



Review

Skin tissue sample collection, sample homogenization, and analyte extraction strategies for liquid chromatographic mass spectrometry quantification of pharmaceutical compounds



Ignace C. Roseboom^{a,b}, Hilde Rosing^a, Jos H. Beijnen^{a,b}, Thomas P.C. Dorlo^{a,*}

^a Department of Pharmacy & Pharmacology, the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands

^b Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands

ARTICLE INFO

Article history:

Received 10 April 2020

Accepted 22 August 2020

Available online 28 August 2020

Keywords:

Liquid chromatography tandem mass spectrometry

Dermal

Tissue homogenization

Sample preparation

Bioanalysis

Quantification

ABSTRACT

Quantification of pharmaceutical compounds in skin tissue is challenging because of low expected concentrations, small typical sample volumes, and the hard nature of the skin structure itself. This review provides a comprehensive overview of sample collection, sample homogenization and analyte extraction methods that have been used to quantify pharmaceutical compounds in skin tissue, obtained from animals and humans, using liquid chromatography-mass spectrometry. For each step in the process of sample collection to sample extraction, methods are compared to discuss challenges and provide practical guidance. Furthermore, liquid chromatographic-mass spectrometry considerations regarding the quality and complexity of skin tissue sample measurements are discussed, with emphasis on analyte recovery and matrix effects. Given that the true recovery of analytes from skin tissue is difficult to assess, the extent of homogenization plays a crucial role in the accuracy of quantification. Chemical or enzymatic solubilization of skin tissue samples would therefore be preferable as homogenization method.

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Contents

| | |
|---|---|
| 1. Introduction | 2 |
| 2. Methodology | 2 |
| 3. Results | 2 |
| 3.1. Skin tissue composition | 2 |
| 3.2. Sample collection methods | 2 |
| 3.2.1. Tape stripping method | 3 |
| 3.2.2. Skin punch biopsy | 4 |
| 3.2.3. Shaving methods | 4 |
| 3.2.4. Separation of dermis and epidermis | 4 |
| 3.3. Sample homogenization methods | 4 |
| 3.3.1. Centrifugal precipitation | 5 |
| 3.3.2. Mechanical rotor-stator homogenization | 5 |
| 3.3.3. Pulverization and grinding | 5 |
| 3.3.4. Bead homogenization | 5 |
| 3.3.5. Chemical and enzymatic solubilization | 5 |
| 3.4. Analyte extraction methods | 5 |
| 3.5. Matrix effects and recovery in skin tissue samples | 6 |
| 3.5.1. Matrix effects | 6 |

* Corresponding authors at: Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

E-mail addresses: i.roseboom@nki.nl (I.C. Roseboom), t.dorlo@nki.nl (T.P.C. Dorlo).

| | |
|--|---|
| 3.5.2. Recovery | 6 |
| 4. Discussion & conclusion | 7 |
| Declaration of interests | 8 |
| Funding | 8 |
| CRedit authorship contribution statement | 8 |
| References | 8 |

1. Introduction

Increased focus on target-site pharmacokinetics (PK) and pharmacodynamics (PD) in drug development has led to a growing interest and demand for bioanalytical drug quantification in tissues [1,2]. However, practical and ethical constraints, associated with the invasive sampling procedures required to obtain a sample, restrict its applicability. Establishing target-site PK/PD relationships for drugs is nevertheless important to assess and improve drug target strategies [3].

The aim of this review is to provide a comprehensive overview of general sampling methods and corresponding pre-treatment and extraction methods of skin tissue samples collected in *in vivo* studies for the quantification of small molecules through liquid chromatography coupled to mass spectrometry (LC-MS). The measurement of distribution of drugs from plasma into the skin has clinical importance e.g. in order to improve therapeutic strategies for skin infections. Uptake of drugs in the skin is dependent on the physicochemical properties of the drug and the route of administration used. Topical administration is effective for local treatment of skin-related diseases, e.g. anthralin cream and topical steroids for the treatment of psoriasis [4]. Systemic administration is e.g. used in the application of oral fluconazole for the treatment of fungal skin infections [5] or injection-based biologicals like adalimumab for the treatment of psoriasis [6].

The invasiveness of skin sampling techniques as well as the nature of the resulting skin samples has various implications for the bioanalytical procedures to extract, detect and quantify analytes in these samples. Tissue samples are typically only small in volume, which in turn requires a relatively low limit of quantification of the bioanalytical assay compared to plasma samples. Skin tissue is classified as 'hard' tissue, meaning that samples require more powerful sample preparation compared to tissue classified as 'soft' or 'tough' [2]. Moreover, releasing the analytes of interest from (sub)cellular compartments in these skin samples (stratum corneum, epidermis and dermis layers) requires rigorous sample pre-treatment methods, which at the same time cause the release of many other endogenous matrix components. Matrix effects, therefore, tend to be more pronounced in tissue samples compared to plasma samples [7]. Determining the true recovery of analytes from tissue samples is also more challenging compared to plasma samples [8]. Due to these factors, both ultra-sensitive bioanalytical methods, e.g. using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as well as proper sample preparation and extraction methods are of pivotal importance for the analysis of analytes in skin tissue samples.

As far as we know no other reviews have been published about sample preparation of skin tissue samples for the extraction and quantification of pharmaceutical compounds. This review further elaborates on bioanalytical considerations regarding the quality and complexity of tissue sample measurements.

2. Methodology

Quantification methods of analytes in skin tissue samples including sample collection methods, sample pre-treatment and

extraction methods of the analytes were identified using a PubMed database search, restricted to the English language, using the following search terms for title and abstract contents: ((skin OR dermal) AND (liquid chromatography AND quantification AND mass spectrometry OR bioanalytical OR lcms OR lc-ms OR lc-ms/ms OR lcms ms OR lc-esi-ms/ms OR lc-esi-msms OR lc-apci-ms/ms OR lc apci ms ms)) NOT (DNA OR proteomics OR immunologic techniques OR peptides OR glycan OR permeation). If the title or abstract contained information suggesting that skin tissue was sampled from *in vivo* experiments or clinical trials and was used for the quantification of analytes via LC-MS/MS, the publication was included in this review. Both animal and human studies were included, except studies involving fish species because of significant differences in skin structure compared to mammals. Studies that focused on protein analysis in skin tissue were excluded since sample preparation is performed differently compared to small molecules. Given our focus on samples from *in vivo* studies, *in vitro* and *ex vivo* studies, such as permeation studies, were excluded from this analysis.

3. Results

The literature search resulted in 641 publications, of which 30 studies were identified as relevant and included in this review (search date 19 March 2020). Included studies are comprehensively summarized in Tables 1 through 3.

3.1. Skin tissue composition

The skin consists roughly of three different cellular layers, with each their own function and cellular composition (Fig. 1). The outermost layer of the skin tissue, the epidermis is covered by the stratum corneum. The epidermis consists mainly (around 90%) of keratinocytes and is responsible for the protection against environmental hazards, like infections [9,10]. Underneath the epidermis lies the dermis, which is connected through fibers known as the basement membrane. The dermis is more complex than the epidermis as blood vessels, sweat glands, hair follicles and nerve endings are in the dermis and not in the epidermis, broadening the range of cell diversity. Underneath the dermis lies the hypodermis which consists of subcutaneous fat.

3.2. Sample collection methods

Skin tissue samples are typically obtained by three different methodologies with variable levels of invasiveness: tape stripping (Section 3.2.1) can be considered less invasive, while skin punch biopsies (Section 3.2.2) and shaving methods (Section 3.2.3) are more invasive. Applying these different methodologies result in the isolation of different skin layers. For some applications the dermis and epidermis should be separated (Section 3.2.4) before sample homogenization can take place to release the analytes from the biomatrix. Other methodologies to determine *in vivo* skin drug concentrations, such as microdialysis techniques, do not involve sampling of skin tissue and are out of the scope of this review.

Table 1
An overview of homogenization methods for skin tissue samples described in the selected studies.

| Homogenization method | Homogenization specifications | Sampling method | Species | Analyte | Ref |
|--|---|--|---------------|--|--------------|
| Centrifugal precipitation | Centrifugation and supernatant extraction | Skin biopsy | Mouse | Digitoxigenin | [32] |
| | Cutting | Skin biopsy | Pig | Levamisole Clavulanic Acid | [40] [50] |
| Mechanical rotor-stator homogenization | Rotation by tissue tearer | Stratum corneum tape stripping and skin biopsy | Mouse | Gefitinib | [41] |
| | | | Rat | Capsaicin | [38] |
| | | | Rat | Avobenzone | [42] |
| Pulverization and grinding | Mortar and pestle | Skin biopsy | Chicken | Ampicillin | [43] |
| | | | Human | Pegcantratinib | [28] |
| | | | Rat | Diclofenac | [35] |
| Bead homogenization | 1.8- and 2.4-mm beads | 10 stripping tapes with stratum corneum | Human and rat | Tigecycline | [31] |
| | | | Human | 5-Methyltetrahydrofolate | [29] |
| Chemical/enzymatic solubilization | 0.9–2.0 mm beads 2M ammonium hydroxide in 30% hydrogen peroxide Collagenase in HEPES buffer | Separated dermis and epidermis using 1 M NaCl incubation | Pig | Lidocaine and 2,6-Dimethylaniline | [20] |
| | | | Human | LEO 29102, Tofacitinib, Ruxolitinib and Tacrolimus | [22] |
| | | | Rat | Diclofenac | [35] |
| Chemical/enzymatic solubilization | 0.9–2.0 mm beads 2M ammonium hydroxide in 30% hydrogen peroxide Collagenase in HEPES buffer | Skin biopsy | Human | Eumelanin and Pheomelanin | [24] |
| | | | Rat | Diclofenac | [35] |

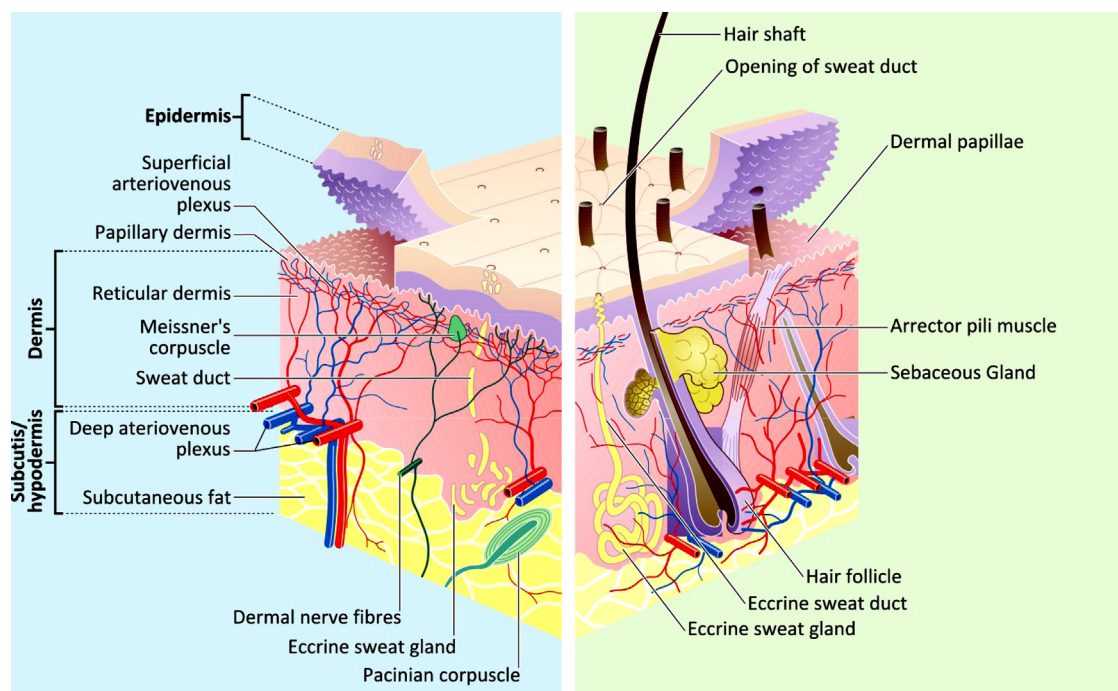


Fig. 1. A cross-section of human skin tissue [65].

3.2.1. Tape stripping method

Dermal tape stripping is performed using stripping tapes with a pre-specified surface area that peel off superficial layers of the skin of subjects by applying pressure (Fig. 2). It is used for the identification or quantification of analytes in the stratum corneum. The stratum corneum is peeled away using a number of tapes [11,12]. The tapes are weighed prior to and after the stripping procedure to assess the amount of skin tissue adhering to each tape strip [13]. Commonly, the stratum corneum is stripped, while the dermis and epidermis remain. A typical amount of skin tissue obtained by

skin-stripping tapes from washed skin is approximately $50 \mu\text{g}/\text{cm}^2$, unwashed skin contains more debris which increases the typical mass per cm^2 [14–16]. Bioanalytical studies on tape strip samples were used for human skin [17–19] and porcine skin [20] to quantify 1,6-hexamethylene diisocyanate in relation to toxicity studies in the skin, acetylsalicylic acid and pyrrolidone carboxylic acid in cosmetics, and lidocaine local anesthetics. Obtaining layers of skin by dermal tape stripping is labor-intensive because the procedure needs to be done by hand using numerous tapes combined as a single sample. Analytes can be extracted from a stripping tape, e.g.

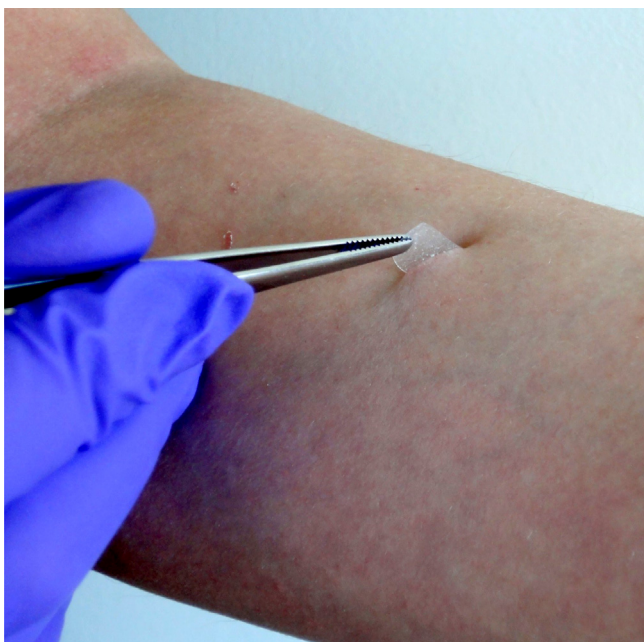


Fig. 2. Peeling off the stratum corneum using the tape stripping method [66].

by soaking the tapes in organic solvents followed by scraping the corneocytes from the tapes [19], or by more rigorous methods like bead homogenization of the tapes [17,18,20].

3.2.2. Skin punch biopsy

Various techniques have been applied to obtain a skin biopsy, which is usually obtained under local anesthesia. The skin punch biopsy is retrieved using a circular blade that ranges from 1 mm to 8 mm in diameter that is rotated by manual force through the skin layers until the hypodermis (fat layer) is reached and is retrieved by cutting through the subcutaneous fat layer (Fig. 3) [21]. The skin punch biopsy is therefore cylindrical in shape. Typically, a 3 mm punch biopsy is used to obtain the biopsy, after which the resulting wound can be closed with a suture [21]. Smaller diameters often do not require suturing. Bioanalytical studies on skin punch biopsy samples were identified for human skin [22–24].

3.2.3. Shaving methods

The shave biopsy technique is the most commonly used type of skin tissue sampling because of its simplicity [25]. Depending on the purpose, either a superficial shave biopsy or a saucerization biopsy can be used. Both methods shave the skin using a blade with a variable depth. Superficial shave biopsies yield the epidermis and the upper dermis, while saucerization biopsies yield a full-thickness biopsy, including dermis and possibly a fraction of subcutaneous fat [26]. Biopsies can be further categorized as incisional or excisional, for example removing a whole tumor is considered excisional, while removing a portion of the tumor is considered incisional. Such biopsies are usually taken with a scalpel and can be equally deep as skin punch biopsies, but lack the cylindrical shape [27]. Bioanalytical studies on shave biopsies were identified for both human [28–31] and animal skin [20,32–43].

3.2.4. Separation of dermis and epidermis

For some bioanalytical applications, there is an interest in the separation of the epidermis from the dermis after skin biopsy collection. This can be done by incubating the skin tissue for 1 h in saline buffered water at 37 °C [44]. Other methods involve incubation at 4 °C with enzymatic solvents containing trypsin [45] or dispase [46] for 18 h and incubating for 72 h in 1M NaCl and phenyl-

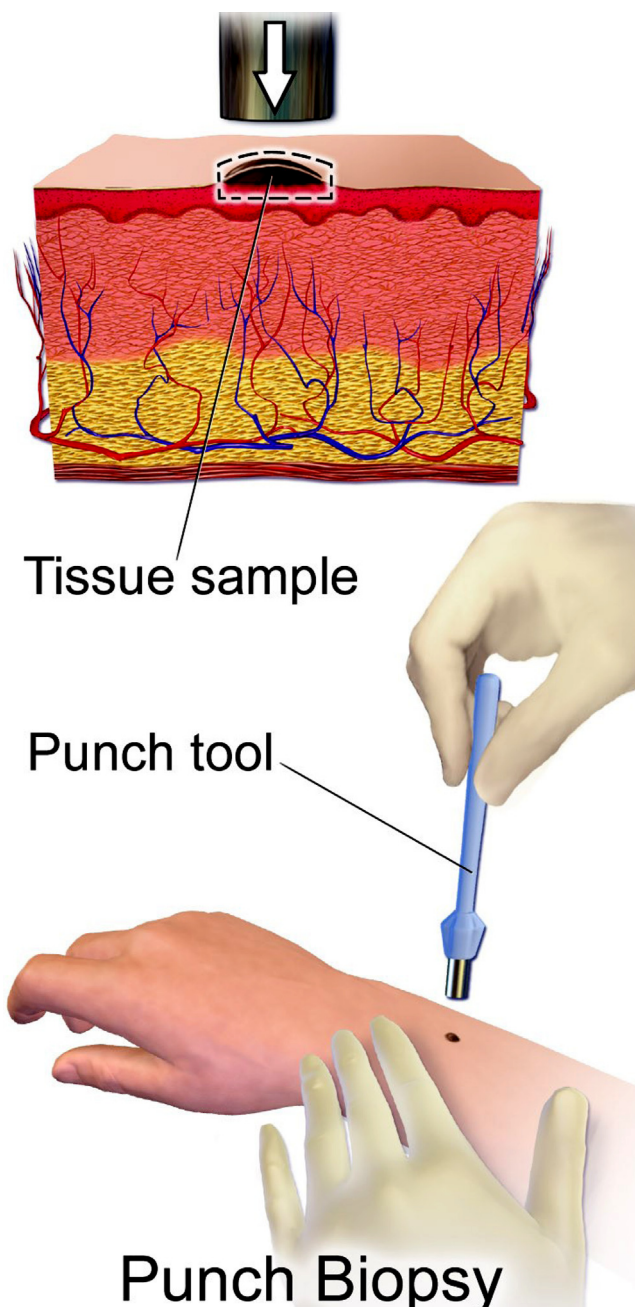


Fig. 3. Skin punch biopsy method using a circular punch biopter [67].

methylsulfonylfluoride (PMSF, a protease inhibitor) at 4 °C [47]. After incubation, the epidermis and dermis are separated using forceps.

3.3. Sample homogenization methods

Homogenization of the tissue samples is necessary in order to release interstitial and intracellular analytes, prior to their extraction and quantification. Various methodologies for the homogenization of skin tissue have been described, aiming at a high level of cell disruption and a homogeneous suspension of cell constituents. The homogenization should ensure the transition of the skin tissue to a semisolid/suspension state [48] and preferably result in disruption of the cell structures. In total, 5 different homogenization methods were identified (Table 1), which are individually discussed below. Homogenization methods should preferably be

amenable for high throughput and thus low in labor intensity and reliable, illustrated by low inter-sample variability.

3.3.1. Centrifugal precipitation

Precipitation is the simplest pre-treatment method with high throughput. A matrix is added to the skin and after mixing and centrifugation, the supernatant is used for analyte detection and quantification. Skin cell membranes are not disrupted by rotational force, which may lead to poor recovery of compounds that are mainly distributed intracellularly/interstitially and are thus not extracted in the precipitation matrix. The sample homogenization method has been used on mouse [32] skin biopsy samples using methanol/water (50/50, v/v) as matrix, the supernatant is then considered the skin tissue homogenate. Reproducibility might be dependent on the rotational force during centrifugation leading to variability in cell separation and analyte recovery [49].

3.3.2. Mechanical rotor-stator homogenization

Mincing of skin tissue is performed by using, for instance, a grater to cut skin tissue into a fine pulp. Rotational homogenization is a combination of mincing and pulverizing, showing similarities with bead homogenization (discussed later). A fast-rotating head shreds the skin tissue into smaller pieces using high-velocity rotational and collision forces. This homogenization procedure can be performed using a rotor-stator homogenizer or mixer, typically in a solvent such as blank plasma. Skin tissue has also been homogenized using less specialized equipment, such as a cutter-mixer, a common kitchen appliance that has been used for bigger quantities of skin tissue samples. Animal skin tissue samples from pig [40,50], mouse [41], rabbit [38], rat [31,35,42], and chicken [43], as well as human [28,31], have all been homogenized using this method. The method of rotational homogenization is not always done high throughput since the speed depends on the size and thickness of the skin tissue and is performed using one sample at a time but it enables disruption of cell membranes by its high-velocity collisional force. In potential, a temperature-controlled environment will decrease variability in recovery given that the large acceleration energies might result in high uncontrolled local temperature differences, possibly affecting stability of the analyte.

3.3.3. Pulverization and grinding

Pulverization and grinding are more conventional homogenization methods and are performed using a tissue pulverizer or simply a mortar and pestle. The method has been applied to frozen human skin tissue samples from a full-thickness skin biopsy of which dermis and epidermis were separated from the stratum corneum [29]. A so-called Bessman tissue pulverizer makes use of a mortar and pestle, which fit tightly together to create a closed system. To increase performance, liquid nitrogen or dry ice can be added to the skin tissue sample in a tissue pulverizer and a hammer can be used to crush the frozen tissues into a powder-like form. Simple mortar and pestle methods can also efficiently break small frozen pieces of skin tissue to a powder form. This is performed simply with a pestle, grinding the frozen tissue into powdered form. Powdered skin tissue is suspended in solution for further analyte extraction. These methods are, however, highly labor-intensive and require extensive washing after each sample to prevent carryover and cross-contamination.

3.3.4. Bead homogenization

Bead homogenization is a combination of grinding and centrifugation. It is performed using stainless steel, zirconium or ceramic grinding balls, available in various sizes, in combination with a lysing solvent to disrupt the cells or plasma. Specialized cups and centrifuges are used in combination with these beads. Through high-velocity collision forces, the beads grind and disrupt the cell

membranes, potentially resulting in the release of intracellular fluid. This methodology is commonly used for the homogenization of soft tissue samples. Although it is likely less effective for tough tissues, like skin, the method has been applied to human [22] as well as pig [20], mouse [37] and rat skin tissue [35]. This methodology can be considered versatile, as it can be applied to any type of skin tissue sample (tape stripped skin, full-thickness skin biopsy, and separated dermis and epidermis) and can be automated. The supernatant of the resulting homogeneous mixture is used for further extraction of the analyte. Bead homogenization is not labor-intensive and can be performed on multiple samples simultaneously.

3.3.5. Chemical and enzymatic solubilization

Chemical solubilization of skin tissue has been achieved using several techniques. Alkaline solvents can solubilize tissue structures due to hydrolysis mechanisms, while acidic solvents can lead to oxidation of the tissue structures [51]. Alkaline solubilization using 30% hydrogen peroxide in combination with 2 M ammonium hydroxide has been applied to human skin tissue [24]. Chemical tissue solubilization is typically performed under extremely high (e.g. ammonium hydroxide in hydrogen peroxide [24] or quaternary ammonium hydroxide compounds typically used in scintillation studies) or low pH (e.g. aqua regia) conditions, which means that the stability of analytes in these environments has to be assured. Enzymatic digestion using collagenase has also been used as a homogenization method and was performed on rat skin tissue [35]. Both chemical solubilization and enzymatic digestion of the tissue will, in theory, lead to a more complete release of intracellular and interstitial fluids than any of the other homogenization methods.

3.4. Analyte extraction methods

Extraction and purification of analytes can be performed by protein precipitation. This extraction procedure is conducted by dispersing homogenized skin tissue samples in precipitation agents, followed by centrifugation and separation of the supernatant and precipitant. The following analytes were extracted using this method: digitoxigenin [32], 4-n-butyl resorcinol [52], desmopressin acetate [30], gefitinib [41], avobenzone [42], 1,6-hexamethylene diisocyanate monomers and oligomers [17], pegcantratinib [28], acetylaspartic acid [18], tigecycline [31] and pyrrolidone carboxylic acid [19]. The solubility profile of the analyte determines the choice of sample collection homogenization precipitation agent. Filtration is often an addition to protein precipitation, separating filtrate from the rest of the homogenate in a semi-solid state. Solid particles will not pass the membrane of the filter and the filtrate is collected. Ultrafiltration employs semi-permeable membranes with specific molecular weight cut-offs, which can be used as an analyte extraction procedure [53,54] and can also be implemented for proteomic analysis [55]. Examples of analytes in skin tissue that were recovered after ultrafiltration were clavulanic acid [50] (using 30 kDa cut-off filters), 2,6-dimethylaniline [20] (using 10 kDa cut-off filters), ampicillin [43] (using 10 kDa cut-off filters). This extraction method was performed on full-thickness skin biopsies, homogenized by cutting, rotation by a tissue tearer and by bead homogenization methods. LLE or acid-base extraction methods have also been used to retrieve the following analytes from full-thickness skin biopsy homogenates and separated dermis and epidermis: capsaicin [38], levamisole [40], dexamethasone acetate [30], indomethacin [39] and diclofenac [35]. Centrifugal precipitation, cutting and bead homogenization were used as homogenization methods prior to LLE. SPE has been applied to skin tissue homogenates through (analyte-specific) ion-exchange mechanics, e.g. using a strong cation exchanger as stationary phase (levamisole [40] and lidocaine

Table 2
Analyte extraction methods used in combination with different sample collection and homogenization methods for skin tissue samples.

| Analyte extraction method | Sample collection method | Homogenization method | Species | Analyte | Ref |
|---------------------------|--|-----------------------------------|--------------------------------|--------------------------------|------|
| Protein precipitation | Skin biopsy | Centrifugal precipitation | Mouse | Digitoxigenin | [32] |
| | Stratum corneum tape stripping, dermis and epidermis | – | Human | 4-n-Butyl Resorcinol | [52] |
| | Skin tissue | – | Human | Desmopressin Acetate | [30] |
| | | Cutting | Mouse | Gefitinib | [41] |
| | Stratum corneum tape stripping and skin biopsy | Rotation by tissue tearer | Rat | Avobenzone | [42] |
| | Tape stripped skin tissue | – | Human | 1,6-Hexamethylene Diisocyanate | [17] |
| | Skin biopsy | Rotation by tissue tearer | Human | Pegcantratinib | [28] |
| | Tape stripped skin tissue | – | Human | Acetylaspartic Acid | [18] |
| | Human | Human | Pyrrolidone Carboxylic Acid | [19] | |
| | Skin biopsy | Rotation by tissue tearer | Human & rat | Tigecycline | [31] |
| Ultrafiltration | Skin biopsy | Cutting | Pig | Clavulanic acid | [50] |
| | | Bead homogenization | Pig | 2,6-Dimethylaniline | [20] |
| | Rotation by tissue tearer | Chicken | Ampicillin | [43] | |
| | Centrifugal precipitation | Rabbit | Capsaicin and Dihydrocapsaicin | [38] | |
| | – | – | – | – | – |
| LLE/acid-base extraction | Skin biopsy | Cutting | Pig | Levamisole | [40] |
| | | Centrifugal precipitation | Mouse | Indomethacin | [39] |
| | Chemical/enzymatic solubilization, bead homogenization and rotation by tissue tearer | Rat | Diclofenac | [35] | |
| SPE | Skin biopsy | Cutting | Pig | Levamisole | [40] |
| | Dermal stripping tapes | Bead homogenization | Pig | Lidocaine | [20] |
| | Epidermis | Chemical/enzymatic solubilization | Human | Eumelanin and Pheomelanin | [24] |

[20]) or a weak anion exchanger (eumelanin and pheomelanin [24]). SPE is generally more labor-intensive compared to other analyte extractions; however, automated systems are available. Analyte selectivity and specificity are usually high due to the wide variety of sorbents that are available and various washing steps, producing relatively clean final extracts. SPE was used for different types of skin tissue samples; skin biopsies, and tape strips, either processed by mincing, cutting or bead homogenization [20,40]. An overview of analyte extraction methods of pre-treated skin tissue samples is provided (Table 2).

3.5. Matrix effects and recovery in skin tissue samples

The (sub-)cellular endogenous compounds released from the tissue cells during the homogenization procedures might give rise to increased matrix effects when using mass spectrometry detection. Matrix effects are typically more pronounced and challenging for tissue samples compared to plasma samples. Matrix effects and extraction recovery can be determined in multiple ways [2,7,56]. Sample homogenization and analyte extraction methods may influence the matrix effects and the recovery of the analytes of interest, and ultimately on the lower limit of quantification and/or reproducibility of the analytical method. Therefore, we have focused on reported matrix effects and recovery of analytes after skin tissue sample collection, homogenization and extraction.

3.5.1. Matrix effects

Matrix effects are changes in the analytical signal that most often result from competition of analyte and undetected co-eluting factors from the sample matrix, causing ion suppression or enhancement compared to the analyte signal from non-biological matrices [7,57,58]. This theory is widely accepted, yet the origin and mechanisms are not fully understood [57–60]. Matrix effects are expressed as absolute matrix effect from the analyte peak area or as normalized IS matrix effects from the analyte/IS peak ratio. The skin tissue matrix consists of a diverse range of tissue constituents (in the solid and liquid phase), leading to a heterogeneous

type of sample, in contrast to homogeneous plasma samples [7,9]. Increasing the complexity of the matrix may in theory increase matrix effects due to an increased diversity of endogenous substances in the sample, although this might be highly analyte- and tissue-dependent [61]. Absolute matrix effects of biological samples are likely to decrease when increasing the sample cleanup process steps such as extractions, as well as the separation power of the LC system. Reported absolute or IS-normalized matrix effects for the identified skin sample preparation methods appeared to be minimal (Table 3), showing no absolute or IS-normalized matrix effects for any of the reported homogenization/extraction methods or skin sources [20,28,31,32,35,38,42]. This IS-normalized matrix effect requires to be $\leq 15\%$ CV in the validation of a bioanalytical assay according to the U.S. Food and Drug Administration (FDA) or European Medicines Agency (EMA) guidelines [62–64].

3.5.2. Recovery

Assessing the true recovery of analytes from tissue samples is challenging as pre-extraction spiking of the matrix is complicated, given that the analyte does not necessarily distribute to the intracellular/interstitial space as it would do in *in vivo* settings. Extraction recovery can be assessed by spiking tissue/plasma homogenates prior to extraction and compare this to post-extraction spiked matrix samples [8,62,64]. Establishing the overall recovery from plasma samples is less challenging compared to the overall recovery using tissue samples. Spiking analyte working solution to tissue homogenates pre- and post-extraction might give recovery from the extraction process but will not establish recovery during the homogenization process. The real concentration inside the tissue is unknown and the reproducibility of tissue sample treatment remains unidentified, which means true recovery cannot be determined. In pre-clinical studies, assessing true recovery could e.g. be done by administering a radiolabeled analyte [3,8]. None of the identified studies reported true recovery but rather established extraction recovery by spiking the skin tissue homogenates with analyte [17,20,24,28,31,32,35,38,39,41–43,50,62–64]. Centrifugal precipitation sample pre-treatment in combination with several

Table 3

Extraction recoveries and matrix effects established in the selected studies after applying different analyte extraction method and homogenization methods to skin tissue samples. Matrix factors are shown as % or numerical as the literature stated.

| Extraction method | Homogenization method | Extraction recovery | IS-normalized matrix effect | Absolute matrix effect | Analyte | Ref |
|-----------------------|--|---------------------|-----------------------------|------------------------|---|------|
| LLE | Centrifugal precipitation | 70.7%–78.6% | – | 90.5%–107% | Capsaicin | [38] |
| | | 63.1%–68.0% | – | 91.2%–107.2% | Dihydrocapsaicin | |
| | | 49.53% | – | – | Indomethacin | [39] |
| SPE | Enzymatic digestion, bead homogenization and rotation by tissue tearer | 64.5%–68.4% | 0.90–1.10 | 0.97–1.02 | Diclofenac | [35] |
| | | 78.2%–98.0% | 0.99–1.06 | 1.06–1.14 | Lidocaine | [20] |
| | | 79.3% | – | – | Eumelanin and Pheomelanin | [24] |
| Protein precipitation | Centrifugal precipitation | 65.3%–79.5% | – | 90.3%–104.1% | Digitoxigenin | [32] |
| | | 93.7%–106.5% | – | 93.5%–110.4% | Avobenzone | [42] |
| | Mincing/Cutting/Rotation by tissue tearer | 86.4%–93.7% | 0.91–1.02 | 0.92–1.05% | Pegcantratinib | [28] |
| | | 87.6%–100.9% | – | – | Gefitinib | [41] |
| | – | 92.2% | – | 94.5%–105.5% | Tigecycline | [31] |
| | | 106–116% | – | – | 1,6-Hexamethylene Diisocyanate Mono/Oligomers | [17] |
| Ultrafiltration | Mincing/Cutting/Rotation by tissue tearer | 95.5%–102.2% | – | – | Clavulanic acid | [50] |
| | | 93.0% | – | – | Ampicillin | [43] |

extraction procedures resulted in the lowest extraction recovery (between 49.5 and 78.6%, Table 3), possibly due to a lack of tissue disruption and subsequent absence of matrix constituents in the supernatant. Adding analytes to non-homogenized samples for the determination of extraction recovery for a longer period could lead to diffusion into the tissue cells by unintentional incubation. If the tissue sample is not properly disrupted afterward this might result in an underestimation of extraction recovery. All the other homogenization methods led to extraction recoveries >78.2% (Table 3).

4. Discussion & conclusion

This review provides a comprehensive overview of general sampling methods and corresponding pre-treatment and analyte extraction methods for skin tissue samples collected in *in vivo* clinical and pre-clinical studies for the quantification of small molecules through liquid chromatography coupled to mass spectrometry (LC-MS).

Skin tissue samples are collected using invasive sampling methods such as punch or shaving biopsies, while tape stripping is a less invasive methodology. After collection, skin tissue samples require homogenization to disrupt the cell membranes of the skin tissue cells to release intracellular and interstitial constituents [48]. Different mechanical skin tissue homogenization methods have been applied, as well as chemical solubilization methods, e.g. using 30% hydrogen peroxide in combination with 2M ammonium hydroxide or enzymatic digestion methods using collagenase. Matrix effects and recovery experiments were conducted for skin tissue homogenization methods in combination with analyte extraction procedures. In none of the studies, significant matrix effects were observed (C.V. > 15%), neither in terms of IS-normalized or absolute analyte response (Table 3). This might indicate that endogenous constituents from the skin matrix generally have no substantial effect on the absolute analyte response in bioanalytical assays.

Extraction recovery was on the other hand not always optimal. Skin tissue centrifugal precipitation as a homogenization technique exhibited relatively low extraction recoveries compared to other homogenization methods (Table 3). This could be caused by the diffusion of the spiked analyte into the intact skin tissue matrix, which remains later unrecovered due to inefficient homogenization. This tissue partitioning is dependent on physical chemical properties, indicating that recovery may be highly analyte dependent. Additionally, LLE demonstrated consistently relatively low extraction recoveries as well. Given the low extraction recover-

ies, either centrifugal precipitation as homogenization or LLE as extraction, or a combination of both, are not recommended. No substantial difference in extraction recovery was found for the other homogenization methods and extraction methods.

Disruptiveness of the skin tissue homogenization technique appears defining for the extraction recovery but might also be pivotal for the homogenization efficiency and thus true recovery. While extraction recovery can be determined for the skin tissue matrix, true recovery (including homogenization recovery) is typically not possible to assess. It is therefore crucial that homogenization efficiency is evaluated as much as possible by alternative means, e.g. through visual inspection or by evaluating the recovery of a known endogenous marker in the tissue, such as the total amount of protein present after homogenization. However, except from the tigecycline study [31], no other study in our review reported on these aspects explicitly or mentioned the bias in true-ness due to missing true recovery data. Chemical solubilization or enzymatic degradation homogenization methods may lead, theoretically, to the highest degree of homogenization or disruption of the tissue and would, therefore, be preferred in terms of homogenization efficiency. Although true recovery cannot be assessed, in theory these methodologies should result in a more complete release of compounds from the interstitial and intracellular space. However, stability of the analyte of interest under these conditions must be assured. Stability, matrix effects and extraction recovery need to be assessed, especially when chemical solubilizers (extreme high/low pH) or elevated incubation temperatures (collagenase) are used, which are necessary to completely homogenize or dissolve the skin tissue. In addition, the compatibility of these solubilizers with the subsequent extraction and chromatography methodologies need to be fully evaluated. Method development may, therefore, be more complex than for other sample preparation methods.

Given that true recovery of the analyte of interest is difficult to assess, emphasis should be given to the extent of homogenization during the development of sample preparation and extraction methods of skin tissue samples. Therefore, homogenization plays a crucial role in the accuracy of quantification if the true recovery cannot be evaluated. Chemical or enzymatic solubilization of skin tissue samples is preferable, because of the complete release of intracellular and extracellular components into the solubilization matrix. The inability to assess true recovery may lead to estimations of the pharmaceutical compound concentration in skin tissue instead of accurate quantifications. However, if the method

is reproducible, it will be able to measure the relative distribution of drugs from plasma into the skin and this is of clinical importance to improve formulations or to optimize dosing strategies.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

TD was supported by a personal grant from the Dutch Research Council (NWO)/ZonMw (Veni project no. 91617140) and by the EDCTP2 programme supported by the European Union (grant number RIA2016S-1635-AfrikADIA).

CRediT authorship contribution statement

Ignace C. Roseboom: Methodology, Investigation, Writing - original draft, Writing - review & editing. **Hilde Rosing:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Jos H. Beijnen:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Thomas P.C. Dorlo:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

References

- [1] M. Rizk, L. Zou, R. Savic, K. Dooley, Importance of drug pharmacokinetics at the site of action, *Clin. Transl. Sci.* 10 (2017) 133–142, <http://dx.doi.org/10.1111/cts.12448>.
- [2] Y.-J. Xue, H. Gao, Q.C. Ji, Z. Lam, X. Fang, Z. Lin, M. Hoffman, D. Schulz-Jander, N. Weng, Bioanalysis of drug in tissue: current status and challenges, *Bioanalysis* 4 (2012) 2637–2653, <http://dx.doi.org/10.4155/bio.12.252>.
- [3] H. Neubert, S. Fountain, L. King, T. Clark, Y. Weng, D.M. O'Hara, W. Li, S. Leung, C. Ray, J. Palandra, M.F. Ocaña, J. Chen, C. Ji, M. Wang, K. Long, B. Gorovits, E. Fluhler, Tissue bioanalysis of biotherapeutics and drug targets to support PK/PD, *Bioanalysis* 4 (2012) 2589–2604, <http://dx.doi.org/10.4155/bio.12.234>.
- [4] N.J. Lowe, R.E. Ashton, H. Koudsi, M. Verschoore, H. Schaefer, Anthralin for psoriasis: short-contact anthralin therapy compared with topical steroid and conventional anthralin, *J. Am. Acad. Dermatol.* 10 (1984) 69–72, [http://dx.doi.org/10.1016/S0190-9622\(84\)80046-8](http://dx.doi.org/10.1016/S0190-9622(84)80046-8).
- [5] J.L. Leshner, Oral therapy of common superficial fungal infections of the skin, *J. Am. Acad. Dermatol.* 40 (1999) S31–S34, [http://dx.doi.org/10.1016/S0190-9622\(99\)70395-6](http://dx.doi.org/10.1016/S0190-9622(99)70395-6).
- [6] E.A. Alwawi, S.L. Mehls, K.B. Gordon, Treating psoriasis with adalimumab, *Ther. Clin. Risk Manag.* 4 (2008) 345–351.
- [7] S. Ho, Challenges of atypical matrix effects in tissue, *Bioanalysis* 5 (2013) 2333–2335, <http://dx.doi.org/10.4155/bio.13.209>.
- [8] L. Yuan, L. Ma, L. Dillon, R.M. Fancher, H. Sun, M. Zhu, L. Lehman-McKeeman, A.-F. Aubry, Q.C. Ji, Investigation of the "true" extraction recovery of analytes from multiple types of tissues and its impact on tissue bioanalysis using two model compounds, *Anal. Chim. Acta* 945 (2016) 57–66, <http://dx.doi.org/10.1016/j.aca.2016.09.039>.
- [9] B. Alberts, D. Bray, K. Hopkins, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Essential Cell Biology*, 3rd ed., 2009, New York.
- [10] I. McColl, Rook's textbook of dermatology, *Australas. J. Dermatol.* 46 (2005), <http://dx.doi.org/10.1111/j.1440-0960.2005.00184.x>, pp. 3.1–3.6.
- [11] C. Surber, F.P. Schwarb, E.W. Smith, Tape-stripping technique, *J. Toxicol. Cutan. Ocul. Toxicol.* 20 (2001) 461–474, <http://dx.doi.org/10.1081/CUS-120001870>.
- [12] N.V. Sheth, M.B. McKeough, S.L. Spruance, Measurement of the stratum corneum drug reservoir to predict the therapeutic efficacy of topical iododeoxyuridine for herpes simplex virus infection, *J. Invest. Dermatol.* 89 (1987) 598–602, <http://dx.doi.org/10.1111/1523-1747.ep12461357>.
- [13] J.J. Escobar-Chavez, V. Merino-Sanjuán, M. López-Cervantes, Z. Urban-Morlan, E. Piñón-Segundo, D. Quintanar-Guerrero, A. Ganem-Quintanar, The tape-stripping technique as a method for drug quantification in skin, *J. Pharm. Pharm. Sci.* 11 (2008) 104, <http://dx.doi.org/10.18433/j3201Z>.
- [14] H.-J. Weigmann, U. Lindemann, C. Antoniou, G.N. Tsirikas, A.I. Stratigos, A. Katsambas, W. Sterry, J. Lademann, UV/VIS absorbance allows rapid, accurate, and reproducible mass determination of corneocytes removed by tape stripping, *Skin Pharmacol. Physiol.* 16 (2003) 217–227, <http://dx.doi.org/10.1159/000070844>.
- [15] H.-J. Weigmann, J. Lademann, H. Meffert, H. Schaefer, W. Sterry, Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption, *Skin Pharmacol. Physiol.* 12 (1999) 34–45, <http://dx.doi.org/10.1159/000029844>.
- [16] E. Marttin, M.T.A. Neelissen-Subnel, F.H.N. De Haan, H.E. Boddé, A critical comparison of methods to quantify stratum corneum removed by tape stripping, *Skin Pharmacol. Physiol.* 9 (1996) 69–77, <http://dx.doi.org/10.1159/000211392>.
- [17] K.W. Fent, K. Jayaraj, L.M. Ball, L.A. Nylander-French, Quantitative monitoring of dermal and inhalation exposure to 1,6-hexamethylene diisocyanate monomer and oligomers, *J. Environ. Monit.* 10 (2008) 500, <http://dx.doi.org/10.1039/b715605g>.
- [18] L. Duracher, L. Visdal-Johnsen, A. Mavon, In vitro and in vivo dermal absorption assessment of acetyl aspartic acid: a compartmental study, *Int. J. Cosmet. Sci.* 37 (2015) 34–40, <http://dx.doi.org/10.1111/ics.12255>.
- [19] M. Jung, J. Choi, S.-A. Lee, H. Kim, J. Hwang, E.H. Choi, Pyrrolidone carboxylic acid levels or caspase-14 expression in the corneocytes of lesional skin correlates with clinical severity, skin barrier function and lesional inflammation in atopic dermatitis, *J. Dermatol. Sci.* 76 (2014) 231–239, <http://dx.doi.org/10.1016/j.jdermsci.2014.09.004>.
- [20] Q. Li, T. Magers, B. King, B.J. Engel, R. Bakhtiar, C. Green, R. Shoup, Measurement of lidocaine and 2,6-dimethylaniline in minipig plasma, skin, and dermal tapes using UHPLC with electrospray MS/MS, *J. Chromatogr. B.* 1087–1088 (2018) 158–172, <http://dx.doi.org/10.1016/j.jchromb.2018.04.030>.
- [21] Thomas J. Zuber, Punch biopsy of the skin - American family physician, *Am. Fam. Physician* (2002) 1155–1158, <http://www.aafp.org/aafp/2002/0315/p1155.html>.
- [22] I.S. Sørensen, C. Janfelt, M.M.B. Nielsen, R.W. Mortensen, N.Ø. Knudsen, A.H. Eriksson, A.J. Pedersen, K.T. Nielsen, Combination of MALDI-MSI and cassette dosing for evaluation of drug distribution in human skin explant, *Anal. Bioanal. Chem.* 409 (2017) 4993–5005, <http://dx.doi.org/10.1007/s00216-017-0443-2>.
- [23] G. Szekeley-Klepser, K. Wade, D. Woolson, R. Brown, S. Fountain, E. Kindt, A validated LC/MS/MS method for the quantification of pyrrole-2,3,5-tricarboxylic acid (PTCA), a eumelanin specific biomarker, in human skin punch biopsies, *J. Chromatogr. B.* 826 (2005) 31–40, <http://dx.doi.org/10.1016/j.jchromb.2005.08.002>.
- [24] C.M. Lerche, P. Olsen, C.V. Nissen, P.A. Philipsen, H.C. Wulf, A novel LC-MS/MS method to quantify eumelanin and pheomelanin and their relation to UVR sensitivity - a study on human skin biopsies, *Pigment Cell Melanoma Res.* 32 (2019) 809–816, <http://dx.doi.org/10.1111/pcmr.12805>.
- [25] K.T. Tran, N.A. Wright, C.J. Cockerell, Biopsy of the pigmented lesion—when and how, *J. Am. Acad. Dermatol.* 59 (2008) 852–871, <http://dx.doi.org/10.1016/j.jaad.2008.05.027>.
- [26] P.C. Ng, D.A. Barzilai, S.A. Ismail, R.L. Averitte, A.C. Gilliam, Evaluating invasive cutaneous melanoma: is the initial biopsy representative of the final depth? *J. Am. Acad. Dermatol.* 48 (2003) 420–424, <http://dx.doi.org/10.1067/mjd.2003.106>.
- [27] H. Pickett, N. Air, F. Base, F. Medicine, M.O. Callaghan, F. Hospital, N. Air, F. Base, *Shave and Punch Biopsy for Skin Lesions*, 2011, pp. 995–1002.
- [28] M. Zangarini, N. Rajan, M. Danilenko, P. Berry, S. Traversa, G.J. Veal, Development and validation of LC-MS/MS with in-source collision-induced dissociation for the quantification of pegcatinib in human skin tumors, *Bioanalysis* 9 (2017) 279–288, <http://dx.doi.org/10.4155/bio-2016-0199>.
- [29] L.Z. Hasoun, S.W. Bailey, K.K. Outlaw, J.E. Ayling, Effect of serum folate status on total folate and 5-methyltetrahydrofolate in human skin, *Am. J. Clin. Nutr.* 98 (2013) 42–48, <http://dx.doi.org/10.3945/ajcn.112.057562>.
- [30] M. Getie, R.H. Neubert, LC-MS determination of desmopressin acetate in human skin samples, *J. Pharm. Biomed. Anal.* 35 (2004) 921–927, <http://dx.doi.org/10.1016/j.jpba.2004.02.009>.
- [31] A. Ji Ji, J.P. Saunders, P. Amorusi, G. Stein, N.D. Wadgaonkar, K.P. O'Leary, M. Leal, E.N. Fluhler, Determination of tigeocycline in human skin using a novel validated LC-MS/MS method, *Bioanalysis* 2 (2010) 81–94, <http://dx.doi.org/10.4155/bio.09.159>.
- [32] X. Feng, J. Turley, Z. Xie, S.V. Pierre, H. Koc, M.O. Khan, J. Hao, An LC-MS/MS method for the determination of digitoxigenin in skin samples and its application to skin permeation and metabolic stability studies, *J. Pharm. Biomed. Anal.* 138 (2017) 378–385, <http://dx.doi.org/10.1016/j.jpba.2016.12.029>.
- [33] E.J. Park, M.S. Kim, Y.L. Choi, Y.-H. Shin, H.S. Lee, D.H. Na, Liquid chromatography-tandem mass spectrometry to determine the stability of collagen pentapeptide (KTTKS) in rat skin, *J. Chromatogr. B.* 905 (2012) 113–117, <http://dx.doi.org/10.1016/j.jchromb.2012.08.010>.
- [34] L. Li, P. Ma, J. Wei, K. Qian, L. Tao, LC-ESI-MS method for the determination of dexamethasone acetate in skin of nude mouse, *J. Chromatogr. B.* 933 (2013) 44–49, <http://dx.doi.org/10.1016/j.jchromb.2013.06.024>.
- [35] R. Nirogi, N.S.P. Padala, R.K. Boggavarapu, I. Kalaikadhiban, D.R. Ajjala, G. Bhyrapuneni, N.R. Muddana, Skin sample preparation by collagenase digestion for diclofenac quantification using LC-MS/MS after topical application, *Bioanalysis* 8 (2016) 1251–1263, <http://dx.doi.org/10.4155/bio-2016-0031>.

- [36] X.M.R. van Wijk, M.J. Vallen, E.M. van de Westerlo, A. Oosterhof, W. Hao, E.M. Versteeg, J. Raben, R.G. Wismans, T.F.C.M. Smetsers, H.B.P.M. Dijkman, J. Schalkwijk, T.H. van Kuppevelt, Extraction and structural analysis of glycosaminoglycans from formalin-fixed, paraffin-embedded tissues, *Glycobiology* 22 (2012) 1666–1672, <http://dx.doi.org/10.1093/glycob/cws119>.
- [37] J. Franco, C. Ferreira, T.J. Paschoal Sobreira, J.P. Sundberg, H. HogenEsch, Profiling of epidermal lipids in a mouse model of dermatitis: identification of potential biomarkers, *PLoS One* 13 (2018), e0196595, <http://dx.doi.org/10.1371/journal.pone.0196595>.
- [38] D. Wang, F. Meng, L. Yu, L. Sun, L. Sun, J. Guo, A sensitive LC-MS/MS method for quantifying capsaicin and dihydrocapsaicin in rabbit plasma and tissue: application to a pharmacokinetic study, *Biomed. Chromatogr.* 29 (2015) 496–503, <http://dx.doi.org/10.1002/bmc.3302>.
- [39] J. Ma, Y. Gao, Y. Sun, D. Ding, Q. Zhang, B. Sun, M. Wang, J. Sun, Z. He, Tissue distribution and dermal drug determination of indomethacin transdermal-absorption patches, *Drug Deliv. Transl. Res.* 7 (2017) 617–624, <http://dx.doi.org/10.1007/s13346-017-0392-5>.
- [40] M. Cherlet, S. De Baere, S. Croubels, P. De Backer, Quantitative analysis of levamisole in porcine tissues by high-performance liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry, *J. Chromatogr. B Biomed. Sci. Appl.* 742 (2000) 283–293, [http://dx.doi.org/10.1016/S0378-4347\(00\)00171-7](http://dx.doi.org/10.1016/S0378-4347(00)00171-7).
- [41] M. Zhao, C. Hartke, A. Jimeno, J. Li, P. He, Y. Zabelina, M. Hidalgo, S.D. Baker, Specific method for determination of gefitinib in human plasma, mouse plasma and tissues using high performance liquid chromatography coupled to tandem mass spectrometry, *J. Chromatogr. B* 819 (2005) 73–80, <http://dx.doi.org/10.1016/j.jchromb.2005.01.027>.
- [42] M.G. Kim, T.H. Kim, B.S. Shin, M.G. Kim, S.H. Seok, K.-B. Kim, J.B. Lee, H.G. Choi, Y.S. Lee, S.D. Yoo, A sensitive LC-ESI-MS/MS method for the quantification of avobenzone in rat plasma and skin layers: application to a topical administration study, *J. Chromatogr. B* 1003 (2015) 41–46, <http://dx.doi.org/10.1016/j.jchromb.2015.09.014>.
- [43] K. Hamamoto, Y. Mizuno, LC-MS/MS measurement of ampicillin residue in chicken tissues at 2 days after in-feed administration, *J. Vet. Med. Sci.* 79 (2017) 474–478, <http://dx.doi.org/10.1292/jvms.15-0350>.
- [44] M. Regnier, M. Prunieras, D. Woodley, Growth and differentiation of adult human epidermal cells on dermal substrates, *Front. Matrix Biol.* 9 (1981) 4–35.
- [45] P.B. Medawar, Sheets of pure epidermal epithelium from human skin, *Nature* 148 (1941) 783, <http://dx.doi.org/10.1038/148783a0>.
- [46] Y. Kitano, N. Okada, Separation of the epidermal sheet by dispase, *Br. J. Dermatol.* 108 (1983) 555–560, <http://dx.doi.org/10.1111/j.1365-2133.1983.tb01056.x>.
- [47] L.J. Scaletta, J.C. Occhino, D.K. MacCallum, J.H. Lillie, Isolation and immunologic identification of basement membrane zone antigens from human skin, *Laboratory Invest.* 39 (1978) 1–9.
- [48] C. Yu, L.H. Cohen, Not the same old grind, sample prep, *Perspect. LC-GC Eur.* 17 (2004) 5.
- [49] C. de Duve, J. Berthet, Reproducibility of differential centrifugation experiments in tissue fractionation, *Nature* 172 (1953) 1142, <http://dx.doi.org/10.1038/1721142a0>.
- [50] T. Reyns, S. De Boever, S. De Baere, P. De Backer, S. Croubels, Quantitative analysis of clavulanic acid in porcine tissues by liquid chromatography combined with electrospray ionization tandem mass spectrometry, *Anal. Chim. Acta* 597 (2007) 282–289, <http://dx.doi.org/10.1016/j.aca.2007.06.056>.
- [51] PerkinElmer, Application note: LSC Sample Preparation by Solubilization, (2008).
- [52] W. Wargniez, E. Jungman, S. Wilkinson, N. Seyler, S. Grégoire, Inter-laboratory skin distribution study of 4-n-butyl resorcinol: the importance of liquid chromatography/mass spectrometry (HPLC-MS/MS) bioanalytical validation, *J. Chromatogr. B* 1060 (2017) 416–423, <http://dx.doi.org/10.1016/j.jchromb.2017.05.026>.
- [53] J. Ueyama, K. Kitaichi, M. Iwase, K. Takagi, K. Takagi, T. Hasegawa, Application of ultrafiltration method to measurement of catecholamines in plasma of human and rodents by high-performance liquid chromatography, *J. Chromatogr. B* 798 (2003) 35–41, <http://dx.doi.org/10.1016/j.jchromb.2003.08.045>.
- [54] F.-C. Cheng, J.-S. Kuo, L.-G. Chia, T.-H. Tsai, C.-F. Chen, Rapid measurement of the monoamine content in small volumes of rat plasma, *J. Chromatogr. B Biomed. Sci. Appl.* 654 (1994) 177–183, [http://dx.doi.org/10.1016/0378-4347\(94\)00002-6](http://dx.doi.org/10.1016/0378-4347(94)00002-6).
- [55] E. Chernokalskaya, S. Gutierrez, A.M. Pitt, J.T. Leonard, Ultrafiltration for proteomic sample preparation, *Electrophoresis* 25 (2004) 2461–2468, <http://dx.doi.org/10.1002/elps.200405998>.
- [56] F.L.S.T. Wenkui Li, Jie Zhang, *Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols, and Regulations*, John Wiley & Sons, Inc., Hoboken, New Jersey, 2013.
- [57] P. Kebarle, L. Tang, From ions in solution to ions in the gas phase - the mechanism of electrospray mass spectrometry, *Anal. Chem.* 65 (1993), <http://dx.doi.org/10.1021/ac00070a001>, 972A–986A.
- [58] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, *Anal. Chem.* 75 (2003) 3019–3030, <http://dx.doi.org/10.1021/ac020361s>.
- [59] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, Mechanistic investigation of ionization suppression in electrospray ionization, *J. Am. Soc. Mass Spectrom.* 11 (2000) 942–950, [http://dx.doi.org/10.1016/S1044-0305\(00\)00163-X](http://dx.doi.org/10.1016/S1044-0305(00)00163-X).
- [60] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, Validation of bioanalytical LC-MS/MS assays: evaluation of matrix effects, *J. Chromatogr. B* 877 (2009) 2198–2207, <http://dx.doi.org/10.1016/j.jchromb.2009.01.003>.
- [61] W. Lv, Investigation of the effects of 24 bio-matrices on the LC-MS/MS analysis of morinidazole, *Talanta* 80 (2010) 1406–1412, <http://dx.doi.org/10.1016/j.talanta.2009.09.043>.
- [62] Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation Guidance for Industry Bioanalytical Method Validation Center for Veterinary Medicine (CVM) Contains Nonbinding Recommendations, 2013 <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm%0Ahttp://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm>.
- [63] European Medicines Agency, Committee for Medicinal Products for Human Use, Guideline on Bioanalytical Method Validation, EMA, 2012, Reference number EMEA/CHMP/EWP/192217/2009 <https://www.ema.europa.eu/en/bioanalytical-method-validation>.
- [64] N. Kadian, K.S.R. Raju, M. Rashid, M.Y. Malik, I. Taneja, M. Wahajuddin, Comparative assessment of bioanalytical method validation guidelines for pharmaceutical industry, *J. Pharm. Biomed. Anal.* 126 (2016) 83–97, <http://dx.doi.org/10.1016/j.jpba.2016.03.052>.
- [65] M. M.Komorniczak, File: Skin_Layers - Wikimedia Commons, 2012 https://commons.wikimedia.org/wiki/File:Skin_Layers.svg.
- [66] T. Sadowski, C. Klose, M.J. Gerl, A. Wójcik-Maciejewicz, R. Herzog, K. Simons, A. Reich, M.A. Surma, Large-scale human skin lipidomics by quantitative, high-throughput shotgun mass spectrometry, *Sci. Rep.* 7 (2017) 43761, <http://dx.doi.org/10.1038/srep43761>.
- [67] BruceBlaus, File:Skin Punch Biopsy.png - Wikimedia Commons, 2017 https://commons.wikimedia.org/wiki/File:Skin_Punch_Biopsy.png.