since smoking is known to be unsafe, due to inhalation of harmful substances like tar and carbon monoxide. It could be argued that most addicts smoke tobacco cigarettes anyway, and that their common practice of drug use on the streets is not very safe either, but ethically the medical prescription and dispensing of an unsafe pharmaceutical product is unacceptable. Efficacy of smokable diacetylmorphine will probably also suffer from the 'burning' aspect of this mode of administration, since diacetylmorphine is likely to burn and degrade during the smoking process in which very high temperatures are reached.

The oldest study into the pharmacokinetics of smoked heroin is a comparison of 'chasing the dragon' and a procedure called 'ack ack', in which cigarettes dipped in street heroin are smoked [21]. The authors tested bioavailability via determination of total morphine concentrations after smoking and injecting heroin (68% of heroin dose recovered as morphine in urine) and found chasing to be a more efficient smoking method (26%) than 'ack ack' (14%) [21]. This could be explained by a more extensive degradation of heroin smoked via cigarettes, since much higher temperatures are involved. Moreover, heroin could have burnt up, which is unlikely to occur in 'chasing the dragon' as that technique is aimed at applying just enough heat for volatilisation and preventing burning. Furthermore, in this study street heroin containing diacetylmorphine base was used for 'chasing the dragon', while 'ack ack' involved dipping a cigarette in street heroin containing diacetylmorphine hydrochloride, which is known to be less suitable for smoking [21,56].

Despite the disadvantages of smoking diacetylmorphine mentioned above, a Swiss study into pharmaceutical smokable heroin for prescription to addicts was performed [87]. These cigarettes have also been dispensed to addicts in heroin-assisted treatment, because no better alternative was available [91]. Special impregnated woodruff cigarettes (without nicotine) were developed, that contained 100 mg diacetylmorphine base (from a 200 mg/mL solution in dichloromethane). These cigarettes showed low recoveries of diacetylmorphine and 6-acetylmorphine in an vitro smoking experiment: 2.2% and 5.5%, respectively [87]. Smoking efficiency depended on the duration and number of inhalations per min. Stability of diacetylmorphine base in the woodruff cigarettes was limited: after storage for 60 days at room temperature (in the dark, vacuum packed), the diacetylmorphine content declined to 88.6% [87].
Analysis of the smoke (using gas chromatography with mass spectrometric detection) showed 6-acetylmorphine, morphine, 3-acetylmorphine and N,6-diacetylnormorphine as degradation products of diacetylmorphine [87]. In vivo pharmacokinetic studies were performed in two female addicts that were selected from the population in heroin-assisted treatment. They each smoked five cigarettes in a standardised way: 4 inhalations of 5 sec each per min. After smoking one cigarette (100 mg), diacetylmorphine and 6-acetylmorphine AUCs were 0.08 and 0.07 hr·µmol/L, respectively (Table 1). These exposures were relatively high, compared to the other methods, which was unexpected, considering the low recoveries of these analytes in the smoke. This might be explained by the fact that the Swiss researchers collected 5 plasma samples in the first 5 min after the start of smoking [87]. In the other studies, fewer samples from this period were available, resulting in underestimation of the AUCs of diacetylmorphine and 6-acetylmorphine (Table 1). Exposures to morphine (1.6 hr·µmol/L) and morphine-3- and –6-glucuronide (9.1 and 3.2 hr·µmol/L, respectively) after 5 woodruff cigarettes (500 mg) were only 27-55% of those found after 'chasing the dragon' by Rook et al. (dose 200-300 mg, [53]), suggesting that smoking diacetylmorphine cigarettes is very inefficient.

Inhalation after volatilisation

Inhalation of diacetylmorphine after volatilisation is probably safer than smoking of diacetylmorphine, since no burning is involved, thereby avoiding inhalation of carbon monoxide and tar or soot. On the other hand, high temperatures are needed for efficient volatilisation, which could lead to formation of (possibly toxic) degradation products that could be inhaled alongside with diacetylmorphine. However, formation of toxins is more likely on heating a mixture of substances, such as street heroin, because the constituents could interact chemically. This has also been considered as an explanation for the rare occurrences of spongiform leukoencephalopathy in addicts inhaling heroin vapours (see *Street heroin*). Considering the above, diacetylmorphine for inhalation after volatilisation can be regarded as an option for the development of non-injectable pharmaceutical heroin for prescription to heroin-dependent patients.

Higher efficiency is expected for inhalation of volatilised diacetylmorphine compared to smoking, as the temperatures involved in volatilisation will be lower, with less decomposition of diacetylmorphine. Furthermore, volatilisation of diacetylmorphine base is more efficient than diacetylmorphine hydrochloride. Thermal analysis and in vitro studies simulating 'chasing the dragon' have shown that diacetylmorphine base is less susceptible to degradation upon heating and its volatilisation results in more unchanged diacetylmorphine in vapours [56](Table 2). Moreover, as mentioned under *Street heroin*, additives could influence inhalation efficiency positively: recovery of unchanged diacetylmorphine in the vapours after volatilisation increased in samples containing caffeine, barbital, or methaqualone [56](Table 2). Even though the sedative properties of the latter are probably also appreciated by users of street heroin, in diacetylmorphine for inhalation after volatilisation additives without synergistic pharmacological activity would be preferred.

The efficacy of inhalation of diacetylmorphine vapours is also likely to depend on the method used to heat the product. Specific techniques exist for 'chasing the dragon', suggesting that heating diacetylmorphine for inhalation of the vapours requires certain skills. Many heroin addicts have developed tricks and habits in their 'chasing technique' that serve to minimise the loss of heroin vapour through charring, combustion and fumes escaping inhalation via the straw. Mimicking these street habits (that have evolved over decades) could be a suitable starting point for development of a method for volatilisation of pharmaceutical heroin that is acceptable to the users. However, it would be difficult to develop a heating device that would incorporate all these 'tricks of the trade', especially the movement of the molten substance. Addicts testing a heating device for inhalation of diacetylmorphine after volatilisation expressed concerns of loss of vapour, due to the lack of movement [89]. Apparently, moving the molten substance is a way of ensuring a controlled release of vapour in the form of a neat 'dragon's tail', that is easy to inhale completely. Volatilisation without movement caused the vapours to appear as a broad smoke column or cloud, that was difficult to inhale efficiently [89].

Table 2: Results of in vitro experiments on the recovery of heroin (% unchanged) after volatilisation.

Sample	Temperature	Recovery	Study
Diacetylmorphine hydrochloride	200°C	28%	Jenkins et al. [54]
	2-300°C	10%	Cook et al. [101]
	-	17%	Huizer et al. [56]
with caffeine (1:1)	-	36%	Huizer et al. [56]
with barbital (1:1)	-	33%	Huizer et al. [56]
Diacetylmorphine base	200°C	89%	Jenkins et al. [54]
	2-300°C	65%	Cook et al. [101]
	>400°C	<30%	Cook et al. [101]
	-	62%	Huizer et al. [56]
with caffeine (1:1)	-	76%	Huizer et al. [56]
with methaqualone (1:1)	-	55%	Huizer et al. [56]

Furthermore, movement of the molten heroin might prevent overheating and subsequent decomposition of the drug.

Only two pharmacokinetic studies have been performed with addicts using heroin via 'chasing the dragon' (Table 1). An early study compared three modes of administration: injecting, 'chasing the dragon' and 'ack ack' (smoking heroin via a tobacco cigarette) by addicts using the corresponding types of street heroin [21]. Administering heroin via 'chasing the dragon' was found to be about 1/3 as effective as intravenous heroin and about twice as effective as smoking heroin from a cigarette, based on total morphine concentrations in urine. More recent pharmacokinetic studies, comparing intravenous administration to inhalation via 'chasing the dragon' found 52% bioavailability for the latter [53]. Addicts in this study inhaled pharmaceutical inhalable heroin (a 75% w/w diacetylmorphine base / 25% caffeine anhydrate powder mixture) instead of street heroin containing diacetylmorphine hydrochloride in the early study. No statistically significant differences in the half-lives of diacetylmorphine, 6-acetylmorphine, morphine and morphine-3- and -6-glucuronide in plasma were found between injected or inhaled diacetylmorphine [53]. Surprisingly, no difference was found in the subjective appreciation of the diacetylmorphine between the groups, even though equal doses were used and plasma concentrations of diacetylmorphine and metabolites were much lower in the inhalation compared to the injecting group. The authors suggest that this was due to the lack of cross-over comparison of administration methods; each patient used diacetylmorphine via his usual route of administration and therefore also rated its effect compared to what he was used to. Both methods showed dose-related craving and appreciation upon double-blinded variation of dose (dose range 66-150% of regular dose). Population pharmacokinetic models for

plasma concentrations of diacetylmorphine and its metabolites after intravenous use and after inhalation were published by the same group [53].

Use of a heating device by addicts as an alternative for 'chasing the dragon' was described in three studies (Table 1). In the first of these studies, a computer-controlled device with a nichrome heating coil was used to heat 2.6-10.5 mg diacetylmorphine base to be inhaled by two healthy volunteers [54]. The whole dose was administered in one puff and maximum concentrations of diacetylmorphine and 6-acetylmorphine achieved were 0.045-0.809 µmol/L and 0.043-0.428 µmol/L, respectively, depending on the smoked dose. The diacetylmorphine AUC at the 10.5 mg dose level was also quite high (0.041 hr·µmol/L), considering the low doses that were administered. 'Chasing' a 20-30 times higher dose yielded a diacetylmorphine AUC that was only 10 times higher than with this heating device (Rook et al., Table 1). The apparent efficiency of this method might be explained by the high in vitro recovery of diacetylmorphine from the smoking device (89%, Table 1) [54].
Two Swiss addicts, selected from participants of the heroin-assisted treatment program, were asked to inhale the fumes that resulted from heating 100/50 mg tablets of diacetylmorphine base and caffeine in a TAS-oven apparatus, fitted with an insulated mouthpiece. Caffeine anhydrate was the only additive present in the tablets, and it was added to diacetylmorphine base for its positive influence on volatilisation. Tablets were manufactured manually from granulate prepared using a diacetylmorphine/caffeine powder mixture and a 2% w/w caffeine solution in water. In vitro recovery of diacetylmorphine from heating these tablets in the TAS-oven was 53%. One patient smoked one tablet, the other three; smoking sessions lasted 16-22 min per tablet [88]. The bioavailability was similar to the in vitro recovery (37%) and 'flash' and 'high' effects were achieved. However, maximum concentrations of diacetylmorphine were lower than in the study by Rook et al. [53](Table 1), as was the AUC of morphine, while bioavailability and dosages were similar, indicating that the TAS-oven procedure is not quite as efficient as 'chasing the dragon'. The third study using a heating device for volatilisation of diacetylmorphine reported a bioavailability of the same order (38-45%), based on measurements of total morphine in urine (Table 1) [89].

Nebulisation

In the Swiss study by Speich and co-workers, inhalation after volatilisation and nebulisation were compared in *in vivo* and *in vitro* experiments [88]. *In vitro* tests on 100 and 200 mg/mL solutions of diacetylmorphine hydrochloride in distilled water showed that they were antimicrobially active and stable for 7 days when stored at 4°C in the dark. These concentrated solutions were hyperosmolar, and had pH values of 3-4, near the lower limit of the range suitable for solutions for inhalation (3-8.5 [102]). However, as large doses are required for heroin-assisted treatment and the volume of the aqueous solution to be nebulised is limited (1-3 mL), the authors decided that use of concentrated solutions could not be avoided. Three types of nebuliser were tested, a Pari IS-2 jet nebuliser, a Pari LC-plus jet nebuliser and an

Omron ultrasonic nebuliser. The first was found to result in an aerosol with a mean particle size of 2.4-2.6 µm and 80% of the particles had a size between 0.8-4.8 µm, which led the authors to conclude that it was suitable for delivery to the peripheral parts of the lungs. The other two types of nebuliser resulted in larger and more variable particle sizes. A mean release of 45% diacetylmorphine from the solution in the nebuliser was achieved using the Pari IS-2 apparatus. In vivo tests were limited to a single patient inhaling 536 mg (effective dose: 240 mg) of diacetylmorphine as a 200 mg/mL solution from a Pari IS-2 jet nebuliser (Table 1). The inhalation session lasted for 95 min (with 43 min of actual inhaling), because many breaks were necessary due to the very bitter taste of the inhalation solution. Even though the total exposures to diacetylmorphine and morphine were not much lower than those measured after 'chasing the dragon', the time to reach the first maximum concentration of diacetylmorphine was quite long (5.6 min), while a second C_{max} was measured at 12.7 min after a 6 min break. This pharmacokinetic profile, combined with the bitter taste of the inhalation solution will probably not contribute to the acceptability of this inhalation method in a heroin-assisted treatment program, especially since the latter was reported to lead to nausea and retching [88]. It is likely that the bitter taste of the diacetylmorphine solution is associated with its high concentration, but solutions with lower concentrations cannot deliver large diacetylmorphine doses in a volume suitable for nebulisation. Therefore, nebulisation of diacetylmorphine is not likely to be pursued further for use in heroin-assisted treatment of heroin dependent patients.

Intranasal diacetylmorphine

Intranasal use of diacetylmorphine could be acceptable to addicts in a heroin-assisted treatment program, since it is a well-known route of administration on the streets as well. However, snorting heroin does not give the user the same intense 'rush' feeling that injecting does, the 'high' begins more gradually [69], which might hinder its acceptance by chronic addicts. There is no reason to question the safety of intranasal use of diacetylmorphine, as it has been used clinically, even in children (see *Clinical use*). Diacetylmorphine hydrochloride is considered very suitable for intranasal application, since it is highly water soluble and it is much more lipid soluble than morphine in unionised form [31]. Therefore, only very small volumes are needed to administer a dose intranasally and rapid and good absorption through the nasal mucosa is expected. Subsequently, its lipophilicity will assist distribution into the brain [31]. However, liquid preparations for nasal use by addicts in heroin-assisted treatment are less feasible, due to the large doses needed (up to 300-400 mg). The maximum volume for nasal spray or nasal drops is about 0.1-0.2 mL, since larger volumes are increasingly likely to leak down the back of the nose and be swallowed. Solutions containing 300-400 mg in such a small volume cannot be prepared (diacetylmorphine hydrochloride is soluble in two parts water) and if they were, they would be very viscous and unsuitable for spraying.
Therefore, intranasal use of diacetylmorphine by addicts requires a solid pharmaceutical dosage form, e.g., diacetylmorphine hydrochloride powder, which

can be snorted through a straw in the nose. The only pharmacokinetic studies (in opioid dependent patients) on intranasal use of powdered diacetylmorphine concern quite small doses, mixed with lactose for blinding reasons [68,103,104](Table 3). Snorting diacetylmorphine powder was found to be about half as efficient as intramuscular administration, based on the behavioural and physiological effects [68] as well as based on the ratio of morphine-3-glucuronide AUCs for both routes of administration [103]. Intranasal compared to intravenous administration of diacetylmorphine resulted in 30 times lower diacetylmorphine peak concentrations and longer T_{max}: 4 and 10 min for diacetylmorphine and 6-acetylmorphine respectively, compared to 2 min for both after intravenous administration [104]. Concentrations of diacetylmorphine and 6-acetylmorphine were elevated 3-4 times longer than after intravenous administration. A fourfold difference in potency between the two routes of administration was observed with several pharmacodynamic parameters: i.e. visual analog scale (VAS) ratings of 'high', 'good drug effect', 'drug liking', and 'sedated' [104].

Further investigation of intranasal administration of diacetylmorphine for prescription to addicts is required to clarify the pharmacokinetics of larger doses. Furthermore, intranasal administration might become more acceptable if its pharmacokinetic profile could be modified to be more similar to that of injectable diacetylmorphine, for example by using controlled release techniques. Such a technique was successfully used to achieve an optimal pulsatile and sustained plasma nicotine profile by controlled release of nicotine from a nasal formulation [105]. This research group also reported improving nasal administration of morphine by formulating it as a powder or a solution with chitosan as an excipient (absorption-promoter) [106]. Such absorption-promoters might be used to achieve a 'rush' effect after nasal administration of diacetylmorphine similar to that after injection, which would make intranasal administration much more acceptable to chronic addicts.

Table 3: Comparison of studies on intranasal use of diacetylmorphine powder (snorting).

Study	**Cone et al. [68]**	**Cone et al. [68]**	**Skopp et al. [103]**	**Skopp et al. [103]**	**Comer et al. [104]**
Number of patients	6	6	4	4	6
Dose *(mg)*	6*	12*	6*	12*	50 (12.5-100)*
C_{max} *(µmol/L)*	0.025	0.043	0.042	0.097	0.144
T_{max} *(min)*	<5	<5	<5	<5	4
AUC *(hr·µmol/L)*			0.0066	0.0138	
T½ *(min)*	5.4	4.2	6	4.8	
AUC_M *(hr·µmol/L)*			0.0287	0.0926	

* diacetylmorphine hydrochloride, with lactose added to a total weight of 100 mg; total dose inhaled divided between two nostrils.

Oral diacetylmorphine

Oral administration of diacetylmorphine (as tablets or potion) is known to be a safe and effective route of administration of diacetylmorphine for analgetic purposes (see *Clinical use*). An early study described testing a 1 mg/mL diacetylmorphine solution for stabilisation of opiate abusers and found it to be as effective as oral methadone (1 mg/mL) when used on demand to suppress withdrawal symptoms [107]. Doctors licensed to prescribe diacetylmorphine to addicts in the UK commonly prescribe tablets (32% of them does), probably because it is the most obvious (marketed) alternative for patients that need an alternative to injecting (due to damaged veins). A Spanish protocol for heroin-assisted treatment proposes to study oral diacetylmorphine versus oral methadone [6] and oral formulations of diacetylmorphine were tested for use in the Swiss heroin-assisted treatment program [91].

Capsules, controlled release tablets and rectally administered diacetylmorphine hydrochloride were tested for use by addicts in a two-patient pilot study [52,108]. The origin, composition or exact purpose of the controlled release tablets were not specified, but the dosing schedule suggested that the aim was to achieve delayed release (230 mg, dose interval 12 hours) compared to the capsule formulation (200 mg, dose interval 6 hours) [52]. More details on the dosage forms were provided in the thesis that also described this study [108]. Gelatin capsules were filled with diacetylmorphine hydrochloride powder with mannitol and Aerosil as excipients; the controlled release tablets were designed for delayed release of diacetylmorphine hydrochloride via diffusion from a porous matrix with a film coating, and the diacetylmorphine suppositories contained 400 mg diacetylmorphine hydrochloride in a fatty basis (adeps solidus) [108]. Interestingly, even though no diacetylmorphine or 6-acetylmorphine was detected in plasma after oral administration of diacetylmorphine, 'flash' and 'high' effects were experienced, although peak effects occurred much later (60-120 min after administration) and were less intense (up to ±60% of the VAS scale) than after intravenous administration (±15 min; ±90% of VAS scale). Rectal administration reportedly also resulted in 'flash' and 'high' effects, although the authors admitted that apparently rectal administration had not succeeded in (partly) avoiding first-pass metabolism, as hoped, since neither diacetylmorphine nor 6-acetylmorphine was detectable in blood [52]. These pharmacodynamic results deviate so much from what is known about the pharmacokinetics of heroin in relation to the occurrence of 'flash' effects that they should be verified in double blind studies with more patients, before pharmaceutical development of (oral and rectal) diacetylmorphine for prescription to addicts can extend its focus to formulations that are unable to deliver unchanged diacetylmorphine to systemic circulation.

Two other pharmacokinetic studies were published on oral diacetylmorphine [27,109]. The first study compared the pharmacokinetics of low doses (26-52 mg) of oral diacetylmorphine and oral morphine in healthy volunteers and found that diacetylmorphine resulted in 80% lower bioavailability than morphine

(bioavailability 38%)[27]. However, the pharmacokinetics after oral administration of large doses to addicts appeared to be very different [109]. In both studies, neither diacetylmorphine nor 6-acetylmorphine was detected in plasma, and therefore morphine bioavailability was calculated from oral diacetylmorphine in both studies. Mean bioavailability of morphine after ingestion of up to 600 mg of diacetylmorphine by opioid dependent patients was much higher (67 ± 19%) than expected based on the study in healthy volunteers [109]. Furthermore, morphine absorption from oral diacetylmorphine was more rapid and more complete than absorption from concomitantly administered morphine-d3 [109]. The authors suggest that intestinal metabolic or transporter alterations could have occurred in tolerant persons, which could explain these findings.
Future studies into oral formulations of diacetylmorphine could attempt to avoid first-pass metabolism via the buccal mucosa: a bioadhesive buccal tablet (as developed for morphine [110]) or a chewing gum formulation (as developed for methadone [111]) might be able to deliver a sufficient amount of diacetylmorphine or 6-acetylmorphine into the systemic circulation to achieve the desired 'flash' effect. However, these attempts might be hindered by the bitter taste of diacetylmorphine that was responsible for abandoning nebulised diacetylmorphine [88].

Conclusion

Heroin dependent patients in heroin-assisted treatment will often prefer diacetylmorphine for injection as the prescribed drug, as it is the most efficient way to achieve their goals of feeling an almost instantaneous 'flash' followed by more sustained euphoria. Acceptability of this form of pharmaceutical heroin will therefore be high. Safety can only be ensured by strict dosing schemes to prevent overdose, by supervision following the first 10-15 min after use, and by providing a high quality product for injection and clean needles and syringes for its administration. However, alternative formulations are necessary for those that want change routes of administration to minimise the risk of overdose or that have to, due to damaged veins. Diacetylmorphine for inhalation is an obvious candidate, because many addicts in Europe already use heroin this way, and studies indicate that especially inhalation after volatilisation ('chasing the dragon') could be an effective route of administration: first-pass metabolism is avoided and rapid peak concentrations of diacetylmorphine and 6-acetylmorphine are achieved. Intranasal diacetylmorphine could also be a safe and effective alternative, but further research into enhanced absorption techniques and the pharmacokinetics and pharmacodynamics of large doses is required. The same could be said for oral administration, which is however theoretically less likely to be acceptable to treatment-resistant addicts, due to its failure to deliver diacetylmorphine or 6-acetylmorphine to the systemic circulation.

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2 Diacetylmorphine for injection

Chapter 2.1

Pharmaceutical development of an intravenous dosage form of diacetylmorphine hydrochloride

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Abstract

A solid dosage form for multiple use was developed for parenteral administration of diacetylmorphine in a clinical trial on co-prescription of heroin to heroin addicts. A 300 mg/mL diacetylmorphine hydrochloride solution was lyophilised as 10 mL aliquots in 30 mL glass vials, to be reconstituted to 150 mg/mL with Water for Injection before use. Addition of bulking agents for improvement of the cake structure of the lyophilised product appeared unnecessary. Stability studies indicated good stability of the lyophilised product under prescribed storage conditions (25°C, 60% RH) and under more extreme conditions (40°C, 75% RH). The reconstituted product was found to be stable for six days at room temperature. Suitability of the product for multiple use was supported by the fact that the reconstituted product was found to be antimicrobially active.

Abbreviations

cfu = colony forming units; HCl = hydrochloride; HPLC-UV = high performance liquid chromatography with ultraviolet detection; MDSC = modulated differential scanning calorimetry; P_c = chamber pressure; Ph.Eur. = European Pharmacopoeia; RH = relative humidity; sd = standard deviation; T_g = glass transition temperature; $T_{product}$ = product temperature; T_{shelf} = shelf temperature.

Introduction

3,6-Diacetylmorphine (heroin, diamorphine) is a synthetic morphine derivative used as an analgesic in some countries. However, it is better known as a drug of abuse that leads to strong dependence. In 1998, a clinical trial was started in The Netherlands to evaluate the effect of co-prescription of heroin to chronic treatment-resistant heroin addicts in a methadone maintenance program [1]. For this study, a dosage form for diacetylmorphine for intravenous injection was required.
Diacetylmorphine HCl for injection is available as a lyophilised product in the United Kingdom, in 5, 10, 30, 100, and 500 mg ampoules [2]. The 500 mg ampoule size was also used in Switzerland in the program for medical prescription of narcotics (PROVE), but they were replaced with 10 g ampoules for multiple use, because higher dosages and a simpler injection preparation procedure were found necessary [3]. In the Dutch trial, a maximum dose per administration of 400 mg was set, but the dosages were expected to be subject to much variation. Considering the estimated number of patients injecting diacetylmorphine daily and the expected variation in dosage and dosing frequency, a content of 3 g of diacetylmorphine per dosage unit for multiple use seemed appropriate.

Diacetylmorphine hydrochloride (HCl) is preferred for parenteral dosage forms since it has a much higher solubility in water than diacetylmorphine base [4,5]. Diacetylmorphine in aqueous solution is known to be susceptible to hydrolysis. Stability was reported in literature to be dependent on pH, temperature, excipients used, and concentration [5-10]. Lyophilisation of diacetylmorphine solutions was considered as an obvious and feasible pharmaceutical option, and it was decided to develop a solid dosage form for multiple use by freeze-drying a concentrated aqueous solution of diacetylmorphine HCl. In this paper, we describe the pre-formulation studies, the lyophilisation process and quality control of diacetylmorphine HCl for injection, as well as the results of stability studies and an antimicrobial effectiveness study.

Methods and materials

Chemicals

Diacetylmorphine HCl ($C_{21}H_{23}NO_5$.HCl.H_2O) drug substance (quality conform British Pharmacopoeia 2002) was obtained through the Central Committee on the Treatment of Heroin Addicts. Sterile Water for Injection was purchased from Braun NPBI Medical ('s Hertogenbosch, The Netherlands). Acetonitrile (Biosolve, Valkenswaard, The Netherlands), phosphoric acid, hydrochloric acid, potassium bromide, and potassium biphosphate (Merck, Amsterdam, The Netherlands) were of analytical grade and used without further purification.

Formulation process

A solution of diacetylmorphine HCl in sterile Water for Injection was prepared aseptically, filtered through a sterile 0.22 µm Millipak 40 filter (Millipore, Milford, MA, USA) and dispensed (10 mL) into washed and sterilized glass vials (30 mL, hydrolytic class 1, Münnerstadt Glaswarenfabrik GmbH, Münnerstadt, Germany) using a peristaltic dispenser pump (Model 505Dz/RL, Watson Marlow). After filling, vials were partially closed with washed and sterilised siliconized grey bromobutyl rubber stoppers (type FM 157/1, Helvoet Pharma NV, Alken, Belgium) and lyophilised in a Model Lyovac GT4 freeze-dryer (STERIS, Hürth, Germany). After completion of the program, vials were pneumatically closed under vacuum, sealed with aluminium caps (Bico Pharma GmbH, Neuss, Germany), and labelled.

The aseptic manufacturing process was controlled by checking filter efficacy (filter integrity after use, bioburden before filtration, and sterility testing of finished product), weight variation of the fill volume, and formulation solution content before and after filtration. In-process controls during lyophilisation consisted of temperature monitoring of product, shelf and condenser, and monitoring of the chamber pressure. All manipulations took place in a class 100 down-flow cabinet inside a class 100 clean room (Interflow, Wieringerwerf, The Netherlands). Air particle counts in the critical areas and microbiological contamination of area and personnel were routinely monitored during the manufacturing process.

Stability studies

A stability study was performed according to ICH guideline Q1A R [11], using three batches of lyophilised product. For long-term stability, the lyophilised product was stored at 25 ± 2 °C and 60 ± 5 % relative humidity (RH) for two years. Content, purity and residual moisture content were determined after 0, 3, 6, 9, 12, 18 and 24 months. The storage condition for the accelerated stability study was 40 ± 2 °C and 75 ± 5 % RH. Content, purity and residual moisture content were determined at 0, 1, 2, and 3 months after storage.

Product stability after reconstitution was determined in three vials per batch of lyophilised product (n = 2). These vials were reconstituted to 150 mg/mL with sterile Water for Injection and the concentration and purity measured after storage for 0, 6, 22, 28, 48 and 144 h at room temperature using the HPLC-UV method described below.

Antimicrobial effectiveness study

The antimicrobial activity of the reconstituted product was tested according to the European Pharmacopoeia § 5.1.3 ‘Efficacy of antimicrobial preservation’ [12]. The reconstituted product was inoculated with 10^5-10^6 colony forming units (cfu) per ml product of the following microorganisms: *Candida albicans* (ATCC no. 10231), *Escherischia coli* (ATCC no. 8739), *Pseudomonas aeruginosa* (ATCC no. 9027), and *Staphylococcus aure*us (ATCC no. 6538). *Aspergillus niger* is also prescribed for this test, but was replaced by *Escherischia coli* because of hospital hygiene regulations. The inoculated product samples were prepared in duplicate and then incubated at

22.5 ± 2.5°C, protected from light. At 0, 6, 24 h and 7, 14 and 28 days, a 200 µL sample was taken after gentle homogenisation and the number of cfu determined by membrane filtration. Mixed cellulose ester membrane filters (0.45 µm) were used, that were washed three times with 100 mL of NaCl 0.9%, before transferring them onto tryptic soy agar plates (or Sabouraud-dextrose plates for *C. albicans*). This procedure was performed in duplicate for each inoculated vial. The agar plates were incubated for 1-2 days at 37°C, after which a stable colony count could be obtained.

Quality control of diacetylmorphine HCl lyophilised product

The quality control of diacetylmorphine HCl 3 g/vial lyophilised product comprised the following tests: a visual inspection of appearance and colour of the product; determination of the reconstitution characteristics and pH of the reconstituted product; determination of mean content and purity by HPLC-UV analysis; measurement of residual moisture content with the Karl Fisher titration method; determination of uniformity of mass (§ 2.9.5), a Limulus Amoebocyte Lysate test for bacterial endotoxins (§ 2.6.14) and a filtration test for sterility of the product (§ 2.6.1) [12].

Analytical methods

HPLC analysis

Diacetylmorphine HCl was assayed by a validated, stability-indicating reversed-phase HPLC-UV method. The HPLC system consisted of a P1000 pump, an AS3000 autosampler and a UV1000 UV-VIS detector (Spectra Physics, Santa Clara, USA), using Chromquest software for data acquisition and management (Thermoquest Inc., San Jose CA, USA). A Zorbax SB-C_{18} analytical column (4.6 mm ID x 7.5 cm, particle size 3.5 µm, Rockland Technologies Inc., Newport, DE, USA) was used for separation. The mobile phase consisted of 85% v/v 0.05 M phosphate buffer, pH = 6.0, mixed with 15% v/v acetonitrile. Analyses were performed using a 1.0 mL/min flow rate, an injection volume of 20 µL, and 214 nm as the detection wavelength. Capacity factors of diacetylmorphine and its main degradation products, 6-acetylmorphine and morphine in this system were 19, 4.6, and 0.9, respectively. Independently prepared (by separate weighing of reference standard) stock solutions were used to prepare a series of standard solutions containing 5-50 µg/mL diacetylmorphine HCl and a series of quality control solutions in the same concentration range. The solvent used for these dilutions and in the last dilution step for the quality control samples was similar to the mobile phase but with pH 4.0 instead of 6.0. Diacetylmorphine content was determined in triplicate using the six-point calibration line obtained ($r^2 > 0.999$). Concentrations calculated for quality control samples did not deviate more than 5% from the nominal concentrations. Purity was calculated by dividing diacetylmorphine peak area by the total peak area.

Residual moisture content

Residual moisture levels in diacetylmorphine HCl 3 g/vial lyophilised product were determined with the Karl Fisher titration method. The content of each vial was quantitatively transferred to the titration unit of a Model 658KF titrino apparatus (Metrohm, Herisau, Switzerland) with previously dried methanol, and subsequently titrated using Hydranal® Titrant 2.0 mg H_2O/mL (Riedel-de Haen, The Netherlands). The end-point of the titration was determined biamperometrically.

Modulated differential scanning calorimetry (MDSC)

Glass transition temperatures (T_g) of the formulation solution were determined using a DSC2920 apparatus (TA Instruments, New Castle, DE, USA), equipped with a liquid nitrogen cooling accessory for low temperatures. Samples of ca. 20 µL were placed in 5 mm aluminium pans (Netzsch Thermal Analysis, Burlington, MA, USA), which were sealed immediately. An empty pan was used as reference and analyses were performed under a helium purge. Temperature scale and heat flux were calibrated with indium. Sample temperature was lowered from 20°C to −40°C (0.2°C/min) and, at a rate of 0.1°C/min, temperature was subsequently raised to 0°C, mimicking the primary drying phase of the freeze drying cycle. T_g values were determined by taking the half height between the baseline below and above the temperature range of the glass transition.

X-ray diffraction

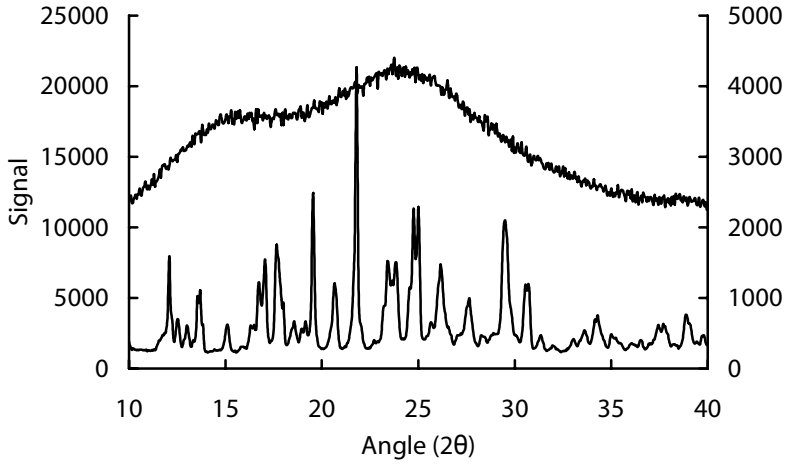
X-ray diffraction was performed with a Model PW 3710 PC-APD diffractometer (Philips, The Netherlands) at atmospheric humidity over a 2-theta (θ) range of 5-40° (where theta is the scattering angle). Samples (0.2-0.5 g) were placed in open rectangular aluminium sample holders (100 x 80 mm). The CuK_α radiation from the anode operating at 40 kV and 50 mA was monochromised with a 15 µm Ni foil. Scan step size was 0.02° and step time 4.0 s.

Results and discussion

Analytical characterisation of diacetylmorphine HCl drug substance

Diacetylmorphine HCl drug substance was supplied accompanied with a certificate of analysis stating its compliance with the specifications of the British Pharmacopoeia 2002 [13]. In-house quality control of the drug substance was limited to confirmation of identity via infrared spectroscopy and a determination of purity by HPLC-UV analysis. The major absorption bands in the infrared spectrum of the drug substance (2640, 1765, 1740, 1450, 1370, 1250, 1180 cm^{-1}) were always identical to those in the reference substance and in correspondence with literature data [14,15]. Mean chromatographic purity of the drug substance was 98.9 ± 0.45% (n = 4 batches), with 6-acetylmorphine as the main impurity. The x-ray diffraction pattern of diacetylmorphine HCl drug substance showed several sharp peaks, indicative of its crystalline nature (Figure 1).

Figure 1: X-ray diffraction patterns of diacetylmorphine hydrochloride drug substance and diacetylmorphine hydrochloride lyophilised product (upper graph).



Preformulation studies

The purpose of the formulated product was to administer up to 400 mg of diacetylmorphine HCl as an intravenous bolus injection, therefore, a highly concentrated solution was required. Literature data indicate that stability of aqueous diacetylmorphine solutions is dependent on pH, temperature, and concentration. Stability testing of 1 and 20 mg/mL solutions in saline showed that 90% of the diacetylmorphine content remained after storage at 4°C for 15 days. When stored at room temperature, stability of these solutions was limited to 7 and 12 days, respectively [10]. Results from more concentrated aqueous solutions (31.2 and 250 mg/mL) showed 4-5% and 14-18% losses of diacetylmorphine after 8 weeks of storage at 4°C and 21°C, respectively. Furthermore, after 2 weeks of storage, concentrations above 15.6 mg/mL were reported to show turbidity or even precipitation [9]. According to these data, the shelf-life of an aqueous solution would not be sufficient for diacetylmorphine HCl to be formulated as a ready-to-use solution for injection.

A preliminary test was performed to assess stability of a 300 mg/mL solution when sterilised by moist heat (15 min 121°C). During this procedure, 6-acetylmorphine content in the formulation solution increased from 2.2% to 9.0%. Furthermore, upon storage of the autoclaved solution at room temperature, a decrease in pH to 3.5, further degradation of diacetylmorphine HCl into 6-acetylmorphine and morphine, and formation of a precipitate were observed. It was decided to solve possible stability problems by using an aseptic manufacturing process with sterile filtration as the sterilisation method, and by developing a solid lyophilised dosage form to be reconstituted prior to use.

In a preliminary test, 9 formulation solutions were lyophilised; these contained 100, 200 and 300 mg/mL diacetylmorphine HCl in sterile Water for Injection, 2.5% w/v lactose solution, or 2.5% w/v mannitol solution. Visual inspection of the resulting products showed best results for the 300 mg/mL solutions with and without excipients. No meltback of ice and thereby collapse of the product was observed. These results indicate that addition of bulking agents is not necessary, since solid off-white cakes with excellent appearance were obtained without excipients. Furthermore, Poochikian et al. showed that addition of mannitol accelerates the degradation of diacetylmorphine HCl lyophilised product [8]. Thus, for further development we selected a 300 mg/mL diacetylmorphine HCl formulation solution.
Optimal pH value for minimal hydrolysis of diacetylmorphine HCl in aqueous solution is known to be between pH 4.0-4.5 [5]. Adjusting pH of diacetylmorphine HCl formulation solution (300 mg/mL) was not necessary, since it was 4.5 [16]. Furthermore, use of a buffer in a diacetylmorphine HCl formulation solution was reported to result in catalysed hydrolysis, as well as in discoloured cakes and accelerated degradation in the freeze-dried product [8].
A 300 mg/mL diacetylmorphine HCl solution in Water for Injection was selected as the formulation solution used to prepare the 3 g per vial freeze-dried product, to be reconstituted with sterile Water for Injection to a 150 mg/mL injectable solution.

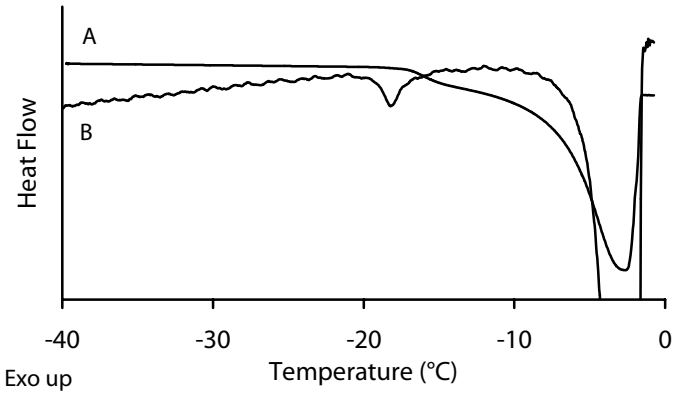
Modulated Differential Scanning Calorimetry

Transition temperature(s) and freezing characteristics of diacetylmorphine HCl formulation solutions were studied using MDSC. The thermogram of the formulation solution showed two thermal events, a baseline shift at −16.2°C and a melting endotherm with an extrapolated onset temperature of −3.2°C (Figure 1). The baseline shift with an onset temperature of −17.1°C, observed in the reversing heat flow signal, indicates that a glass transition occurred in the frozen formulation solution. The signal was slightly obscured in the total heat flow signal by a non-reversible endothermic peak at −18.4°C, probably representing enthalpic relaxation. The glass transition was taken into consideration in the development of the freeze-drying cycle.

Lyophilisation of diacetylmorphine HCl

The product temperature ($T_{product}$) as a function of the shelf temperature (T_{shelf}) was determined at a chamber pressure (P_c) of 0.2 mbar. Vials containing 10 mL of formulation solution were frozen to −45°C in 3 h, followed by a freeze-hold lasting half an hour to ensure complete freezing of the vials contents. $T_{product}$ was determined using three Pt-100 probes, placed in the vials. After reducing P_c to 0.2 mbar, T_{shelf} was raised in 10°C steps from −30 to 30°C (at a rate of 0.67°C/min). After each rise of T_{shelf}, the temperature was kept constant for 2 h, after which mean $T_{product}$ was determined. Diacetylmorphine HCl showed a linear increase in $T_{product}$ with T_{shelf} ($r^2 > 0.99$) (Figure 2). A maximum T_{shelf} of 13.7°C (at P_c 0.2 mbar) was derived from this plot, considering a maximal $T_{product}$ of −17°C, corresponding to T_g onset. It was known from experience that in our freeze-dryer, a 4.5°C temperature difference

Figure 2: DSC thermogram of diacetylmorphine HCl formulation solution 300 mg/mL. The heat flow signal is split into the reversing heat flow (A) and the non-reversing heat flow (B).

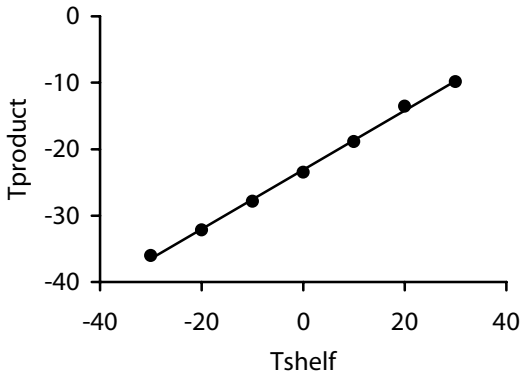


existed between vials placed in an optimal position (in the middle of the middle shelf) and those placed in the worst position in terms of isolation (in the corner positions on the upper shelf near the door). In order to avoid problems of meltback and collapse of less optimally positioned vials, a maximum $T_{product}$ of −21.5°C was used in the calculation of T_{shelf} and 4°C was chosen as a safe T_{shelf} for the primary drying phase.

The mean sublimation rate during the primary drying phase was determined in 10 vials, containing 10 mL formulation solution using the selected T_{shelf} (4°C) and P_c (0.2 mbar). The vials were placed in the optimal position in the freeze dryer, surrounded with two insulating layers of vials filled with 10 mL sterile Water for Injection. After freezing the vials at the above-mentioned cooling rate, they were dried until about 30% w/w of the water content had sublimated and the sublimation rate could be calculated from the weight loss. Sublimation rate was found to be 217 ± 12 mg/h, resulting in a mean primary drying time of 39 ± 2 h.

The following lyophilisation cycle was therefore selected for the manufacturing of diacetylmorphine HCl 3 g/vial lyophilised product. The formulation solution was frozen to −35°C in 2½ h, followed by a freeze-hold for 3 h. The primary drying phase started at a chamber pressure of 0.2 mbar, while temperature rose to 4°C in 1 h. This temperature and pressure were kept constant for 41 h. Then the secondary drying phase started, in which temperature rose linearly to 25°C in 10 h and pressure was lowered to its minimum value and maintained for 6 h. Lyophilisation of the formulation solution using this 62.5-h program resulted in a solid off-white cake with excellent appearance and a residual moisture content of about 0.25% w/w. The crystalline structure present in diacetylmorphine HCl drug substance was completely lost in the lyophilised product: its x-ray diffraction pattern (Figure 1) lacked all the sharp peaks that could be observed in the drug substance.

Figure 3: Product temperature as a function of shelf temperature at 0.2 mbar chamber pressure.



Manufacture and quality control

In-process control tests were performed routinely to assure the quality of the aseptic manufacturing process. In preparation for lyophilisation, the formulation was sterilised via membrane filtration, after which the formulation solution was dispensed into vials by a peristaltic pump. There was no evidence of adsorption of diacetylmorphine HCl from the solution onto the filter membrane or other manufacturing equipment, since the content of the formulation solution before and after filtration was not significantly different (in-process control data from 10 batches, mean ± sd: content before filtration 102.0 ± 5.4%; after filtration: 100.7 ± 4.6%). Chromatograms of these solutions show no significant increase in peak area for the main degradation product, 6-acetylmorphine (3.0 ± 0.6% and 3.2 ± 0.9%, respectively), indicating that the solution was sufficiently stable during the manufacturing process (about 5 h at controlled room temperature).

Bioburden of the formulation solution was tested routinely and counts were found to be acceptable (0-10^3 cfu, n = 10), considering the retention capacity of the filter used (2.10^9 cfu). The results and specifications of the quality control of 10 batches of diacetylmorphine HCl 3 g/vial lyophilised product are presented in Table 1. All batches were well within specifications.

Antimicrobial effectiveness study

Although there has been considerable interest in the microbiological status of (street) heroin and its association with problems of infection in intravenous drug users [17-19], little is known about the antimicrobial effects of diacetylmorphine solutions. In a recent investigation into the microbiological safety of solutions for epidural infusion, bupivacaine solutions (0.5%) were found to be rapidly bactericidal for *E. coli*, *Ps. aeruginosa*, *E. faecalis*, and a coagulase negative *Staphylococcus* species; increasing diacetylmorphine concentrations (0.01, 0.1 or 1%) acted synergistically [20]. Another study showed antimicrobial effects of street heroin against *S. aureus* and *B. cereus*

Table 1: Quality control results of 10 batches of diacetylmorphine HCl 3 g/vial lyophilised product. The mean value (sd) of the test result is given where possible.

Test item	**Specification**	**Result**
Visual inspection	off-white, lyophilised cake	conforms
Content	95-105% of labelled content	99.6% (1.8)
Purity	> 97.5%	98.2 (0.3)
Uniformity of mass	Ph.Eur.IV. § 2.9.5	conforms, 2.97 g (0.06)
pH after reconstitution	4-6	4.96 (0.62)
Residual water	< 2.0 %	0.23% (0.04)
LAL-test	< 50 EU/vial	< 24 EU/vial
Sterility	Sterile	conforms

Table 2: Results of antimicrobial effectiveness study. The viable count per microorganism is given in cfu/vial.

Time		***B. subtilis***	***C. albicans***	***E. coli***	***Ps. aeruginosa***	***S. aureus***
0	vial A	6.10^4	3.10^4	2.10^4	3.10^2	5.10^5
	vial B	6.10^4	4.10^4	2.10^4	1.10^2	5.10^5
6 h	vial A	1.10^2	7.10^2	-	-	3.10^5
	vial B	-	1.10^3	-	-	3.10^5
24 h	vial A	-	-	-	-	2.10^4
	vial B	-	-	-	-	1.10^5
7 days	vial A	-	-	-	-	-
	vial B	-	-	-	-	-
14 days	vial A	-	-	-	-	-
	vial B	-	-	-	-	-
28 days	vial A	-	-	-	-	-
	vial B	-	-	-	-	-

Criteria of acceptance A for parenteral preparations (Ph.Eur.IV): 2 log reductions after 6 h and 3 after 24 h, with no recover after 28 days for bacteria and 2 log reductions after 7 days, with no recover after 28 days for fungi. Criteria of acceptance B: 1 log reduction after 24 h and 3 after 7 days, with no increase after 28 days for bacteria.

(not against *Ps. aeruginosa*), but this could also be attributed to the quinine content of the samples [21].
We added *Bacillus subtilis* (ATCC no. 6633) to the range of standard micro-organisms for the antimicrobial effectiveness study because of reports of infections with *Bacillus* species. [17-19,21]. The results of this study show a substantial antimicrobial effect of the reconstituted 150 mg/mL diacetylmorphine HCl solution (Table 2). The bactericidal effect seems to affect *C. albicans* and *Ps. aeruginosa* most, but is evident for all tested species. The criteria of acceptance A (Table 2) for parenteral preparations of the European Pharmacopoeia were met for all species, except for *S. aureus*, that showed no log reductions after 6 h and only one after 24 h, but that did show 5 log reductions after 7 days. This is consistent with findings in literature of growth inhibition of *S. aureus* and inhibition of its protein and nucleic acid synthesis when cultured in nutrient medium containing 30-60 mM diacetylmorphine [22,23].

Stability studies

The pH of the reconstituted product (diacetylmorphine HCl 150 mg/mL in Water for Injection) was between 4.2 and 5.5, which is within the optimal pH range with respect to stability [5]. The volume of the lyophilised cake was determined to be 2.3 mL, so reconstitution with 18 mL sterile Water for Injection is required to obtain a 150 mg/mL solution of diacetylmorphine HCl. The reconstituted product was found to be chemically stable for six days at room temperature. After six days, 6-acetylmorphine concentrations (given as percentages of diacetylmorphine peak area) had increased from 1.7 to 3.5%, while diacetylmorphine content did not decrease significantly.
Long-term stability studies of the lyophilised product showed that after 103 weeks (24 months) 95.6 ± 4.1% of the initial content remained when stored at 25 ± 2°C and 60 ± 5% RH (Table 3). Chromatograms showed no increase in peak area for the main

Table 3: Long-term stability studies at 25±2°C and 60±5% RH. Mean values are given, with standard deviations in parentheses.

Time[1]	Content[2]	6-MAM[3]	Residual water[4]
3	98.4 (2.8)	2.0 (0.1)	0.21 (0.00)
15	99.5 (4.1)	1.8 (0.1)	0.23 (0.01)
27	94.3 (1.9)	2.0 (0.1)	0.25 (0.01)
36	99.5 (2.3)	2.1 (0.5)	0.25 (0.02)
51	98.0 (2.2)	2.1 (0.1)	0.25 (0.00)
77	94.9 (1.8)	2.0 (0.0)	0.27 (0.01)
103	95.6 (4.1)	3.3 (1.1)	0.24 (0.01)

[1] time in weeks after manufacturing date; [2] mean content as % of nominal content; [3] 6-MAM = 6-acetylmorphine peak area as a percentage of diacetylmorphine peak area; [4] residual water content as % w/w.

Table 4: Accelerated stability studies at 40±2°C and 75±5% RH. Mean values are given, with standard deviations in parentheses.

Time[1]	Content[2]	6-MAM[3]	Residual water[4]
0	100.4 (3.0)	1.6 (0.5)	0.35 (0.17)
5	100.1 (2.1)	1.6 (0.5)	0.37 (0.14)
9	101.0 (3.5)	1.6 (0.5)	0.39 (0.15)
14	98.5 (2.5)	1.9 (0.6)	0.39 (0.17)

[1] time in weeks; [2] mean content as % of nominal content; [3] peak area 6-MAM = 6-acetylmorphine as a percentage of peak area of diacetylmorphine; [4] residual water content as % w/w.

degradation product (6-acetylmorphine) within 103 weeks. Residual moisture increased only marginally and stayed well within specifications during this period (Table 3). Accelerated stability studies show no significant decrease in mean content, nor any significant increase in 6-acetylmorphine peak area (Table 4). Further stability studies are ongoing.

Considering the results of the stability studies and the antimicrobial effectiveness study, it was decided to label diacetylmorphine HCl 3 g/vial lyophilised product with a shelf life of two years, when stored at 15-25°C. After reconstitution, a maximum storage period of 12 h at room temperature was considered acceptable, since reconstitution of the product and subsequent preparation of syringes for use are performed aseptically under laminar air flow conditions.

Conclusion

A dosage form for multiple use has been developed for diacetylmorphine for injection to be used in a clinical study of co-prescription of heroin to heroin-dependent patients in a methadone maintenance program. A 300 mg/mL solution of diacetylmorphine HCl in Water for Injection has been selected to be aseptically processed, sterilised by membrane filtration and filled into glass vials. Subsequently, this solution was freeze-dried according to a carefully selected lyophilisation program that produced diacetylmorphine HCl 3 g/vial, a lyophilised product with excellent appearance and stability. After reconstitution with sterile Water for Injection, diacetylmorphine HCl 3 g/vial was found to be stable for six days at room temperature. Suitability of the product for multiple use was supported by the fact that the reconstituted product was found to be antimicrobially active.

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3 Diacetylmorphine for inhalation: pharmaceutical development

Chapter 3.1

Pharmaceutical heroin for inhalation: thermal analysis and recovery experiments after volatilisation

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Submitted for publication

Abstract

Pharmaceutical heroin for inhalation was developed for a clinical trial on co-prescription of heroin and methadone to chronic treatment-resistant heroin addicts. Diacetylmorphine base was selected as the active pharmaceutical ingredient for this product, with caffeine anhydrate added as an excipient. Differential Scanning Analysis and Thermogravimetric Analysis showed that addition of caffeine resulted in a lower melting temperature and a higher volatilisation rate for the mixture than for diacetylmorphine base alone. Recovery experiments showed that 40.8 ± 5.3% of diacetylmorphine base could be found in smoke condensate after volatilisation of diacety-morphine for inhalation. All of the caffeine from each tablet was recovered unchanged in the fumes, while 85.6% of the diacetylmorphine from each tablet was recovered, either unchanged in the fumes or as non-volatilised residue. Recovery was found to be reproducible and only small differences were found between the tablet types. The experimental set-up was found to efficiently collect the vapours resulting from heating the powder. Under the tested experimental conditions, no evidence was found that degradation products of diacetylmorphine or caffeine, other than 6-acetylmorphine (5.9%) had volatilised, even though a decomposed residue was present after heating diacetylmorphine/caffeine samples. Diacetylmorphine/caffeine was found to be a suitable basis for pharmaceutical heroin to be used by 'chasing the dragon'.

Abbreviations

DSC = differential scanning calorimetry; TGA = thermogravimetric analysis; HCl = hydrochloride; HPLC-UV = high performance liquid chromatography with ultraviolet detection.

Introduction

Heroin is a well-known drug of abuse, that is usually administered intravenously, but smoking heroin has gained popularity since it was first described in Shanghai in the 1920s [1]. After some refinement, the use of a smoking procedure called 'chasing the dragon' spread to South East Asia, India and some parts of Europe in 1960-1980 [1]. In this procedure, drug users heat heroin powder on a piece of aluminium foil with a cigarette lighter until it melts and evaporates. The fumes are subsequently inhaled through a straw.

A clinical trial was conducted in the Netherlands to evaluate the effect of medical co-prescription of heroin and methadone on mental and physical health and social functioning of chronic treatment-resistant heroin dependent patients. Since in the Netherlands, 75-85% of the heroin addicts use heroin by 'chasing the dragon' [2], two separate study protocols were developed, one trial testing the efficacy of the prescription of an inhalable form of heroin and another trial testing the efficacy of the prescription of injectable heroin. In preparation for the first trial, an inhalable form of pharmaceutical heroin was developed, containing diacetylmorphine base and caffeine anhydrate in tablets, obtained via direct compression. The formulation of this product was restricted by the unpredictable (adverse) effects that excipients could have when the product was heated and the resulting vapours inhaled. Therefore, no excipients (except for caffeine) were added. Caffeine was considered acceptable, because it is commonly found in street heroin samples [3-6] and has been shown to improve the volatilisation of heroin [7]. It was considered important for patient compliance to offer a product that could be used without interfering too much with the habits and rituals the subjects had developed over the years. Alternative dosage forms, like orally, nasally, or rectally administered heroin were therefore not considered. In this article, pharmaceutical heroin to be used via the procedure of 'chasing the dragon' is referred to as diacetylmorphine for inhalation.

The primary goal for the development of diacetylmorphine for inhalation was to ascertain that its use would result in an acceptable and reproducible level of diacetylmorphine in the inhaled fumes. Furthermore, thermal analysis was used to gain insight in the volatilisation process that occurs when using heroin in the abovementioned way. Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) analyses were used to study melting and volatilisation of diacetylmorphine base and the hydrochloride salt in the absence and presence of different proportions of caffeine. A simple *in vitro* model for the procedure of 'chasing the dragon' was developed and used to study the recovery of diacetylmorphine from the pharmaceutical product after volatilisation.

Materials and Methods

Chemicals

Diacetylmorphine base and diacetylmorphine hydrochloride (HCl) (British Pharmacopoeia quality) were manufactured specifically for the clinical trial and obtained through the Central Committee on the Treatment of Heroin Addicts. Caffeine anhydrate was purchased from Bufa (Uitgeest, The Netherlands), 6-acetylmorphine hydrochloride from Sigma Aldrich Co. Ltd. (Zwijndrecht, The Netherlands). Potassium dihydrogen phosphate, phosphoric acid 85% and hydrochloric acid 25% g/g were analytical grade and originated from Merck (Amsterdam, The Netherlands). Acetonitrile was HPLC grade and came from Biosolve (Amsterdam, The Netherlands).

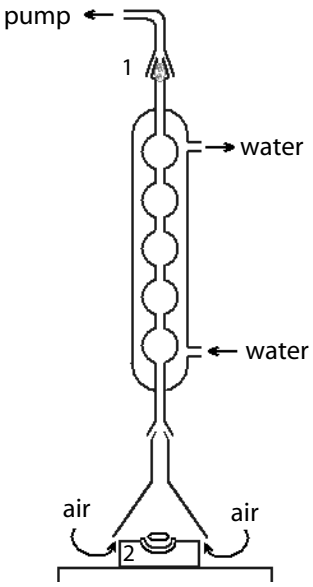
Differential Scanning Calorimetry (DSC)

DSC measurements were performed on a DSC Q1000 v.2.5 Differential Scanning Calorimeter (TA Instruments Inc., New Castle (DE), USA). Samples were punched out of a powder bed and weighed into aluminium pans (diameter: 5 mm, TA Instruments) that were hermetically sealed. An empty pan was used as a reference and indium was used for temperature and heat calibration. Thermal Advantage™ software (version 1.3.0.179 for Q-Series™, TA Instruments) was used for data acquisition and Universal Analysis 2000™ software (version 3.0G, TA Instruments Inc.) was used for data analysis. Simple heating experiments were performed, measuring heat flow at a temperature of 50°C, rising to 300°C at a rate of 10°C/min. Additional experiments concerned repeated cycles of heating (10°C/min) and cooling (50°C/min was the maximum rate). Diacetylmorphine base, diacetylmorphine HCl and caffeine anhydrate were tested, as well as 11 different physical mixtures of diacetylmorphine base and a 1:1 mixture of diacetylmorphine HCl and caffeine. The mixtures were prepared by weighing the two components and mixing them by stirring with a spatula and shaking the powder in its container.
Additional visual information on the heating process was obtained by performing experiments, using the same temperature ramp as in the DSC measurements, in a melting point apparatus (Büchi B-540, Mettler-Toledo, Tiel, the Netherlands).

Thermogravimetric Analysis (TGA)

A TGA 51 apparatus (TA Instruments, New Castle (DE), USA) was used for thermogravimetric analysis of diacetylmorphine base and diacetylmorphine/caffeine mixtures. It measures the decrease of sample weight in time, at a set temperature (under a nitrogen flow). Calciumoxalate monohydrate was used for calibration. Samples (10-20 mg) were brought into platinum pans and put in the oven at 30°C. Temperature rose at 10°C/min to the set temperature, which was then kept constant until sample weight was minimal. The experiment was performed under a constant nitrogen flow. The volatilisation time was derived from the resulting thermogravimetric curves.

Figure 1: Schematic of the set-up of the recovery experiment, showing (top to bottom): outlet to pump, (1) location of the cotton plug, reflux condenser, funnel, brass block surrounding the crucible (2), top of the heating device. Arrows indicate the direction of the flow of water and air.



Recovery after volatilisation of diacetylmorphine for inhalation

Tablets of diacetylmorphine for inhalation from test batches were used for the recovery experiments. The tablets contained 25, 50 or 100 mg of diacetylmorphine base and 100 mg of caffeine anhydrate, without other additives, and they were manufactured via direct compression. Samples were heated in a porcelain crucible, placed in a brass block on a heating device (IKAMAG RCT Basic, IKA-Werke GmbH, Staufen, Germany). The resulting fumes were collected in a reflux condenser, fitted with a funnel that covered the sample on one side, and a water pump on the other side (Figure 1). To prevent loss of vapours into the pump, a cotton plug was placed in the top of the condenser.

The heating device was set at a temperature of 300°C and the brass block and crucible were pre-heated for 30 min before the tablet was inserted. Samples were heated to achieve complete volatilisation, which was defined as the moment that no more fumes arise from the heated samples. Complete volatilisation was achieved within 7 min for tablets containing 25 or 50 mg diacetylmorphine base and within 15 min for tablets with 100 mg diacetylmorphine. The water pump and condenser were started 5 min prior to the start of the experiment and stopped 5 min after the end. After volatilisation, the condenser, the funnel and cotton plug were rinsed with 80 mL 0.1 N HCl. The rinse fluid was collected, sonicated using an ultrasonic bath, filtered, diluted to 100 mL with 0.1 N HCl and diluted further with mobile phase for injection into the HPLC system. The experiment was repeated 3-4 times per tablet

type. Tablet and crucible were weighed before and after the experiment, so that the size of the residue could be determined.

High performance liquid chromatography

The recovery of diacetylmorphine, caffeine, and degradation product 6-acetylmorphine in the vapour condensate after heating diacetylmorphine/caffeine tablets was determined using a HPLC-UV method. The system consisted of a Zorbax SB-C_{18} analytical column (75 mm x 4.6 mm ID, particle size 3.5 µm; Rockland Technologies Inc., Newport, DE, USA), connected to a P1000 pump (Spectra Physics, San Jose, USA), a Model U6K injection system, and a Model 441 Absorbance detector (Waters Associates, Milford, MA, USA). A DataJet integrator (Thermo Separation Products, Fremont, CA, USA) calculated the peak areas. The flow was 1.0 mL/min, the injection volume was 10 µL, and the detection wavelength was set at 214 nm. The mobile phase consisted of 85% v/v 0.05 M KH_2PO_4 buffer, brought to pH = 3 with phosphoric acid and 15% v/v acetonitrile. Samples were quantified using calibration curves of diacetylmorphine, caffeine, and 6-acetylmorphine. Standard solutions were prepared by dissolving diacetylmorphine base, 6-acetylmorphine and caffeine in 0.1 N HCl and diluting to concentrations ranging from 30-125 µg/mL, 0.5-50 µg/mL and 15-85 µg/mL, respectively.

Results

Differential Scanning Calorimetry

The thermogram for diacetylmorphine HCl showed two endotherms on heating (50-300°C 10°C/min, Figure 2A) at ±172°C and 210-220°C, that are probably attributable to solid transitions, degradation, and/or dehydration processes. At 150.4-154.3°C, a glass transition occurred (see insert in Figure 2). An exothermic signal with an onset temperature of 252°C occurred where the melting point was expected (243-4°C [8]. This signal most likely represents a combination of melting, boiling, and decomposition. A sample that was heated from 20-255°C at 10°C/min (followed by rapid cooling) did show a melting endotherm at 243.4°C (Figure 2B), indicating that melting, boiling, and decomposition occur in a narrow temperature range and could be competitive processes. When diacetylmorphine HCl samples were reheated, none of the thermal events reappeared, except the exothermic degradation signal. When the 20-300°C 10°C/min temperature gradient was used to heat a sample of diacetylmorphine HCl in the melting point apparatus, discolouration was observed when sample temperature exceeded 180°C. Melting was observed at 247°C, soon followed by signs of boiling and extensive degradation.

The DSC thermogram for the diacetylmorphine HCl / caffeine mixture (1:1 w/w) showed a large endotherm at 160.8°C and a small one at 204.1°C (Figure 2C). An exothermic process occurred above 250°C, but less pronounced than in the diacetylmorphine HCl samples. A heating cycle experiment conducted with the

Figure 2: DSC thermograms of: A. diacetylmorphine hydrochloride (temperature range: 50-300°C, heating rate 10°C/min); B. diacetylmorphine hydrochloride (50-255°C, 10°C/min, followed by rapid cooling); C. 1:1 mixture of caffeine and diacetylmorphine hydrochloride (50-300°C, 10°C/min). The insert shows the glass transitions in curve A and B.

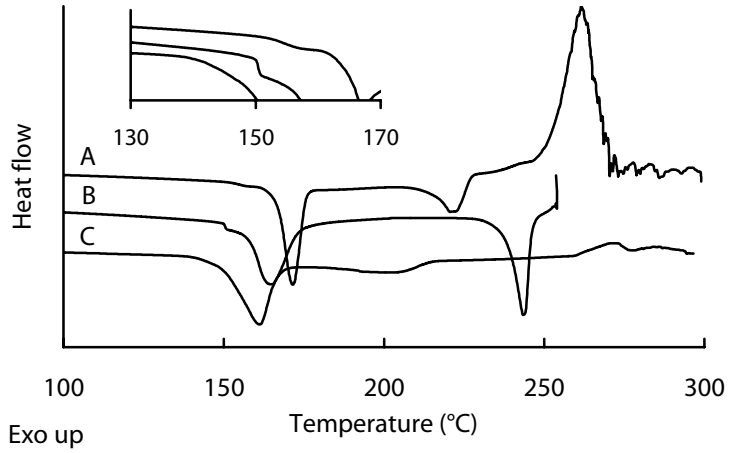


Figure 3: DSC thermograms of a physical mixture (3:1) of diacetylmorphine base and caffeine anhydrate (A), diacetylmorphine base (B) and caffeine anhydrate (C) (heating rate 20°C/min 50-250°C and 10°C/min 250-400°C). The baseline shift at 250°C was caused by a programmed change in heating rate.

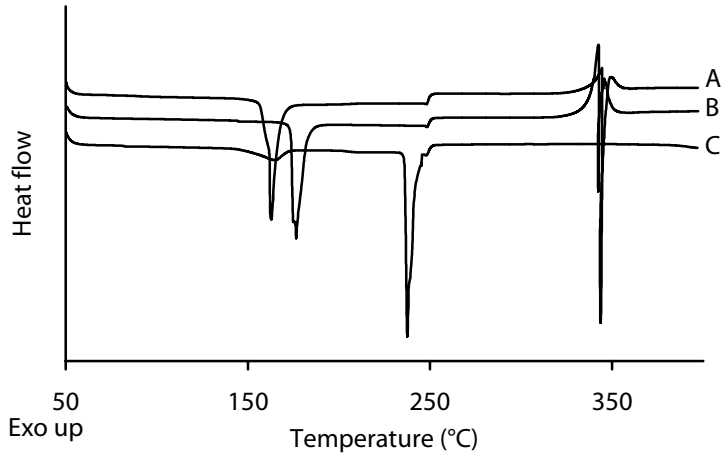


Figure 4: DSC thermograms of of physical mixtures of diacetylmorphine base and caffeine anhydrate (temperature range: 50-300°C, heating rate 10°C/min), showing (from left to right): the melting signal of the eutectic mixture, the melting signal for (excess) diacetylmorphine base (DAM) and the melting signal for the (excess) caffeine anhydrate.

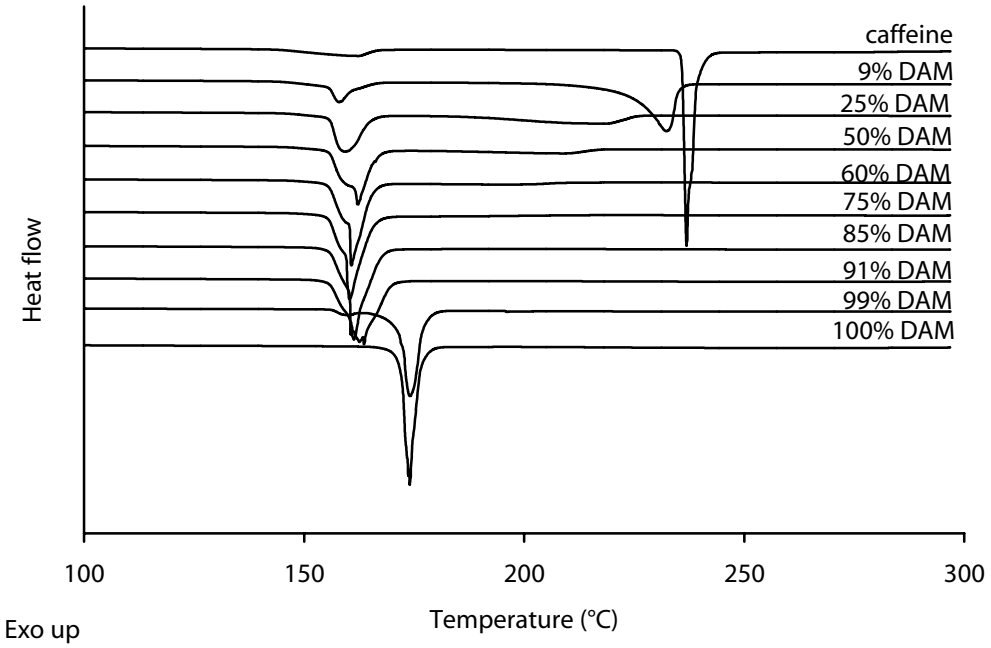


Table 1: Repeated DSC heat cycle experiments on diacetylmorphine base, caffeine anhydrate and mixture samples (3:1 and 1:1 w/w diacetylmorphine and caffeine); Melting temperatures are given (in °C) for each heat cycle (1, 2, 3), with the corresponding heats of fusion (in J/g).

Heat cycle	**1**		**2**		**3**	
Diacetylmorphine base	174.5°C	89.3 J/g	169.7°C	78.8 J/g	167.0°C	80.8 J/g
Caffeine	237.0	99.8	238.5	99.1	237.7	97.6
3:1 Mixture	160.1	85.6	154.0	72.6	151.1	74.2
1:1 Mixture	158.3	67.2	152.0	45.9	150.7	43.3

Temperature range: 50-300°C, heating rate: 10°C/min, cooling rate: 50°C/min.

mixture showed a re-crystallisation exotherm at 174.3°C during cooling, but only a small ±200°C endotherm reappeared in the second heating cycle. Visual observation of the heated diacetylmorphine HCl / caffeine mixture showed similar behaviour of discolouration and boiling and a lower melting temperature (215-224°C) than for diacetylmorphine HCl.
DSC thermograms of diacetylmorphine base and caffeine (heated 50-300°C at 10°C/min) showed sharp melting endotherms at the expected temperatures (174.4°C and 236.6°C, respectively, Figure 3B and C). In the thermogram for caffeine anhydrate there was also a small endotherm at 161.4°C that did not reappear on reheating, indicating that it might represent an irreversible process, like dehydration or a solid transition (β→α modification [9]). When samples were heated to 350 or 400°C, diacetylmorphine base (as well as the 3:1 diacetylmorphine/caffeine mixture) showed a mixed endo- and exothermic process with an onset temperature of 335°C, possibly representing boiling or evaporation (Figure 3A and B). Caffeine samples did not show any thermal events between 250-400°C (Figure 3C).

DSC analysis of the physical mixtures of diacetylmorphine base and caffeine showed that a eutectic mixture was formed, that melted at 159.8 ± 0.96°C (Figure 4). The excess component melting in the mixtures took place at a temperature that was lower than the melting temperature of the pure substance and melting temperature decreased with the proportion in excess. From the thermograms of several mixtures of diacetylmorphine base and caffeine (Figure 4), a phase diagram was constructed (Figure 5). It shows that the eutectic mixture contains 90-94% diacetylmorphine base; other mixtures of diacetylmorphine and caffeine need temperatures above 160°C for complete melting.

Figure 5: Phase diagram, obtained from the results of the DSC experiments. ○ = mean temperature of the eutectic signal; ● = mean temperature of the excess caffeine signal; Δ = mean temperature of the excess diacetylmorphine signal; error bars indicate standard deviations.

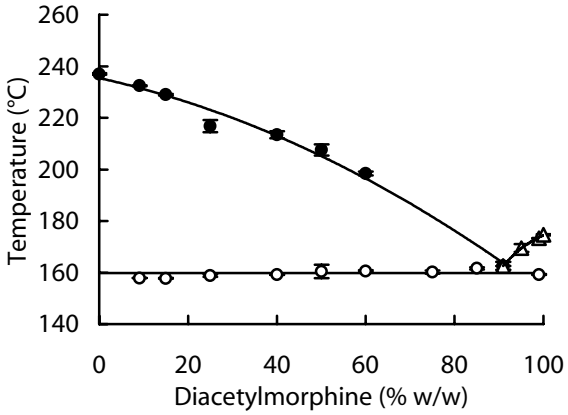
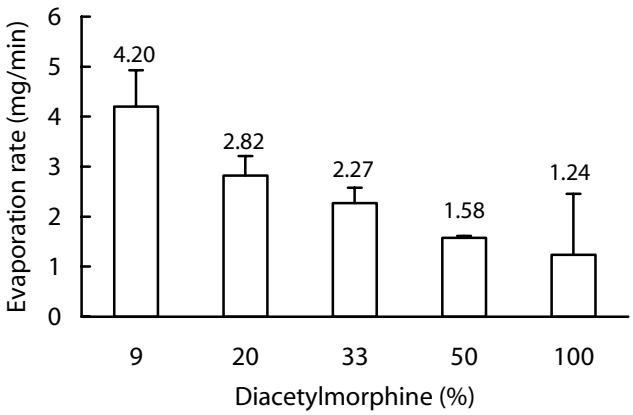


Figure 6: Results of TGA experiments on different diacetylmorphine/caffeine mixtures. The bars represent the mean volatilisation rate (at 230-275°C) with error bars indicating the standard deviation.



Experiments using heat/cool cycles showed identical behaviour for caffeine anhydrate samples when it was reheated or reheated twice (Table 1). Furthermore, the melting endotherm observed during heating was compensated during the cooling phase by a re-crystallisation exotherm at 231.5°C (102.2 J/g). On reheating diacetylmorphine base and the 50% and 75% diacetylmorphine base/caffeine mixtures however, melting temperature and associated heat of fusion values (Table 1) decreased, especially in the second heating cycle. Re-crystallisation exotherms occurred in the first cooling phase (at 99°C) for some diacetylmorphine base samples, but most diacetylmorphine base and mixture samples exhibited some degree of super-cooling, only re-crystallising in the following heating phase (at 82-101°C). Heats of fusion of the re-crystallisation exotherm(s) did not equal those of the melting endotherms, possibly indicating some degree of contamination of the sample with degradation products.

Thermogravimetric Analysis

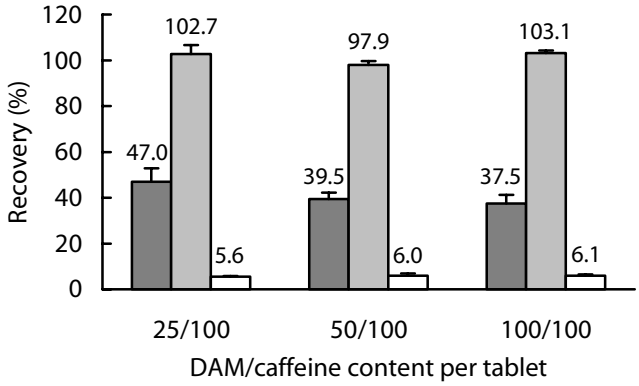
TGA experiments were started at a temperature of 165°C, slightly higher than the melting point of the eutectic in the diacetylmorphine/caffeine mixtures found in the DSC experiments. However, since at temperatures below 205°C complete volatilisation took one hour or more, experiments were conducted at 230°C, 250°C, and 275°C. The resulting thermograms were used to determine the volatilisation rate and the onset temperature for volatilisation. Mean volatilisation rate (slope of the thermogram) was found to depend on the proportion of caffeine in the mixture (Figure 6): increasing proportions of caffeine increased the volatilisation rate. No correlation between volatilisation rate and temperature was found. Onset temperature for volatilisation was higher for diacetylmorphine base (184.0 ± 7.8°C) than for the different diacetylmorphine base/caffeine mixtures (155.0 ± 4.4°C), which is consistent with the difference in melting point found in the DSC experiments (Table 1).

Volatilisation seems to start at slightly lower temperatures than melting of the eutectic mixture in mixture samples, while for pure diacetylmorphine base the onset temperature for volatilisation is higher than the melting temperature.

Recovery after volatilisation of diacetylmorphine for inhalation

Volatilisation of diacetylmorphine for inhalation was not complete; a small residue remained in the crucible after fumes had ceased to arise from the sample at the end of the experiment. The recovery of diacetylmorphine from the tablets in the fumes collected by the condenser system was found to be 40.8 ± 5.3%, *versus* 101.1 ± 3.2% for caffeine (Figure 7). Since all of the caffeine was recovered in the condenser system, the residue in the crucible was assumed to have originated from diacetylmorphine in the tablet. The size of the residue was therefore expressed as a percentage (% w/w) of the amount of diacetylmorphine in the tablet, similar to the 6-acetylmorphine that was found in the condensate in small quantities (5.9 ± 0.6% w/w) (Figure 7). Overall, 83.5-88.4% w/w of diacetylmorphine from the tablet was recovered as volatilised diacetylmorphine and 6-acetylmorphine (in mg) or as residue in the crucible. Mean diacetylmorphine recovery from a 25/100 mg diacetylmorphine/caffeine tablet was slightly higher (47.0 ± 5.2%) than the recovery from a 50/100 mg tablet (39.5 ± 2.4%, p = 0.046) and a 100/100 mg tablet (37.5 ± 3.9%, p = 0.038); similarly, the mean residue (30.8 ± 3.4%) was significantly smaller (42.9 ± 4.9%, p = 0.024 and 41.4 ± 2.5%, p = 0.005, respectively).

Figure 7: Results of recovery experiments. Mean percentages are given (error bars: standard deviation) for recoveries in condensate as well as for the size of the foil residue. Recovered caffeine (% w/w, black bars) is given relative to the total amount of caffeine in the tablet; diacetylmorphine (dark grey bars), 6-acetylmorphine (light gray) and the residue (white) are given as percentages (% w/w) of the amount of diacetylmorphine in the sample.



Discussion

Diacetylmorphine is available as the free base and as the hydrochloride salt. The latter is theoretically less suitable for inhalation after volatilisation, since it has a higher melting point (243-244°C) than the free base (173°C) [8]. Furthermore, diacetylmorphine HCl was found to be more sensitive to degradation on heating [7]. Our DSC experiments supported this: diacetylmorphine HCl thermograms showed extensive degradation, large exothermic signals appeared at relatively low temperatures in the first heating cycle and none of the non-degradation-associated signals reappeared on reheating. Diacetylmorphine base samples also showed signs of degradation: a small decrease in melting temperature and melting energy on reheating, discolouration on heating, and exothermic signals suggesting decomposition. However, this seemed to occur at higher temperatures (335°C *versus* 240-250°C) and to a lesser extent than observed for diacetylmorphine HCl.

Addition of caffeine has been suggested to increase the recovery of diacetylmorphine HCl and diacetylmorphine base after volatilisation and to reduce degradation upon heating [7]. DSC experiments with caffeine indeed showed more stable thermal behaviour on heating and reheating than diacetylmorphine base and diacetylmorphine HCl. The main effects of the addition of caffeine to diacetylmorphine base seemed to be the formation of a eutectic mixture with a 14°C lower melting point and increasing volatilisation rates for mixtures with larger proportions of caffeine. DSC thermograms for diacetylmorphine base and mixtures of diacetylmorphine base and caffeine did not show obvious differences in the events associated with degradation. However, it is likely that a lower melting point and an earlier onset of volatilisation benefit the stability of diacetylmorphine during heating. This might explain why the recovery experiments showed slightly larger diacetylmorphine recoveries as well as slightly smaller residues for the 25/100 mg diacetylmorphine/caffeine tablets than for the tablet types with smaller proportions of caffeine. Furthermore, the increased recovery of diacetylmorphine from mixtures with caffeine might be due to an increased volatility of the mixture: the vapour pressure of diacetylmorphine for inhalation would be expected to increase after addition of caffeine (vapour pressure $9x10^{-4}$ Torr at 25°C) to diacetylmorphine base (vapour pressure $5x10^{-8}$ Torr at 25°C)[10].

Caffeine is commonly used as a diluent in street heroin [3,5-7] and has never been associated with any adverse effects as far as we know. It was therefore considered to be relatively safe to use as an excipient in pharmaceutical heroin for inhalation. Unlike some of the other diluents found in street heroin, caffeine does not exhibit strong pharmacological effects that could interfere in the evaluation of the effect of diacetylmorphine. Having considered the arguments mentioned above, we decided to continue the development of a dosage form for diacetylmorphine for inhalation with a combination of diacetylmorphine base and caffeine.

In the process of 'chasing the dragon' or smoking heroin, temperature is a very important variable, influencing recovery of the unchanged drug in smoke and thereby influencing bioavailability. An *in vitro* test on tobacco cigarettes containing diacetylmorphine HCl found 12-19% recovery as unchanged heroin [11]. Similarly, *in vitro* recovery from woodruff cigarettes containing diacetylmorphine base was reported as 5-14%, expressed as total opiates [12]. These results could be explained by extensive degradation caused by the high temperature occurring at the tip of a cigarette (400-700°C). This suggestion was supported by findings of Cook et al. [13] that showed rapidly decreasing recoveries with increasing temperatures after pyrolysis of diacetylmorphine HCl (from 89% at 200°C to 8% at 300°C) and diacetylmorphine base (±70% at 2-300°C, 30% and decreasing from 400°C upward). A pharmacokinetic study using a computer-controlled smoking device to administer diacetylmorphine base to human volunteers [14] showed much better results. The device volatilised small doses (0-10 mg) at a temperature of ±200°C, which were then inhaled as a single puff. In the smoke condensate 89% heroin was found.
These findings suggest that heating diacetylmorphine at lower temperatures (closer to its melting temperature) is beneficial and will produce more unchanged drug in the smoke. The technique heroin users on the street apply also shows several aspects that could be considered to aim to control the temperature of the drug. The substance is generally heated intermittently and the resulting liquid is moved around to prevent it becoming too hot and char. The ideal method for volatilising diacetylmorphine might have to incorporate several of the abovementioned parameters to optimise the temperature of the sample.
For this reason, our recovery experiments were conducted at the lowest possible temperature (±300°C) that could ensure a reasonable volatilisation time for the relatively large amount of diacetylmorphine. The results show a reasonable 40.8% recovery, which was however less than reported by others [13,14]. This could be explained by the different volatilisation temperatures used, as well as increased degradation in our samples due to overheating part of the sample by continuously heating the tablets. Using a powder instead of a compressed sample could prevent large temperature differences within the sample.

There was no evidence, however, for the presence of potential degradation products of diacetylmorphine or caffeine (besides 6-acetylmorphine) in the vapours collected after complete volatilisation of diacetylmorphine for inhalation. All of the caffeine from the tablets was recovered unchanged in the vapours, indicating that A) the method used to collect the vapours was quite efficient in collecting volatilised solids, and B) caffeine did not degrade upon heating and volatilisation. Furthermore, only 11.6-16.5% w/w of diacetylmorphine from the tablets was unaccounted for after volatilisation, the rest was recovered as volatilised diacetylmorphine and 6-acetylmorphine or as residue in the crucible. The diacetylmorphine not accounted for could have decomposed to substances that escaped the fume collection system (gases) or that could not be detected by our HPLC-UV system. There was no evidence for any formation of toxic products vaporising in significant quantities, especially not

from the excipient, caffeine anhydrate. However, it is possible that products of degradation and pyrolysis of diacetylmorphine were missed in the analysis of the fumes. Therefore, formation of these (possibly toxic) products in the volatilisation process cannot be completely ruled out and requires further investigation.
As mentioned above, 6-acetylmorphine was the only degradation product in the chromatograms of the condensate from the recovery experiments of diacetylmorphine for inhalation. This is consistent with findings in condensate from diacetylmorphine base heated with a computer controlled smoking device [14] and from other *in vitro* experiments imitating 'chasing the dragon' [7]. The latter also identified N,6-diacetylnormorphine and N,3,6-triacetylnormorphine in the fumes. Cook et al. showed that heating diacetylmorphine base for 10 min at 250°C produced all of the aforementioned pyrolysis products and 3,4-diacetoxyphenanthrene [15].
As can easily be predicted from the ester-structure of diacetylmorphine, 6-acetylmorphine has also been found an important metabolite *in vivo*, produced under the influence of esterases [16-18]. In *in vitro*- and animal studies 6-acetylmorphine was found to be pharmacologically active [19-21] and it is even considered to be the active metabolite responsible for the actions of heroin [22]. Detection of 6-acetylmorphine as a degradation product of volatilisation of diacetylmorphine for inhalation was therefore not considered a safety problem.

Conclusion

Diacetylmorphine base in combination with caffeine was found to be a suitable basis for a pharmaceutical dosage form for heroin to be inhaled after volatilisation ('chasing the dragon'). Thermal analysis showed these substances, as well as their mixtures, to have better thermal stability than diacetylmorphine hydrochloride. Recovery experiments showed that 40.8 ± 5.3% of diacetylmorphine base could be found in smoke condensate after volatilisation of diacetylmorphine for inhalation. All of the caffeine and 85.6% of the diacetylmorphine from each tablet was recovered, either unchanged in the fumes or as non-volatilised residue. Under the tested experimental conditions, no degradation of caffeine was observed and only small amounts of one degradation product (6-acetylmorphine) of diacetylmorphine appeared to volatilise with the main components. However, further research into possible toxic degradation products is necessary to ensure safe use of diacetylmorphine for inhalation after volatilisation.

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Chapter 3.2

Volatilisation of diacetylmorphine: *in vitro* simulation of 'chasing the dragon'

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Abstract

In preparation for a trial on co-prescription of heroin to chronic treatment-resistant heroin addicts, a pharmaceutical dosage form for smokable heroin was developed, consisting of a powder mixture of diacetylmorphine base and caffeine anhydrate. During the development of this dosage form, in vitro experiments were performed simulating 'chasing the dragon': the technique used by addicts for inhalation of heroin after volatilisation. Samples were heated on aluminium foil using a heating device and the resulting vapours were collected and analysed using a suitable HPLC-UV method. The recovery of diacetylmorphine and caffeine in vapours was studied after volatilisation of pure drug substances and mixture samples, at temperatures between 200-350°C. Furthermore, this volatilisation set-up was combined with an Andersen sampler collecting the vapours to determine the sizes of aerosol particles. Only small differences in recovery of diacetylmorphine and caffeine were found between temperatures and between powder mixtures: 46-62% of diacetylmorphine from the sample was recovered in vapour and 65-83% of caffeine. The only degradation product detected in vapour was 6-acetyl-morphine (4.1-7.1% of diacetylmorphine in the sample). In the temperature range studied, temperature mainly influenced the volatilisation rate. Mass median aerodynamic diameters of aerosols from diacetylmorphine containing samples ranged from 1.8-4.1 µm and 45-60% of each sample was recovered as aerosol particles < 5 µm. The described experiment set-up was successfully used for simulation of 'chasing the dragon'. Volatilising pharmaceutical smokable heroin resulted in sufficient amounts of diacetylmorphine in vapour and in particles small enough for effective deposition in the lungs.

Abbreviations

CAF = caffeine; DAM = diacetylmorphine; GSD = geometric standard deviation; HPLC = high performance liquid chromatography, with DAD = diode array detection, or UV = ultraviolet detection; MAM = 6-acetylmorphine; MMAD = mass median aerodynamic diameter.

Introduction

Heroin (3,6-diacetylmorphine) is a well-known drug of abuse that is usually administered intravenously. However, smoking heroin has gained popularity since it was first described in Shanghai in the 1920s [1]. After some refinement, the use of an inhalation procedure called 'chasing the dragon' spread to South East Asia, India, and some parts of Europe in 1960-1980 [1]. In this procedure, addicts heat heroin powder on a piece of aluminium foil with a cigarette lighter until it melts and evaporates. The fumes are subsequently inhaled through a straw in the mouth.
A clinical trial was performed in the Netherlands to evaluate the effect of medical co-prescription of heroin and methadone on mental and physical health and social functioning of chronic, treatment-resistant, heroin-dependent patients [2]. Since in the Netherlands 75-85% of the heroin addicts use heroin by 'chasing the dragon' [3], two separate study protocols were developed; in one trial patients received injectable heroin, in the other they were prescribed pharmaceutical heroin to be inhaled after volatilisation. For the latter, we developed a dosage form (diacetylmorphine for inhalation) that consisted of a mixture of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate [4,5]. Diacetylmorphine base was preferred to diacetylmorphine hydrochloride, since the base has a lower melting point (173°C) than the hydrochloride salt (243-244°C) [6] and because of its relative insensitivity to degradation [7]. Caffeine was added as it was suggested to increase the recovery of diacetylmorphine base and hydrochloride after volatilisation and to reduce degradation upon heating [7]. Furthermore, it is commonly used as a diluent in street heroin [7-10] and has never been associated with any adverse events as far as we know. It was therefore considered to be relatively safe to use as an excipient in pharmaceutical heroin for inhalation.

In this study, we describe a standardised method for *in vitro* simulation of the process of 'chasing the dragon'. This method was used to study the recovery of diacetylmorphine and caffeine from samples of diacetylmorphine base mixed with varying proportions of caffeine anhydrate at different temperatures. Furthermore, since in preparations for inhalation aerosol properties are important for lung penetration and bioavailability, particle-sizing experiments were performed on the aerosols that were formed after volatilisation of diacetylmorphine, caffeine, and mixtures thereof.

Materials and methods

Chemicals

Diacetylmorphine base was manufactured specifically for the clinical trial and obtained through the Central Committee on the Treatment of Heroin Addicts. Caffeine anhydrate and morphine hydrochloride were purchased from Bufa (Uitgeest, The Netherlands), and 6-acetylmorphine hydrochloride was obtained from Sigma Aldrich Co. Ltd. (Zwijndrecht, The Netherlands).

Analysis

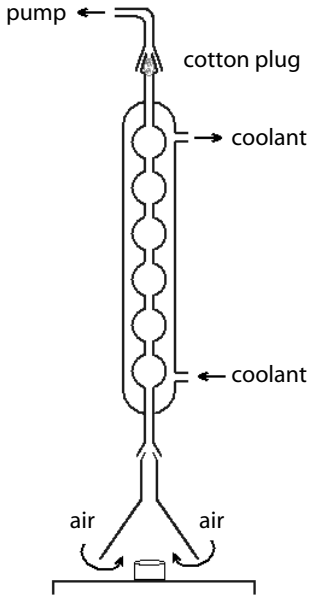
A high performance liquid chromatography system with diode array detection (HPLC-DAD) was used to quantify the recoveries of diacetylmorphine, caffeine, and degradation products of diacetylmorphine, in condensates and residues obtained from the *in vitro* volatilisation procedure. The system consisted of an 1100 Series binary HPLC pump, Model G1312A (Agilent Technologies, Amstelveen, The Netherlands), a SpectraSERIES Model AS3000 automatic sample injection device, equipped with a 100 µL sample loop (Thermo Separation Products, Breda, The Netherlands), and a photodiode array detector Model Waters™ 996 (Waters Chromatography B.V., Etten-Leur, The Netherlands). Chromatograms were processed using Chromeleon® software (Dionex Corporation, Sunnyvale, CA, USA). In the liquid chromatography system, separation was achieved using a Zorbax Bonus RP analytical column (4.6 mm ID x 15 cm, particle size 5 µm, Rockland Technologies Inc., Newport, DE, USA), protected by a Chromguard RP column (10x3 mm ID, Chrompack, Middelburg, The Netherlands). The mobile phase consisted of a 5 mM ammonium acetate buffer pH 5.7, mixed with acetonitrile according to a programmed gradient: 0-2 min 3% acetonitrile, 2-2.6 min a linear rise from 3-13%, 2.6-8 min 13-15.5%, 8-15 min 15.5-80%, 15.1-24 min 3% acetonitrile. Quantification of diacetylmorphine, caffeine, 6-acetylmorphine, and morphine was performed using a 6-point calibration curve in the following respective concentration ranges: 1-50 µg/mL, 1-40 µg/mL, 0.5-5 µg/mL and 1-10 µg/mL.

In vitro volatilisation

The powder samples were heated in sample holders, shaped from aluminium foil (Ø 3 cm, height 0.5-1.5 cm, flat bottom), which were placed on a heating device (IKA Werke RH Basic, Staufen, Germany). The desired temperatures were set using an infrared thermometer (Fluke Model 65, Fluke Corporation Europe, Eindhoven, The Netherlands) to check the exact surface temperature the sample was exposed to. Fumes emitted from the volatilising sample were directed through a 40-cm ball condenser, fitted with a funnel (Ø 9 cm) above the sample and with a vacuum pump (Type N022 AT18, KNF Neuberger, Vleuten, The Netherlands) on the other side (Figure 1). To prevent the fumes being sucked into the pump, a cotton plug was placed in the top of the condenser. Condenser temperature was kept at –5°C by a cooling bath (Haake GH Fisons D8, Karlsruhe, Germany), filled with coolant (1:1 ethylene glycol:water). The sample was weighed accurately into the tared sample holder, to enable determination of the size of the residue by weighing it again after the experiment.

Diacetylmorphine base and caffeine were volatilised at 250°C, 300°C and 350°C. Three different mixtures of diacetylmorphine base and caffeine (containing 25%, 50%, and 75% w/w diacetylmorphine) were tested at 250°C and 300°C, while the 75% diacetylmorphine mixture was also tested at 225°C, 275°C, 325°C and 350°C. Volatilisation was said to be complete when the sample was heated until no more

Figure 1: Experimental set-up for in vitro volatilisation of samples of diacetylmorphine and caffeine. From top to bottom: air outlet towards vacuum pump, cotton plug, ball condenser (40 cm) with inlet and outlet for coolant (−5°C), funnel (Ø 9 cm), aluminium sample holder on a heating device.



fumes were emitted. The heating process was easily controllable, since the thin aluminium sample holder allowed the heating process to start directly after placing it onto the preheated device and to stop instantly after its removal. This enabled us to test the 75% diacetylmorphine base/caffeine mixture using a fixed heating time (3 min) and variable temperature (200°C, 225°C, 250°C, 265°C, 285°C, 300°C). Each experiment was repeated 4 times. In order to determine the composition of the foil residue, samples (±30 mg) of 50% and 75% diacetylmorphine mixtures were heated at 250°C for 0, 30, 60, 90, etc. to 360 s.

After volatilisation of the sample, the condenser, the funnel and the cotton plug were rinsed with 1:1 v/v mixture of 5 mM ammonium acetate buffer pH4 and acetonitrile. The rinsing fluid was collected and diluted to 100.0 mL. The aluminium sample holder was sonicated for 15 min in 25 mL of the abovementioned solvent that was diluted to 50.0 mL after removal of the sample holder. The condensate and residue samples were diluted with 5 mM ammonium acetate buffer pH 5.7 before analysis. Diacetylmorphine and caffeine recoveries in vapour (condensate) or residue were calculated relative to the respective amounts present in the powder sample (percentage w/w). 6-Acetylmorphine recovery was calculated relative to the amount of diacetylmorphine present in the original sample, using a correction for the difference in molecular weight.

Particle size

The particle size of the aerosols, generated after *in vitro* volatilisation of (mixtures of) diacetylmorphine and caffeine powder samples were determined using an eight-stage Andersen sizing sampler (apparatus D, [11]. This sampler consists of 8 aluminium stages, each designed to collect airborne particles in a specific size range (< 0.4 µm, 0.4-0.7 µm, 0.7-1.1 µm, 1.1-2.1 µm, 2.1-3.2 µm, 3.2-4.7 µm, 4.7-5.8 µm and 5.8-9.0 µm). Separation of particles is achieved via the principle of inertial impaction. The Andersen sampler was fitted with an inductor port [11], that was positioned ± 1.5 cm above and directly next to the sample holder to minimise the loss of vapours. Glass fibre filters (grade 934 AH, Ø 82 mm, 1.5 µm, Whatman via VWR International, Amsterdam, The Netherlands) were placed on the collection plates on each stage of the Andersen sampler, in order to limit particle bounce. The powder samples were volatilised in the aluminium sample holders on the heating device, as described under *In vitro volatilisation*. A Becker pump, attached to the sizing sampler, was set to generate a 28.3 L/min air flow rate at the induction port, sucking the vapours into the sizing sampler.

After complete volatilisation of the 100-150 mg powder samples, the inductor port, the 8 stages, and the final filter were analysed using the abovementioned HPLC method with UV detection (λ = 214 nm). Each of these components was washed and diluted with a mixture of 15% v/v acetonitrile and 85% v/v 5 mM ammonium acetate buffer pH 4 before injecting 20 µL in the HPLC-system. The resulting data (analyte mass on each of the stages) were used to determine the mass median aerodynamic diameter (MMAD, in µm) and geometric standard deviation (GSD), via a log-probability plot of the cumulative mass fraction per stage versus the cut-off diameter of each stage [11]. Furthermore, this plot was used to calculate the fine particle fraction: the fraction of the sample mass (% w/w) that was recovered as aerosol particles < 5 µm. Concentration data from the lower 6 stages of the Andersen sampler were used to calculate the composition of the aerosol particles < 4.7 µm (in % w/w).

Diacetylmorphine base, caffeine anhydrate, and 75% diacetylmorphine mixture samples were volatilised at 300°C (in duplicate), as well as a 25% and a 50% diacetylmorphine mixture. Temperature effects were tested in 75% mixtures, volatilised at 250°C, 300°C and 350°C. This mixture was also volatilised via intermittent heating (20 s on, 10 s off) at 300°C.

Results

Volatilisation of pure drug substance samples

The results of the complete volatilisation of drug substance samples are given in Table 1. Most of the caffeine from the pure drug substance samples was recovered unchanged from the collected fumes (68.2 ± 7.8% w/w (n = 12)), only very little (0-1.4%) was left in the sample holder. No carbonisation of these samples was observed, nor were unidentified peaks in the chromatograms from the condenser, or

Table 1: Recovery results after complete volatilisation of pure diacetylmorphine base and pure caffeine anhydrate at different temperatures (°C). Recoveries (as unchanged diacetylmorphine or caffeine) in condensate, in residue and overall are given as mean % w/w of sample mass, with standard deviation between parentheses. Residue sizes are given expressed as % w/w of sample mass.

	Caffeine			Diacetylmorphine base		
Temperature	**250**	**300**	**350**	**250**	**300**	**350**
Recovery (%)						
Condensate	65.0 (5.9)	66.2 (2.3)	72.1 (11.7)	55.2 (1.9)	48.5 (9.6)	45.1 (8.9)
Residue	1.4 (0.6)	0.0 (-)	0.1 (0.2)	0.9 (0.6)	8.7 (9.8)	2.4 (1.6)
Overall	66.4 (6.3)	66.2 (2.3)	72.2 (11.8)	56.1 (2.1)	57.2 (10.2)	47.5 (9.6)
Residue size (%)	2.9 (0.4)	0.0 (0.0)	0.1 (0.9)	11.4 (1.4)	16.5 (14.9)	7.5 (1.7)

from the aluminium foil, which means that 31.8% w/w of the sample was unaccounted for. Diacetylmorphine recoveries in the fumes emitted from diacetylmorphine base were lower, 52.9 ± 8.8% (n = 12) (Table 1). No significant differences were found between the three temperatures tested for either drug substance, but a trend of decreasing diacetylmorphine recoveries in condensate with increasing temperature was observed (Table 1). Even though diacetylmorphine base samples left carbonised residues after volatilisation and unidentified peaks were present in residue chromatograms, no signs of decomposition were detected in the chromatograms of the corresponding condensate samples. The major degradation product found in both condensate and residue samples after volatilisation of diacetylmorphine base was 6-acetylmorphine: 3.5-5.2% w/w (relative to diacetylmorphine in the sample, corrected for molecular weight) in condensate and 0.3-0.6% in residue. No morphine was detected in condensate or residue chromatograms. Mean overall recovery of a diacetylmorphine base sample as diacetylmorphine or 6-acetylmorphine in vapours or as residue weight was found to be 64.2 ± 11.4% w/w, indicating that 35.8% of the sample was not accounted for.

Volatilisation of diacetylmorphine/caffeine mixture samples

The results of the volatilisation experiments with diacetylmorphine/caffeine mixtures are given in Figure 2. Mean diacetylmorphine recovery from the mixture samples was not significantly different between the two temperatures tested: 55.0 ± 8.2% w/w at 250°C and 56.2 ± 6.7% at 300°C. The same was true for 6-acetylmorphine recovery (5.6 ± 1.3 and 5.9 ± 1.5%) and caffeine (76.0 ± 9.4 and 76.9 ± 6.0%, respectively). Some significant differences, however, were found between temperatures for specific mixture samples: diacetylmorphine recovery in condensate from 25% diacetylmorphine mixture samples was higher ($p = 0.043$) at 250°C (60.0 ± 4.9%, n = 4) than

at 300°C (49.0 ± 6.7%, n = 4), while the opposite was found for 50% diacetylmorphine mixtures ($p < 0.035$, 250°C: 45.6 ± 2.7%, n = 4; 300°C: 57.4 ± 8.4%, n = 3) (Figure 2). These differences were not reflected in the respective caffeine and 6-acetylmorphine recoveries (Figure 2), or in diacetylmorphine, 6-acetylmorphine, or caffeine recoveries from 75% diacetylmorphine mixtures (Figure 2). There was no difference in recovery of these substances from 75% mixtures between temperatures of 250°C and above, even though 6-acetylmorphine recovery showed a slight increase with temperature (Figure 3). The mean recovery of 6-acetylmorphine from the 75% diacetylmorphine samples (4.8 ± 0.4%, n = 8) was significantly ($p < 0.002$) lower than from the other two mixtures (50%: 6.6 ± 1.2%, n = 7; 25%: 5.9 ± 1.2%, n = 8) (Figure 2). Overall, 62.9-80.3% of the mixture samples' weight was accounted for as diacetylmorphine, caffeine, or 6-acetylmorphine in condensate or as residue mass left on the aluminium foil. No morphine or any unknown extra peaks were detected in condensate chromatograms from volatilisation of diacetylmorphine/caffeine mixtures. Thus, 19.7-37.1% of the mixture samples was unaccounted for.

The ratio (w/w) of diacetylmorphine/caffeine had changed from 3 in the 75% diacetylmorphine powder mixture to 2.2 ± 0.2 in the condensate. Similarly, a decrease in the diacetylmorphine/caffeine ratio was observed with the 50% and 25% diacetylmorphine mixtures, from 1 and 0.33 in the powder mixture to 0.70 and 0.24 in the condensate, respectively. In residues, left after complete volatilisation of 75% diacetylmorphine mixtures, no caffeine was recovered and the amount of diacetylmorphine base that remained decreased when the temperature increased (2.9%, 1.8%, 1.0% and 0.1% at 225°C, 250°C, 275°C and 300°C respectively).

The volatilisation rate of 75% diacetylmorphine mixture samples was found to depend on temperature: complete volatilisation took 25-34 min at 200-225°C, 4.5-7.5 min at 250-275°C and 2.5-3.1 min above 300°C. Furthermore, complete volatilisation was shown to take more time when a sample consisted of a larger proportion of diacetylmorphine (Figure 4).
The influence of temperature on the volatilisation process was also illustrated by the results from the experiment with a fixed heating time (Figure 5). It is obvious from these graphs that heating the sample for 3 min at 200°C results in volatilisation of only a small amount of caffeine and almost no diacetylmorphine (Figure 5a), while heating for 3 min above 285°C results in maximum recovery of both components in vapour and a negligible recovery in residue (Figure 5b). A difference in volatilisation rate for both components of the residue can also be observed, since heating the sample for 3 min at temperatures below 250°C results in higher recoveries of caffeine in condensate than diacetylmorphine. Figure 5 also shows a decrease in overall recovery with temperature: after 3 min at 200°C 91.6% was recovered as diacetylmorphine, 6-acetylmorphine, or caffeine in condensate or residue, while above 250°C mean overall recovery was 55.6 ± 2.8%.

Figure 2: Results of volatilisation of 3 different diacetylmorphine/caffeine mixtures (25%-50%-75%) at 250°C (A) and 300°C (B). Mean recoveries are given (% w/w) with error bars indicating standard deviations. Caffeine recovery (white bars) is given relative to the amount of caffeine in the powder sample; diacetylmorphine (light gray bars), 6-acetylmorphine (dark grey) and residue size (black) are given relative to the amount of diacetylmorphine in the sample (6-acetylmorphine % w/w corrected for molecular weight).

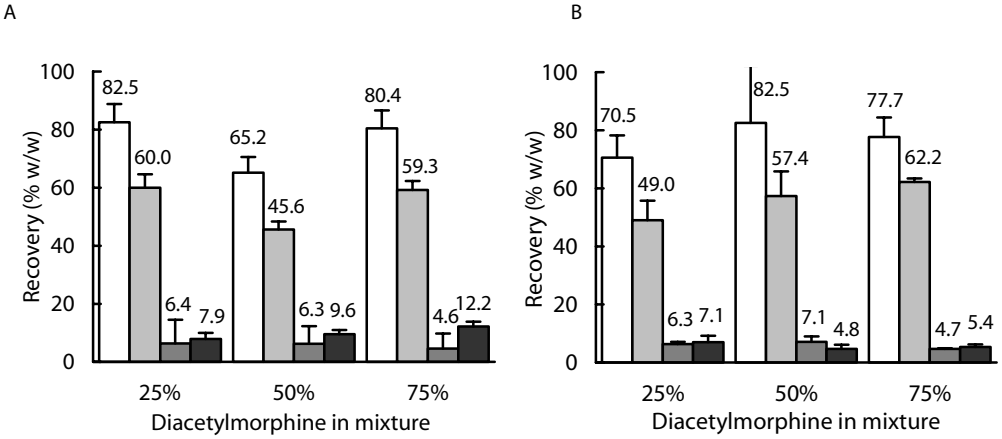


Figure 3: Mean recoveries of diacetylmorphine (DAM, closed bullets), caffeine (CAF, open bullets) and 6-acetylmorphine (MAM, open triangles, right y-axis) after complete volatilisation of 75% diacetylmorphine mixtures at different temperatures (recoveries given as % w/w of the original amount in the sample, 6-acetylmorphine as % w/w of diacetylmorphine in the sample, corrected for molecular weight; error bars indicate standard deviations).

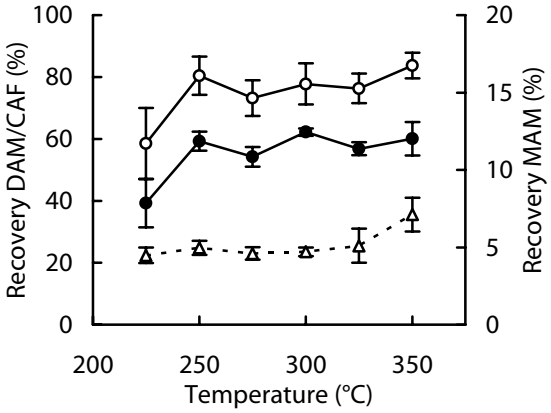


Figure 4: Mean time needed for complete volatilisation for different sample types. The solid line represents the volatilisation time at 250°C, the dashed line at 300°C; error bars indicate standard deviation.

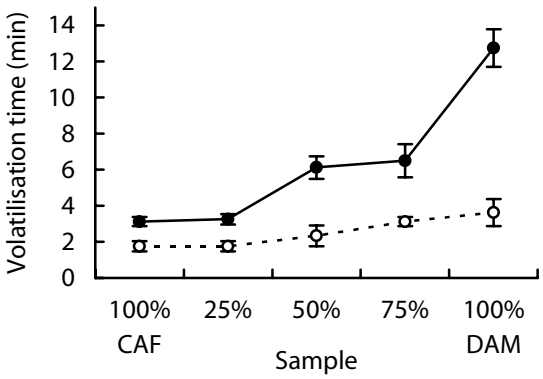
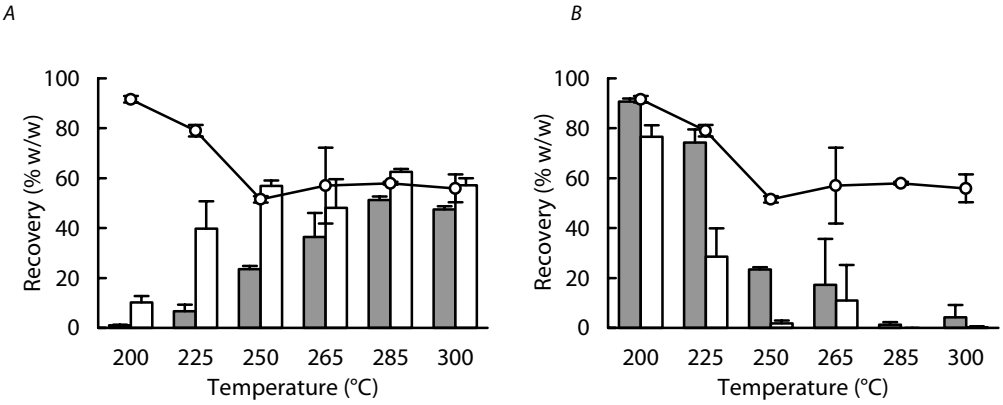


Figure 5: Mean recoveries of diacetylmorphine and caffeine from a 75% diacetylmorphine mixture samples after heating them for 3 min at different temperatures. Bars represent recoveries in condensate (A) and residue (B): grey: diacetylmorphine, white: caffeine; the solid line represents overall recovery of the sample (as diacetylmorphine, 6-acetylmorphine or caffeine in condensate or residue).



Analysis of the residues left in the aluminium sample holders after volatilisation of 50% and 75% diacetylmorphine mixtures show a decreasing proportion of caffeine in time for both mixtures (Figure 6). Furthermore, the graphs show signs of increasing degradation in the residue when volatilisation times increase: the proportion of 6-acetylmorphine in the sample increases (especially in the 75% samples), as well as the 'unidentified' proportion of the residue (not diacetylmorphine, 6-acetylmorphine, or caffeine).

Particle size

The results of the particle sizing experiments are shown in Table 2. When diacetylmorphine base and caffeine anhydrate were volatilised at 300°C, the resulting aerosols showed very different particle sizes (MMAD 2.4 ± 0.2 µm and 6.2 ± 0.5 µm, respectively). Mixture samples containing 75% diacetylmorphine showed similar MMAD values (2.8 µm) as for diacetylmorphine base at 300°C; samples with larger proportions of caffeine showed larger MMAD values. Furthermore, samples with more caffeine showed slightly more impaction in the inductor port of the Andersen sampler, reflecting the situation *in vivo*, where larger particles would be expected to deposit in the mouth and throat (for which the inductor port is a model). Within the diacetylmorphine/caffeine mixture samples, there were only small differences between $MMAD_{diacetylmorphine}$ and $MMAD_{caffeine}$. 6-Acetylmorphine seemed to be consistently more abundant in the smaller particles of the mixture aerosols, as indicated by the small $MMAD_{6\text{-}acetylmorphine}$ values (Table 2).
Temperature seemed to affect the particle size of the aerosol from the 75% mixture: volatilisation at 250°C yielded smaller aerosol particles (MMAD 1.8 µm) than at 300 or 350°C (MMAD 2.8 and 3.8 µm, respectively). Geometric standard deviations showed little variation between samples or between temperatures. The results from the 75% sample that was heated at 300°C intermittently were similar (MMAD 2.2 µm) to the aerosol from the sample that was continuously heated at 250°C (1.8 µm).

The aerosol recovery (% w/w of sample mass recovered in Andersen sampler and inductor port) of the caffeine samples was 88.9% (Table 2), indicating that the particle sizing experimental set-up was able to efficiently collect the vapours arising after volatilisation. The other sample types show lower aerosol recoveries (59.0-81.7%). The fine particle fraction (% sample mass recovered as aerosol particles < 5 µm) ranged from 41.4-59.9% w/w; no correlation with sample composition was observed, but increasing temperatures did result in much lower fine particle fractions for 75% mixture samples. The best results were obtained after volatilisation of a 75% mixture sample at 250°C: 59.9% of the sample was recovered as aerosol particles < 5 µm. This fraction of the aerosol was found to consist mainly of unchanged diacetylmorphine (61.3% w/w) with 7.6% 6-acetylmorphine and 31.1% caffeine. Similar aerosol compositions were observed after volatilisation of a 75% mixture via intermittent heating at 300°C. Higher volatilisation temperatures resulted in larger proportions of 6-acetylmorphine in this fraction of the aerosol.

Table 2: Results of aerosol particle size measurements: for each experiment, temperature, particle size parameters and recovery parameters are given.

Sample	100% CAF	25% DAM	50% DAM	75% DAM				100% DAM
Temperature (°C)	300	300	300	250	300	350	300*	300
MMAD (µm)	6.2	3.4	4.1	1.8	2.8	3.8	2.2	2.5
GSD	2.9	3.2	2.8	2.9	2.8	2.6	2.8	2.5
$MMAD_{diacetylmorphine}$		2.6	4.0	1.7	2.9	4.1	2.1	2.5
$MMAD_{caffeine}$		3.8	4.2	2.2	2.8	3.7	2.3	
$MMAD_{6\text{-}acetylmorphine}$		1.3	2.6	1.3	2.3	3.3	1.8	2.3
Aerosol recovery	88.9	81.7	80.6	72.3	63.2	63.4	64.2	59.0
Recovery in inductor port	7.1	6.6	9.2	0.9	3.7	5.0	3.1	3.0
Fine particle fraction	41.4	51.2	46.5	59.9	45.2	35.4	50.8	46.5
Aerosol <4.7 µm content								
Diacetylmorphine		29.1	43.2	61.3	55.2	49.7	62.6	88.7
6-Acetylmorphine		2.8	3.4	7.6	10.6	10.3	7.6	11.3
Caffeine	100	68.1	54.0	31.1	34.2	40.0	29.7	

DAM = diacetylmorphine base; CAF = caffeine anhydrate; * = heated intermittently; MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation. Aerosol recovery (mass in inductor port and in Andersen sampler) and recovery in inductor port are given as % w/w of sample mass; Fine particle fraction = fraction (% w/w) of sample mass recovered as aerosol particles <5 µm; Aerosol <4.7 µm contents are given as % w/w of total mass of aerosol <4.7 µm.

Discussion

In this paper, we describe an experimental set-up for *in vitro* experiments simulating heroin smoking via 'chasing the dragon'. The sample was heated in an aluminium foil sample holder, for accurate simulation of street practice. But for standardisation purposes, a heating device was preferred to the cigarette lighter used by addicts. Combining the excellent heat conducting properties of the aluminium foil sample holder with the heating device enabled us to subject the samples to exactly the desired temperature (set accurately using an infrared thermometer) and made temperature easy to control. This was considered important, as varying results of volatilisation studies in literature often might be explained by different volatilisation temperatures.

Our results for the recovery of diacetylmorphine in vapour after volatilisation of diacetylmorphine base (45.1-55.2%) resembled those found in a study in which a cigarette lighter was used to (intermittently) heat the samples: 57-69% [7].

Volatilisation temperature could account for the small difference between the outcomes, assuming that intermittent use of a lighter (maximum temperature about 600°C [7]) results in average volatilisation temperatures below 300°C. Similarly, larger recoveries were found in a Swiss study (70% diacetylmorphine base), because in this case the cigarette lighter reportedly only heated the samples to 250°C [12]. Different volatilisation temperatures could not explain the recovery of 69% of a diacetylmorphine base sample after heating it (at 300°C) in a quartz furnace [13]. However, it was not clear if complete volatilisation was attempted, or if this value indicated overall recovery or recovery in vapour only. The largest recovery of unchanged diacetylmorphine base in vapour (89%) was found after heating 3-11 mg using a diacetylmorphine coated wire coil at 200°C [14]. However, although elegant, this set-up did not mimic 'chasing the dragon', nor would it be suitable for administration of diacetylmorphine to addicts in the quantities they need (100-300 mg), which is the purpose of the product studied here. The influence of temperature was limited in the temperature range studied in our volatilisation experiments. Increasing volatilisation temperature seemed to slightly (but non-significantly) decrease the recovery of diacetylmorphine in vapour from samples of diacetyl-morphine base and a slight increase in 6-acetylmorphine recovery from 75% mixture samples was observed when temperature increased (Figure 3). Diacetyl-morphine/caffeine mixtures showed little change in recovery of diacetyl-morphine on changes of temperature (Figure 3); some statistically significant differences were found for 25% and 50% mixtures, the first showed a higher recovery at 250°C than at 300°C, the other a lower diacetylmorphine recovery (Figure 2).

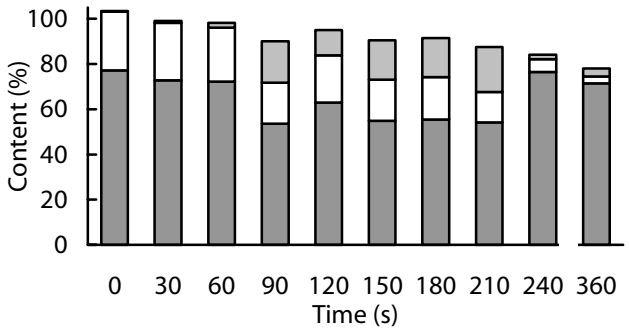
It has been suggested by Huizer that the presence of caffeine in a sample will protect diacetylmorphine from degradation when volatilised [7]. Experiments on intermittent heating of 50% mixtures of diacetylmorphine base or hydrochloride with caffeine yielded higher diacetylmorphine recoveries with caffeine (76% and 36%) than without (62% and 17%, respectively). Caffeine recovery was found to decrease when less volatile substances were admixed [7]. This could be explained thermodynamically: sublimation and volatilisation of caffeine utilise part of the energy supplied by the heat source and 'divert' it from volatilisation and degradation of diacetylmorphine in the sample. In our mixture experiments, where samples were heated continuously rather than intermittently [7], no protective effect was observed. It is possible that continuous heating leads to an excessive supply of heat to the volatilising sample, masking a possible protective effect of caffeine. However, we considered continuous heating of the samples necessary in order to standardise the heating process, which Huizer admitted was as important as it was variable [7].
The only result suggesting a protective effect of caffeine was the finding that 75% diacetylmorphine mixture residues soon contain a larger proportion of 6-acetylmorphine than 50% diacetylmorphine mixtures (Figure 6). However, since overall residue sizes decreased in time and the foil residue compositions in Figure 6 are relative figures, the absolute amount of 6-acetylmorphine in residue would have

decreased during the time needed for complete volatilisation. Moreover, proportions of analytes in residue samples do not necessarily predict proportions in vapour: no difference in 6-acetylmorphine recovery in vapour was found between 75% and 50% diacetylmorphine/caffeine mixtures after complete volatilisation (Figure 2).

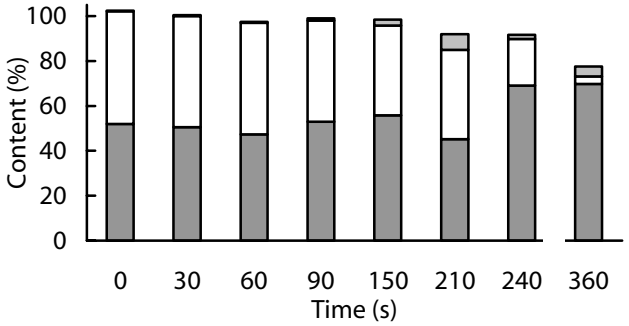
Another positive effect of addition of caffeine to a sample was the increase in volatilisation rate of diacetylmorphine/caffeine mixtures (Figure 4). This could be explained by an increase in vapour pressure of the mixture, increasing its volatility (caffeine vapour pressure: 9.10^{-4} Torr at 25°C; diacetylmorphine base: $6x10^{-8}$ Torr at 25°C [15]). Moreover, it is known that caffeine has a sublimation temperature (178°C) below its melting temperature (238°C) [6]. These properties could cause a distillation effect in diacetylmorphine/caffeine mixtures, resulting in a decreasing proportion of caffeine in the sample (residue) and an increasing proportion of caffeine in the vapours. This is illustrated by our findings in the experiments with a fixed heating time (Figure 5): a larger proportion of caffeine volatilises from the 75% mixture in 3 min, especially at lower temperatures. The analysis of the composition of the foil residues shows a decrease in the proportion of caffeine in time, consistent with this hypothesis (Figure 6). This distillation effect could cause the beneficial effect of caffeine on onset and rate of volatilisation of diacetylmorphine to decrease during the time needed for volatilisation, but this does not seem very likely, since thermal analysis has shown that the eutectic mixture contains only 6-10% caffeine (and 90-94% diacetylmorphine) [16].

The most obvious (visual) difference between diacetylmorphine and caffeine during volatilisation seems to be the extent of degradation of the sample: none was observed for caffeine, while carbonised residues and detection of 6-acetylmorphine in the vapour were clear indications of degradation of diacetylmorphine samples. Apparently, degradation of diacetylmorphine to 6-acetylmorphine occurs readily on heating, similar to the process of hydrolysis in aqueous solutions [17]. However, there is no evidence for the next step of hydrolysis of diacetylmorphine occurring on heating: no morphine was detected in condensate or in residue samples. Apparently, the rate of carbonisation and pyrolysis of diacetylmorphine (and 6-acetylmorphine) is higher than the rate of conversion to morphine. Carbonisation and pyrolysis could account for part of the loss of sample that was observed in the volatilisation experiments (mean loss: 31.8% of caffeine samples, 35.8% of diacetylmorphine samples and 19.7-37.1% of mixture samples). Diacetylmorphine could have decomposed to substances that escaped the vapour collection system (gases) or that could not be detected by our HPLC-UV system. However, the HPLC-UV system was designed to enable detection of compounds in a wide polarity range (gradient 3-80% acetonitrile) and used an aspecific detection wavelength (214 nm), it is therefore not very likely that degradation products would escape detection, unless they were present in very small quantities.

Figure 6: Composition of the foil residue versus sample heating time (at 250°C). Proportions (% w/w) of diacetylmorphine (dark grey), caffeine (white) and 6-acetylmorphine (light grey) are given found in residues left after volatilising A. 75% and B. 50% diacetylmorphine mixtures.



A



B

The particle sizing experiments showed that volatilisation of diacetylmorphine (mixture) samples using our standardised *in vitro* set-up resulted in aerosols with small MMADs: 1.8-3.8 µm, small enough to reach the primary, secondary, and terminal bronchi (product information Andersen sampler). The fine particle fraction of these samples was found to be 35.4-59.9% w/w, indicating that approximately half of the volatilised sample was recovered as aerosol particles < 5 µm that are able to penetrate to the tracheobronchial area of the lungs and beyond (product information Andersen sampler). The experiment with a 75% mixture sample heated at 250°C resulted in the largest fine particle fraction (59.9%), which contained 61.3% diacetylmorphine and 7.6% w/w 6-acetylmorphine. Furthermore, it can be derived from the log-normal distributions of the aerosol particles that 16% w/w of the particles from a diacetylmorphine base aerosol will be smaller than (MMAD/GSD) 0.96 µm, small enough to reach the alveoli. That is even true for the diacetylmorphine sample with the largest MMAD (50% diacetylmorphine), since 16% was smaller than 1.5 µm. Addition of caffeine to diacetylmorphine did not seem to influence deposition of diacetylmorphine samples negatively, even though caffeine

samples showed a relatively large MMAD (6.2 µm) and a relatively small fine particle fraction (41.4%). In summary, our *in vitro* simulation of 'chasing the dragon' indicates that inhalation of diacetylmorphine after volatilisation could deliver a sufficiently large dose of diacetylmorphine to the airways for rapid absorption (Table 2). The experiment set-up was considered to be a reasonably accurate simulation, even though the (prescribed) air flow rate (28.3 L/min) in these experiments was not powerful enough to trap all of the vapours in the Andersen sampler and continuous heating was used instead of intermittent heating. *In vivo*, it is also impossible for addicts to inhale all of the vapours they generate, and there is no reason to assume that the Andersen sampler 'inhaled' a non-representative proportion of the vapours. The bioavailability found for diacetylmorphine for inhalation used via 'chasing the dragon' by addicts (52.2%, [18]) was similar to the fine particle fraction found in our *in vitro* studies, which supports the validity of the simulation set-up.
Our results are similar to earlier (*in vitro*) findings for cocaine: powdered cocaine base smoked from a glass pipe was found to result in airborne cocaine particles with MMAD of 2.05-2.87 µm (GSD 1.68-2.22) [19]. These findings add to the explanation of the success of heroin and cocaine as smokable drugs of abuse. The obvious pharmaceutical alternative, an aerosol generated from an aqueous solution, was tested in Switzerland: 50, 100 or 200 mg/mL aqueous solutions of diacetylmorphine HCl were nebulised using different types of nebulisers (jet-nebulisers Pari IS-2 and Pari LC-Plus, and ultrasonic nebuliser Omron U1)[20]. The particle size was found to depend on the nebuliser, and ranged from MMAD 2.4-2.6 µm to between 3.9-4.1 µm and 7.6-21.5 µm, respectively. However, this method was not found to be suitable for administering diacetylmorphine to addicts: inhalation of an effective dose of 240 mg (= 3.1 mL = 536 mg) took a patient in a pilot study 95 min and caused nausea and retching, due to the extreme bitterness of the solution [20].

Summarising, volatilisation experiments showed little influence of the amount of caffeine in the mixture on the recovery of diacetylmorphine in vapour, or on degradation of diacetylmorphine to 6-acetylmorphine. Moreover, particle sizing experiments showed that adding more than 50% caffeine yielded larger aerosol particles and would result in larger deposition of caffeine in the lungs, as 54-68% of aerosol particles < 5 µm consisted of caffeine. Since patients in the trial on co-prescription of heroin use diacetylmorphine doses up to 1000 mg per day, the use of 25% or 50% diacetylmorphine mixtures as medication would result in deposition of the equivalent of 5-9 cups of coffee in the lungs (at ± 80 mg caffeine per cup). The 75% diacetylmorphine/caffeine mixtures seemed to profit from beneficial effects of caffeine as an excipient (facilitating volatilisation and possibly protecting diacetylmorphine when it is heated intermittently by 'chasing the dragon'), without the disadvantage of co-depositing large doses of caffeine in the lungs. Therefore, a mixture of 75% w/w diacetylmorphine with 25% w/w caffeine was preferred for the pharmaceutical product 'diacetylmorphine for inhalation after volatilisation'. This product has been used successfully in the Dutch clinical trial on medical co-

prescription of heroin and methadone [2] and further pharmaceutical development studies were performed in preparation for market authorisation [4,5].

Conclusion

Volatilisation of 25, 50 and 75% diacetylmorphine/caffeine mixtures at 250 and 300°C resulted in about 45.6-62.2% recovery of unchanged diacetylmorphine in the collected vapours. In the temperature range studied (200-350°C), the main effect of increasing volatilisation temperature was an increasing volatilisation rate. Degradation of diacetylmorphine upon volatilisation was limited to conversion of 4.1-7.1% to 6-acetylmorphine. Particle sizes of aerosols from volatilised diacetylmorphine base and diacetylmorphine/caffeine mixtures were found to be very suitable for effective deposition of the active substance in the lungs: MMAD values ranged from 1.8-4.1 µm, and 45-60% of each sample was recovered as aerosol particles < 5 µm. Samples with more caffeine showed larger particle sizes and increasing volatilisation temperature also increased particle sizes. The 75% diacetylmorphine/25% caffeine mixture was preferred for the pharmaceutical development of diacetylmorphine for inhalation after volatilisation, since sufficient recoveries of unchanged diacetylmorphine in vapour were obtained, combined with little degradation to 6-acetylmorphine and acceptable amounts of caffeine co-depositing in the lungs.

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Chapter 3.3

Process characterisation, optimisation and validation of production of diacetylmorphine/caffeine sachets: a design of experiments approach

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Abstract

Powder filled sachets containing a 3:1 w/w powder mixture of diacetylmorphine base and caffeine anhydrate were developed as a dosage form for smokable heroin used for the treatment of chronic, treatment-resistant heroin addicts. The powder mixture was filled into sachets using a micro dose auger filler machine. The goal of this study was to identify the most important process variables that influence precision of dosing. Five variables were tested: auger speed, agitator speed, hopper fill level, dose interval, and dose. An experimental design was used to study the effects of each of these variables, including possible non-linear and interaction effects. A 9-term regression model was constructed, explaining 94% of the observed variation in dose weight variation coefficient. Dose, agitator speed and hopper fill level were the most important variables. The regression model was used to identify optimal settings of the variables for four sachet doses intended for routine manufacture. The results of four test batches manufactured with these optimised settings showed that accurate (accuracy: 99.0-101.0%) and precise (CV: 3.2-5.3%) filling of diacetylmorphine/caffeine sachets is possible using the micro dose auger filler machine.

Abbreviations

AoR = angle of repose; AgS = agitator speed; AuS = auger speed; CCI = Carr's compressibility index; D = dose; DI = dose interval; DoE = design of experiment; d_p = poured density; Dt = dosing time; d_t = tapped density; F = hopper fill level; Ph.Eur. = European Pharmacopoeia; rpm = revolutions per minute; SS = sachet speed, number of sachets made per minute; USP = United States Pharmacopeia.

Introduction

In 1998, two clinical trials were initiated in the Netherlands to evaluate the effect of co-prescription of heroin (3,6-diacetylmorphine) and methadone on mental and physical health and social functioning of chronic, treatment-resistant, heroin-dependent patients [1]. In the Netherlands, only 15-25% of the heroin addicts inject heroin, the remaining 75-85% inhale the heroin fumes that arise after heating heroin on aluminium foil until it evaporates ('chasing the dragon' [2]). Therefore, one of the two trials concerned co-prescription of inhalable heroin as the experimental intervention. As no pharmaceutical dosage form for inhalable heroin was available, it had to be developed specially for this trial. An important requirement was to avoid problems of patient non-compliance, by ensuring that the product could be used according to the long-established habits of the patients in the trial. A powder formulation was therefore preferred and a 3:1 w/w mixture of diacetylmorphine base and caffeine anhydrate was found to be a suitable basis for pharmaceutical smokable heroin. Diacetylmorphine base is more appropriate than diacetylmorphine hydrochloride, because it showed less degradation and larger recoveries after volatilisation [3]. Caffeine was added because it is commonly found in street heroin samples [4-7] and because it has been shown to improve the volatilisation of diacetylmorphine [3]. Addition of excipients to alter the properties of the 3:1 w/w diacetylmorphine/caffeine powder mixture was considered undesirable, because of the possibility of adverse effects arising from volatilising and inhaling these substances. Therefore, four types of powder filled sachets were developed for the clinical trial, containing 75/25 mg, 100/33 mg, 150/50 mg, or 200/67 mg diacetylmorphine/caffeine [8]. In the manufacturing process, a micro dose auger filler is used to fill the powder mixture into sachets. A long and narrow auger was designed specifically to accurately fill small amounts of powder by mechanically forced transport (ejection of several milligrams with each revolution of the auger). This principle of dosing is flexible with respect to dose, without the need to add excipients or alter excipient concentration in the powder mixture in order to obtain specific flow properties. The powder portions were packaged into sachets formed on-line from packaging foil, consisting of aluminium, paper, and polyethylene layers.

Powder filled sachets are not a common dosage form in the pharmaceutical industry, especially not for small doses (< 1 gram of powder). No literature was available on formulation issues in auger filling of powders. Furthermore, no scientific information could be found on the influence of process variables on accuracy and precision of dosing using a micro dose auger filler. It has become common practice, however, to identify important variables and subsequently optimise manufacturing processes using experimental design, especially when complex pharmaceutical processes are concerned. Granulation processes for example, have been studied extensively using design of experiments (DoE) [9-12]. Response surface methodology (an effective tool in DoE to demonstrate interaction effects between factors) has been used to study many other complex formulation issues: tablet coating [13], preparation of

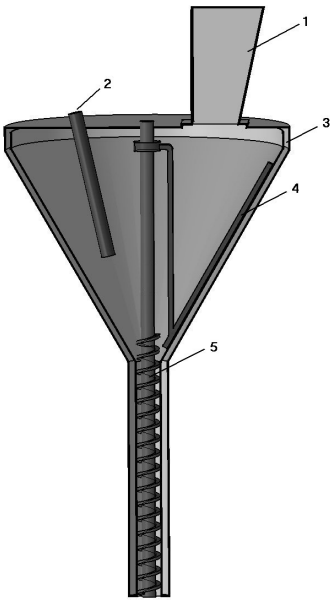
nanoparticles [14], or self-nanoemulsifying tablets [15], and drug release from controlled release formulations [16,17].
Design of experiments and response surface methodology have therefore also been employed in this study. Our first goal was to identify important process variables that influence precision of diacetylmorphine/caffeine dosing by the micro dose auger filler machine. Our second goal was to optimise the manufacturing process for each of the four diacetylmorphine/caffeine dosages intended for routine production.

Materials and Methods

Materials

Diacetylmorphine base was obtained through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands) and caffeine anhydrate was purchased from Bufa (Uitgeest, The Netherlands). The formulation to be used in this validation experiment is a 3:1 w/w powder mixture of diacetylmorphine base and caffeine anhydrate. The powder mixture was prepared by mixing three parts of diacetylmorphine with one part of caffeine using a Model UM12 Stephan mixer (Stephan Electronic 2011, Hameln, Germany).

Figure 1: Schematic representation of the micro dose auger filler (type SD1, Optima).



1. opening with funnel for filling powder into hopper; 2, product sensor; 3. plexiglass hopper. 4. agitator; 5. auger

Table 1: Study variables with selected ranges.

Variable	Range	Units
Dosage (D)	50-300	mg
Auger speed (AuS)	300-1100	rpm
Agitator speed (AgS)	10-90	rpm
Hopper fill level (F)	10-90	%
Dosing interval (DI)	500-5000	ms

Equipment

Dosing of the powder mixture was performed using a micro dose auger filler machine (type SD1, Optima, Schwäbisch Hall, Germany). The machine (Figure 1) consists of a 5 L hopper (plexiglass), fitted with a dosing funnel, an agitator, a capacitive product sensor, and a 340 mm auger (diameter 5 mm, pitch 5 mm), all constructed from stainless steel. It is operated by a microcomputer that enables the operator to control the process via a touch screen.
The auger filler is mounted vertically on top of a packaging unit (type EU1N1, Boato Pack, Staranzano, Italy) that forms sachets from foil simultaneous with dosing. The packaging foil consisted of 50 g/m^2 clay-coated paper on the outside, followed by a layer of 12 g/m^2 low-density polyethylene (LDPE), 7 µm aluminium foil, and a LDPE coating (23 g/m^2) on the inside.

Powder properties

The angle of repose (AoR) of the powder mixture was determined before and after the experiment series using a granulate flow tester (Type GTB, Erweka, Heusenstamm, Germany). Poured (d_p) and tapped (d_t) densities were determined before and after the experiment series and before every experiment run, using a tapped volumeter (Type SVM12, Erweka, Heusenstamm, Germany) according to the procedure in § 2.9.15 of the European Pharmacopoeia [18]. Carr's compressibility index (CCI) was calculated from these densities (difference between d_p and d_t as a percentage of d_p).

Experiment design

Five variables were included in the experimental design: dose (D), auger speed (AuS), agitator speed (AgS), hopper fill level (F), and dose interval (DI). Ranges for the variables are given in Table 1. An experimental design was selected to study the effect of each of the five variables, including possible non-linear effects and interaction effects (in which the effect of a variable depends on the level of a second variable). The final design (Table 2) was generated using D.o.E. Fusion Pro™ software (version 7.3.20, by S-Matrix Corp., Eureka, CA, USA). It consisted of 24 runs and contained two *centre points* (runs 11 and 16), two *factorial points to be replicated* (runs 3/17 and 4/5) and *five degrees of freedom points*. The centre points and replicate runs were used to calculate the experimental error. The coefficient of variation (CV) within the sets of sample weights was selected as a response parameter.

Sampling

Every run started with machine set-up: the hopper was filled with the desired amount of the diacetylmorphine/caffeine powder mixture and the powder was transported into the auger using standardised settings (AuS 700 rpm, AgS 50 rpm, D 300 mg for 30 doses). After the appropriate test values for D, AuS and AgS were entered into the

Table 2: Experiment design matrix (replicate runs: 3/17, 4/5, 11/16).

Run No.	D	AuS	AgS	F	DI
1	1	−1	−1	−1	−1
2	0.5	−0.5	−0.5	−0.5	0.5
3	−1	1	−1	1	−1
4	1	1	−1	1	−1
5	1	1	−1	1	−1
6	−1	1	1	−1	−1
7	0.5	−0.5	0.5	−0.5	0.5
8	−1	−1	−1	1	1
9	0	−1	1	−1	1
10	−0.5	0.5	0.5	−0.5	0.5
11	0	0	0	0	0
12	−0.5	−0.5	0.5	−0.5	0.5
13	1	1	1	1	1
14	1	1	1	−1	−1
15	1	−1	−1	1	1
16	0	0	0	0	0
17	−1	1	−1	1	−1
18	1	1	−1	−1	1
19	0.5	0.5	0.5	−0.5	0.5
20	−1	1	1	1	1
21	−1	−1	1	1	−1
22	−1	−1	−1	−1	−1
23	1	−1	1	1	−1
24	−1	1	−1	−1	1

D = dose; AuS = auger speed; AgS = agitator speed; F = hopper fill level; DI = dose interval. Numbers represent the coded parameter settings: 1 for the maximum of the selected range, -1 for the minimum of the selected range, etcetera.

auger filler computer, the accuracy of filling was checked. Three doses were weighed and filling was corrected by entering the mean fill weight into the auger filler computer as feedback on its performance. When the mean filled dose was within ± 5 mg of the design value for D, DI was set by using the resulting dosing time (Dt) to calculate the suitable sachet speed setting (SS, number of sachets made per minute). Since it was known that it could take some time for the filling performance to stabilise (especially with large doses), it was decided to include a 200 doses stabilisation period in the preparation for every experiment. After this period, accuracy of filling was checked again and if a correction of D was necessary, DI and SS were also corrected before the experiment was started. During each experiment run, samples were collected in 8 mL glass vials (that were immediately closed with grey butyl rubber stoppers) every 40 doses during a total of 1000 doses (25 dose weights per run). The glass vials were weighed before and after sampling on a type PM480 balance (Mettler-Toledo, Tiel, The Netherlands, accuracy 0.1 mg) and dose weights were calculated and analysed statistically using spreadsheet software (Microsoft Excel) and D.o.E. Fusion Pro™ software.

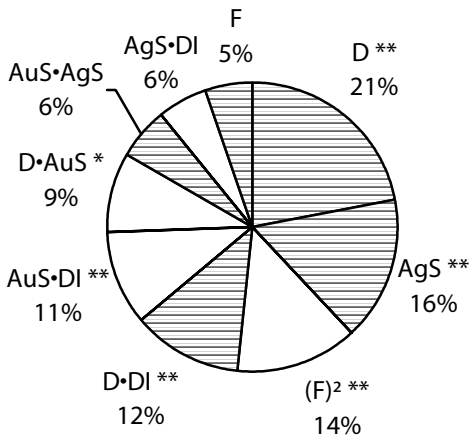
The sampling procedure in the test batches was different, as the powder was filled into sachets, making it impossible to collect the powder portions in pre-weighed sample holders. During the test batches, one in every 100 sachets was emptied to determine the delivered weight (weight of powder contents shaken out of a sachet). This procedure did not take into account the powder residue remaining on the inside of the sachets. This residue was known to be small and reproducible (8.93 ± 1.67 mg, n = 19 batches, 20 sachets each) and independent of the sachet content. It was therefore considered a necessary surplus to deliver to the user the amount of powder claimed on the sachet label; it was decided to routinely calibrate the auger filler using feedback from the determinations of the delivered weight, disregarding the residue [8].

Results & Discussion

Experiment design

All machine settings that were not dependent on properties of powder or hardware were included in the experiment design. This resulted in the five variables given in Table 1; ranges for the variables were selected on the basis of technical and practical limitations. For example, for D the technical limits were 0.05-50 mL (equalling 0.021-21 g diacetylmorphine powder mixture), but since our purpose for the machine was to fill quantities of 50-300 mg, this range was selected. For AuS, technical limits were 0-2000 revolutions per minute (rpm), however, it was known from experience that speeds over 1100 rpm could cause problems involving friction heat and that speeds smaller than 300 rpm caused unacceptably long dosing times, therefore a 300-1100 rpm range was used. DI can be considered a dependent variable, since it is a result of

Figure 2: Experiment variable ranking for the regression model of dose weight coefficient of variation (CV).



The pie chart shows the relative effect of each experiment variable across its range as a percentage of the total combined effects of all variables across their ranges. (* $p < 0.01$; ** $p < 0.001$). Shaded areas indicate a negative effect on CV. D = dose; AuS = auger speed; AgS = agitator speed; F = hopper fill level; DI = dose interval.

$$CV = 0.0188 - 0.00961 \cdot D - 0.00709 \cdot AgS - 0.00229 \cdot F + 0.0121 \cdot F^2 + 0.00394(D \cdot AuS)$$
$$- 0.00528(D \cdot DI) - 0.00250(AuS \cdot AgS) + 0.00487(AuS \cdot DI) + 0.00248(AgS \cdot DI)$$

CV can be calculated from the regression model by entering parameter values, after coding them by rescaling their tested range to –1.0 to 1.0 and calculating the corresponding coded value.

the sachet speed setting (number of sachets made per minute) of the packaging unit and the dosing time necessary to deliver the desired amount of powder. DI will preferably be as small as possible for efficient manufacturing, but because the experiment required manual sampling, its lower limit (500 ms) was based on an estimated limit of human reaction time. The selected 5000 ms upper limit was arbitrary. As F is not constant during an experiment run, the mean hopper fill level within each run was used as a variable. The powder mixture that had passed the dosing auger was not reused in the experiments, to prevent bias from changing powder properties due to (for example) grinding.

Due to technical limitations, some deviations from the design settings (Table 2) were necessary for three variables. In run 4 and 5 (replicates), AgS was set at 13 instead of 10 rpm, and in run 23, F was 74% instead of 90. Since DI is a dependent variable that was set via SS, it was not possible to set it at exactly the levels defined by the experimental design (mean deviation: −2.9%; range −47.7-23.4%). However, all deviations were entered into the design model matrix and the actual settings were used in the statistical analysis.

Table 3: Powder flow properties of the 3:1 diacetylmorphine/caffeine mixture.

Powder	d_p	d_t	CCI		AoR	
	(mg/mL)	*(mg/mL)*	*(%)*	*n*	*(°)*	*n*
Just before use	433.8 (8.9)	582.4 (6.3)	34.3 (2.6)	24	52.8 (1.2)	6
From the hopper	443.6 (5.3)	581.2 (2.3)	31.0 (1.1)*	3	49.6 (1.9)*	6
After passing auger	408.8 (14.8)*	567.0 (4.1)*	38.8 (4.3)	3	50.9 (2.4)	6

The mean values are given (with their standard deviation within parentheses) for the powder mixture just before use in an experiment run, for the powder from the hopper and for the powder collected after passing the auger. Values differing significantly ($p<0.05$) from the powder just before use are marked by *. d_p = poured density; d_t = tapped density; CCI = Carr's compressibility index; AoR = angle of repose.

Powder properties

Powder properties were determined before, during, and after performing the design of experiment runs. The high values found for CCI and AoR (Table 3) illustrate the very poor flowability of the diacetylmorphine/caffeine mixture. Poor flowability of the powder mixture might to a certain extent be advantageous in the process of auger filling, as it is essential for dosing accuracy and precision that the ejection of powder stops as soon as the auger stops moving. But more importantly, no attempts were made to adjust powder flowability by adding excipients to avoid toxicity during volatilisation and inhalation of the product.

Significant differences were found for the d_p and d_t just before use and after the powder mixture had passed the auger. The AoR and the CCI of the powder remaining in the hopper after the experiment were both significantly lower than just before use. After the powder passed the auger, these properties seemed to return to their initial level. The observed differences were very small and were not considered to have a significant impact on dosing accuracy or precision. Therefore, statistical bias from these differences seems unlikely, especially since none of the powder properties showed drift or time effects, nor was any confounding with study variables (D, AuS, AgS, DI, F) found.

In order to check for segregation of the powder mixture during the experiments, diacetylmorphine content in each first and last powder sample of every experiment run was determined using a HPLC-UV method described elsewhere [8]. No difference (paired t-test: $p = 0.895$) was found in diacetylmorphine content (in % w/w): mean content before the experiment 74.2 ± 1.2% w/w, after the experiment 74.2 ± 1.1% w/w. This proves that no separation of the diacetylmorphine/caffeine mixture takes place during the filling process.

Accuracy and precision

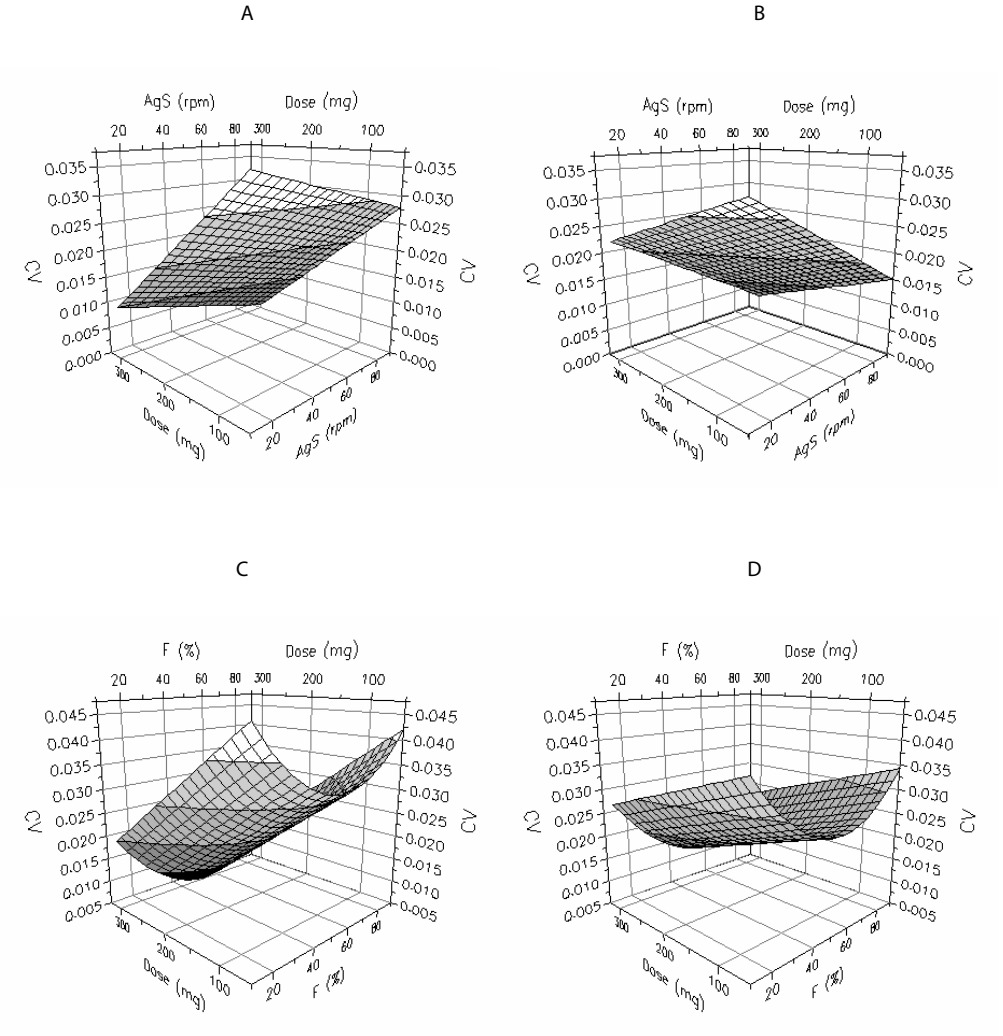
The finished product was required to comply with specifications for Uniformity of Mass [19] and/or Uniformity of Dosage Units [20]. Since we know that the

Table 4: Design of experiment with tested values for independent variables and dose weight statistics per run (n=25 dose weights per run).

Run	*D*	*AuS*	*AgS*	*F*	*DI*	mean	SD	CV	Dev 10/15	Dev 15/25
	(mg)	*(rpm)*	*(rpm)*	*(%)*	*(ms)*	(mg)	(mg)	(%)		
1	*300*	*300*	*10*	*10*	*617*	287.2	9.1	3.2	0/0	0/0
2	*237.5*	*500*	*30*	*30*	*4146*	246.8	4.8	1.9	0/0	0/0
3	*50*	*1100*	*10*	*90*	*498*	57.6	2.1	3.7	0/0	13/0
4	*300*	*1100*	*13*	*90*	*505*	302.4	9.7	3.2	0/0	0/0
5	*300*	*1100*	*13*	*90*	*479*	310.0	11.2	3.6	0/0	0/0
6	*50*	*1100*	*90*	*10*	*502*	53.2	0.8	1.6	0/0	0/0
7	*237.5*	*500*	*70*	*30*	*4145*	240.7	3.8	1.6	0/0	0/0
8	*50*	*300*	*10*	*90*	*4947*	53.9	2.3	4.2	0/0	1/0
9	*175*	*300*	*90*	*10*	*4085*	178.9	4.3	2.4	0/0	0/0
10	*112.5*	*900*	*70*	*30*	*3806*	117.2	2.5	2.2	0/0	0/0
11	*175*	*700*	*50*	*50*	*2713*	184.1	3.0	1.6	0/0	0/0
12	*112.5*	*500*	*70*	*30*	*4063*	117.6	3.0	2.5	0/0	0/0
13	*300*	*1100*	*90*	*90*	*5132*	305.7	3.4	1.1	0/0	0/0
14	*300*	*1100*	*90*	*10*	*531*	304.5	6.1	2.0	0/0	0/0
15	*300*	*300*	*10*	*90*	*2891*	303.6	5.4	1.8	0/0	0/0
16	*175*	*700*	*50*	*50*	*2719*	177.3	3.9	2.2	0/0	0/0
17	*50*	*1100*	*10*	*90*	*507*	52.6	2.0	3.9	0/0	0/0
18	*300*	*1100*	*10*	*10*	*4772*	301.7	11.8	3.9	0/0	0/0
19	*237.5*	*900*	*70*	*30*	*3884*	248.3	3.8	1.5	0/0	0/0
20	*50*	*1100*	*90*	*90*	*4899*	53.4	2.4	4.4	1/0	1/0
21	*50*	*300*	*90*	*90*	*508*	55.0	1.9	3.4	0/0	4/0
22	*50*	*300*	*10*	*10*	*510*	51.1	2.7	5.4	1/1	1/0
23	*300*	*300*	*90*	*74*	*261*	305.3	2.9	0.9	0/0	0/0
24	*50*	*1100*	*10*	*10*	*4832*	54.3	2.9	5.4	1/0	4/0

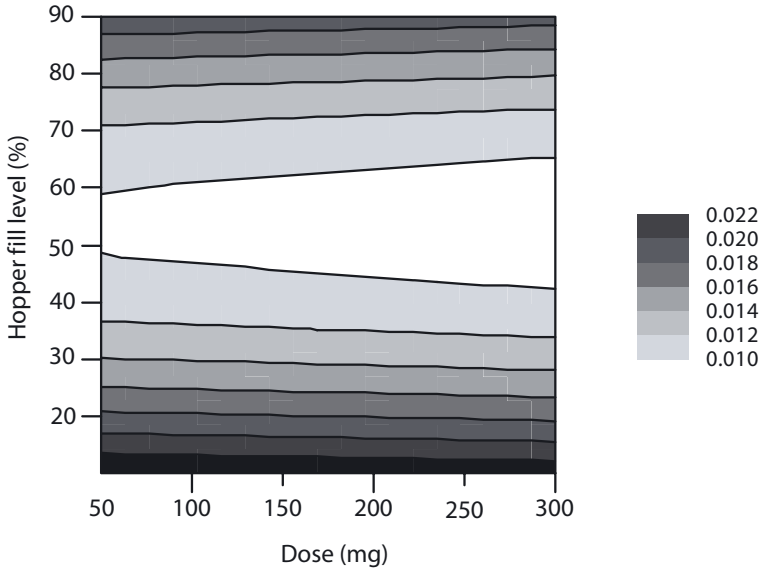
D = dose; AuS = auger speed; AgS = agitator speed; F = hopper fill level; DI = dose interval; mean = mean dose weight; SD= standard deviation; CV = coefficient of variation; Dev10/15 = number of weights deviating more than 10/15% from the mean weight, respectively; Dev 15/25 = number of weights deviating more than 15/25% from D.

Figure 3: Response surface plots for the effects of dose, agitator speed (AgS) and hopper fill level (F) on dose weight coefficient of variation (CV), at minimum auger speed (AuS, A and C) and at maximum AuS (B and D).



diacetylmorphine content of the filled powder was constant (see *Powder properties*), we could use dose weights to evaluate content uniformity. In that case, the specifications from the United States Pharmacopeia (USP, [20]) and the European Pharmacopoeia (Ph.Eur., [19]) would be similar: both state that a maximum of 3 out of 30 units deviates outside 85-115% from the label claim and none deviate outside 75-125%. However, the USP also requires the relative standard deviation (equal to CV) to be ≤7.8% and relates the percentages to the label claim, whereas in Ph.Eur. percentages relate to the average content. The specifications for Uniformity of Mass in Ph.Eur. [19] are more stringent, but also relate deviation percentages to mean mass instead of the label claim. The consequences of these differences for the results of the experiment runs are demonstrated in the last columns in Table 4, where the number of weights deviating >10% and >15% from the mean weight are given (origin: Ph.Eur.IV, 2002 [19]), as well as the number of weights deviating >15% and >25% from D (label claim; origin: USP XXIV, 2000 [20]). The difference in sample size as prescribed by Ph.Eur. (n=20) and USP (n=30) to the sample size tested (n=25) should be taken into account when interpreting these data, but it is obvious that only run 22 does not conform to the specifications in Ph.Eur., whereas it does conform to USP specifications. The opposite is true for the runs 3, 21 and 24; they do not conform to USP, but do conform to Ph.Eur. specifications. None of the runs in Table 4 show CV values that exceed or even approach the 7.8% limit [20].

Figure 4: Two-dimensional contour plot of CV as a function of dose and hopper fill level, at AuS = 1100 rpm , AgS = 65 rpm and DI = 261 ms. CV ranges from 0.8-1.0% in the middle, and to 1.8-2.0 and 2.2-2.4% in the upper and lower part of the graph, respectively.



Considering that both the Uniformity of Mass specifications from the Ph.Eur. and the CV limit from the USP primarily test precision of dosing, it can be concluded that the micro dose auger filler is suitable for precise filling of the diacetylmorphine/caffeine mixture. However, some problems with accuracy of dosing were observed: three out of eight runs with the minimum dose did not comply with USP specifications. This might be explained by the absence of dosing checks and dose correcting feedback into the machine during the experiments. They were not included in the sampling procedure to avoid possible bias in precision data, caused by these manipulations. Dose correcting feedback might be extra important when filling the 50 mg dose, as this is close to the lower technical limit of the auger filler (0.05 mL ≈ 22 mg diacetylmorphine/caffeine mixture).

The results for dose weight CV from the experimental design were analysed statistically, resulting in a 9-term regression model (Figure 2) with an R^2 of 0.9403 (adjusted R^2 0.9020), indicating that the regression model explained 94% of the observed variation in CV. One quadratic term and five interactions factors were required to adequately describe the variation in CV, as can be seen in Figure 2. Dose, AgS and F show the most important main effects on the precision of dosing, whereas DI is only involved via interaction effects with these parameters and AuS. The effects of the main response factors (D, AgS and F) on CV are presented in response surface plots in (Figure 3). Plots A and B show that a combination of high D and high AgS will result in low CV. No interaction between D and AgS is evident, since the slopes of the individual effects are independent of each other in both plots. However, when plots A and B are compared, there is an obvious difference in the slopes of both factors, indicating both parameters show an interaction with AuS. Dose level in particular shows more effect on CV when AuS is low (plot A and C) than when it is high (plot B and D, Figure 3). The influence of dose on filling precision is easily understood, as CV is a relative measure and a given deviation from the target weight will have less impact on CV when a high dose is filled. Agitator speed probably influences filling precision by achieving optimal aeration of the powder mixture in the hopper at higher agitator speeds, resulting in uniform filling of the auger and reproducible fill weights. The influence of F on precision of dosing is illustrated in Figure 3 C and D: intermediate levels of F are optimal in both plots. The increased CV that is observed at large F values might be caused by sub-optimal performance of the agitator with very large amounts of powder. Increased CV at small values for F might result from sub-optimal filling of the auger, due to the decreasing influence of gravity feeding the powder mass into the auger. In summary, a complex regression model was constructed that accurately predicts dosing precision under the experimental conditions. The multidimensional character of the auger filling process was illustrated by the number of terms involved in the model, many of which were however readily explicable in view of process characteristics.

Table 5: Optimisation results for minimising the dose weight coefficient of variation (CV): predicted optimal settings and mean predicted values for CV are given, with their 95% confidence interval.

Dose (mg)	DI (ms)	AuS (rpm)	AgS (rpm)	F (%)	CV (%)
50	261	1100	66	54	1.07 (1.0-1.1)
100	261	1100	66	54	0.93 (0.8-1.1)
133	261	1100	65	54	0.95 (0.8-1.1)
200	261	1100	65	54	0.94 (0.6-1.3)
267	261	1100	64	54	0.94 (0.5-1.3)
300	261	1100	64	54	0.93 (0.5-1.4)

DI = dose interval; AuS = auger speed; AgS = agitator speed; F = hopper fill level.

Table 6: Results of routine manufacturing using optimised settings. Statistics for delivered weights are given, the mean filling accuracy as a percentage of the label claim, as well as the number of dose corrections performed and the batch size. IPC = in-process control.

Dose (mg)	75/25	100/33	150/50	200/67
Accuracy (%)	101.0	99.0	99.5	99.8
Number deviating >10%from label claim (%)	6.3	0.5	0.6	0.0
Number deviating >15% from label claim (%)	0	0	0	0
Standard deviation (mg)	5.3	5.0	6.1	8.5
Coefficient of variation (%)	5.3	3.8	3.1	3.2
Number of sachets in IPC	191	212	157	175
Number of dose corrections	3	4	2	6
Batch size	18019	20220	15240	15723
Dose Interval (ms)	813	572	513	438
End of batch hopper fill level (%)	11	3	1	4

Optimisation

The regression model for CV was used to optimise the machine settings for the minimum and maximum dose in the tested dose range and the four dose unit contents that were selected for manufacture. The range chosen for DI in the optimisation procedure was 261 (minimum DI tested) - 500 ms, because DI will preferably be as low as possible in routine production for optimal manufacturing efficiency. The optimisation goal was to minimise CV; the results are given in Table 5, including the predicted values for CV with their 95% confidence intervals. The optimal settings for DI and AuS are ideally compatible with efficient manufacturing, since maximum AuS and minimum DI will result in the maximal sachetting speed for each dose (Table 5). The optimal value for F was found to be 54%, but F is not a constant value during routine manufacturing, therefore, its influence on dosing precision was visualised in a two-dimensional contour plot in Figure 4. It can be derived from this plot that, when filling a 300 mg dose (at the optimised settings for AuS, AgS and DI), a decrease in F from 50% to 10% would increase CV from 0.8-1.0% to 2.2-2.4%. Thus it is not likely that variation in hopper fill level during manufacture alone would compromise dosing precision.

Test batches

The optimised settings were tested in routine manufacturing: one test batch (15-20,000 sachets) was produced for each of the four doses selected for the clinical trial (75/25 mg, 100/33 mg, 150/50 mg, and 200/67 mg diacetylmorphine/caffeine). AuS and AgS were set at their optimised levels (Table 5) and DI was calculated from the sachet speed used and the dosing time. F was maintained between 30-70% during most of the batch, but was allowed to decrease below 10% near the end of the batch. Every 100 sachets, one sachet was emptied to determine the delivered weight (weight of powder contents shaken out).

To ensure filling accuracy, the operator was allowed to give dose correcting feedback (mean of last 2-3 weights) to the auger filler when the delivered weight consistently deviated >5% from the label claim; feedback was required on consistent (repeated two to three times) deviations >10%. When the delivered weight deviated >15% from the label claim, the sachets concerned were discarded.

Results for accuracy and precision of dosing in routine manufacturing are given in Table 6. The CV values found in the test batches exceed the predicted levels from the optimisation experiment. Extra variation is probably introduced because the weight delivered by the sachets was determined instead of the weight of the powder portions. Furthermore, in routine manufacturing it was not possible to set the optimised settings for DI and F exactly or to maintain them. Other factors possibly influencing dose weight variation are: the larger number of samples, the different sampling interval, and the inclusion of dose correcting feedback in the in-process control procedure. However, the results show that the micro dose auger filler can fill the four doses into sachets precisely using the optimised machine settings. Only few

dose corrections were necessary to ensure excellent filling accuracy (99-101% of set dose). No sachets were discarded due to deviation >15% from the set dose.

Conclusion

The complex pharmaceutical manufacturing process of micro dose auger filling of diacetylmorphine/caffeine powder was successfully characterised using design of experiments. All parameters tested in the experiment design, but especially dose, agitator speed and hopper fill level were found to affect dosing precision either through linear, quadratic or interaction effects. A regression model was obtained that explained 94% of the observed variation in the dose weight CV. This model was used to optimise the manufacturing processes of four types of diacetylmorphine/caffeine sachets. It was found to be necessary to include dose-correcting feedback in the in-process controls to ensure dosing accuracy. Four pilot batches showed that routine manufacturing using the optimised process resulted in a precise (e.g., CV: 3.2-5.3%) and accurate (e.g., accuracy: 99.0-101.0%) filling of diacetylmorphine/caffeine sachets.

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Chapter 3.4

Development and manufacture of diacetylmorphine/caffeine sachets for inhalation via 'chasing the dragon' by heroin addicts

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Abstract

In 1998, two clinical trials were started in the Netherlands to evaluate the effect of co-prescription of heroin and methadone on mental and physical health and social functioning of chronic treatment-resistant heroin dependent patients [1]. Since 75-85% of the heroin addicts in the Netherlands use their heroin by 'chasing the dragon' [2], one of the two study arms concerned the co-prescription of inhalable heroin. A pharmaceutical dosage form for inhalable heroin was developed for this trial, consisting of a 3:1 powder mixture of diacetylmorphine base and caffeine anhydrate.

We describe the manufacturing process that was developed for filling sachets with this mixture in four dosages using a micro dose auger filler. In order to control product quality, in-process controls were developed to monitor the filling process and quality control tests were performed on the finished product. In-process control results have shown the filling process to be accurate and precise. The diacetylmorphine/caffeine sachets were shown to comply with the specifications for content and uniformity of mass. The finished product was found to be stable for six months when stored at 40°C, 75% relative humidity.

Abbreviations

AoR = angle of repose; CCI = Carr's compressibility index; d_p = poured density; d_t = tapped density; FR = flow rate; HPLC-UV = high perfor-mance liquid chromatography with ultraviolet detection; ICH = Inter-national Commission on Harmonization; IR = infrared spectroscopy; LDPE = low-density polyethylene; OOS = out of specification; Ph.Eur. = European Pharmacopoeia; RH = relative humidity; RSD = relative standard deviation; UV/VIS = ultraviolet and visual light absorption.

Introduction

Heroin (3,6-diacetylmorphine) is a well-known drug of abuse that is usually administered intravenously. However, smoking heroin has gained popularity in many parts of the world since it was first described in Shanghai in the 1920s [3]. In a procedure called 'chasing the dragon' addicts typically inhale heroin fumes resulting from heating heroin powder on aluminium foil with a cigarette lighter until it melts and evaporates.

In 1998, two clinical trials were started in the Netherlands to evaluate the effect of co-prescription of heroin and methadone on mental and physical health and social functioning of chronic, treatment-resistant, heroin-dependent patients [4]. Since 75-85% of the heroin addicts in The Netherlands use their heroin by 'chasing the dragon' [2], one of the two study arms concerned the co-prescription of inhalable heroin. As no pharmaceutical dosage form for inhalable heroin was available, it had to be developed specially for this trial. An important requirement was to avoid problems of patient non-compliance, by ensuring that the product could be used according to the long-established habits of the patients in the trial.

A 3:1 w/w mixture of diacetylmorphine base and caffeine anhydrate was found to be an appropriate basis for a pharmaceutical form of inhalable heroin. Caffeine is commonly found in street heroin samples [5-8] and has been shown to improve the volatilisation of diacetylmorphine [9]. Furthermore, diacetylmorphine base was more suitable than diacetylmorphine hydrochloride, because it showed less degradation and larger recoveries after volatilisation [9]. This pharmaceutical form of inhalable heroin was suitable for use by 'chasing the dragon': patients placed the powder mixture on aluminium foil, and heated it from below with a cigarette lighter until the powder melted. They subsequently moved the melted mass across the surface of the foil, still carefully heating it with the lighter, while inhaling the arising fumes through a plastic straw in the mouth.

For the clinical trial, four dosage units were desired, containing 75/25 mg, 100/33 mg, 150/50mg, or 200/67 mg diacetylmorphine/caffeine. It was decided to use mechanically forced transport, using a micro dose auger filler machine, to fill sachets with the above amounts of powder. Powder-filled sachets, however, are not a common dosage form in pharmaceutical practice, especially not for small doses (< 1 g of powder). We could not find any literature on formulation and manufacturing in auger filling of powders. Therefore, in this paper the selection and development of dosage form and production process, as well as methods for in-process controls and quality control of the finished product are discussed.

Materials and Methods

Materials

Diacetylmorphine base was obtained through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands). The manufacturer used quality specifications that were derived from the British Pharmacopoeia Monograph

for Diacetylmorphine Hydrochloride [10]. In-house quality control consisted of infrared (IR) spectroscopy (identity) and high performance liquid chromatography (HPLC) analysis with ultraviolet (UV) detection (identity and purity). Caffeine anhydrate [European Pharmacopoeia (Ph.Eur.) quality] was purchased from Bufa (Uitgeest, The Netherlands). In-house quality control consisted of thin layer chromatography analysis (identity) and UV/VIS-spectroscopy (identity, content). All other chemicals used were of analytical grade and used without further purification.

Powder Properties

Poured (d_p) and tapped (d_t) densities were determined using a tapped volumeter (Type SVM12, Erweka, Heusenstamm, Germany), according to the procedure in Ph.Eur.Ed.IV § 2.9.15 [11]. Carr's compressibility index (CCI) was calculated from these densities (difference between d_p and d_t as a percentage of d_p). The angle of repose (AoR) and the flow rate (FR) (tested according to Ph.Eur.Ed.IV § 2.9.16 [12]) were determined using a granulate flow tester (Type GTB, Erweka, Heusenstamm, Germany), fitted with a 25 mm nozzle and an agitator (operated at speed setting 4).

Manufacture

A micro dose auger filler machine (Type SD1, Optima, Schwäbisch Hall, Germany) was used for filling sachets with 3:1 diacetylmorphine/caffeine powder mixture. This machine consists of a 5 L hopper (plexiglass), fitted with a product sensor, a dosing funnel, an agitator, and a 340 mm auger (diameter 5 mm, pitch 5 mm), the latter three all being constructed from stainless steel. The dosing principle of the filling machine is based on transportation of powder into the sachet by rotating the dosing auger. It is operated using a touch screen on a computer that displays settings and process data and enables the operator to adjust filling during manufacturing. The auger filler is mounted vertically on top of a packaging unit (Type EU1N1, Boato Pack, Staranzano, Italy) that forms heat-sealed sachets from a foil material. This foil consists of (from the inside out) a low-density polyethylene (LDPE) coating (23 g/m^2 LDPE), a 7-µm aluminium foil layer, a second layer of LDPE (12 g/m^2) and a layer of claycoated paper (50 g/m^2), printed with the desired label text. The packaging machine is fitted with an in-line printer for batch number and expiration date and an in-line labelling unit for tear-off labels for drug accountability purposes. During manufacturing, dosing accuracy was checked by weighing ejected powder portions (during start-up), sachet contents (powder shaken out, every 100 sachets) and filled sachets (total sachet weight, every 500 sachets) on a type PM480 balance (Mettler-Toledo, Tiel, The Netherlands).

For each manufacture run, the diacetylmorphine/caffeine mixture 3:1 w/w was prepared by mixing three parts of diacetylmorphine with one part of caffeine using a Model UM12 Stephan mixer (Stephan Electronic 2011, Hameln, Germany). Four different sachet types were produced, containing 100 mg of powder per sachet (75/25 mg diacetylmorphine/caffeine), 133 mg (100/33 mg), 200 mg (150/50 mg) and 267 mg (200/67 mg). Batch sizes ranged from 9,000-17,000 sachets, depending on the

dose. Sachets were packaged per 50 in labelled cardboard boxes (60 x 60 x 146 mm, OPG, Utrecht, The Netherlands).

High Performance Liquid Chromatography

For the analysis of diacetylmorphine and caffeine, a validated, stability-indicating, reversed-phase HPLC-UV method was used. The HPLC system consisted of a model AS3000 SpectraSystems autosampler, connected to a model P1000 SpectraSystems HPLC pump, and a UV1000 SpectraSeries detector (Thermo Separation Products, Fremont, CA, USA). Chromatograms were processed using Chromeleon® software (Dionex Corporation, Sunnyvale, CA, USA). Separation was achieved using a Zorbax Bonus RP analytical column (4.6 mm ID x 15 cm, particle size 5 µm, Rockland Technologies Inc., Newport, DE, USA). The mobile phase consisted of 85% v/v 0.05 M phosphate buffer pH = 6, mixed with 15% v/v acetonitrile. Detection wavelength was 214 nm, flow was 1.0 mL/min and injection volume was 20 µL. Samples and standard solutions were prepared using a 85/15% v/v mixture of 0.05 M phosphate buffer pH = 4 and acetonitrile as a solvent. Calibration lines for diacetylmorphine and caffeine were linear ($r^2 > 0.999$) in the concentration ranges of 20-60 µg/mL diacetylmorphine and 8-24 µg/mL caffeine. The relative diacetylmorphine (% w/w) content of the sachets was calculated from the diacetylmorphine and caffeine content (determined in triplicate). Identity of diacetylmorphine and caffeine was confirmed by comparison of retention times with those of reference standards. The chromatographic purity of diacetylmorphine was determined by dividing the peak area of the diacetylmorphine peak by the sum of the peak areas of all peaks, except the caffeine peak and the solvent peak.

Uniformity of Mass

For the test on Uniformity of Mass (Ph.Eur.Ed.IV § 2.9.5 [13]) the weight of the contents of 20 sachets was calculated by subtracting the weight of the emptied sachet from the total sachet weight. The amount of powder remaining in the sachet after shaking out the contents (residue) was calculated from the weight of the emptied sachet before and after removal of the residue.

Stability Studies

Long-term and accelerated stability studies were performed according to International Commission on Harmonization (ICH) guidelines [14]. To assess long-term stability, samples from three batches per dosage were stored at 25 ± 2°C, 60 ± 5% relative humidity (RH) in their secondary packaging in a HEKK0057 climate chamber (Weiss Technik Ltd., Buckinghamshire, UK). Mean content and purity were determined at 6, 12, 18, and 24 months using the aforementioned HPLC-UV method. For accelerated stability studies, three batches of 100/33 mg, 150/50 mg, and 200/67 mg sachets and one batch of 75/25 mg sachets were stored at 40 ± 2°C, 75 ± 5% RH in their secondary packaging in a HEKK0057 climate chamber. Mean content and purity were determined after 1, 2, 3, and 6 months.

Results and discussion

Selection of Dosage Form

It was considered important for patient compliance to develop a dosage form for pharmaceutical smokable heroin that could be used according to the long-established habits of the chronic heroin addicts in the clinical trial. Considering this requirement, powder formulations were preferred for their similarity to street heroin. Powder flow tests were performed on the drug substances used in smokable heroin, diacetylmorphine base and caffeine anhydrate, as well as on the 3:1 w/w diacetylmorphine/caffeine mixture (Table 1). Their poor flowability is demonstrated by their large angle of repose and their Carr's compressiblity index exceeding 30% [15]. Powder flow rate was also slow, and in most cases the entire sample even failed to flow through.

During the pilot phase of the clinical trial, capsules containing the 3:1 w/w diacetylmorphine/caffeine mixture were manufactured manually on a small scale; hence the poor flow properties of the 3:1 w/w powder mixture did not pose a major problem. The nursing staff opened these capsules before administering the contents to the patients to be smoked under supervision. This procedure resulted in symptoms of contact dermatitis in several members of staff [16]. In order to avoid such problems in the next phase of the trial, a pharmaceutical dosage form was required that was not contaminated with the diacetylmorphine/caffeine mixture on the outside. Furthermore, it was considered undesirable to add excipients other than caffeine anhydrate, since the sachet's contents were to be heated and the resulting vapours inhaled ('chasing the dragon') by the patients; possible adverse effects of

Table 1: Powder flow properties.

Property	Diacetylmorphine base	Caffeine	3:1 Mixture
Poured density (mg/ml)	393.7 (23.4)	420.3 (16.0)	426.2 (20.9)
Tapped density (mg/ml)	527.5 (31.5)	557.6 (10.9)	572.7 (25.0)
CCI (%)	34.0 (2.2)	32.8 (4.4)	34.5 (4.9)
N	4	2	4
Angle of Repose (°)	49.2 (2.3)	46.0 (4.1)	49.4 (3.6)
N	3	2	3
Flow rate EP (s/100 g)	11.3-∞*	3.3-∞*	9.9-∞*
N	3	2	3

CCI = Carr´s compressibility index, N = number of batches or mixtures tested, Flow rate EP = flow rate according to Ph.Eur.Ed.IV §2.9.16; densities tested in triplicate, flow rate and angle of repose measurements repeated 4-10 times; mean values are given, with standard deviations within parentheses, both for the pooled data of all batches; ranges (for pooled data) are given for flow rate. * Entire sample failed to flow through.

excipients would be unpredictable under these circumstances. Therefore, capsule or tablet formulations requiring additives like glidants, fillers, and binders were not pursued.

It was decided to develop a powder formulation filled in sachets, containing diacetylmorphine combined with only caffeine anhydrate as an excipient. Poor powder flow properties were not expected to interfere with the selected dosing principle of mechanically forced transport by a micro dose auger filler. The sachets would be easy to open and empty by the nursing staff before administration and contact dermatitis would be less likely, since the outside of the packaging material would not come into contact with the powder mixture during manufacturing.

Manufacturing Process

The manufacturing process described in this paper involves a micro dose auger filler. A long and narrow vertical auger was specifically designed to accurately fill small amounts of powder by mechanically forced transport (each revolution of the auger results in ejection of several milligrams). This principle of dosing is flexible with respect to dose, without the need to add excipients or alter excipient concentration in the powder mixture in order to obtain specific flow properties. The powder portions are subsequently packaged into sachets formed in-line from packaging foil. The process of auger filling diacetylmorphine/caffeine sachets was characterised and optimised using an experimental design approach. This study showed that regular checks of the fill weight were required to ensure accuracy of dosing. Therefore, two in-process control tests were selected: determination of the delivered weight and the total weight of a sachet.

Table 2: Specifications for in-process controls and actions upon deviation.

Test	Specification	Action on deviation
Delivered weight	Within ±10% of label claim (derived from Ph.Eur.IV §2.9.5)	Repeated test required, if deviation is repeated, a fill correction is required (Alert level)
Delivered weight	Outside ±10% but within ±15% of label claim (derived from Ph.Eur.IV §2.9.5)	Sachets concerned are rejected, a fill correction is required (Action level)
Total sachet weight	Weight difference within 10 sachets <30% of label claim (based on ±15% in delivered weight)	Determine delivered weight of sachets with largest and smallest total sachet weight, act on deviations outside 10 or 15% of label claim as described above

Delivered weights are judged individually, total sachet weights as part of a set of 10 sachets.

In-Process Controls: delivered weight

The delivered weight was defined as the weight of the powder shaken out of a sachet. It was determined every 100 sachets, to enable the operator to correct for deviations in time, thereby limiting the loss of sachets that are out of specification (OOS) and improving overall dosing accuracy. Furthermore, an accurate estimate of the mean delivered weight was necessary for reconciliation purposes (see below). Results for a typical 150/50 mg batch are shown in Figure 1. Alert levels and action levels were defined for the delivered weight (Table 2), that were based on the maximal deviation percentages mentioned in the Ph.Eur. test for uniformity of mass of capsules weighing less than 300 mg [13]: 10% deviation from label claim (alert level) and 15% deviation from label claim (action level).The results of 19 batches (Table 3) show that all four dosages could be filled accurately.

No significant difference in mean delivered weight (as a percentage of the label claim)(Table 3) was observed between the four different doses. The mean number of sachets deviating more than 10% from the label claim (alert level) was higher in sachet batches with a lower fill weight. This is easily explained by the fact that small absolute deviations will exceed the 10% alert level sooner when total fill weight is small. None of the batches showed delivered weights deviating more than 15% from the label claim; all batches were within specifications.

During manufacture, feedback from the operator on dosing accuracy to the auger filler computer was based on the delivered weight, which underestimates the fill weight, since a residue was left on the interior walls of the sachet.

Figure 1: Results of in-process control 'delivered weight' of a representative 150/50 mg sachet batch. Closed bullets represent delivered weights of these sachets (right y-axis) during manufacture, x-markings represent the total weight of the filled sachets before emptying (left y-axis). Grey lines represent alert levels (±10% of label claim), black lines represent action levels (±15% of label claim).

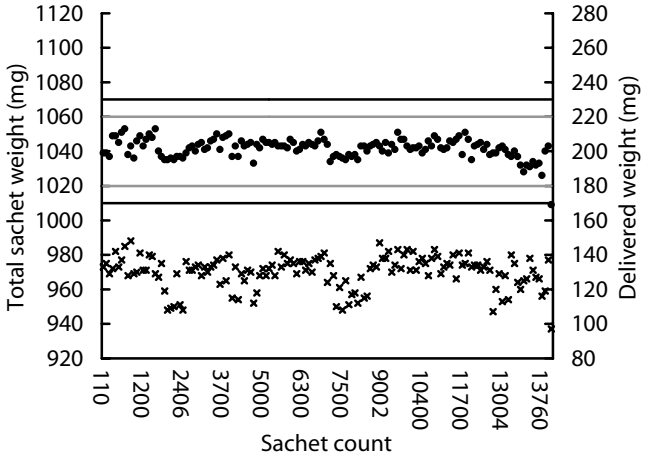

Table 3: Results of in-process weight checks and determinations of the size of the residue in the sachet.

Dose	Delivered weight (%)	N>10%	Batch size	Residue (mg)	n
75/25 mg	101.6 (0.9)	7.5 (6.5)	13205 (6368)	7.15 (1.18)	2
100/33 mg	101.1 (1.4)	2.3 (1.5)	10789 (4056)	9.97 (2.50)	4
150/50 mg	100.9 (0.6)	3.6 (4.6)	11512 (3672)	9.22 (1.33)	5
200/67 mg	100.8 (0.9)	2.8 (3.2)	11749 (3091)	8.68 (1.25)	8

Mean values are given for all parameters, with sd in parentheses. Delivered weight given as a percentage of label claim; N>10% = number of delivered weights deviating more than 10% from label claim; Batch size = number of sachets manufactured per batch; Residue = amount of powder remaining inside sachet after shaking out contents; n = number of batches.

This surplus was considered necessary to ensure that the desired amount of drug was delivered to the patient. The amount of residue remaining in the sachet after shaking out the powder mixture was quantified routinely (Table 3) and was found to be 8.93 ± 1.67 mg, independent of the sachet content.

In-Process Controls: total sachet weight

It was known from experience that certain combinations of machine settings (auger speed, dose and sachetting/packaging speed) could cause the pause between the powder portions to decrease below a critical level, resulting in full and partly filled sachets to be ejected alternately. Furthermore, it was known that every time something caused the machine to stop, the 4^{th} or 5^{th} sachet after restart might be empty. Therefore, a test of the total weight of 10 successive sachets was introduced to screen for outliers and empty sachets. The test of total sachet weight was performed every 500 sachets and after every machine stop; a difference between the smallest and largest total sachet weight $>30\%$ of the label claim was defined as indicative of the presence of an outlier or an empty sachet (Table 2).

The mean weight of an empty sachet including its tear-off label was determined using 10-20 empty sachets per batch from 19 batches: it was found to be 738.4-765.4 mg, with a standard deviation varying from 2.7-7.6 mg between batches. Since the powder contents of a sachet would form only 12-26% of the total weight of a filled sachet, it was likely that small deviations in the weight of the contents would be attributed to normal variation in sachet and/or label weight. However, ejection of an empty sachet would certainly be noticed, as well as deviations outside $\pm 15\%$ of the label claim in 150/50 mg and 200/67 mg sachets.

In routine samples from 19 batches, the mean difference between the smallest and the largest total sachet weight was found to be 14-30 mg, independent of the dose filled. This difference was attributed to the variation in weight of the packaging

Table 4: Results for quality control of 19 batches of finished product.

Quality Control Item	**Specification**	**75/25 mg (n=2)**	**100/33 mg (n=4)**	**150/50 mg (n=5)**	**200/67 mg (n=8)**
Appearance	Intact sachets, filled with a white to light yellow/pink powder mixture	conform	conform	conform	conform
Identity (HPLC-UV)	Rt DAM sample = Rt DAM reference standard	conform	conform	conform	conform
	Rt CAF sample = Rt CAF reference standard	conform	conform	conform	conform
Content (HPLC-UV)	97.5-102.5% of label claim = 73.1-76.9% w/w DAM	74.62 (0.81)	74.70 (0.63)	74.74 (0.57)	75.03 (0.43)
Purity (HPLC-UV)	>95%	99.47 (0.08)	99.10 (0.31)	98.99 (0.48)	98.95 (0.28)
Uniformity of Mass	Ph.Eur.Ed.IV § 2.9.5	conform	conform	conform	conform
RSD (IPC)	≤7.8% (derived from USP <905>)	4.81 (0.66)	3.91 (0.27)	3.78 (1.64)	3.91 (1.20)
N>15% (IPC)	not more than 1 deviates >15% from label claim	none	none	none	none

Mean values are given, with sd in parentheses. DAM = diacetylmorphine base, CAF = caffeine anhydrate, IPC = in-process controls, Ph.Eur. = European Pharmacopoeia, RSD = relative standard deviation, N = number of delivered weights, n= number of batches.

material combined with the normal variation in fill weight. Deviations from the mean difference always occurred in samples tested directly after a machine stop and amounted to mean differences of 118 mg, 161 mg, 226 mg, and 298 mg (for 75/25 mg, 100/33 mg, 150/50 mg, and 200/67 mg sachets, respectively). This indicated that a completely empty sachet was ejected, because the auger filler skipped a dose at the moment of the stop and dosing and packaging were resynchronised after restart. Intermediate size differences did not occur, proving the filling process to be very constant unless an (emergency) stop was triggered.

Reconciliation

Meeting the requirements of the Dutch Narcotics Law was an important aspect of the manufacturing process. Weighing and counting checks were developed for accurate reconciliation of the amount of bulk drug with the amount of finished product, accounting for the lost powder mixture and/or sachets. Two strategies were employed, aimed at the reconciliation of 1) the number of sachets and 2) the amount of diacetylmorphine/caffeine powder.

Reconciliation of the number of sachets means that the (electronic) sachet count by the packaging unit during manufacturing must be in close agreement with the (manual) sachet count that takes place after packaging. Empty or OOS sachets were recorded on the production protocol, so they could be accounted for. In most batches the manual count slightly exceeded electronic count: in 19 batches (9,000-18,000 sachets per batch), the mean deviation was 8 ± 12 sachets (range 8-35). These deviations could be caused by human errors in the manual count or by errors in the electronic count. Human errors were minimised by having a second person double-check the contents of every box of sachets. Errors in the electronic count, however unlikely, might arise from the (emergency) machine stops that may occur during manufacturing (see also In-Process Controls: total sachet weight). Reconciliation of the amount of diacetylmorphine/caffeine powder involved subtracting several 'types' of processed powder from the amount taken into production.

Some types of processed powder could simply be weighed, like the powder remaining in the machine hopper after manufacturing and the powder shaken out of the sachets during the in-process controls (delivered weight). For the powder that was processed into sachets, another strategy was used to determine the amount involved. The weight of the contents of the sachets was calculated by multiplying the mean content of a sachet (mean delivered weight + mean residue size) by the number of sachets. Furthermore, some of the sachets were discarded during manufacturing, for being OOS (for fill weight, quality of the seals or appearance, for example). Since the mean content of these sachets was unknown, the weight of their contents was calculated by determining the combined weight of the sachets and subtracting the mean weight of an empty sachet multiplied by the number of discarded sachets. The mean amount of powder that was not accounted for using the abovementioned calculations was 27.9 ± 22.3 g per batch ($n = 19$, 1.4 ± 1.1% of the amount taken into production). This is caused by powder loss during manufacturing, due to adhesion of the powder mixture onto the manufacturing equipment (mixer, auger/hopper of the filling

machine). However, the result of these reconciliation calculations also depends on the accuracy of the determined values for mean delivered weight (n = 150-200), mean residue size (n = 20), and mean weight of an empty sachet (n = 20). Small deviations in these factors are multiplied and contribute to the observed (variation in) loss of powder.

Quality Control of the Finished Product

For quality control of the finished product, the following tests were selected: inspection of product appearance, determination of uniformity of mass (according to Ph.Eur.IV § 2.9.5 [13]), and HPLC-UV analysis. The combined results from in-process controls and the test for uniformity of mass were evaluated to ensure accuracy and precision of the filling process. Results of the quality control of 19 batches are shown in Table 4.

High performance liquid chromatography-UV analysis was used for confirmation of the identity of diacetylmorphine and caffeine and determination of purity and relative content of diacetylmorphine. Relative content was defined as the % w/w of diacetylmorphine in the powder mixture and it was calculated from the absolute contents of diacetylmorphine and caffeine. Interestingly, when relative content was determined in a sample of the powder mixture removed from the sachets (n = 20) used for the determination of uniformity of mass, it was consistently lower (T-test: $p = 0.0007$) in 75/25 mg sachets (72.2 ± 1.5% w/w) than in 200/67 mg sachets (74.0 ± 0.9% w/w). This could be explained by a stronger adhesion of diacetylmorphine to the LDPE insides of the sachets relative to caffeine. When the contents of a sachet were flushed out quantitatively (n = 3) instead of shaken out, relative diacetylmorphine content was consistently close to the label claim for all doses (74.7 ± 0.5% w/w). Therefore, the latter method was used in the determination of diacetylmorphine content. Initially, a specification of 90-110% of label claim (67.5-82.5% w/w diacetylmorphine) was used. Based on the results of 17 batches and evaluation of the risk of batch failure per dose (100/33 mg: $2x10^{-28}$%, 150/50 mg: $8x10^{-36}$%, 200/67 mg: $6x10^{-67}$%, calculated according to Stafford et al. [17]), this specification was tightened to 97.5-102.5% (73.1-76.9% w/w diacetylmorphine, risks of batch failure: $6x10^{-1}$, $2x10^{-1}$, and $9x10^{-4}$%, respectively). The batches of 75/25 mg sachets could not be evaluated statistically, since there were only two. However, the content of both batches was within the tightened specifications.

The HPLC-UV method was shown to separate diacetylmorphine, caffeine and the main degradation products of diacetylmorphine, 6-acetylmorphine and morphine (Figure 2). 6-Acetylmorphine was found to be the main degradation product of diacetylmorphine in the finished product with levels equivalent to the drug substance used for manufacture (peak area 0.3-1.6% of diacetylmorphine peak area). All 19 batches conformed to the specification for chromatographic purity (> 95%) (Table 4).

Figure 2: Representative chromatograms, for a standard solution (left) containing morphine (1), caffeine (2), 6-acetylmorphine (3) and diacetylmorphine (4), and a sachet sample (right).

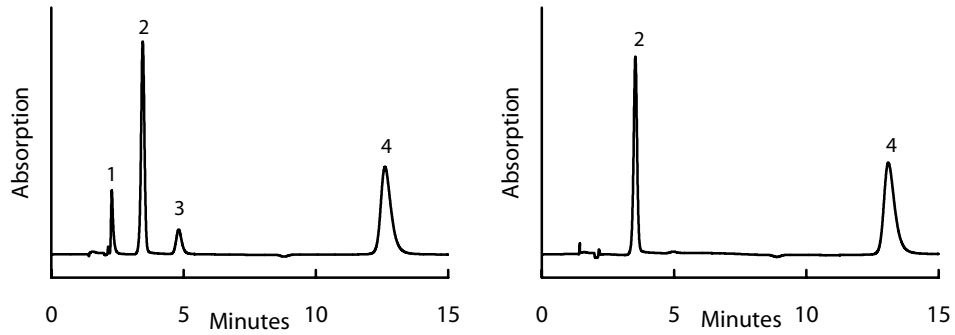

Table 5: Long term stability of diacetylmorphine/caffeine sachets (n= 3 batches/dosage) upon storage at 25±2°C, 60±5% RH.

Storage time (months)		**0**	**6**	**9**	**12**	**18**	**24**
Dose	**Test item**						
75/25 mg	Content	73.6 (1.1)	72.8 (3.4)	73.3 (2.2)	73.5 (1.5)	75.1 (0.5)	73.7 (1.5)
	Purity	99.1 (0.2)	99.0 (0.4)	99.2 (0.7)	98.8 (0.2)	98.0 (0.4)	97.4 (0.8)
100/33 mg	Content	72.5 (1.1)	71.5 (0.6)	74.4 (2.4)	73.1 (2.0)	74.3 (0.3)	74.1 (0.6)
	Purity	99.3 (0.4)	n.d.	99.0 (0.9)	98.7 (0.2)	98.0 (0.4)	97.3 (0.5)
150/50 mg	Content	74.0 (0.3)	73.8 (0.5)	72.2 (4.8)	74.7 (0.5)	74.6 (0.5)	74.5 (0.3)
	Purity	99.2 (0.5)	98.8 (-)	99.4 (0.8)	98.8 (0.1)	97.3 (0.4)	97.1 (0.5)
200/67 mg	Content	74.6 (1.3)	74.2 (1.0)	72.5 (4.3)	74.6 (0.2)	75.3 (0.8)	75.2 (0.5)
	Purity	98.8 (0.2)	98.6 (0.3)	99.1 (0.8)	98.7 (0.2)	97.6 (0.5)	97.0 (0.6)

Mean relative diacetylmorphine content (% w/w) and mean chromatographic purity (%) are given, with sd in parentheses. Diacetylmorphine content was determined in powder mixture instead of in quantitatively flushed out sachet contents up to 9 months into the study. n.d. = not determined.

After completion of the batch, the in-process control results for delivered weight were evaluated. Mean, standard deviation, and relative standard deviation (RSD) were calculated and the number of weights exceeding 15% deviation from the label claim was determined. A RSD ≤7.8% was used as a specification, based on the maximum RSD used in the USP test for Uniformity of Dosage Units <905> performed on 30 units [18]. The results in Table 3 show that all four dosages could be filled precisely, no significant difference in mean RSD was observed between the four different doses and none of the batches showed delivered weights in the in-process controls deviating more than 15% from the label claim (Table 4).

Stability Studies

Long-term stability results for diacetylmorphine/caffeine sachets are given in Table 5. The powder mixture shaken out of the sachets was used for determination of diacetylmorphine content instead of the sachet contents flushed out quantitatively, up to 9 months into the stability study. Therefore, in this period, smaller dosages show lower diacetylmorphine contents due to adhesion of diacetylmorphine to the inside of the sachet. The final results however, show that diacetylmorphine/caffeine sachets are stable for two years when stored at 25 ± 2°C, 60 ± 5% RH. No change in diacetylmorphine content was observed and chromatographic purity remained well above 95%, with 6-acetylmorphine appearing as the only degradation product.

Table 6: Accelerated stability of diacetylmorphine/caffeine sachets upon storage at 40±2°C, 75±5% RH (n= 3 batches/dosage, 1 batch of 75/25 mg).

Storage time (months)		**0**	**1**	**2**	**3**	**6**
Dose	**Test item**					
75/25 mg	Content	74.48 (-)	75.52 (-)	76.12 (-)	75.29 (-)	74.68 (-)
	Purity	99.41 (-)	98.46 (-)	98.83 (-)	99.05 (-)	98.70 (-)
100/33 mg	Content	74.86 (0.68)	74.98 (0.10)	75.29 (0.08)	74.79 (0.53)	74.38 (0.21)
	Purity	99.05 (0.36)	98.40 (0.07)	98.86 (0.03)	98.94 (0.07)	98.98 (0.13)
150/50 mg	Content	74.88 (0.51)	75.32 (0.22)	75.63 (0.29)	74.60 (034)	74.36 (1.19)
	Purity	99.13 (0.07)	98.32 (0.05)	99.03 (0.09)	98.75 (0.12)	99.01 (0.14)
200/67 mg	Content	75.09 (0.57)	74.94 (0.22)	75.48 (0.63)	74.80 (0.73)	74.45 (0.61)
	Purity	98.84 (0.17)	98.18 (0.04)	99.13 (0.01)	98.63 (0.03)	98.83 (0.11)

Mean relative diacetylmorphine content (% w/w) and mean chromatographic purity (%) are given, with sd in parentheses.

The results of the accelerated stability studies (Table 6) indicate that all types of diacetylmorphine/caffeine sachet can withstand storage at 40 ± 2°C, 75 ± 5% RH for 6 months. 6-Acetylmorphine was found to be the main degradation product (mean peak area 1.28 ± 0.40% of diacetylmorphine peak area), while morphine was mostly undetectable (peak area < 0.25% of diacetylmorphine peak area).

Conclusions

A dosage form was selected for pharmaceutical smokable heroin (3:1 w/w diacetylmorphine base/caffeine anhydrate). A micro dose auger filler was used in the manufacturing process, which was developed for filling four sachet doses, containing 75/25 mg, 100/33 mg, 150/50 mg, and 200/67 mg diacetylmorphine/caffeine. In-process controls were developed to monitor the filling process as well as quality control tests on the finished product. In-process control results were within specifications for all doses. The resulting powder filled sachets were shown to comply with the specifications for content and uniformity of mass. The diacetylmorphine/caffeine sachets were found to be stable for two years at 25°C, 60% RH and for 6 months at 40°C, 75% RH.

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4 Diacetylmorphine for inhalation: administration

Chapter 4.1

Pharmacokinetic comparison of two methods of heroin smoking: 'chasing the dragon' *versus* the use of a heating device

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Abstract

In preparation for a trial on co-prescription of inhalable heroin and methadone, two methods for inhalation of heroin/caffeine tablets were compared: the commonly used method of 'chasing the dragon' and a standardised procedure for inhalation of volatilised heroin, using a heating device. Five male addicts inhaled a tablet of smokable heroin daily on five days, alternating the inhalation method. Plasma concentrations of heroin, 6-acetylmorphine, morphine and morphine-3- and –6-glucuronide were determined using a liquid chromatography method with tandem mass spectrometric detection. The exposure to heroin and its metabolites (expressed as areas under the concentration-time curve) was significantly lower after smoking via the heating device than after 'chasing the dragon': heroin 80% and 6-acetylmorphine 73% lower ($p < 0.05$). Maximal concentrations of heroin and 6-acetylmorphine were also 80 and 70% lower ($p < 0.02$) after using the heating device. 'Chasing the dragon' is a more efficient inhalation method than inhalation via the heating device.

Abbreviations

AUC = area under the concentration-time curve; C_0 = plasma concentration at baseline ($t = 0$); C_{max} = maximum plasma concentration; DSM-IV = Diagnostic and Statistical Manual of mental disorders, fourth edition; HPLC-MS/MS = high performance liquid chromatography with tandem mass spectrometric detection; k_e = elimination constant; LLQ = lower limit of quantitation; M3G = morphine-3-glucuronide; M6G = morphine-6-glucuronide T½ = elimination half-life; T_{max} = time of maximum plasma concentration; ULQ = upper limit of quantitation.

Introduction

Heroin (3,6-diacetylmorphine) is a well-known drug of abuse, that is usually administered intravenously, but smoking heroin has gained popularity since it was first described in Shanghai in the 1920s [1]. After some refinement, the use of an inhalation procedure called 'chasing the dragon' spread to South East Asia, India and some parts of Europe in 1960-1980 [1]. In this procedure, drug users heat heroin powder on a piece of aluminium foil with a cigarette lighter until it melts and evaporates. The fumes are subsequently inhaled through a straw.

A clinical trial was performed in the Netherlands to evaluate the effect of medical co-prescription of heroin and methadone on mental and physical health and social functioning of chronic treatment-resistant heroin-dependent patients [2]. Since in the Netherlands 75-85% of the heroin addicts use heroin by 'chasing the dragon' [3], two separate study protocols were developed: one trial testing the efficacy of the prescription of an inhalable form of heroin and another trial testing the efficacy of the prescription of injectable heroin. In preparation for the first trial, a inhalable form of pharmaceutical heroin was developed to be used by 'chasing the dragon', containing 50 mg diacetylmorphine base and 100 mg caffeine anhydrate in tablets, obtained via direct compression. Caffeine was added because it is commonly found in street heroin samples [4-7] and has been shown to improve the volatilisation of heroin [8]. Since 'chasing the dragon' might not be the most effective and reproducible method for inhalation of volatilised heroin, an alternative method was developed. A heating device fitted with a sample holder was proposed as a method for inhaling heroin that allowed for improved control of the volatilisation temperature and thereby for standardisation of the inhalation process. The latter was considered important for the future acceptance of inhalable heroin prescription as an authorised treatment for heroin-dependent patients. The method of preference would be used in the abovementioned clinical study and in the future in heroin prescription programs (after obtaining market authorisation for diacetylmorphine for inhalation after volatilisation).

A pharmacodynamic comparison of 'chasing the dragon' and inhalation via the heating device has been reported by Hendriks *et al.*: no significant differences in physiological and behavioural effects were found between the two inhalation methods, but participants expressed a strong preference for 'chasing the dragon' [3]. In this paper, the pharmacokinetics of inhalation of volatilised diacetylmorphine/ caffeine tablets by addicts via 'chasing the dragon' and via a heating device were compared.

Experimental procedures

Inhalation methods

On five consecutive days, the patients alternately used pharmaceutical heroin for inhalation via 'chasing the dragon' (the commonly used method on the street) or via the heating device. The maximum time allowed for inhalation was 10 min. In the procedure of 'chasing the dragon', patients placed the heroin tablet on a piece of aluminium foil and heated it carefully with a lighter until it melted and evaporated. The liquefied substance was moved around on the aluminium foil and patients followed the 'dragon's tail' of fumes it left behind to inhale through a straw in their mouth. This process was regularly interrupted to enjoy the effect, allowing the liquid heroin to solidify between subsequent inhalations.

The alternative method for heroin inhalation involved inhaling the fumes emitted from a tablet heated on a heating device. The tablet was placed on a piece of aluminium foil that was shaped to fit in an indentation in a brass block, placed on top of a preheated heating device (30 min at 300°C, RCT Basic, IKA Werke, Staufen, Germany). The fumes were inhaled in the same way (using a straw in the mouth) and intermittent inhalation was achieved by removing the aluminium foil from the brass block between subsequent inhalations. However, no movement of the liquid heroin was possible.

Patients and medication

Five male patients were recruited from the patient population of the methadone maintenance treatment facility of the Municipal Health Service in Amsterdam. The inclusion criteria were similar to the criteria used in the randomised clinical trial testing the effectiveness of co-prescribed heroin in chronic treatment-resistant addicts [2]; in short: minimum 5 years history of DSM-IV heroin dependence, minimum age 25 years, inhalation as the predominant route of heroin administration, substantial daily or nearly daily use of illicit heroin and current treatment in a methadone maintenance program. Subjects were excluded if they had a minimum of 2 months of voluntary abstinence from heroin in the previous year. The patients were admitted to a closed clinical research unit for a period of six days. The use of alcohol, cannabis, cocaine and opiates besides trial medication was not allowed during this period. Care was taken to prevent concomitant use of illicit drugs during the study. The study was conducted under the provisions of the Declaration of Helsinki, as amended in Hong Kong (1989). The study was approved by the Medical Ethics Committee of the Academic Medical Centre in Amsterdam. All subjects received extensive oral and written information about the study and provided written informed consent.

Prescribed medication consisted of a daily dose of oral methadone and on day 2-6 a daily dose of pharmaceutical smokable heroin, consisting of a tablet containing 50 mg diacetylmorphine base and 100 mg caffeine anhydrate. Three of the five patients

started with inhaling heroin via 'chasing the dragon', alternating this method with the heating device method; the other two followed the opposite schedule. In order to minimise bias from comparing a very common route of self-administration to a completely new route, participants practised both inhalation methods on the first day by inhaling a 150 mg caffeine tablet via 'chasing the dragon' and via the heating device.

Sampling and analysis

Blood samples were collected via an intravenous canulla in the underarm, taking a first sample before the start of the inhalation session and at 1, 2, 5, 7.5, 10, 15, 22.5, 30, 45, 60, 120, 240 and 480 min after the end of the inhalation session. In order to prevent degradation of diacetylmorphine, sodium fluoride was added to the sample tubes and samples were quickly frozen at −30°C after centrifugation and collection of the plasma fraction. Concentrations of diacetylmorphine, 6-acetylmorphine, morphine, and morphine-3- and 6-glucuronide were determined using a validated high performance liquid chromatography method with tandem mass spectrometry detection (HPLC-MS/MS), that has been described elsewhere [9]. Plasma samples were pre-treated by solid phase extraction through Oasis MCX sorbent columns and deuterated compounds were used as internal standards. Lower limit of quantification (LLQ) was 5 ng/mL, upper limit of quantification (ULQ) was 500 ng/mL for all quantified analytes. Inter-assay accuracy of this method was $<5\%$ and inter-assay precision was $<11\%$ for all quantified analytes. The HPLC-MS/MS method was also suitable for detection of concomitant use of illicit drugs, by screening for the presence of codeine and 6-acetylcodeine (common constituents of street heroin), and for cocaine and its metabolites benzoylecgonine and norcocaine [10].

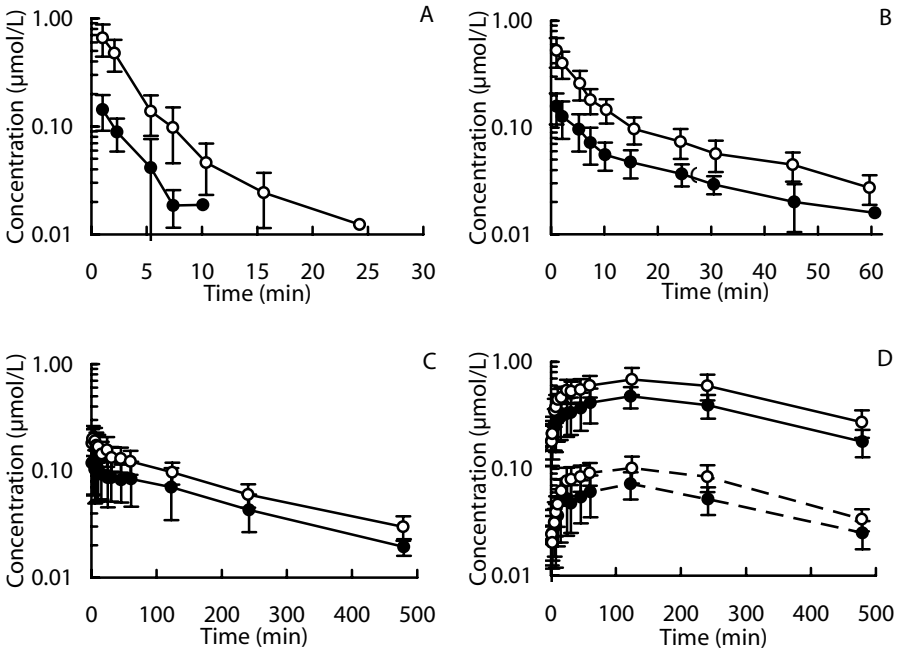
Pharmacokinetic parameters for all compounds were calculated by non-compartmental analysis, using WinNonlin software (Professional Ed., Version 4.1, Pharsight, Mountain View, CA, USA). The area under the curve of the observed concentrations (AUC) was determined by the log-linear interpolated trapezoid rule, with extrapolation to infinity by the elimination constant k_e (slope of terminal part of the concentration-time curve on a semi-log scale). The elimination constant was also used to calculate the elimination half-life (T½) for all analytes. When plasma concentrations were above the LLQ at the baseline measurement (C_0), AUC was corrected by subtracting C_0/k_e. Maximal plasma concentrations (C_{max}) and the time these concentrations were measured (T_{max}) were derived from experimental data. Inter- and intra-patient variabilities for the two administration methods were calculated by dividing the standard deviations in C_{max} and AUC values by the corresponding means. Differences in pharmacokinetic parameters between the two inhalation methods were tested using paired t-tests.

Results

Patients

Five male heroin addicts were included in the study, four of them were Caucasian, one subject was Asian. The mean age was 36.2 years (range 30-48) and the mean body weight was 65 kg (range 51-89 kg). All subjects used methadone once daily; mean dose 78 mg (range 45-100 mg). No evidence for co-use of illicit drugs during the study was found: none of the plasma samples contained 6-acetylcodeine, codeine, cocaine, norcocaine, or benzoylecgonine. No plasma samples were obtained for patient B on day 2 and 6 (first and last day of diacetylmorphine administration). A total of 23 plasma concentration-time curves were available for analysis: 13 after 'chasing the dragon', 10 after inhaling via heating device. In one sample series (patient E, day 5, inhaling via heating device), no diacetylmorphine was detectable; in another (patient D, day 1, inhaling via heating device) diacetylmorphine was only detected at t = 1 min. In both cases only the concentrations of diacetylmorphine metabolites were included in the pharmacokinetic and statistical analyses.

Figure 1: Mean plasma concentration-time curves of diacetylmorphine (A), 6-acetylmorphine (B), morphine (C), morphine-3-glucuronide (D: solid lines) and morphine-6-glucuronide (D: dashed lines) after smoking 50 mg heroin tablets using the method of 'chasing the dragon' (open bullets) or using the heating device (closed bullets). Error bars indicate the 95% confidence interval.



Pharmacokinetics

Mean plasma concentration-time curves for diacetylmorphine, 6-acetylmorphine, morphine and morphine-3- and –6-glucuronide resulting from the two inhalation methods are given in Figure 1. Distinct differences between the two inhalation methods could be observed in these plots: concentration-time curves appeared to be lower for diacetylmorphine and 6-acetylmorphine and variation in morphine and morphine glucuronide concentrations appeared to be larger in the first hour after inhaling via the heating device. The first observation was reflected in the pharmacokinetic data (Table 1), which indeed showed 80 and 73% lower AUC values ($p = 0.044$ and 0.012) after using the heating device for diacetylmorphine and 6-acetylmorphine, respectively. Although the differences were less obvious in the concentration-time curves, AUCs for morphine and the glucuronides were also significantly lower (38-42%, $p = 0.0016$-0.020). The same pattern was observed in diacetylmorphine and 6-acetylmorphine C_{max} (80 and 70% lower, $p = 0.019$ and 0.0010) as compared to morphine and its metabolites (27-38% lower), but in this case the latter differences did not all reach statistical significance. No significant differences were found in the T½ values of the analytes between the two inhalation methods (Table 1).

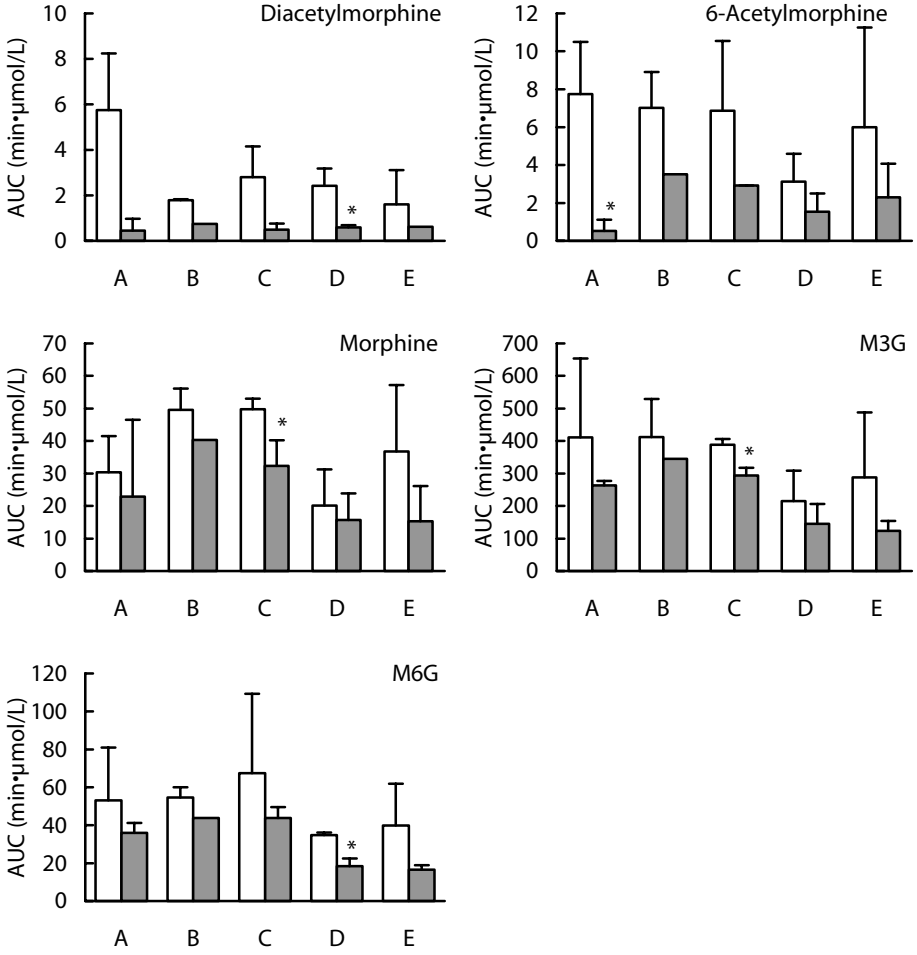
The second observation from the concentration-time plots, concerning the differences in variation observed in the morphine and morphine-glucuronide concentrations in the first hour, was not reflected as variability in the AUCs of these analytes.

Table 1: Pharmacokinetic parameters for diacetylmorphine and metabolites after smoking 50 mg heroin tablets via 'chasing the dragon' or via the heating device.

Analyte	**AUC** *(min·µmol/L)*	**Cmax** *(µmol/L)*	**T½** *(min)*
'Chasing the dragon'			
Diacetylmorphine	2.99 (2.11)	0.61 (0.36)	2.1 (0.8)
6-Acetylmorphine	6.31 (3.32)	0.53 (0.28)	11.4 (3.5)
Morphine	37.71 (15.23)	0.21 (0.12)	143.0 (67.9)
Morphine-3-glucuronide	347.5 (156.3)	0.74 (0.36)	280.1 (81.7)
Morphine-6-glucuronide	50.77 (25.52)	0.11 (0.05)	269.9 (123.4)
Heating device			
Diacetylmorphine	*0.56 (0.24)	*0.12 (0.06)	2.1 (0.7)
6-Acetylmorphine	*1.96 (1.27)	**0.16 (0.08)	9.0 (4.4)
Morphine	*22.82 (13.44)	**0.13 (0.09)	127.0 (36.7)
Morphine-3-glucuronide	**214.1 (91.3)	0.50 (0.20)	252.0 (47.6)
Morphine-6-glucuronide	**29.15 (12.91)	*0.08 (0.04)	216.2 (35.0)

Mean values are given, with standard deviations in parentheses. Asterisks indicate significantly lower values for the heating device (* $p<0.05$, ** $p<0.01$)

Figure 2: Exposure to diacetylmorphine, 6-acetylmorphine, morphine and the morphine glucuronides, for all five patients (A-E) after smoking 50 mg heroin tablets: white bars represent area under the curve (AUC) after 'chasing the dragon', grey bars after using the heating device. Error bars indicate the standard deviation, the asterisks (*) indicate a significant difference between smoking methods within a patient.



Both smoking methods show similar inter-patient variability (ranging from 26% to 58% for 'chasing the dragon' and from 15% to 57% for the heating device, depicted in Figure 2) and intra-patient variability (7-93% and 8-115%; depicted by the error bars in Figure 2) in AUCs of diacetylmorphine and its metabolites. However, the inter-patient variation in C_{max} of morphine and morphine-3- and –6-glucuronide were higher (56%, 42%, 53%) after using the heating device compared to 'chasing the dragon' (47%, 30%, and 34%, respectively), while intra-patient variabilities were equal. The differences were however not statistically significant (F-test, $p = 0.51$-0.94).

Diacetylmorphine and 6-acetylmorphine T_{max} was consistently equal to the first time point, one min after the end of the inhalation session, for both inhalation methods. The time to morphine peak concentrations was two min and morphine-3- and –6-glucuronide T_{max} occurred about two hours after the end of the inhalation session with both methods.

Baseline concentrations were below LLQ in all curves for diacetylmorphine, 6-acetylmorphine and morphine. Morphine-3-glucuronide baseline concentrations were above the LLQ in 18 (out of 23) curves: mean 0.065 µmol/L (range 0.027-0.130 µmol/L). Morphine-6-glucuronide baseline concentrations were above LLQ in 5 curves: mean 0.014 µmol/L (range 0.010-0.016 µmol/L). In these cases, AUC values were corrected as described in the *Experimental procedures* section.

Discussion

The alternative inhalation device was developed as part of the preparation for the Dutch Heroin Trial [2]. It was considered important to provide a safe, controllable and standardised method for administration of smokable heroin to the patients in the experimental condition of the inhalation trial. Since temperature is known to be important in degradation as well as recovery of volatilised heroin [8,11,12], controlling the volatilisation temperature was considered essential; hence the simple solution of the heating device with a preheated brass sample holder. It might be argumented that both smoking methods are potentially dangerous, as smoking heroin has been associated with progressive spongiform leukoencephalopathy ([13-16]). However, to date no toxin has been identified as the cause for this condition. Diacetylmorphine is not the most likely cause, considering that all cases published until now have occurred in users 'chasing' street heroin, which is known to be a very variable mixture of substances. The presence of diacetylmorphine in their street heroin is most likely one of the few similarities between users that develop spongiform leukoencephalopathy and those who do not.

The simple, temperature-controlled, standardised alternative for 'chasing the dragon' turned out to be less efficient, possibly because other important aspects of smoking heroin were not controlled. Many heroin addicts have developed tricks and habits in their chasing technique that serve to minimise the loss of heroin vapour through charring, combustion, and fumes escaping the straw. The heating device method did not allow optimal use of these 'tricks of the trade', which could have resulted in sub-optimal inhalation efficiency. An important difference between the inhalation methods was the absence of movement of the liquefied heroin on the heating device. This hampered efficient inhalation of volatilised heroin, since the fumes did not appear as a neat 'dragon's tail', but rather as a broad smoke column or cloud. Furthermore, movement of liquid heroin might contribute to controlling the temperature of the drug, preventing overheating and subsequent decomposition. The volatilisation rate of the tablet could also differ between inhalation methods; during

the study, patients complained of a slow volatilisation rate when inhaling using the heating device, reportedly even resulting in incomplete volatilisation of the tablet within the 10 min inhalation period [3]. All of these aspects could have contributed to the low exposures to diacetylmorphine and metabolites after inhaling via the heating device. However, it is likely that they also caused variation in the plasma concentrations of diacetylmorphine and 6-acetylmorphine during the inhalation session. The short half-lives of these substances, combined with the absence of samples taken during the inhalation session could have resulted in underestimation of AUC and C_{max} values for diacetylmorphine and 6-acetylmorphine in this study. This means that the actual difference in efficiency between the inhalation methods might be reflected more accurately by the AUCs of morphine and its glucuronides.

Considering the pharmacokinetics and pharmacodynamics of diacetylmorphine, it is reasonable to assume that peak concentrations of diacetylmorphine and 6-acetylmorphine are responsible for the initial 'flash' effect and exposure to morphine and its metabolites for the more prolonged euphoria [17]. Therefore, our findings of significantly higher peak concentrations of diacetylmorphine and 6-acetylmorphine after 'chasing the dragon' could explain the trend towards improved subjective drug effects after 'chasing the dragon' compared to the heating device observed in the pharmacodynamic comparison study [3]. However, since no plasma samples were obtained during the 10 min inhalation session, due to the intensive assessment schedule for the pharmacodynamic parameters [3], it is not possible to link the actual diacetylmorphine and 6-acetylmorphine concentrations to the corresponding pharmacodynamic effects.

Interestingly, Hendriks *et al.* reported no difference in bioavailability between the two inhalation methods, based on the total excretion of morphine (conjugated and free morphine) in 24-h urine samples [3], while our plasma data indicate that the heating device yields ±40% lower AUCs for morphine and its glucuronides than 'chasing the dragon'. It would be expected that these differences in plasma concentrations would be reflected (with some delay) in urine concentrations. It is possible that the combination of the alternated use of the inhalation methods and the long circulation time of morphine and its glucuronides has generated a carry-over effect, obscuring the difference between the smoking methods in the 24-h urine samples. The finding that baseline morphine-3-glucuronide concentrations were above LLQ on 18 out of 23 patient days supports this proposition. The determination of the bioavailability of inhaled heroin via measurements of total morphine (morphine + conjugated morphine) in urine must be considered a rough estimate, since it has been found that after intravenous administration of heroin, only 68% of the heroin dose was recovered as total morphine in urine [18]. In this study, an accurate determination of the absolute bioavailability using the plasma concentrations of diacetylmorphine and its metabolites was not possible, as no intra-patient comparison with intravenous administration was available.

Differences in inhalation technique, as well as (pharmacokinetic) patient characteristics contribute to the inter-patient variability in AUC (Figure 2). The fact that intra-patient variability (error bars in Figure 2) was similar to the inter-patient variability might indicate variability in inhalation efficiency. The observation that even experienced heroin chasers sometimes burn their eyebrows from accidental combustion of their heroin illustrates the existence of such inter-occasion variability.
No time effects were observed in the AUCs of any of the analytes, indicating that there was no apparent learning effect, neither for using the alternative inhalation method nor for inhaling a tablet rather than street heroin (powder or granules). Moreover, AUC values in our study were comparable (after dose correction) to those found by Rook *et al.* in a pharmacokinetic study among Dutch heroin addicts inhaling 200-300 mg of pharmaceutical heroin powder via 'chasing the dragon' [10]. This was true for all analytes except for diacetylmorphine, of which the AUC was 65% higher in the high-dose study. This could have been a result of sampling during the inhalation session, which was not possible in our study. This could also explain the shorter half-lives we found for diacetylmorphine and 6-acetylmorphine (2.1 and 11.4 min) compared to Rook *et al.* (3.2 and 25.6 min, respectively), while morphine and morphine glucuronide half-lives were comparable [10]. No differences were found in any of the T½ values of the analytes between the inhalation methods in our study; no difference was expected, since in both methods heroin is administered via inhalation and it has been shown that T½ does not depend on route of administration [10].

Conclusion

In conclusion, 'chasing the dragon' was found to be a more efficient method for inhalation of diacetylmorphine/caffeine tablets than use of a heating device. Exposure to diacetylmorphine and its metabolites was found to be significantly higher, as well as peak concentrations of diacetylmorphine and 6-acetylmorphine. These differences could be explained by the technique that experienced heroin 'chasers' have developed to avoid degradation and loss of heroin fumes that cannot be applied when using the heating device. This may lead to degradation due to overheating of the sample and to decreased inhalation efficiency. In summary, we conclude that 'chasing the dragon' is a more effective method for inhaling heroin than the heating device used in this study.

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Chapter 4.2

Deuterodiacetylmorphine as a marker for use of illicit heroin by addicts in a heroin-assisted treatment program

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Abstract

In preparation for a treatment program concerning the medical co-prescription of heroin and methadone to treatment-resistant addicts in the Netherlands, we studied a novel strategy for monitoring co-use of illicit (non-prescribed) heroin. A deuterated analogue of heroin was added (1:20) to pharmaceutical smokable heroin (a powder mixture of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate), to be used by inhalation after volatilisation ('chasing the dragon').

Plasma and urine samples were collected of nine male patients who had used pharmaceutical smokable heroin during a four-day stay in a closed clinical research unit, and these samples were analysed by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS). Ratios of deuterated and undeuterated diacetylmorphine and 6-acetylmorphine (MAM/MAM-d3) in plasma and urine were calculated from peak areas of these substances in the respective chromatograms.

The MAM/MAM-d3 ratios in plasma and urine were normally distributed (with small standard deviations) and independent from concentrations of 6-acetylmorphine and from time after use of pharmaceutical heroin. A MAM/MAM-d3 ratio in urine above 32.8 was considered indicative of co-use of illicit heroin, and this value was associated with a percentage false positives of only 1% (95% confidence interval: –1-3%). The MAM/MAM-d3 ratio was detectable in urine for 4-9½ h after use of pharmaceutical smokable heroin.

Addition of stable isotopically labelled heroin to pharmaceutical smokable heroin was shown to be a feasible strategy for detection of co-use of illicit heroin by patients in heroin-assisted treatment.

Abbreviations

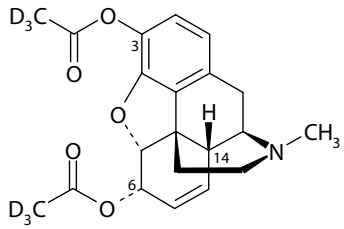
DAM = diacetylmorphine; DAM/DAM-d6 = ratio of diacetylmorphine to deuterated diacetylmorphine; LC-MS/MS = high performance liquid chromatography with tandem mass spectrometric detection; MAM = 6-acetylmorphine; MAM/MAM-d3 = ratio of 6-acetylmorphine to deuterated 6-acetylmorphine.

Introduction

In the Netherlands, a trial on the medical co-prescription of heroin to treatment-resistant heroin dependent patients in a methadone maintenance program was performed to evaluate its effect on mental and physical health and social functioning of the participants [1]. Since the results were positive, this is expected to lead to the introduction of a heroin prescription program intended to treat a specific subgroup of treatment-resistant addicts undergoing methadone maintenance treatment. Optimal results of this treatment can only be expected when prescribed heroin completely substitutes the addict's use of illicit (non-prescribed) heroin, therefore monitoring of any illicit heroin consumption is required.
To date, the search for suitable markers for (concomitant) use of illicit heroin has been limited to common alkaloid constituents of illicit heroin, like 6-acetylcodeine, noscapine, papaverine and their metabolites. Several studies on detection of these substances in hair [2] and urine [3-7] have been conducted. The main problems of this approach, however, are false negative results due to the variable composition of illicit heroin and the small amounts of these alkaloids present, and false positive results due to consumption of medicines or food containing these or related alkaloids. 6-Acetylcodeine is the alkaloid most commonly used. It is found in most samples of illicit heroin, but usually only in small amounts: 1-5% [7], <5% [8], 3-11% [9], or 4.3-7.4% [10]. A specific problem of this marker for routine monitoring in a heroin-assisted treatment program is the possibility of transacetylation from high doses of diacetylmorphine to co-used oral codeine to form 6-acetylcodeine [7]. Papaverine and noscapine are less common in street heroin and quantities vary greatly: up to 3 and 19% were found, respectively, in one study [8], while another found 1.2-2.5% papaverine and 0.7-8.5% noscapine [10]. Another general limitation of these markers is that they cannot be used to determine how much illicit heroin has been co-used, while this is potentially possible (with some knowledge on the time of co-use) if a deuterated marker is used. A stable isotope of heroin could be added to pharmaceutical heroin, which would be expected to show similar effects and side-effects as heroin, as well as negligible additional safety risks for the patient [11,12]. The ratio of the labelled and unlabelled compound (or metabolite) in plasma and urine samples can be expected to be constant, so that a shift in this ratio would be indicative of co-use of illicit heroin.

This approach was proposed by Gyr *et al.* in a study on pharmaceutical heroin for prescription to addicts [13], but to our knowledge, no paper on this subject has been published to date. We studied the suitability of deuterated diacetylmorphine as a marker, by adding it to pharmaceutical smokable heroin, a product intended for inhalation after volatilisation. Five percent of the diacetylmorphine base in the 3:1 w/w mixture of diacetylmorphine base and caffeine anhydrate was replaced by diacetylmorphine-d6 (Figure 1). The stable isotopically labelled diacetylmorphine contains three deuterium atoms in each acetyl group, resulting in a deuterated

Figure 1: Chemical structure of deuterodiacetylmorphine



metabolite (6-acetylmorphine-d3) and non-deuterated morphine after hydrolysis. Diacetylmorphine, 6-acetylmorphine, their deuterated analogues and morphine were measured in plasma and urine samples of patients using pharmaceutical heroin by 'chasing the dragon' in a closed clinical research unit. These data were used to study the feasibility of using a deuteration ratio in plasma or urine samples as potential proof for co-use of illicit heroin by addicts in heroin-assisted treatment.

Experimental

Materials

The pharmaceutical heroin to be used by 'chasing the dragon' was prepared by mixing diacetylmorphine-d6 (Figure 1) with diacetylmorphine base (DAM) and caffeine anhydrate, resulting in a 3:1 w/w powder mixture of diacetylmorphine/caffeine, with a diacetylmorphine/diacetylmorphine-d6 w/w (DAM/DAM-d6) ratio of 20. Diacetyl-morphine and diacetylmorphine-d6 were provided through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands) and caffeine anhydrate was purchased from Bufa (Uitgeest, The Netherlands).

Methods

LC-MS/MS analysis

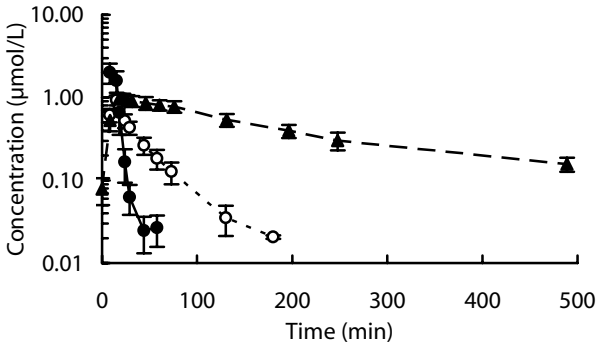
A previously described liquid chromatography method with tandem mass spectrometric detection (LC-MS/MS) was used for quantification of diacetylmorphine, 6-acetylmorphine (MAM), morphine, and morphine-3- and -6-glucuronide in plasma samples [14]. It was modified slightly, using morphine-d3 instead of diacetylmorphine-d6 as an internal standard for quantification of diacetylmorphine and 6-acetylmorphine, to avoid interference in the calculation of DAM/DAM-d6 and 6-acetylmorphine/6-acetylmorphine-d3 (MAM/MAM-d3) ratios. Plasma samples were simultaneously screened for the presence of 6-acetylcodeine, codeine, cocaine, and its metabolites benzoylecgonine and norcocaine [14]. Urine samples were analysed using essentially the same method with some modifications in the sample pre-treatment procedure. For quantification of morphine and the morphine glucuronides, the solid phase extraction procedure was substituted for dilution with blank urine and mobile phase followed by direct injection into the LC-MS/MS system. Sample pre-treatment for analysis of 6-acetylmorphine and its deuterated analogue in urine involved a solid

phase extraction procedure: 1 mL of urine (acidified to pH 4 with 1 M HCl) was subjected to the same procedure as the plasma samples. DAM/DAM-d6 and MAM/MAM-d3 ratios were calculated from the peak areas of the respective components. The lower limit of detection of the deuterated compounds was set at a signal-to-noise ratio of 4 and a minimum peak area of 1000 cps (mass spectrometer signal in counts per second).

Sampling

Patients were recruited from the Dutch Heroin Trial [1] and were considered eligible when they had used prescribed heroin for inhalation for at least 12 months and were considered responders in the trial. For this study, nine male patients were admitted to a closed research facility for a period of four days. Prescribed medication consisted of a daily dose of methadone and pharmaceutical smokable heroin twice daily, to be used via 'chasing the dragon' in a maximum of 30 min. The morning dose of heroin was varied; each patient used 66, 100 or 150% of his regular dose (overall dose range 133-450 mg heroin). The use of alcohol, cannabis, cocaine and non-prescribed opiates was not allowed. Blood samples were collected after the morning dose via an intravenous canulla in the underarm, 10 min before the start of the smoking session, 2 min after using 40% of the heroin dose and 2, 5, 10, 15, 30, 45, 60, 115, 180, 240 and 480 min after completing the total dose. Urine samples were collected on patients' demand and analysed as separate fractions instead of 24-h accumulation samples, to avoid dilution to concentrations below the lower limit of quantification. Detailed pharmacokinetic and pharmacodynamic results of this study are described elsewhere [15].

Figure 2: Mean concentration-time curves for diacetylmorphine (closed bullets), 6-acetylmorphine (open bullets) and morphine (closed triangles) in plasma after inhalation of 300 mg pharmaceutical heroin (n=15 curves). Error bars indicate the 95% confidence intervals.



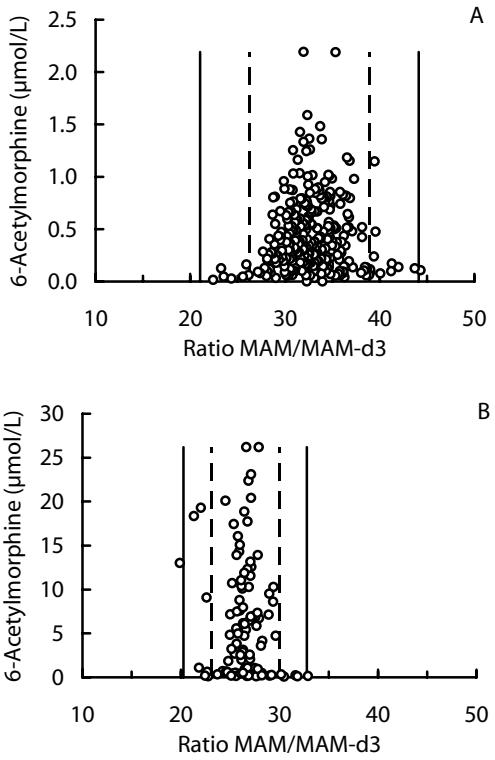
Results

All nine patients completed the pharmacokinetic study, resulting in 36 plasma concentration-time curves for diacetylmorphine, 6-acetylmorphine, morphine, and morphine-3- and –6-glucuronide and 213 urine samples (a mean of 6 samples per patient per day). All plasma samples were screened for the presence of 6-acetylcodeine, codeine, cocaine, and its metabolites benzoylecgonine and norcocaine; all were found negative, except for the samples from the first days of admission to the clinical research unit. Samples from eight out of nine patients contained traces of benzoylecgonine on the first two days of admission to the clinical research unit. Peak areas decreased over time, indicating that cocaine use had occurred prior to entering the facility and had not been repeated. The absence of traces of 6-acetylcodeine and codeine indicated that no co-use of illicit heroin had occurred.

Plasma

Eight patients used a 300 mg diacetylmorphine dose once or twice in the study; the resulting mean plasma concentration-time curves (n = 15 curves) for

Figure 3: MAM/MAM-d3 ratio in plasma (A) and urine (B) as a function of 6-acetylmorphine concentration. The dashed lines represent the 90% (mean±1.645•SD) range around the mean; the solid lines represent the 99.74% range (±3•SD).



diacetylmorphine, 6-acetylmorphine, and morphine are given in Figure 2. These curves illustrate the rapid decrease in the plasma concentrations of diacetylmorphine and 6-acetylmorphine, resulting in half-lives of 3 and 26 min for these compounds, respectively. As a result, the DAM/DAM-d6 ratio could be calculated in only 84 of the 467 plasma samples (18%), due to the rapid decrease of the amount of diacetylmorphine-d6 in plasma to below the lower limit of detection. Diacetylmorphine-d6 was detectable until 5 min after ending the smoking session, resulting in a maximum of 3 deuteration ratios per smoking session. The DAM/DAM-d6 ratio was found to vary from 35.1-193.0, with a mean of 80.4 ± 35.1 (standard deviation). The DAM/DAM-d6 ratios showed large differences between patients and doses, but as expected, no significant linear relation between ratio and dose was found.

Due to the longer half-life of 6-acetylmorphine, ratios of MAM/MAM-d3 could be calculated in 239 (51%) of the plasma samples; they were normally distributed (Kolmogorov-Smirnov test, $p = 0.2$) around a mean of 32.6 ± 2.5 (range 25.9-39.6)(Figure 3A). 6-Acetylmorphine-d3 was detectable until 60 (range 45-180) min after ending the smoking session. Differences in mean plasma MAM/MAM-d3 ratios between individual patients were small, but statistically significant in some cases (ANOVA, Bonferroni post hoc multiple comparisons, $p < 0.001$)(Table 1). A significant difference in MAM/MAM-d3 ratio in plasma was found between the 200 mg and 250 mg doses (ANOVA, Bonferroni post hoc multiple comparisons $p = 0.034$, Table 2), but no significant correlation between ratio and dose was found (Pearson correlation 0.09, $p = 0.174$). Time since the last dose was weakly, but significantly correlated with MAM/MAM-d3 ratio in plasma (Pearson correlation –0.14, $p = 0.03$).

Table 1: MAM/MAM-d3 ratios in plasma and urine samples of patients A-I.

Patient	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	***Overall***
Plasma										
Mean	33.4	31.8	33.9	32.6	34.3	33.2	33.1	30.3*	31.7	*32.6*
SD	2.4	2.2	2.1	1.9	1.8	3.1	2.4	1.8	1.8	*2.5*
N	20	24	32	32	26	22	22	32	29	*239*
Urine										
Mean	27.1	25.8	27.3	23.3*	27.5	27.0	27.3	24.9*	26.7	*26.5*
SD	1.6	0.8	1.5	2.5	2.3	1.4	1.9	1.6	1.8	*2.1*
N	12	9	12	7	15	9	13	12	12	*101*

Mean values are given with standard deviations (SD) per patient, as well as overall statistics. N= number of calculated ratios; * indicates the value differs significantly ($p<0.05$) from other values.

Table 2: MAM/MAM-d3 ratios in plasma and urine samples after inhalation of different doses.

Dose (mg)	133	167	200	250	300	375	450	*Overall*
Plasma								
Mean	31.3	34.1	32.0	34.3	32.5	34.7	32.8	*32.6*
SD	2.8	2.0	2.2	2.0	2.7	1.2	2.1	*2.5*
N	5	6	56	14	103	6	49	*239*
Urine								
Mean	25.1	28.5	25.7	27.0	26.5	29.3	26.6	*26.5*
SD		2.3	1.8	2.3	2.2	0.5	1.4	*2.1*
N	1	2	18	11	58	2	9	*101*

Mean values are given with standard deviations (SD) per dose of heroin, as well as overall statistics. N= number of calculated ratios.

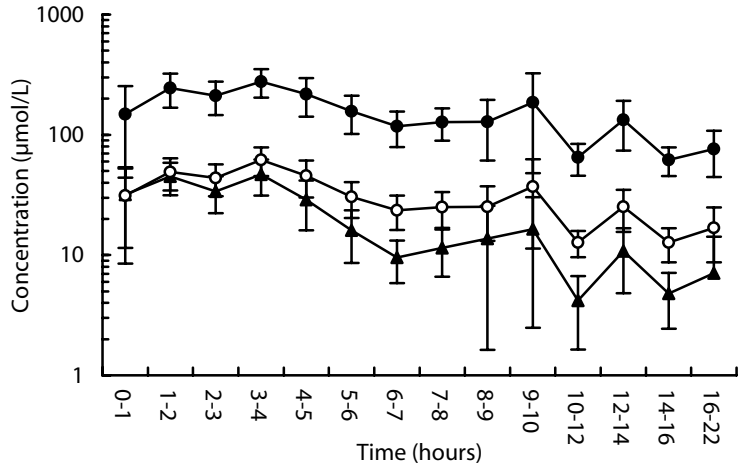
Urine

Concentration-time curves for morphine, morphine-6-glucuronide and morphine-3-glucuronide in urine are given in Figure 4. Diacetylmorphine and diacetylmorphine-d6 were not detected in urine samples. All 213 urine samples were 6-acetylmorphine-positive and in 101 (47%) samples 6-acetylmorphine-d3 was detectable, during a period of 4-9½ h after the end of the smoking session. The MAM/MAM-d3 ratio in urine samples was normally distributed around a mean of 26.5 ± 2.1 (range 19.9-32.9, Kolmogorov-Smirnov test: $p = 0.35$, Figure 3A), differing significantly from the mean value found in plasma (t-test, $p < 0.001$) (Figure 3B). The ratio MAM/MAM-d3 was not significantly correlated with the 6-acetylmorphine concentration (Pearson correlation −0.137, $p = 0.173$) nor was a significantly correlation between ratio and the time after the last dose observed (Pearson correlations 0.138, $p = 0.170$) (Figure 5). No differences in MAM/MAM-d3 ratios were found between doses (ANOVA, $p = 0.165$, Table 2), but statistically significant differences between some of the patients were found (ANOVA, Bonferroni post hoc multiple comparisons, $p < 0.001$) (Table 1).

Discussion

Until now, the search for a marker for use of illicit heroin has focused on common alkaloid constituents of illicit heroin, like 6-acetylcodeine, noscapine, papaverine, and their metabolites. Addition of a marker substance to the prescribed pharmaceutical heroin could be considered a novel approach in the search for an appropriate indicator for co-use of illicit heroin. Our study was performed in a specially selected patient group, that consisted of 'stable' patients from a heroin-assisted treatment program using a heroin dose that was titrated to their needs [1]. Moreover, they were kept closed off from the outside world during the study and were monitored carefully for use of illicit drugs. These circumstances allowed us to determine the feasibility of

Figure 4: Mean concentrations of morphine (triangles), morphine-6-glucuronide (open bullets) and morphine-3-glucuronide (closed bullets) in urine versus time after the last dose of diacetylmorphine for inhalation. Error bars indicate 95% confidence intervals.



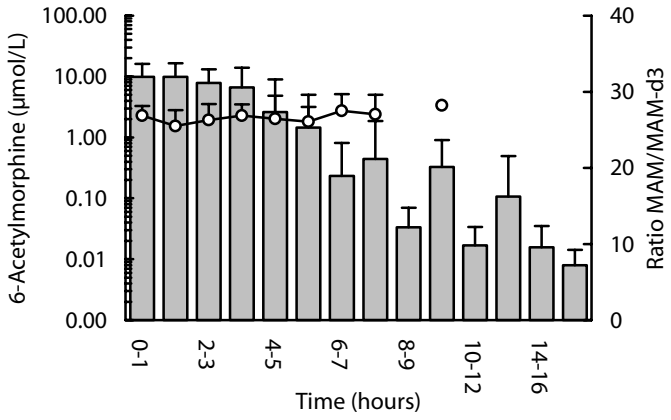
deuterodiacetylmorphine as a marker for co-use of illicit heroin, by studying its pharmacokinetics and the variability of the deuteration ratio in the intended dose range and population. The study design was validated by the fact that there was no evidence for use of illicit heroin by patients during this study: neither 6-acetylcodeine nor codeine was detected in plasma samples. Small amounts of cocaine and benzoylecgonine were present in samples from the first day(s) of the study, indicating that some of the participants used cocaine before they entered the research facility.

The DAM/DAM-d6 ratio in plasma was found to be higher (80.4) than the dose ratio of 20 in the pharmaceutical heroin and quite variable (standard deviation 35.1). This might be explained by small differences between diacetylmorphine-d6 and diacetylmorphine in pharmacokinetic profile (volume of distribution, affinity for metabolic enzymes) and/or in volatilisation properties or bioavailability. Small absolute differences between the compounds could be magnified by the high rates of absorption and hydrolysis. In a later phase of heroin metabolism, the deuteration ratio *in vivo* was closer to the dose ratio: MAM/MAM-d3 in plasma was 32.6 ± 2.5, MAM/MAM-d3 in urine 26.5 ± 2.1. The MAM/MAM-d3 ratios in plasma and urine were found to be normally distributed and independent of the heroin dose, 6-acetylmorphine concentration and time after administration, which would make them suitable parameters for routine monitoring of co-use of illicit heroin by patients in a heroin-assisted treatment program. The observed differences between patients were not considered relevant, the differences were small and the 'deviant' ratios were lower than the mean, indicating that they were unlikely to cause false positive results.

The most important advantage of urine in routine monitoring is the non-invasive sample collection that does require supervision, but no medically trained personnel [16]. Moreover, in this study 6-acetylmorphine-d3 was detectable in plasma for 60 min (45-180 min), while it was detected in urine for 4-9½ h after the end of the smoking session. In general plasma is preferred for quantitative accuracy [16], but the determination of the deuteration ratio could be considered a pseudo-quantitative analysis, which was performed as accurate in urine as in plasma. Moreover, knowledge of the exact 6-acetylmorphine concentrations in urine often will not be necessary to prove co-use of illicit heroin, even though the described method of analysis can easily provide this information. These considerations, combined with the larger detection window and easy sample collection, make urine the preferred matrix for routine monitoring.

When the mean MAM/MAM-d3 ratio in urine as found in this study is considered to be the reference value for a patient who does not co-use illicit heroin, a urine MAM/MAM-d3 ratio larger than 32.8 (mean + 3·SD)(Figure 3) could be considered the lower limit ratio proving co-use of illicit heroin with 99.87% certainty (one-tailed application of the normal distribution). When this limit value was applied to our study population (Figure 3), one false positive sample was found (1%, 95% confidence interval: −1-3%) that had a ratio of 32.9, while it was extremely unlikely that the patient had co-used (illicit) heroin. Ratios of 27.0 and 27.1 were observed in two urine samples from the same patient, collected sooner after use of the same dose; moreover, the false positive result was associated with a low concentration of 6-acetylmorphine in urine (0.15 µmol/L), which might have resulted in a less reliable

Figure 5: Mean concentrations of 6-acetylmorphine (grey bars, left y-axis) found in urine at different time intervals after smoking a dose of diacetylmorphine for inhalation. Mean ratios of 6-MAM/6-MAM-d3 are also given (open bullets, right y-axis). Error bars indicate standard deviations.



ratio calculation (Figure 3B). Similarly, the lower limit for the MAM/MAM-d3 ratio in plasma samples would be 44.1, and no false positive results were observed in our population.
In summary, a MAM/MAM-d3 ratio above 32.8 in urine (and above 44.1 in plasma) could be considered indicative of co-use of illicit heroin in a heroin-assisted treatment program in which the abovementioned pharmaceutical product was used, provided that it was not associated with very low concentrations of 6-acetylmorphine (< 0.25 µmol/L). Another important aspect of monitoring co-use of illicit heroin, the percentage of false negative results, could not be calculated as no urine samples were available from addicts that had co-used labelled pharmaceutical and illicit heroin. However, using the abovementioned an upper limit for the MAM/MAM-d3 ratio in urine means that the presence of a minimum of 23% 'extra' 6-acetylmorphine ([upper limit = 32.8] / [mean ratio = 26.7]) in urine due to use of illicit heroin could be detected. This value is important in estimating the chance of false-negative results, together with the detection window. The MAM/MAM-d3 ratio was detectable in urine for 4-9½ h, which was considered a workable detection window, but some planning would be required in a heroin-assisted treatment program where heroin is administered 2-3 times daily, to ensure that useful urine samples (with detectable amounts of 6-acetylmorphine-d3) are collected. The number of false-negative results will depend on the time of co-use of illicit heroin, compared to the time of use of pharmaceutical heroin and the sampling time.

A larger detection window would greatly increase the flexibility and decrease the chance of false-negative results of the monitoring program for detection of co-use of illicit heroin. We expect that the detection window in urine samples could be improved if a diacetylmorphine isotope was available that contained deuterium atoms in the morphinan structure, as was also proposed by Gyr *et al.* [13]. Use of this isotope would result in deuterated morphine and morphine glucuronides in urine that would be detectable for much longer than 6-acetylmorphine (Figure 4 and Figure 5). However, while our diacetylmorphine-d6 could be manufactured by simply acetylating morphine with deuterated acetic anhydride, the morphine structure is still synthesized most effectively by the opium poppy and is therefore much less easy to manipulate. Even though synthesis of diacetylmorphine-d9 (intended for use as an internal standard in a mass spectrometric analysis) has been described [17], it is likely that this would be a very costly process, especially considering the large quantities that would be required to routinely add to the smokable pharmaceutical heroin used in a heroin-assisted treatment program. Authorities might consider dispensing pharmaceutical heroin containing diacetylmorphine-d6 (or diacetylmorphine-d9) as part of coordinated spot checks, for example in individuals who were suspected of co-use by the nursing staff. This could greatly reduce the cost of such a program, as smaller amounts of deuterated marker are required, while this strategy could be effective in detecting co-use of illicit heroin and have a preventive effect as well.

Conclusion

A novel strategy for monitoring co-use of illicit (non-prescribed) heroin by participants in a heroin-assisted treatment program was tested. Addition of a deuterated analogue of heroin to the pharmaceutical smokable heroin yielded constant ratios of MAM/MAM-d3 in plasma and urine. The ratios were normally distributed (with small standard deviations) and they were independent from plasma/urine concentrations of 6-acetylmorphine or time after use of pharmaceutical heroin. In a program using pharmaceutical heroin with a deuteration ratio of 20, a deuteration ratio of 6-acetylmorphine in urine above 32.8 was considered indicative of co-use of illicit heroin. A percentage false positive results of 1% (95% confidence interval: −1-3%) was observed and the ratio was detectable in urine for 4-9½ h after use of pharmaceutical smokable heroin. The detection window could possibly be improved by using a heroin analogue that contained stable isotopically labelled atoms in the morphinan structure.

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Chapter 4.3

Analysis of diacetylmorphine, caffeine and degradation products after volatilisation of pharmaceutical heroin for inhalation

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Submitted for publication

Abstract

Pharmaceutical smokable heroin was developed for a clinical trial on medical co-prescription of heroin and methadone. This product consisted of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate and it was intended for use via 'chasing the dragon': inhalation after volatilisation. As this procedure involved heating the powder mixture, it was expected to lead to formation of degradation products that could subsequently be inhaled along with the main constituents of the pharmaceutical smokable heroin. We developed a method for analysis of the vapour, using a high performance liquid chromatography system for separation of diacetylmorphine- and caffeine-related compounds in a wide polarity range, which was compatible with photo diode array detection as well as with mass spectrometric detection. This method was used to analyse the plastic drinking straws that were used by patients to inhale the vapours from pharmaceutical heroin used via 'chasing the dragon'. The contents of these straws were considered representative of the vapours the patients inhaled. They contained primarily unchanged diacetylmorphine, its main metabolite 6-acetylmorphine, caffeine and some morphine. Several unidentified peaks of possible degradation products were observed in the straw chromatograms. Proposed structures were presented for nine degradation products. These compounds are morphine derivatives with different substitution patterns of the C_3, C_6 and/or N_{17} positions and comprise 0.4-9.7% of the straw sample residue weight. Activity and toxicity of most of these compounds is unknown and requires further investigation.

Abbreviations

amu = atomic mass unit; DAD = diode array detection; GC = gas chromatography; HPLC = high performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; m/z = mass-to-charge ratio.

Introduction

A clinical trial was conducted in the Netherlands to evaluate the effect of medical co-prescription of heroin and methadone on mental and physical health and social functioning of chronic treatment-resistant heroin-dependent patients [1]. In the Netherlands, 75-85% of the heroin addicts use heroin by 'chasing the dragon' [2]; in this procedure, drug users heat heroin powder on aluminium foil with a cigarette lighter until it melts and evaporates. The vapours are subsequently inhaled through a straw in the mouth. The popularity of this route of administration was the reason that two separate study protocols were developed for the clinical trial; one trial testing the efficacy of the prescription of an inhalable form of heroin and another trial testing the efficacy of the prescription of injectable heroin. In preparation for the first trial, pharmaceutical heroin for inhalation after volatilisation was developed, which is a powder mixture of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate [3,4].

In vitro experiments with this product have shown that the vapours that developed on heating consisted mainly of unchanged diacetylmorphine and caffeine and some 6-acetylmorphine. However, as several authors have reported degradation of heroin and formation of pyrolysis products on heating heroin samples [5-7], it was decided to develop a method of analysis, suitable for separation and identification of the constituents of the volatilised pharmaceutical smokable heroin. A high performance liquid chromatography method was devised for quantification of heroin, caffeine, 6-acetylmorphine, and morphine and separation of known and likely degradation products of diacetylmorphine and caffeine in a wide polarity range. Furthermore, the liquid chromatography system was compatible with both photodiode array detection and mass spectrometric detection, to enable structural identification and confirmation of the analytes. This system was used to analyse paraphernalia (plastic straws and aluminium foils) that were used by heroin addicts in a pharmacokinetic study comparing smoked heroin with injected heroin [8]. These samples could provide reliable information on the exposure of patients to any degradation products after inhaling volatilised heroin; especially since they were obtained from a controlled clinical trial using pharmaceutical heroin, eliminating the possible influence of impurities, diluents, and adulterants present in street heroin.

Materials and Methods

Chemicals

Diacetylmorphine base was manufactured specifically for the clinical trial and obtained through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands). Caffeine anhydrate and morphine hydrochloride were purchased from Bufa (Uitgeest, The Netherlands), 6-acetylmorphine hydrochoride from Sigma Aldrich Co. Ltd. (Zwijndrecht, The Netherlands). Normorphine and 6-acetylcodeine were obtained from Radian International (via Schmidt, Amsterdam, The

Netherlands), while N,3,6-triacetylnormorphine was a gift from Diosynth (Oss, The Netherlands). Other chemicals used were analytical grade or HPLC grade.

Instrumentation

The high performance liquid chromatography (HPLC) system with diode array detection (DAD) consisted of an 1100 Series binary HPLC pump, Model G1312A (Agilent Technologies, Amstelveen, The Netherlands), a SpectraSERIES Model AS3000 automatic sample injection device, equipped with a 100 µL sample loop (Thermo Separation Products, Breda, The Netherlands), and a photodiode array detector Model Waters™ 996 (Waters Chromatography B.V., Etten-Leur, The Netherlands). Chromatograms were processed using Chromeleon® software (Dionex Corporation, Sunnyvale, CA, USA).

The liquid chromatography (LC) system used for mass spectrometric (MS) detection was a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA), consisting of a binary pump, autosampler, degasser, and column oven. The LC flow was split 1/20 before entering an API3000 triple quadrupole mass spectrometer equipped with an electrospray ion source (Sciex, Thornhill, ON, Canada). The quadrupoles were operated in the positive ion mode with unit resolution. The ion spray voltage was 5500 V and the source temperature was set at 400°C. A range of m/z 250-500 amu was scanned for the identification of the unknown degradation products. A step size of 0.1 amu was used, with dwell times of 2 and 5 sec (product ion scans and Q1 scans, respectively).

In both LC systems, separation was achieved using a Zorbax Bonus RP analytical column (4.6 mm ID x 15 cm, particle size 5 µm, Rockland Technologies Inc., Newport, DE, USA) that was protected by a reversed phase guard column (10x3 mm ID, Varian) and kept at 32°C during analysis. The mobile phase consisted of a 5 mM ammonium acetate buffer (pH 5.7), mixed with acetonitrile according to a programmed gradient: 0-2 min 3% acetonitrile, 2-2.6 min a linear rise from 3-13% acetonitrile, 2.6-8 min 13-15.5%, 8-15 min 15.5-80%, 15.1-24 min 3% acetonitrile. Flow was 1 mL/min and the injection volume was 20 µL. Six calibration standards were used to construct a calibration line for quantification of four analytes: the concentration ranges were 1-10 µg/mL for morphine and 6-acetylmorphine, 1-50 µg/mL for caffeine, and 1-100 µg/mL for diacetylmorphine. For optimisation experiments, a mixture of seven reference standards as well as separate standard solutions in 5 mM ammonium acetate buffer pH 4 were prepared (concentrations: 5-10 µg/mL for normorphine, morphine, 6-acetylmorphine, 6-acetylcodeine, and N,3,6-triacetylnormorphine, and ± 28 µg/mL for diacetylmorphine and caffeine).

Paraphernalia samples

We analysed the plastic straws (cut to ± 11 cm) and aluminium foils (10x20 cm) that were used by heroin addicts in a pharmacokinetic study [8]. In this study, ten male patients, participating in the Dutch Heroin trial [1] were admitted to a closed clinical research facility to study the pharmacokinetics and pharmacodynamics of smoked heroin compared to injected heroin. All patients received pharmaceutical heroin twice

a day, on four consecutive days. Pharmaceutical heroin for inhalation after volatilisation consisted of a powder mixture of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate. Maintenance doses (200-300 mg) were used, but the total morning dose was varied double-blindly: each patient used 66, 100 or 150% of their maintenance dose (overall dose range 133-450 mg) and the morning dose was dispensed in two unequal portions (40 and 60% w/w). New paraphernalia (plastic straws and aluminium foil) were dispensed with each new (portion of a) dose.
The plastic straws were placed in 20 mL of a 1:1 v/v mixture of 5 mM ammonium acetate solution pH 4 and acetonitrile and were subsequently sonicated for 15 min. The resulting solutions were diluted to 25.0 mL with the same solvent mixture and either stored at −20°C or diluted further for HPLC-analysis. The aluminium foil samples were cut in smaller pieces before placing them in 20 mL of a 1:1 v/v mixture of 5 mM ammonium acetate solution pH 4 and acetonitrile. Sonication of the samples was replaced by mechanical shaking for 30 min, because the aluminium foil disintegrated when sonicated. The resulting solutions were analysed after dilution to 25.0 mL with the same solvent or stored at −20°C.

Table 1: List of pyrolysis products mentioned in literature.

Sample	Pyrolysis products	References
Diacetylmorphine base	6-acetylmorphine	[5-7,10-12]
	3-acetylmorphine	[6]
	N,6-diacetylnormorphine	[5-7]
	N,3,6-triacetylnormorphine	[5-7]
	morphine	[6,7]
Diacetylmorphine HCl	3-acetylmorphine	[6]
	6-acetylmorphine	[5,7]
	N-acetylnormorphine	[6]
	N,6-diacetylnormorphine	[5,7]
	N,3,6-triacetylnormorphine	[5,7]
	N,3-diacetyl-6-O-methylnormorphine	[6]
	6-acetylcodeine	[6]
	3,4-diacetoxyphenanthrene	[5]
	1,10-diacetoxyphenanthrene	[6]
	9-hydroxyphenanthrene	[6]
	N-(2-phenylethyl)-acetamide	[6]
	isoquinoline	[6]

Pyrolysis products were found after heating diacetylmorphine base or salt at temperatures ranging from 250-400°C, using different heating methods (a quartz boat in a furnace [5], aluminium foil and a lighter [6,7] or a smoking device [11])

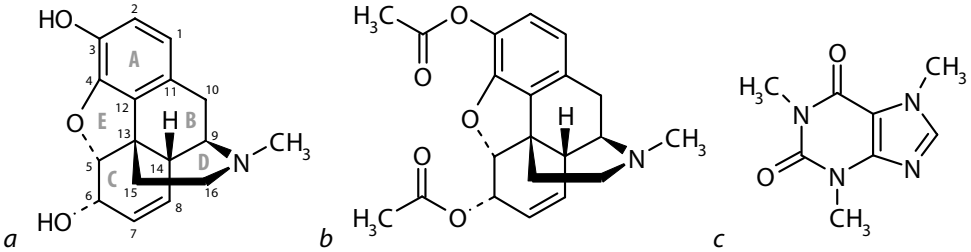
Results and Discussion

HPLC-DAD method

The analysis of heroin for bioanalytical, forensic, or pharmaceutical purposes has been the subject of research for decades and many papers have published on the subject. However, analytical methods that can be used in the study of the volatilisation process of heroin (when it is smoked or 'chased') have received relatively little attention. The earliest study on this subject used an aspecific determination of 'total phenol content' [9], but more recently different chromatographic methods have been developed. Several authors mention the use of high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [5,7,10] or gas chromatograpy - mass spectrometry (GC-MS) [11,12] for quantification en identification of compounds. The studies that use GC-MS methods [6,11,12] may have an inherent problem, since using elevated temperatures (200-300°C) in the analysis (injector, transfer line) could result in on-line degradation of the analyte(s), which could in turn lead to problems identifying the true pyrolysis products.

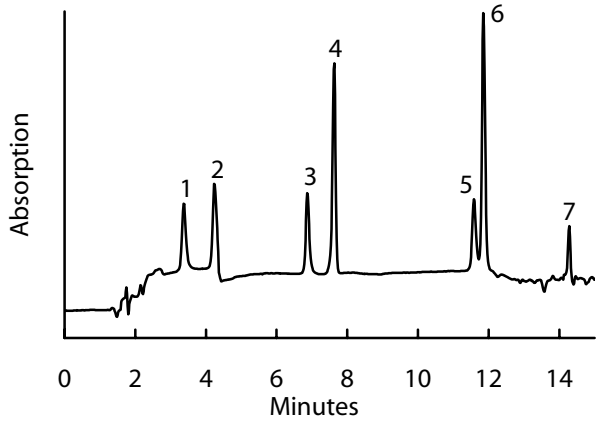
Considering the above, we decided to develop a HPLC method to be used specifically in the study of the volatilisation process of heroin. Easy quantification of heroin, caffeine, 6-acetylmorphine, and morphine was required and the chromatographic system should be able to separate the known and/or likely pyrolysis products from the abovementioned components. Furthermore, the LC-system had to be suitable for both photodiode array detection and mass spectrometric detection, to enable identification of unknown components.
Most degradation products that were reported in literature to have formed on heating pure diacetylmorphine base or its hydrochloride salt were found to be structurally related to morphine, as can be seen in Table 1. The three active sites on the molecule (C_3, C_6 and N_{17}, see Figure 1) appear to be able to either receive or lose methyl (ether) and acetyl groups during the volatilisation process, creating differently substituted morphine analogues. Therefore, we selected reference standards that represented many substitution types (mono-, di- and triacetylation and/or methylation of the morphinan structure), so a large polarity range would be covered and the chance of

Figure 1: Molecular structures of morphine (a), diacetylmorphine (b), and caffeine (c).



a *b* *c*

Figure 2: Chromatogram of a mixture of seven reference standards (blank subtracted). 1. morphine, 2. normorphine, 3. 6-acetylmorphine, 4. caffeine, 5. 6-acetylcodeine, 6. diacetylmorphine, 7. N,3,6-triacetylnormorphine.



missing compounds in the analysis would be minimised. Seven reference substances were available: diacetylmorphine, 6-acetylmorphine, morphine, caffeine, normorphine, 6-acetylcodeine and N,3,6-triacetylnormorphine.

A 5 mM acetate buffer was selected as the polar component of the mobile phase and as the solvent, since it is compatible with MS and has a suitable buffering range (pK_a 4.76) for optimal stability of diacetylmorphine (pH 4-4.5, [13,14]). The mobile phase gradient with acetonitrile was based on the gradient that was used in the bioanalysis of heroin and that was developed in our institution to analyse 16 components in a large polarity range [15]. To achieve optimal separation and peak shape for all of the seven reference standards, optimisation experiments were carried out. The mobile phase gradient was modified and flow, pH and column temperature were subsequently optimised. The resulting LC-system effectively separated the seven reference standards (Figure 2) and calibration lines with $R^2 > 0.999$ were obtained for diacetylmorphine, 6-acetylmorphine, morphine, and caffeine. The highest and lowest calibration solutions showed coefficients of variation $< 1.5\%$ ($n = 6$), indicating that quantification was sufficiently accurate.

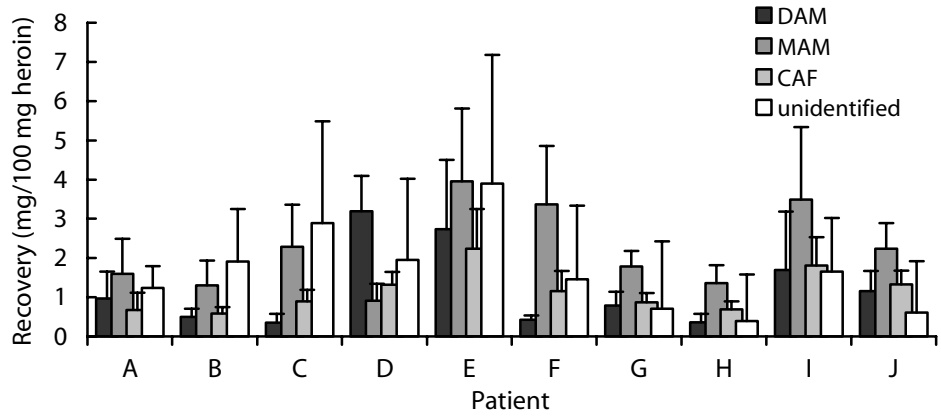
As the LC system was optimised for diacetylmorphine-related compounds, it was considered necessary to ensure that caffeine-related compounds were also separated. Eight reference standards, structurally related to caffeine, were shown to be sufficiently separated in this system: 1-methyl uric acid had a retention time of 5.53 min, 1-methylxanthine 5.73 min, theobromine 5.85 min, 1,7-dimethylxanthine 6.38 min, 1,7-dimethyl uric acid 6.53 min, theofylline 6.60 min, and 5-acetylamino-6-amino-3-methyluracil 3.2 min.

Table 2: Properties of reference standards and unidentified peaks.

Name	T_R	λ_{max}	Relative peak area	Parent m/z	Principal peaks (m/z)
Normorphine	3.4	285		272	165, 181, 153, 209, 121-201
Morphine	4.2	285		286	152, 128, 165, 115, 189
A1	5.7	280	0.05 (0.01-0.28)	286	152, 165, 128, 115, 127, 151
A2	5.7	286		302	
Hydromorphone	5.7	280		286	
B	6.2	282	0.29 (0.01-2.64)	328	268, 193-219, 211, 191, 237, 165, 286
6-Acetylmorphine	6.7	283		328	211, 193, 165, 183-191-209, 201
Caffeine	7.3	272		195	138, 195, 110
C	8.4	285	0.19 (0.01-0.31)	344	268, 215, 162, 165-191-266
D	9.7	284	1.56 (0.01-4.58)	344	268, 162, 215-266, 145
6-Acetylcodeine	11.6	284		342	225, 197, 282
Diacetylmorphine	11.8	279		370	268, 328, 211, 193-237
E	12.3		0.21 (0.02-0.80)	384	225, 283, 207-251, 342
F	12.7	285	0.35 (0.06-0.55)	386	268, 162-215, 327-310
G	13.5		0.71	356	254, 219, 191, 237, 211
Triacetylnormorphine	14.2	279		398	237, 254, 219, 296, 356
H	14.2	279		398	237, 296, 254, 219, 356, 211

T_R (retention time) is given in min; λ_{max} in nm; median values for relative peak area (%) of the unknown peaks in straw sample chromatograms are given, with the range within parentheses; principal peaks are given in order of decreasing intensity, with equally large peaks separated by a hyphen.

Figure 3: Results of the quantitative analysis of straw samples. For each patient, the mean recoveries of diacetylmorphine, 6-acetylmorphine, and caffeine are given, as well as the size of the unidentified part of the residue. All values are corrected for dose (100 mg heroin = 100 mg diacetylmorphine + 33 mg caffeine); error bars indicate standard deviations.

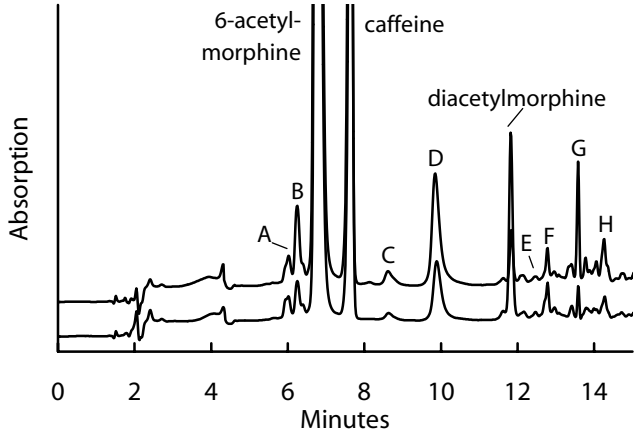


Quantitative analysis of paraphernalia samples

The weight of the residue on the aluminium foil and in the straw samples was calculated from their weight before and after use in the process of 'chasing the dragon'. The mean residue weight in the straw samples was 11.9 ± 9.4 mg (range 0-62 mg, n = 119) and on the aluminium foil samples a mean residue of 4.8 ± 6.2 mg (range −2-61 mg, n = 120) was found. All foil samples showed thick black residues on the side that had been in close contact with the cigarette lighter during the volatilisation of heroin. This is due to the natural inclination of addicts to make optimal use of the dispensed drug: they heated the powder mixture with great care for as long as it took for all signs of vaporisation to cease, creating thick layers of soot on the under side of the foil and very small, carbonised residues on the top. A simple experiment showed that heating a 'blank' piece of aluminium foil for 2 min with a cigarette lighter resulted in a weight increase of 0.6 mg. This indicates that it is likely that most of the weight increase of the foil after use was due to the deposition of soot from the cigarette lighter, since the process of 'chasing the dragon' took 16 min on average, creating 8 times as much soot residue as in the 2-min experiment. This is probably also the explanation for the significant correlation of the weight of the foil residues with the dose of heroin used (Pearson correlation 0.369, $p < 0.001$): more time is needed to volatilise a larger dose, leading to more deposited soot on the aluminium foil. Straw sample residue weight was also significantly correlated with the dose of pharmaceutical heroin used (Pearson correlation = 0.447, $p < 0.001$), which could be explained by increasing amounts of vapour being released from larger doses, depositing more residue on passing through the straw. Considering the above, recoveries will be reported corrected for dose: as mg recovered per 100 mg heroin (100 mg heroin = 100 mg diacetylmorphine base + 33 mg caffeine anhydrate). The corrected mean residue weights were: 6.2 ± 4.2 mg/100 mg heroin (range 0.0-25.0) in straw samples and 2.4 ± 2.3 mg/100 mg heroin (range −1.7-20.3) on aluminium foil samples. Apparently, during 'chasing the dragon' only about 6% of a heroin dose is lost to absorption due to deposition in the straw, indicating that inhalation through a straw during 'chasing the dragon' does not stand in the way of effective inhalation after volatilisation. Efficient inhalation (maximising the amount of vapour reaching the airways) will probably depend mostly on the 'chasing' technique of the patient and on the circumstances (for example: air turbulence disturbing the vapours).

Straw sample chromatograms showed that all residues contained 6-acetylmorphine (2.2 ± 1.5 mg/100 mg heroin, n = 119), caffeine (1.2 ± 0.7 mg/100 mg heroin, n = 119), and diacetylmorphine (1.2 ± 1.3 mg/100 mg heroin, n = 119). Only ten samples showed traces of morphine (0.2 ± 0.1 mg/100 mg heroin). In total, 74% of the residue weight was recovered as diacetylmorphine, caffeine or one of the hydrolysis products of diacetylmorphine, which implies that 26% of the residue weight consists of unknown substances and (probably) soot. Large variations in recovery of known and unknown compounds were observed (Figure 3) between and within patients. We should, however, keep in mind that the quantitative composition of the straw residue is not necessarily similar to the quantitative composition of the inhaled vapours, as

Figure 4: Chromatograms of two straw samples showing distinct degradation peaks (blank subtracted).



the tendency to deposit inside the straw could differ between the constituents of the vapours.

As expected, even the ten foil residues with the largest residue weights (10-61 mg, 3.3-20.3 mg/100 mg heroin) contained only very small amounts of diacetylmorphine, caffeine, or degradation products. Only 0.08-10% w/w of these residues was recovered as one of the four quantified analytes: 6-acetylmorphine (mean 0.12 mg/100 mg heroin, range 0.004-0.48) and diacetylmorphine (0.08 mg/100 mg heroin, range 0.01-0.25) were present in all ten foil samples, while caffeine was found in five samples (0.01 mg/100 mg heroin, 0.004-0.02), and morphine in only one sample (0.02 mg/100 mg heroin).

Identification of unknown peaks

The HPLC-DAD chromatograms of some of the straw samples contained several small, unidentified peaks: two examples are shown in Figure 4. The peaks that were selected for identification (A-H) varied in detection frequency: compound G and C were observed in only 6 and 20 of the 120 straw samples, respectively, while A, B, E, F, D, occurred in 94, 108, 111, 112, and 118 of the samples, respectively. Foil sample chromatograms contained very few unidentified peaks, five of the samples (analysed without prior dilution) contained peak D, and in six samples peak E was detected.

Retention times and relative peak areas of the unknown compounds are listed in Table 2. A UV-spectrum could be obtained for six of the eight unknown peaks, all of which were similar in shape and absorption maximum (λ_{max}) to morphine and morphine-like reference standards (Table 2). Only compound H showed a λ_{max} (279 nm) that resembled the slightly shifted maximum found in diacetylmorphine and N,3,6-triacetylnormorphine. None of the peaks showed UV-spectra comparable to that of caffeine (λ_{max} = 272 nm) or caffeine-related compounds, that generally have lower

λ_{max} (234-271 nm), and higher specific absorbances than morphine-related compounds. Potential m/z values for molecular ions ($[M+H]^+$) in peaks of compounds A-H were obtained from Q1 mass spectra of straw samples and product ion spectra were obtained for each of these parent masses (Figure 5, Table 2).

Compound A

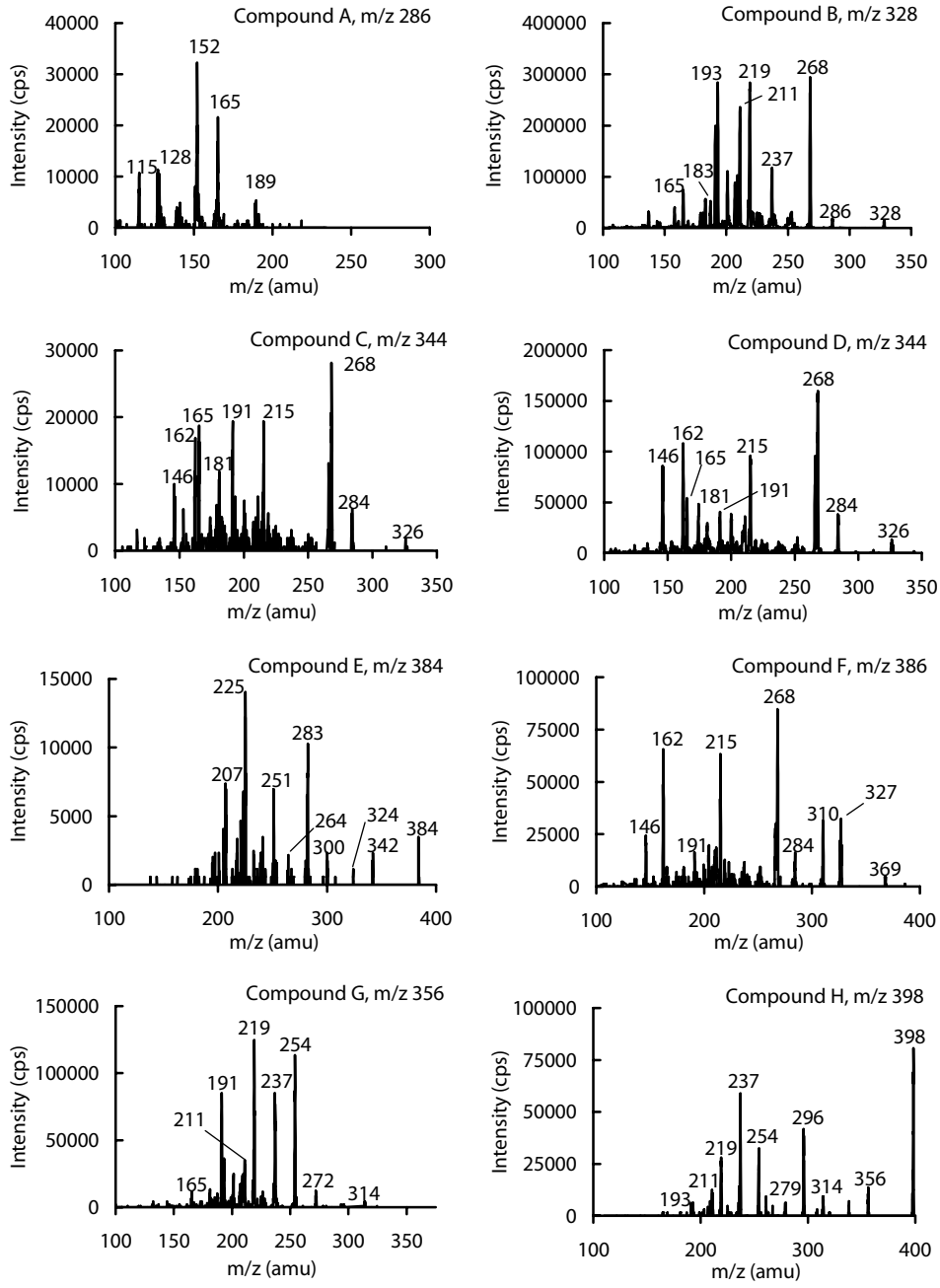
The peak shape of unknown peak A suggests that it is not quite pure (Figure 4) and in some straw samples slightly different absorption maxima could be distinguished for the first (A1) and last (A2) part of the peak: 280 and 286 nm, respectively (Table 2). Both UV-spectra however, were morphine-like in shape and the retention time of 5.7 min was suggestive of slightly less polar morphine derivatives. The Q1 scan of the signal at 5.7 min showed a dominant mass at m/z 286 and a smaller signal at 302. The product ion spectrum of the dominant mass resembled that of the morphine reference standard (Figure 6), which could be explained by the presence of hydromorphone (m/z 286 = $[M+H]^+$) (Figure 7a). A hydromorphone reference standard showed the same retention time and absorption maximum as compound A1, confirming the proposed structure (Table 2).

The second observed Q1 mass, m/z 302 might then have been associated with the second part of unknown peak A, with the absorption maximum of 286 nm. This parent mass suggests the addition of oxygen to morphine, which could indicate formation of for example morphine-N-oxide (Figure 7c), 10-hydroxymorphine (Figure 7d), or 14-hydroxymorphine. Hydroxylation of the fenyl ring of morphine is also possible, but unlikely in this case, as such a structural change would cause a bathochromic shift in the UV spectrum compared to that of morphine. Furthermore, hydroxylation of C_{14} in a morphinan structure was reported to be possible only when C_{14} was unsaturated (like in thebaine) [16], which would suggest C_{10} as the preferred site for hydroxylation. Oxidation is a major mechanism of degradation of morphine in aqueous solutions, and degradation is known to be catalysed by oxygen of air, sunlight, UV irradiation, and organic impurities [17]. Morphine-N-oxide is a well-known oxidation product of morphine in aqueous solutions [17] and it would be expected to have a longer retention time than morphine, as opposed to 10-hydroxymorphine, which is more polar than morphine. Therefore, we propose morphine-N-oxide as a possible structure of compound A2 (Figure 7c).

Compound B

The UV-spectrum of the peak at 6.2 min (compound B) shows a small shift of λ_{max}, compared to morphine, similar to that of the 6-acetylmorphine reference standard (Table 2). The parent mass found at this retention time was also the same as for 6-acetylmorphine (m/z 328, $[M+H]^+$), but the product ion mass spectrum differed slightly: m/z 268 was the base peak instead of 211, and higher relative intensities were observed for the peaks at m/z 193, 219 and 237 (Table 2). Since m/z 268 indicates the loss of acetic acid from the parent mass, it was likely that the main difference between compound B and 6-acetylmorphine was due to the position of the acetyl group, suggesting the presence of 3-acetylmorphine (Figure 7b).

Figure 5: Product ion mass spectra of the unknown compounds A-H.



3-Acetylmorphine was expected to have a very similar UV-spectrum and retention time to 6-acetylmorphine, but no reference standard was available for definite confirmation by direct comparison of 3-acetylmorphine as the proposed structure for compound B.

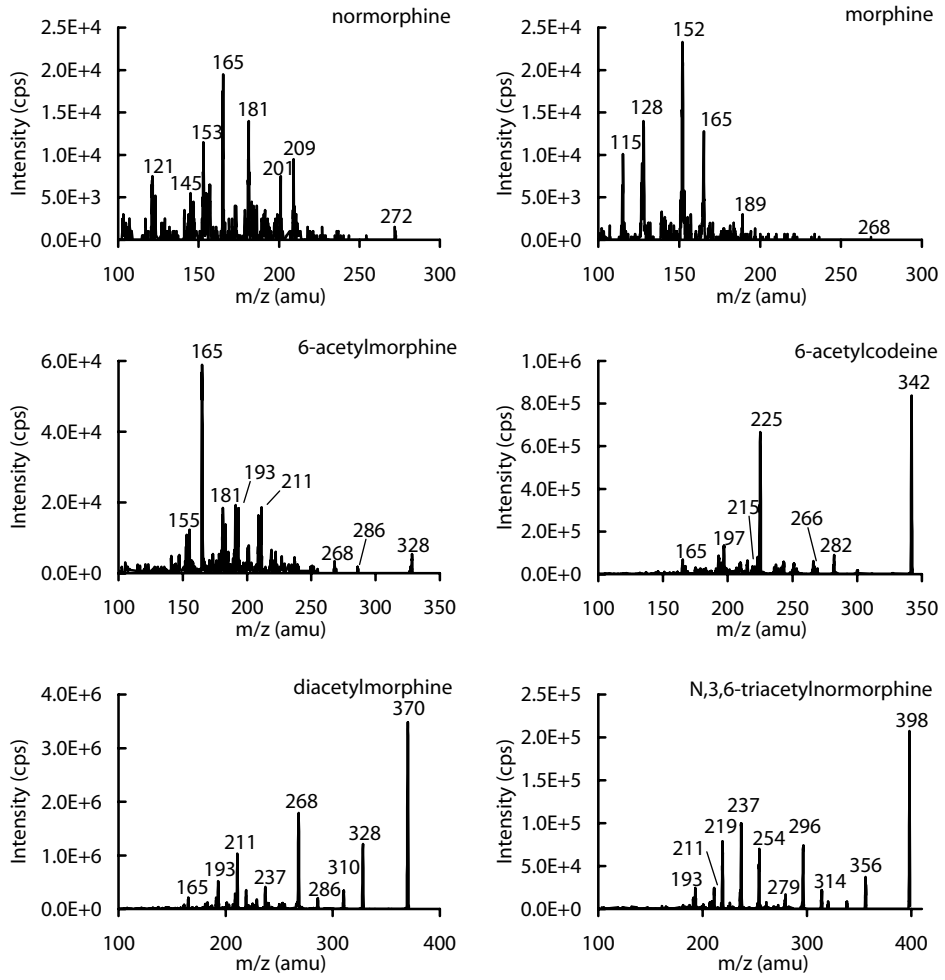
Compounds C and D

Although the retention times of compounds C and D were very different, both had morphine-like UV-spectra and a parent mass at m/z 344 in their Q1 scan. The resulting product ion mass spectra were also very similar, they differed only in the relative intensities of the fragments with m/z 165, 181 and 191 (Figure 5). The parent mass of 344 suggests addition of oxygen to 6-acetylmorphine, which was confirmed by the presence of intense peaks at m/z 266/268 and 284, that suggest the loss of acetic acid from 6-acetylmorphine ($[M+H]^+ = 328$) as well as from $[M+O+H]^+ = 344$. Furthermore, both spectra contain a peak at m/z 326 that could indicate the loss of water from the parent, which was not observed in any of the reference standards (Figure 6), indicating that it might be due to added oxygen. 6-Acetylmorphine is the main degradation product of diacetylmorphine found after volatilisation and unidentified compounds could therefore be the result of degradation of 6-acetylmorphine. Oxidation of 6-acetylmorphine during heating and subsequent volatilisation was considered a potential degradation mechanism, since oxidation is known to be important in degradation of morphine in aqueous solutions [17]. Similar to oxidation of morphine, 6-acetylmorphine could be oxidised to 6-acetylmorphine-N-oxide (Figure 7c). Furthermore, following the same reasoning as under *Compound A*, the parent mass of 344 could also have been the result of hydroxylation of another position of the 6-acetylmorphine molecule, e.g. C_{10} (Figure 7d). 6-Acetyl-10-hydroxymorphine is expected to elute before 6-acetylmorphine-N-oxide. Therefore, 6-acetyl-10-hydroxymorphine is proposed as the structure of compound C, and compound D is proposed to be 6-acetylmorphine-N-oxide.

Compound E

The peak of compound E in the LC-DAD-chromatograms was too small to obtain an UV-spectrum, but it was present in quantities large enough to obtain a parent mass (m/z 384) in a Q1 scan. The product ion mass spectrum (Figure 5) contained fragments suggesting the loss of two acetyl groups from the parent ion (m/z 342 and 300), as well as loss of acetic acid (324 and 264), indicating that compound E could be a 3,6-diacetyl-substituted morphine. The parent mass suggests the addition of CH_2, which is most likely to occur at the tertiary amine, possibly combined with opening of the piperidine ring. This mechanism was proposed by Cook *et al.* to explain a pyrolysis product that they found after volatilisation of diacetylmorphine hydrochloride [18]. However, the product ion mass spectrum also shows a base peak with m/z 225 that was also observed in codeine (data not shown) and 6-acetylcodeine, but not in spectra of other morphine derivatives (Figure 6). However, in the spectra of 6-acetylmorphine, diacetylmorphine, and N,3,6-triacetylnormorphine did show a fragment with m/z 211, corresponding to 225 after loss of CH_2. These fragments could be the result of elimination of the piperidine (D-)ring and elimination

Figure 6: Product ion spectra of six morphine-related reference standards.



of the group at C_6, leading to a structure with intact A, B, and E rings (see Figure 1), a conjugated C ring and either –OH (m/z 211) or $-OCH_3$ (m/z 225) on C_3. The fragment with m/z 283 in the product ion mass spectrum of compound E could then correspond to the 225 fragment, with an acetyl group at C_6. Considering this, the structure in Figure 7e was proposed for compound E. It could have formed from diacetylmorphine during volatilisation by elimination of C-O from the C_3-acetyl group and subsequent opening of the piperidine ring according to the mechanism by Cook *et al.* [18].

Compound F

Compound F combined a morphine-like spectrum with a retention time of 12.7 min. At this retention time, the most abundant mass in the Q1 spectrum was found to be m/z 386, corresponding to addition of oxygen to diacetylmorphine. The product ion mass spectrum was very similar to that of compound D (Figure 5), and the same line

of reasoning as under *Compound A* and *Compounds C and D* might be followed to derive the most likely position of the added oxygen in the morphinan structure. Formation of an N-oxide is more likely than 10-hydroxylation, because of the similarity of the product ion spectra of compound D and F, and because compound D elutes later than diacetylmorphine, indicating that it is less polar. Therefore, the proposed structure for compound D is 3,6-diacetylmorphine-N-oxide (Figure 7c).

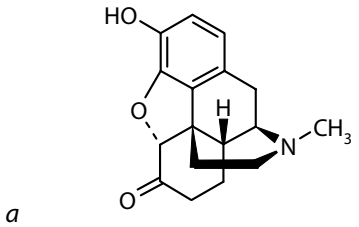
Compound G
The chromatographic peak for compound G was superimposed on a blank peak, which caused interference in its UV-spectrum. However, the Q1 scan clearly showed a single most abundant mass at 13.5 min: m/z 356. The product ion mass spectrum of this parent ion showed peaks indicative of loss of one (m/z 314) or two acetyl (m/z 272) groups as well as one acetyl group and acetic acid (m/z 254). This information suggests that the structure contains two acetyl groups, one of which is positioned on N_{17} in the morphinan structure. Furthermore, since the parent ion mass suggests addition of a carbonyl (C-O) to acetylmorphine, and 6-acetylmorphine is abundantly present as a degradation product during volatilisation, we propose N,6-diacetylnormorphine as the structure of compound G (Figure 7f). This proposal was supported further by the similarity between the product ion spectra of compound G and H, since the latter was also identified as an N-acetylated morphine derivative. N,6-diacetylnormorphine has been reported before as a degradation product occurring after heating diacetylmorphine base or hydrochloride (Table 1).

Compound H
The most likely candidate for the identity of compound H is easily derived from Table 2: N,3,6-triacetylnormorphine. Both compounds showed the same retention time, a similar shift in UV-spectrum, the same parent mass, and similar principal peaks in the product ion scan. We therefore conclude that compound H is N,3,6-triacetyl-normorphine (Figure 7g).

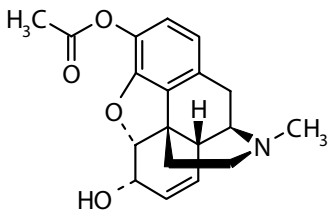
Overall recovery

Morphine-related compounds with different substitution patterns for C_3, C_6 and N (codeine, 6-acetylmorphine, normorphine, dihydromorphine, diacetylmorphine) have similar specific absorbances ($A^{1\%}_{1\,cm} = \pm 50$) [19]. Therefore, the relative peak area of the unidentified peaks in the LC-UV chromatograms could be used to calculate the relative amounts of the unknown compounds present in the straw samples (Table 2). The unidentified compounds had a total peak area of 0.4-9.7% in the straw chromatograms and diacetylmorphine, 6-acetylmorphine, morphine, and caffeine accounted for a mean of 74% of the residue weight in straws, which suggests that the composition of the residue has not been identified completely. Similar to the foil samples, part of the straw residue might also have consisted of soot from the cigarette lighter flame, as over 80% of the samples showed a light brown to black coloured residue. Moreover, many showed signs of melting, indicating that the flame had come close enough to the straws to deposit soot.

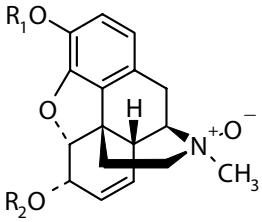
*Figure 7: Structure proposals for compound **A-H**: a. hydromorphone, m/z 286 (**A1**); b. 3-acetylmorphine, m/z 328 (**B**); c. N-oxide formation or d. 10-hydroxy-formation: m/z 302 (R_1=R_2=H)(**A2**), m/z 344 (R_1=H, R_2=$COCH_3$)(**C/D**), m/z 386 (R_1=R_2=$COCH_3$)(**F**); e. N-methylation with opening piperidine ring, m/z 384 (**E**); f. N,6-diacetylnormorphine, m/z 356 (**G**); g. N,3,6-triacetylnormorphine, m/z 398 (**H**).*



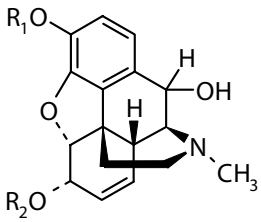
a



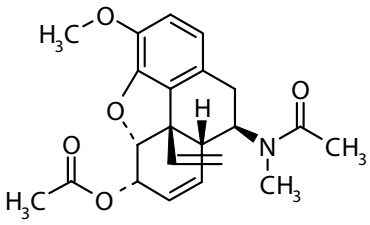
b



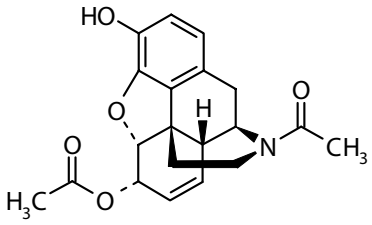
c



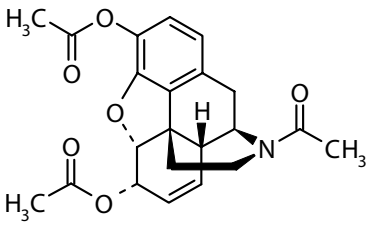
d



e



f



g

The contents of the straw samples that were analysed in this study can be considered representative of the composition of the vapours that patients inhale when they use pharmaceutical smokable heroin by inhalation after volatilisation. These samples were found to contain mainly unchanged constituents of the volatilised pharmaceutical product, as well as the well-known hydrolysis products of diacetylmorphine, that are considered to be its active metabolites (6-acetylmorphine, morphine). The degradation products that we observed in the straw samples were in agreement with those found in *in vitro* experiments involving heated diacetylmorphine base in literature (Table 1), with the exception of the proposed N-oxide and 10-hydroxyl derivatives that were not reported before. The majority of the degradation products were morphine derivatives with different substitution patterns on the C_3, C_6 and N_{17} positions (Figure 1). Only one proposed structure (compound E) showed ring cleavage that could lead to the formation of phenanthrene derivatives (Table 1), which are known to be potentially toxic. Some of the compounds that were detected could bind to μ-receptors and could have analgesic and euphoric effects. Hydromorphone (compound A1) is a well-known active analgesic (trade name Dilaudid) that is considered to be twice as potent as heroin [19-21]. Opiate binding studies have shown that the lack of a free 3-hydroxyl group leads to a low binding affinity to μ-receptors [20,22,23]. Moreover, substitution at N_{17} also influences receptor binding affinity [20]. This means that 3-acetylmorphine (compound A1), N,6-diacetylnormorphine (compound G), and N,3,6-triacetylnormorphine (compound H) are probably not active, even though they could be metabolised to active compounds after systemic absorption. Deacetylation of 6-acetyl-10-hydroxymorphine (compound C) would yield 10-hydroxymorphine, which has been reported to be active [24]. Morphine N-oxide (compound A2) was reported to have weak analgesic effects, weaker subcutaneous and intravenous toxicity than morphine, and no teratogenic effects in mice [17]. In summary, some of the degradation products could have opioid activity, but further research is required to assess their activity and toxicity after chronic administration via inhalation after volatilisation.

Conclusion

We successfully used a HPLC-DAD/MS method to analyse the contents of plastic straws and aluminium foil samples, used by addicts ‘chasing the dragon’ in a clinical study. The residue in the straw samples, that was considered representative for the vapour reaching the patient’s lungs, was found to consist mainly (75%) of unchanged diacetylmorphine, 6-acetylmorphine, caffeine and morphine. Proposed structures were presented for nine degradation products that were found to be morphine derivatives with different substitution patterns of the C_3, C_6 and/or N_{17} positions and that were estimated to comprise 0.4-9.7% of the straw sample residue weight. Activity and toxicity of most of these compounds is unknown and requires further investigation.

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Summary

Introduction

Addiction to opioids, and more specifically, to heroin is a common problem in many countries around the world. Nowadays, addiction has been accepted as a chronic, relapsing psychiatric disorder and pharmacological treatments have been developed to achieve three main goals: crisis intervention, detoxification, and stabilisation or harm reduction. Opioid antagonists (naloxone, naltrexone) and opioid agonists (methadone, buprenorphine) have proven to be useful in the treatment of heroin addiction, but curing the disorder and achieving complete and lasting abstinence remains problematic for many addicts. Therapeutic options for subgroups of chronic, treatment-refractory addicts are limited; therefore, stabilisation of drug use, improvement of well-being, and reduction of drug-related harm have become the focus of therapy for these patients. Presently, there is considerable interest in heroin-assisted treatment: (co-)prescription of heroin to chronic, treatment-refractory opioid dependent patients. Several European countries (Belgium, France, Germany, Spain, Switzerland, The Netherlands, United Kingdom) have planned or ongoing clinical trials on this subject, as do Australia and Canada. These trials, and the routine heroin-assisted treatment programs that might result, need pharmaceutical heroin (diacetylmorphine) to prescribe to the patients.

In this thesis, the development of pharmaceutical preparations of diacetylmorphine is described, intended for prescription to addicts participating in the Dutch clinical trial and the resulting heroin-assisted treatment program. In *Chapter 1*, the existing knowledge on the pharmaceutical and physicochemical properties of diacetylmorphine and the clinically investigated routes of administration is discussed. Furthermore, the properties of street heroin are described, as well as the routes of administration utilised on the street. Pharmaceutical heroin has to comply with the usual requirements of efficacy, safety, and quality of pharmaceutical products, but acceptability to patients is also an important requirement. Especially since heroin-assisted treatment is aimed at treatment-resistant addicts, who often have to be encouraged to participate in a treatment program. This means that the most suitable pharmaceutical dosage form would have a pharmacokinetic profile mimicking that of diacetylmorphine for injection, with rapid peak concentrations of diacetylmorphine and 6-acetylmorphine, ensuring the 'flash' effect and the sustained presence of morphine(-6-glucuronide) creating the prolonged euphoria.

Diacetylmorphine for injection

Injection of heroin is the most widely used mode of administration among addicts: in many European countries, 60-80% of the heroin users in treatment predominantly injected the drug (data 1990-2001). Therefore, *Chapter 2* describes the development of a lyophilised form of diacetylmorphine hydrochloride for injection. Diacetylmorphine is not stable in aqueous solutions, therefore, a freeze-dried dosage form was preferred. No excipients were found necessary to manufacture a stable

product containing 3 grams of diacetylmorphine hydrochloride per vial. The reconstituted product was found to be antimicrobially active and it showed sufficient chemical stability to support its suitability as multi-dose product.

Diacetylmorphine for inhalation: pharmaceutical development

Heroin smoking is the second most popular route of administration by addicts: in 2001, about 45% of the European addicts in treatment predominantly smoked heroin via 'chasing the dragon'. In this procedure, heroin powder is heated on a piece of aluminium foil (using a cigarette lighter), and the vapours that arise after volatilising are subsequently inhaled through a tube or straw in the mouth. This mode of administration was found to result in a 52% bioavailability compared to injected heroin and it has pharmacokinetic and pharmacodynamic profile that would make it acceptable to addicts in heroin-assisted treatment. *Chapter 3* therefore describes the development of diacetylmorphine for inhalation after volatilisation.

Thermal analysis experiments in *Chapter 3.1* showed that addition of caffeine anhydrate to diacetylmorphine base resulted in a lower melting temperature and a higher volatilisation rate for the mixture than for diacetylmorphine base alone. *In vitro* recovery experiments showed that about 41% of diacetylmorphine base could be found in vapour condensate after volatilisation of diacetylmorphine/caffeine tablets. Caffeine seemed to volatilise completely, without degradation, while diacetylmorphine was also recovered as non-volatilised (charred) residue and as 6-acetylmorphine in the vapour condensate.

In *Chapter 3.2*, more elaborate *in vitro* volatilisation experiments showed that different powder mixtures (25%, 50%, and 75% w/w diacetylmorphine) and different temperatures (200-350°C) yielded only minor differences in recovery of diacetylmorphine (46-62%) and caffeine (65-83%). Increasing temperatures mainly resulted in increasing volatilisation rates. Particle sizing experiments showed that the aerosol particles generated on heating diacetylmorphine-containing samples had mass median aerodynamic diameters of 1.8-4.1 µm, and 45-60% of each sample was recovered as aerosol particles < 5 µm. Samples with a larger proportion of caffeine showed larger particle sizes, while increasing volatilisation temperatures also seemed to increase aerosol particle size. Summarising, volatilisation of diacetylmorphine/caffeine mixtures resulted in sufficient amounts of diacetylmorphine in vapour and in particles small enough for efficient deposition in the lungs. The powder mixture containing 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate was preferred for development of pharmaceutical heroin for inhalation after volatilisation.

The next step was the development of a manufacturing process for dosing and packaging of this powder mixture. A micro dose auger filler machine was selected for this purpose and *Chapter 3.3* describes the characterisation, optimisation, and validation of the auger filling process used for manufacturing diacetylmorphine/caffeine sachets. Accurate and precise filling of 50-300 mg portions of diacetylmorphine powder was found to be a complex process; all five tested process variables (auger speed, agitator speed, hopper fill level, dose interval and dose)

influenced precision of dosing, either directly or via interaction effects. A 9-term regression model was constructed, which was used to obtain optimal settings for routine manufacturing. The results of four test batches made with the optimised settings showed that accurate (accuracy 99.0-101.0%) and precise (coefficient of variation: 3.2-5.3%) filling of diacetylmorphine/ caffeine sachets is possible using the micro dose auger filler machine.
Chapter 3.4 describes in more detail the considerations in the early phases of development of diacetylmorphine for inhalation after volatilisation, the manufacturing process and the in-process controls included therein, as well as the quality control of the final product. Several types of weight checks (weighing the complete sachets as well as their contents) were included in the manufacturing process, to ensure the accuracy and precision of dosing. Weighing as well as counting checks are described that were used in the reconciliation of the amount of powder and the number of sachets, which was important for compliance with the Dutch Narcotics Law. Quality control tests on diacetylmorphine and caffeine content and uniformity of mass were selected to supplement the in-process controls in assuring product quality. The diacetylmorphine/caffeine sachets were found to be sufficiently stable under long-term (25 ± 2°C, 60 ± 5% relative humidity) and accelerated stability (40 ± 2°C, 75 ± 5% RH) conditions.

Diacetylmorphine for inhalation: administration

Chapter 4 describes three studies concerning the administration of diacetylmorphine for inhalation after volatilisation.
A pharmacokinetic study comparing two methods for volatilisation of the diacetylmorphine/caffeine tablets (*Chapter 4.1*) was performed in five addicts that alternately used heroin via 'chasing the dragon' or via the standardised method using a heating device. Plasma concentrations of diacetylmorphine, 6-acetylmorphine, morphine and morphine-3- and -6-glucuronide were determined using liquid chromatography with tandem mass spectrometric detection. The exposure to diacetylmorphine and its metabolites was significantly lower after smoking via the heating device than after 'chasing the dragon': diacetylmorphine 80% lower and 6-acetylmorphine 73% lower ($p < 0.05$). Maximal concentrations of diacetylmorphine and 6-acetylmorphine were also 80 and 70% lower ($p < 0.02$) after using the heating device. 'Chasing the dragon' was found to be the more efficient of the two methods for inhalation of diacetylmorphine after volatilisation.
The second study (*Chapter 4.2*) investigated deuterodiacetylmorphine as a marker for use of illicit heroin by patients in heroin-assisted treatment. The success of such treatment depends on complete substitution of the client's illicit heroin use by pharmaceutical heroin; hence the need to monitor patient compliance. Stable isotopically labelled heroin (diacetylmorphine-d6) was added to diacetylmorphine for inhalation after volatilisation that was used by nine patients on four consecutive days. The patients were admitted to a closed research facility and care was taken to eliminate the use of illicit drugs. Urine and plasma samples were collected regularly and analysed quantitatively for diacetylmorphine and metabolites. Furthermore,

deuteration ratios were calculated and the samples were screened for the presence of 6-acetylcodeine, codeine (indicative of use of illicit heroin), and cocaine and its metabolites. No evidence for co-use of illicit drugs was found and plasma samples as well as urine samples showed very constant ratios of deuterated and unlabelled 6-acetylmorphine. These results indicate that monitoring this ratio for deviation from the reference value for deuterated *versus* unlabelled 6-acetylmorphine in urine would be a feasible strategy for detection of co-use of illicit heroin.
In the last study (*Chapter 4.3*), the straws used by addicts 'chasing the dragon' were analysed for the presence of degradation products. Using pharmaceutical heroin via 'chasing the dragon' involves heating a mixture of diacetylmorphine/caffeine powder, which might lead to decomposition of diacetylmorphine and caffeine. Since this procedure involves patients inhaling volatilised product through a plastic straw, the contents of the straw samples were considered to be representative of the vapours the patients inhaled. The samples were analysed using a specifically developed high performance liquid chromatography method for separation of diacetylmorphine- and caffeine-related compounds in a wide polarity range, which was compatible with photo diode array detection as well as with mass spectrometric detection. The straw samples contained mainly (75%) diacetylmorphine, caffeine, 6-acetylmorphine and morphine. Several unidentified peaks were observed in the straw chromatograms. Proposed structures are presented for nine degradation products (comprising 0.4-9.7% of the straw samples). These compounds were identified as morphine derivatives with different substitution patterns of C_3, C_6 and N_{17} positions. Activity and toxicity of these compounds is unknown and requires further investigation.

Concluding remarks

In this thesis, the development of a pharmaceutical dosage form for injectable heroin is described, as well as extensive research into the development of smokable heroin: diacetylmorphine for inhalation after volatilisation. Both resulting dosage forms were developed and manufactured to comply with the strict quality standards for pharmaceutical products for human consumption. Manufacturing processes were validated and controlled via in-process controls and product quality was ensured further by laboratory testing of the final product. Diacetylmorphine for injection, 3 gram/vial lyophilised product and the four dosages of powder filled diacetylmorphine/caffeine sachets for inhalation after volatilisation have been used in the Dutch Heroin Trial, of which a report has since been published in the British Medical Journal (2003;327(7410):310-315). The results were good and the pharmaceutical products will be used in the heroin-assisted treatment program, intended for a subgroup of treatment-resistant addicts that will probably result in the near future (more information is available at www.ccbh.nl).

Samenvatting

Introduction

Verslaving aan morfine-achtige middelen (opioïden), zoals heroïne, is een wereldwijd probleem. Tegenwoordig wordt verslaving gezien als een chronische psychiatrische afwijking, waarbij de patiënt vaak terugvalt in zijn slechte gewoonten. Voor de behandeling van heroïneverslaving zijn farmacologische behandelingen ontwikkeld, met drie hoofddoelen: crisisinterventie, ontgifting (afkicken) en stabilisatie gecombineerd met schadebeperking. Voor crisisinterventie (bijv. na een overdosis) worden geneesmiddelen gebruikt die de effecten van opioïden tegengaan (antagonisten, zoals naloxon en naltrexon), terwijl voor afkicken en stabilisatie van de patiënten meestal juist geneesmiddelen met morfine-achtige werking worden toegepast (opioïd-agonisten, zoals methadon en buprenorfine). Hoe nuttig deze behandelingen ook zijn, verslaving is nog steeds niet te genezen en voor veel chronisch verslaafden blijft het een probleem om langdurig van de drug af te blijven. Daarom richt de behandeling van chronisch heroïneverslaafde patiënten zich tegenwoordig op stabilisatie van illegaal drugsgebruik, verbetering van de algehele gezondheid van de patiënt en het beperken van de schade die gebruik van illegale drugs met zich meebrengt.
In dat verband is tegenwoordig steeds meer interesse voor behandelingsprogramma's waarbij de mogelijkheid bestaat om heroïne voor te schrijven aan chronisch verslaafden, die geen baat hebben gehad bij de andere behandelingsmogelijkheden. Diverse Europese landen (België, Duitsland, Frankrijk, Nederland, Spanje, het Verenigd Koninkrijk en Zwitserland) en ook Australië en Canada hebben plannen voor klinische onderzoeken naar heroïne op medisch voorschrift, of zijn er al mee bezig. Dergelijke onderzoeken zullen farmaceutische vormen van heroïne (diacetylmorfine) nodig hebben om aan hun patiënten te kunnen verstrekken.

Dit proefschrift beschrijft de ontwikkeling van farmaceutische toedieningsvormen voor diacetylmorfine, die bedoeld zijn om voor te schrijven aan chronisch verslaafde patiënten, die meedoen met het Nederlandse onderzoek naar heroïne op medisch voorschrift. In *Hoofdstuk 1* wordt de reeds aanwezige kennis over de farmaceutische en fysisch-chemische eigenschappen van diacetylmorfine besproken, evenals de klinisch toegepaste toedieningswegen (in het Verenigd Koninkrijk wordt diacetylmorfine namelijk toegepast als pijnstiller). Verder worden de eigenschappen van straatheroïne besproken en de manieren van toedienen die verslaafden op straat gebruiken. Farmaceutische heroïne moet voldoen aan de gebruikelijke eisen voor effectiviteit, veiligheid en kwaliteit van farmaceutische producten, maar daarnaast is de acceptatie door de gebruikers ook van groot belang; vooral omdat heroïne op medisch voorschrift bedoeld is voor moeilijk behandelbare verslaafden, die moeten worden gestimuleerd om deel te (blijven) nemen aan een behandelingsprogramma. Verslaafden waarderen vooral het 'flash' effect, dat direct na het spuiten van heroïne optreedt (en dat ontbreekt bij bijv. methadon), dus het is van belang om dat effect ook bij nieuw te ontwikkelen toedieningsvormen te bereiken. Diacetylmorfine wordt

in de bloedbaan omgezet naar 6-acetylmorfine en morfine, die worden gezien als de eigenlijke actieve middelen. Het snel bereiken van hoge concentraties van diacetylmorfine en 6-acetylmorfine is verantwoordelijk voor het 'flash' effect, terwijl morfine en zijn afbraakproducten zorgen voor een langduriger gevoel van euforie.

Diacetylmorfine voor injectie

Heroïne wordt door verslaafden meestal geïnjecteerd, 60-80% van de verslaafden in Europa die onder behandeling zijn, injecteerde de drug (data 1990-2001). Daarom beschrijft *Hoofdstuk 2* de ontwikkeling van een gevriesdroogde vorm van diacetylmorfine hydrochloride voor injectie. Diacetylmorfine is niet stabiel in oplossing, dus werd gekozen voor een droge vorm. Het gevriesdroogde product, dat 3 gram diacetylmorfine hydrochloride per flacon bevat, behoefde geen hulpstoffen en was stabiel bij kamertemperatuur. De inhoud van de flacon wordt opgelost in water voor injecties en de resulterende oplossing bleek antimicrobiële activiteit te hebben, zodat het niet nodig was om conserveermiddel toe te voegen. Ook de chemische stabiliteit van de oplossing was voldoende om meermalig gebruik van het product te rechtvaardigen (meerdere patiëntdoseringen uit één flacon).

Diacetylmorfine voor inhalatie: ontwikkeling

De op-één-na populairste manier om heroïne te gebruiken is roken: in 2001 rookte 45% van de verslaafden in behandeling heroïne. Onder roken wordt in dit verband 'chinezen' ('chasing the dragon') verstaan: heroïnepoeder wordt op een stuk aluminiumfolie verhit met een aansteker tot het smelt en verdampt, zodat de dampen kunnen worden geïnhaleerd via een rietje in de mond. Vergeleken met de injectie heeft deze toedieningsweg een biologische beschikbaarheid van 52% en het heeft een farmacokinetisch en farmacodynamisch profiel (bloedspiegels en effecten) dat acceptabel is voor chronisch verslaafden. *Hoofdstuk 3* beschrijft de ontwikkeling van diacetylmorfine voor inhalatie na verdampen.
In *Hoofdstuk 3.1* laten thermische analyse-experimenten zien dat diacetylmorfine base een lagere smelttemperatuur heeft en sneller verdampt, als coffeïne anhydraat wordt toegevoegd. *In vitro* experimenten, die het 'chinezen' nabootsen, lieten zien dat ongeveer 41% van de diacetylmorfine base uit verdampte diacetylmorfine/coffeïne tabletten wordt teruggevonden in de opgevangen damp. Coffeïne verdampte volledig zonder dat ontleding werd gezien, terwijl diacetylmorfine verdampte, maar ook werd omgezet in 6-acetylmorfine en werd teruggevonden als verkoold residu.
In *Hoofdstuk 3.2* worden meer uitgebreide *in vitro* simulatie-experimenten beschreven. Verschillende verhoudingen van diacetylmorfine en coffeïne in het poeder (25%, 50% of 75% g/g diacetylmorfine) en verschillende temperaturen (200-350°C) bleken maar weinig invloed te hebben op de opbrengst van diacetylmorfine (46-62%) en coffeïne (65-83%) in de damp. Verhoging van de temperatuur leek vooral de verdampingssnelheid te verhogen. De deeltjes in het aërosol dat werd opgevangen na verdampen bleken een 'mass median aerodynamic diameter' van 1.8-4.1 µm te hebben, en 45-60% van elk monster werd teruggevonden als

aërosoldeeltjes < 5 µm. Monsters met meer coffeïne vertoonden grotere aërosoldeeltjes en hogere verdampingstemperaturen leverden ook grotere deeltjes op. De deeltjesgrootte van een aërosol bepaalt hoe diep het kan doordringen in de longen, waar de werkzame stof geabsorbeerd kan worden. Uit deze resultaten kan geconcludeerd worden dat bij het verhitten van een diacetylmorfine/coffeïne mengsel voldoende diacetylmorfine verdampt tot een aërosol waarvan de deeltjes klein genoeg zijn om diep in de longen door te dringen. Het poedermengsel met 75% g/g diacetylmorfine base en 25% g/g coffeïne anhydraat werd gekozen als basis voor farmaceutische heroïne voor inhalatie na verdampen.
De volgende stap was om een productieproces te ontwikkelen om dit poedermengsel te verdelen in porties (patiëntdoses) en te verpakken. Voor dit doel werd een schroefdoseermachine gekozen, die poederporties kan uitvullen in sachets. *Hoofdstuk 3.3* beschrijft hoe het schroefdoseerproces werd gekarakteriseerd, geoptimaliseerd en gevalideerd. Het nauwkeurig uitvullen van de juiste dosis (50-300 mg) diacetylmorfine/coffeïne mengsel bleek een complexe aangelegenheid te zijn. Alle geteste variabelen (machine-instellingen, zoals het toerental van de doseerschroef en de menger, het vulniveau van de trechter, het interval tussen de doses en de dosis) bleken van invloed te zijn op de nauwkeurigheid van uitvullen. Er werd een mathematisch regressiemodel opgesteld met 9 termen (variabelen en interacties tussen variabelen), dat de doseernauwkeurigheid kon voorspellen. Dit model werd gebruikt om optimale instellingen voor de routineproductie te berekenen. Vervolgens werden vier charges sachets gemaakt met deze optimale instellingen, waaruit bleek dat het poedermengsel juistheid van 99.0-101.0% en een nauwkeurigheid van 3.2-5.3% uitgevuld kon worden in sachets.
Hoofdstuk 3.4 beschrijft de overwegingen die zijn gemaakt tijdens de vroege fase van de ontwikkeling van diacetylmorfine voor inhalatie na verdampen. Verder wordt het productieproces in detail beschreven, met de in-procescontroles die daarin zijn opgenomen en de kwaliteitscontrole van het eindproduct. Er zijn verschillende weegcontroles opgenomen in het productieproces (totaalgewicht en gewicht van de inhoud van de sachets) om de juistheid en de nauwkeurigheid van het uitvullen te controleren. Ook worden weegcontroles en tellingen beschreven, die nodig zijn om na te gaan of het afgewogen poeder en alle geproduceerde sachets verantwoord zijn. De Opiumwet vereist namelijk een nauwkeurig bijgehouden boekhouding voor heroïne. Het hoofdstuk beschrijft verder de laboratoriumcontroles van het gehalte diacetylmorfine en coffeïne en een test op gelijkmatigheid van gewicht, die de kwaliteit van het eindproduct verder onderbouwen. Stabiliteitsonderzoek wees uit dat diacetylmorfine/coffeïne sachets voldoende stabiel waren bij 25°C en 60% luchtvochtigheid en zelfs bij extreme omstandigheden: 40°C en 75% luchtvochtigheid.

Diacetylmorfine voor inhalatie: gebruik

Hoofdstuk 4 beschrijft drie studies die te maken hebben met het gebruik van diacetylmorfine voor inhalatie na verdampen.
Twee methodes om heroïne te roken werden vergeleken in een farmacokinetische studie (*Hoofdstuk 4.1*): vijf verslaafden inhaleerden om-en-om diacetylmorfine voor

inhalatie na het verdampt te hebben door te 'chinezen' of met behulp van een verwarmingselement. De concentraties van diacetylmorfine, 6-acetylmorfine, morfine en morfine-3- en -6-glucuronide in plasma werden bepaald met behulp van vloeistofchromatografie met massa-spectrometrische detectie. De blootstelling aan diacetylmorfine en zijn metabolieten was significant lager na het verdampen met het verwarmingselement dan na het 'chinezen': diacetylmorfine 80% lager en 6-acetylmorfine 73% lager. Piekconcentraties van diacetylmorfine en 6-acetylmorfine waren ook 80 en 70% lager na gebruik van het verwarmingselement. 'Chinezen' was dus de meest efficiënte verdampingsmethode voor diacetylmorfine voor inhalatie.

De tweede studie (*Hoofdstuk 4.2*) onderzocht de toepassing van deuterodiacetylmorfine (diacetylmorfine gelabeld met deuterium, een stabiele isotoop van waterstof) als marker voor het detecteren van bijgebruik van illegale heroïne door patiënten die diacetylmorfine voor inhalatie gebruiken. Controle op illegaal bijgebruik is van belang, omdat het succes van de behandeling met heroïne op medisch voorschrift afhangt van het volledig vervangen van straatheroïne door farmaceutische heroïne. In de studie gebruikten negen patiënten gedurende vier dagen diacetylmorfine voor inhalatie, waaraan deuterodiacetylmorfine was toegevoegd. De patiënten waren opgenomen in een gesloten onderzoeksinstituut, waar bijgebruik van drugs verboden was. Vervolgens werden regelmatig urine- en plasmamonsters afgenomen, waarin de hoeveelheden van diacetylmorfine en metabolieten werden bepaald. Verder werden de monsters gescreend op de aanwezigheid van cocaïne en diens metabolieten en van 6-acetylcodeïne en codeïne (die kunnen wijzen op bijgebruik van straatheroïne). Er waren geen aanwijzingen voor bijgebruik van illegale drugs. De verhouding van gelabeld en ongelabeld 6-acetylmorfine in de urine- en plasmamonsters was zeer constant, zoals ook was verwacht, omdat er ook een constante verhouding gelabeld en ongelabeld diacetylmorfine was toegediend. Deze verhouding kan worden gebruikt om bijgebruik van straatheroïne te detecteren, omdat in dat geval de hoeveelheid ongelabeld 6-acetylmorfine in urine zal stijgen, maar de gelabelde hoeveelheid niet, waardoor de verhouding verschuift.

Het laatste Hoofdstuk, 4.3, beschrijft de analyse van de rietjes, die door de verslaafden zijn gebruikt bij het 'chinezen'. Bij het 'chinezen' wordt het diacetylmorfine/coffeïne poeder verhit, waarbij de twee componenten zouden kunnen ontleden tot potentieel toxische stoffen. De aanslag die zich in de rietjes bevond, werd beschouwd als een afspiegeling van de damp die de longen van de patiënt bereikt en daarom werden de rietjes onderzocht op de aanwezigheid van onbekende ontledingsproducten. Daarvoor werd een speciale vloeistof-chromatografische methode ontwikkeld, die geschikt was voor zowel foto-diode-array detectie als massa-spectrometrische detectie. De rietjes bleken vooral (75%) diacetylmorfine, coffeïne, 6-acetylmorfine en morfine te bevatten. Verder werden verschillende onbekende pieken gezien in de chromatogrammen. Voor negen ontledingsproducten werd een structuurvoorstel gedaan; bij elkaar maakten deze negen stoffen 0.4-9.7% van het residue in de rietjes uit. De onbekende stoffen waren allen morfine-derivaten met verschillende substitutiepatronen op de C_3, C_6, en N_{17}

posities in de morfinan-structuur. De activiteit en toxiciteit van deze stoffen is onbekend en vereist nader onderzoek.

Conclusies

In dit proefschrift is de ontwikkeling van een farmaceutische vorm voor injecteerbare heroïne beschreven, evenals uitgebreid onderzoek naar de ontwikkeling van een farmaceutische vorm voor rookbare heroïne. In beide gevallen is een bruikbare toedieningsvorm ontwikkeld, die geproduceerd wordt volgens de strenge kwaliteitseisen voor farmaceutische producten die worden toegepast bij mensen. De productieprocessen zijn gevalideerd en worden gecontroleerd via in-procescontroles en kwaliteitscontrole van het eindproduct om de productkwaliteit te garanderen. Diacetylmorfine voor injectie, 3 gram/flacon en vier sterktes diacetylmorfine/coffeïne sachets (75/25 mg, 100/33 mg, 150/50 mg en 200/66 mg) zijn gebruikt in het Nederlands onderzoek naar heroïne op medisch voorschrift, waarvan de resultaten inmiddels zijn gepubliceerd in het British Medical Journal (2003;327(7410):310-315). De goede resultaten hebben ertoe geleid dat heroïne op medisch voorschrift een reguliere behandeling kan worden voor een subgroep van chronische, moeilijk behandelbare verslaafden in Nederland (meer informatie is beschikbaar op www.ccbh.nl).

Curriculum Vitae

Marjolein Klous werd geboren op 21 augustus 1974 te Amsterdam. In 1992 behaalde zij het VWO-diploma aan de Katholieke Scholengemeenschap Hoofddorp te Hoofddorp. In datzelfde jaar begon zij aan de studie Farmacie aan de Universiteit Utrecht. De doctoraalfase werd in december 1996 afgesloten met een onderzoek naar de meting van intracellulair magnesium in witte bloedcellen, dat plaatsvond op de afdeling Klinische Chemie van het Academisch Medisch Centrum te Amsterdam, onder begeleiding van mevr. dr. ir. R. Sanders, prof. dr. G.T.B. Sanders en prof. dr. J.H.H. Thijssen. In april 1999 behaalde zij het apothekersdiploma, waarna zij als projectapotheker werkzaam was in de Apotheek van het Slotervaartziekenhuis te Amsterdam. In januari 2001 werd in dezelfde instelling, onder begeleiding van prof. dr. J.H. Beijnen, prof. dr. J.M. van Ree en prof. dr. W. van den Brink begonnen met het promotieonderzoek dat is beschreven in dit proefschrift.

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