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RESEARCH ARTICLE

# Pharmaceutical development of an amorphous solid dispersion formulation of elacridar hydrochloride for proof-of-concept clinical studies

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**ABSTRACT**

**Objective:** A novel tablet formulation containing an amorphous solid dispersion (ASD) of elacridar hydrochloride was developed with the purpose to resolve the drug's low solubility in water and to conduct proof-of-concept clinical studies.

**Significance:** Elacridar is highly demanded for proof-of-concept clinical trials that study the drug's suitability to boost brain penetration and bioavailability of numerous anticancer agents. Previously, clinical trials with elacridar were performed with a tablet containing elacridar hydrochloride. However, this tablet formulation resulted in poor and unpredictable absorption which was caused by the low aqueous solubility of elacridar hydrochloride.

**Methods:** Twenty four different ASDs were produced and dissolution was compared to crystalline elacridar hydrochloride and a crystalline physical mixture. The formulation with highest dissolution was characterized for amorphicity. Subsequently, a tablet was developed and monitored for chemical/physical stability for 12 months at +15–25 °C, +2–8 °C and −20 °C.

**Results:** The ASD powder was composed of freeze dried elacridar hydrochloride–povidone K30–sodium dodecyl sulfate (1:6:1, w/w/w), appeared fully amorphous and resulted in complete dissolution whereas crystalline elacridar hydrochloride resulted in only 1% dissolution. The ASD tablets contained 25 mg elacridar hydrochloride and were stable for at least 12 months at –20 °C.

**Conclusions:** The ASD tablet was considered feasible for proof-of-concept clinical studies and is now used as such.

**Abbreviations:** ASD: amorphous solid dispersion; BCRP: breast cancer resistance protein; DMSO: dimethyl sulfoxide; ELAHCL: elacridar hydrochloride; IR: infrared spectroscopy; MDSC: modulated differential scanning calorimetry; P-gp: P-glycoprotein; PVPK30: povidone K30; PVPVA: polyvinylpyrrolidone vinylacetate; SIFsp: simulated intestinal fluid without pancreatic enzymes; SDS: sodium dodecyl sulfate; XRD: X-ray diffractometry

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## Introduction

Elacridar (GF120918, GG918) is an inhibitor of permeability glycoprotein (P-gp) and breast cancer resistance protein (BCRP); drug-efflux pumps that are expressed on cell membranes in the gastro-intestinal tract, blood–brain barrier, stem cells and cancer cells[1,2]. By blocking P-gp and BCRP, the absorption of drugs that are substrates to these drug-efflux pumps can be enhanced. There is a high demand for proof-of-concept clinical trials to evaluate in cancer patients the role of elacridar as an absorption enhancer, e.g. for the treatment of brain tumors, because according to preclinical studies elacridar considerably enhances brain penetration of various anticancer drugs[3–11]. Previous clinical trials demonstrated that elacridar was an effective absorption enhancer for paclitaxel and topotecan when elacridar plasma concentrations of at least 200 ng/mL were achieved. The formulation used for previous clinical trials was a tablet formulation containing elacridar hydrochloride and was administered to cancer patients at oral doses of 100–1000 mg[12–14]. A problem of the previously used tablet formulation was poor and unpredictable absorption caused by the low solubility of elacridar hydrochloride and therefore the minimum effective elacridar plasma concentration of 200 ng/mL was often not achieved in patients[14,15].

To answer the request to conduct proof-of-concept clinical studies, we developed a novel oral tablet formulation of elacridar hydrochloride and the trial for which this formulation was used is registered in the EudraCT database (registration number 2013-001131-47) and recently published[16]. To resolve the drug's low solubility in water and to ensure of achieving the minimum effective elacridar plasma concentration (200 ng/mL), we made an amorphous solid dispersion (ASD).

An ASD is a molecular dispersion of a drug and a biologically inactive hydrophilic amorphous excipient. The amorphous state of the powder, homogeneously mixed up to molecular level, the hydrophilic nature of the excipient and the large particle surface area are mechanisms that induce super-saturated drug dissolution, allowing a time window for increased absorption[17–19]. A common dissolution profile of an ASD is shown in Figure 1. The super-saturation effect is temporary because drug recrystallization eventually takes over, inducing precipitation back to the intrinsic solubility of

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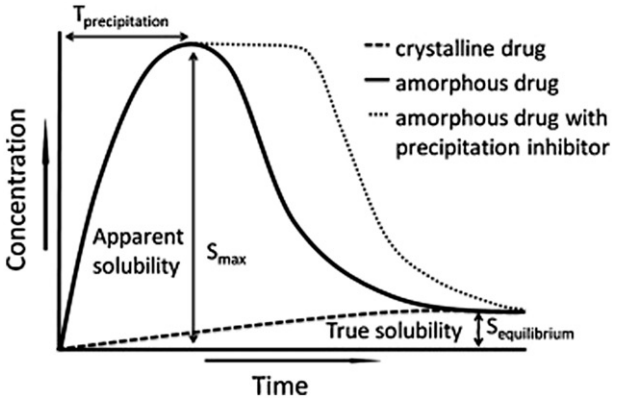


Figure 1. Example dissolution curve of an amorphous solid dispersion, reproduced from Moes et al.[32] with kind permission from Elsevier.

the crystalline drug[20]. The super-saturated state should be as high and as long as possible (the "parachute" effect) and this can be done by careful selection of the amount and type of excipients. Currently there are at least 27 commercialized ASD oral drug formulations, highlighting the successful usability of this formulation method[20]. For example, the oral bioavailability of vemurafenib ASD and regorafenib ASD was increased four and seven times respectively compared to crystalline physical mixtures[21,22].

The pharmaceutical development of an ASD, however, is more time-consuming and complex than that of a conventional pharmaceutical formulation (crystalline physical mixture) because an extensive research on excipient selection, production method and dosage form is required. To facilitate fast and efficient pharmaceutical development, we followed a general systematic formulation procedure for the development of an ASD with elacridar hydrochloride (see Figure 2).

## Materials and methods

### Materials

The drug, elacridar hydrochloride, was synthesized according to a procedure as earlier described[23]. Polyvinylpyrrolidone Vinylacetate 64 co-polymer (PVPVA64) and Soluplus® were kind gifts from BASF Chemtrade (Ludwigshafen, Germany). The following chemicals were purchased: povidone K30 (PVPK30) from BASF Chemtrade (Ludwigshafen, Germany), sodium dodecyl sulfate (SDS), dimethyl sulfoxide (DMSO), dichloromethane, methanol, tert-butanol and potassium dihydrogen phosphate from Merck (Darmstadt, Germany), lactose monohydrate SuperTab® 30GR from DFE Pharma (Goch, Germany), colloidal silicon dioxide and magnesium stearate from Fagron (Capelle a/d Ijssel, The Netherlands), croscarmellose sodium from Caldic (Rotterdam, The Netherlands), demineralized water from B. Braun (Melsungen, Germany), aluminum blister units with polyvinylchloride sealing from Feton (Brussels, Belgium) and hard gelatin capsules size 0 from Capsugel (Morristown, NJ). Simulated intestinal fluid without pancreatic enzymes (SIFsp, pH 6.80) was prepared as in USP-NF[24].

### Methods

#### Thermogravimetric analysis (TGA)

Approximately 10 mg elacridar hydrochloride was weighed on a platinum pan, placed in nitrogen gas and heated from 25 °C to 400 °C at a heating rate of 2 °C/min. Analysis was performed on a TGA Q50 V6.7 instrument (TA Instruments, New Castle, DE).

#### Freeze drying

Drug powder, polymers and SDS were dissolved in DMSO. Solutions were transferred to open stainless steel containers (Gastronorm 1/9) and dried in a Lyovac GT4 freeze dryer (GEA Lyophil, Hürth, Germany) by using a program earlier developed by us[25]. The powder was collected in an amber-colored glass container and airtight-sealed with a polypropylene screw cap and stored at +2 to 8 °C.

#### Spray drying

A Büchi spray drying system consisted of the B-290 spray dryer and B-295 Inert Loop (Flawil, Switzerland) in closed mode and nitrogen as the drying gas. Drug powder, PVPK30 and SDS were dissolved in dichloromethane to an elacridar hydrochloride concentration of 1.3 mg/mL. The inlet temperature was 55 °C, outlet temperature was 44–22 °C, nozzle tip/cap diameter 0.7/1.50 mm, aspirator 90%, pressure of drying gas 35 mm and feed rate of solution 24 mL/min. The powder was stored at +2 to 8 °C.

#### Powder mixing and tablet compaction

Powders were mixed in a Turbula mixer T10B (Muttenz, Switzerland) and pressed on an eccentric press EK0 (Korsch AG, Berlin, Germany). Tablets were stored in aluminum blisters with polyvinylchloride sealing at −20 °C. To minimize hygroscopicity tablets were kept in sealed blisters and warmed up to ambient temperature in a desiccator before the seal was broken.

#### X-ray diffraction (XRD)

X-ray powder diffraction measurements were done with an X'pert pro diffractometer equipped with an X-celerator (PANanalytical, Almelo, The Netherlands). Samples were placed in a 0.5 mm deep metal sample holder which was placed in the diffractometer. Samples were scanned at a current of 30 mA and a tension of 40 kV. The scanning range was 10–45° $2\theta$ with a step size of 0.020° and a scanning speed of 0.002° per second.

#### Modulated differential scanning calorimetry (MDSC)

Reversing heat flow was measured by a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE). Temperature scale and heat flow were calibrated with indium. Samples of approximately 10 mg powder in airtight-sealed $T_{zero}$ aluminum hermetic pans (TA Instruments, New Castle, DE) were placed in the auto sampler. Each sample was equilibrated at −20.00 °C for 5 min, after which the sample was heated to 60.00 °C at a speed of 2.00 °C/min. Modulation was performed every 60 s at ±1.00 °C.

*Residual DMSO* Residual DMSO was determined by gas chromatography. The stationary phase was an Alltech RTX-1301 column of cyanopropylphenyl–dimethylpolysiloxane (6:94, w/w) with dimensions 30 m length ×0.53 mm internal diameter and a pore size of 3.0 µm. The liner was made of glass wool with an internal diameter of 4.0 mm. The carrier gas was helium. The column flow was 2.6 mL/min. The inlet temperature was 230 °C, the pressure was 0.15 bar, split ratio was 5.0, split flow was 20 mL/min and the total flow was ±25 mL/min. Samples were injected by split injection (1 µL was injected into the system). The flame ionization detector is set at 250 °C with a hydrogen gas flow of 40 mL/min, oxygen gas flow of 250 mL/min and make-up nitrogen gas flow of 40 mL/min. The oven temperature was 55 °C, initialization time was 4 min, the heating rate was 40 °C/min and the final

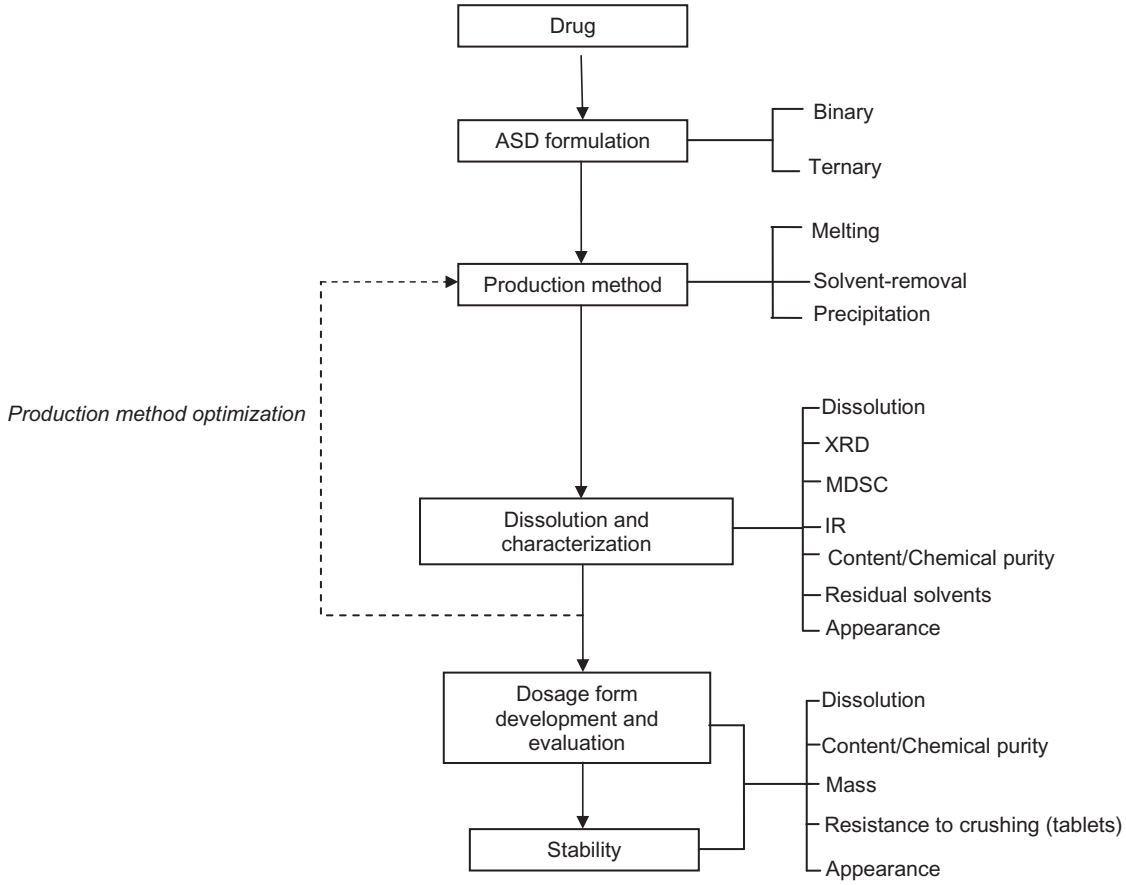


Figure 2. Formulation procedure for the elacridar hydrochloride ASD.

temperature was 200 °C. Total run time was 12 min. Powder samples of approximately 50 mg were dissolved in 5 mL methanol–tert-butanol (90:10, v/v). Tert-butanol was the internal standard. DMSO calibration standards and DMSO quality control standards were prepared on the day of analysis from two DMSO stock solutions (stored at −20 °C).

### *Residual water*

Residual water was measured by a Karl Fischer titration method by using a Metrohm 758 KFD Titrino (Herisau, Switzerland). An amount of 50 mg powder was dissolved in 5 mL preconditioned methanol. The titrant was standardized with 30 mg of demineralized water.

### *Content and chemical purity*

Drug powder, ASD powders and tablets were analyzed and quantified with a validated HPLC-UV method previously described by us[26]. The HPLC system was an 1100 series and consisted of a binary pump (G1312A), autosampler G1367A and a UV detector G1314A (Agilent Technologies, Santa Clara, CA).

### *Dissolution*

A small-scale dissolution test was used to screen formulations for their solubility-enhancing effect. For this, an amount equivalent to 10 mg elacridar hydrochloride was placed in 100 mL SIFsp (37 ± 1 °C) and homogenized at 500 rpm with a magnetic stirrer. One mL was filtrated through a 0.45 µm PVDF filter and immediately diluted with 2 mL DMSO. The duration of the test was 4 h. Samples were analyzed at 409 nm on a spectrophotometer (Shimadzu, Kyoto, Japan). Quantification was done by preparing calibration standards 1–100 µg/mL in SIFsp-DMSO (33:67, v/v).

To study the dissolution of ASD tablets, an USP type II paddle dissolution tester was used according to a method described previously by us[26]. In brief, the dissolution medium was 500 mL of SIFsp (37 °C) homogenized at 100 rpm. One mL sample was filtrated through a 0.45 µm PVDF filter and immediately diluted with 2 mL DMSO and quantified by the validated HPLC-UV method[26].

### *Stability study*

Tablets were stored in aluminum blisters with polyvinylchloride sealing at room temperature (+15 to 25 °C/60% RH), refrigerator (+2 to 8 °C) or freezer (−20 °C) and were analyzed after 0, 3, 6, 9 and 12 months for content, chemical purity, dissolution, appearance, mass and resistance to crushing. The dissolution difference factor ($f_1$) and similarity factor ($f_2$) were calculated up to 60 min according to formulae previously described[27].

## Results and discussion

### *Step 1: ASD formulation*

A vinyl polymer (PVP), a vinyl co-polymer (PVPVA) and a co-polymer (Soluplus®) were selected as candidate hydrophilic carrier excipients. These polymers have a pH-independent solubility and are therefore suitable for dissolution enhancement over the entire gastro-intestinal tract.

PVPK30 and PVPVA64 (vinylpyrrolidone–vinylacetate 60–40% co-polymer) are frequently used excipients for ASD formulations

with $T_g$>100 °C and have good solubility in many organic solvents[20,28]. Soluplus® is a co-polymer with polyethyleneglycol as the hydrophilic backbone and a polyvinylcaprolactam and polyvinylacetate as hydrophobic side chain. Soluplus® is a relatively new excipient but many papers already report promising results regarding super-saturation[29–31].

Another frequently used formulation is a ternary ASD which contains a surfactant as extra excipient (e.g. SDS) and this can increase super-saturation due to its powder-wetting properties and precipitation-inhibition[28,32].

### *Step 2: production method*

There are three widely used production methods for ASDs: melting, precipitation and solvent-removing[18]. Melting is feasible for drugs and excipients that do not decompose during the melting process. However, many drugs have a high melting temperature (>200 °C), often accompanied by decomposition[33,34]. The application of the precipitation method is limited to polymers with a pH-dependent aqueous solubility (such as hydroxypropylmethylcellulose acetate-succinate), the disadvantage being that drug release is not possible in the entire gastro-intestinal tract. In the solvent-removal method, drug and excipient are dissolved in an organic solvent which is then evaporated (e.g. spray drying) or sublimated (e.g. freeze drying).

Elacridar hydrochloride has a high melting point (280 °C, internal data) with decomposition at 200 °C (see Figure 3), thus melting was not the preferred production method. The precipitation method was also unsuitable because this method is restricted to polymeric excipients with a pH-dependent solubility and this does not ensure dissolution over the entire pH range in the gastro-intestinal tract. Solvent-removal was a suitable production method because elacridar hydrochloride has good solubility in DMSO, a solvent that was previously successfully removed by a freeze drying method[25]. Therefore, the preexisting freeze drying program was used to produce formulations described in step 1. The preparation method and the composition of the formulations are shown in Table 1. Formulations A–X and formulation Z were freeze dried in a stainless steel container (36 mL solution per container).

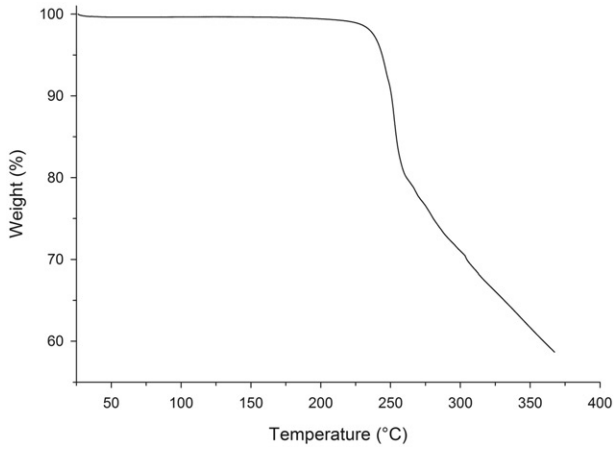


Figure 3. Thermogravimetric analysis of elacridar hydrochloride.

Table 1. Components, weight ratios and preparation methods of elacridar hydrochloride formulations.

| | | | | Freeze drying settings | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Formulation type | Excipients | Drug–excipients weight ratio | Elacridar hydrochloride (mg/mL) | PVPK30 (mg/mL) | PVPVA64 (mg/mL) | Soluplus (mg/mL) | SDS (mg/mL) | Total drug + excipients (mg/mL) |
| A | Binary ASD | PVPK30 | 1:12 | 10 | 120 | – | – | – | 130 |
| B | Binary ASD | PVPK30 | 1:9 | 10 | 90 | – | – | – | 100 |
| C | Binary ASD | PVPK30 | 1:6 | 10 | 60 | – | – | – | 70 |
| D | Binary ASD | PVPK30 | 1:3 | 10 | 30 | – | – | – | 40 |
| E | Binary ASD | PVPVA64 | 1:12 | 10 | – | 120 | – | – | 130 |
| F | Binary ASD | PVPVA64 | 1:9 | 10 | – | 90 | – | – | 100 |
| G | Binary ASD | PVPVA64 | 1:6 | 10 | – | 60 | – | – | 70 |
| H | Binary ASD | PVPVA64 | 1:3 | 10 | – | 30 | – | – | 40 |
| I | Binary ASD | SOLUPLUS | 1:12 | 10 | – | – | 120 | – | 130 |
| J | Binary ASD | SOLUPLUS | 1:9 | 10 | – | – | 90 | – | 100 |
| K | Binary ASD | SOLUPLUS | 1:6 | 10 | – | – | 60 | – | 70 |
| L | Binary ASD | SOLUPLUS | 1:3 | 10 | – | – | 30 | – | 40 |
| M | Ternary ASD | PVPK30, SDS | 1:12:1 | 10 | 120 | – | – | 10 | 140 |
| N | Ternary ASD | PVPK30, SDS | 1:9:1 | 10 | 90 | – | – | 10 | 110 |
| O | Ternary ASD | PVPK30, SDS | 1:6:1 | 10 | 60 | – | – | 10 | 80 |
| P | Ternary ASD | PVPK30, SDS | 1:3:1 | 10 | 30 | – | – | 10 | 50 |
| Q | Ternary ASD | PVPVA64, SDS | 1:12:1 | 10 | – | 120 | – | 10 | 140 |
| R | Ternary ASD | PVPVA64, SDS | 1:9:1 | 10 | – | 90 | – | 10 | 110 |
| S | Ternary ASD | PVPVA64, SDS | 1:6:1 | 10 | – | 60 | – | 10 | 80 |
| T | Ternary ASD | PVPVA64, SDS | 1:3:1 | 10 | – | 30 | – | 10 | 50 |
| U | Ternary ASD | SOLUPLUS, SDS | 1:12:1 | 10 | – | – | 120 | 10 | 140 |
| V | Ternary ASD | SOLUPLUS, SDS | 1:9:1 | 10 | – | – | 90 | 10 | 110 |
| W | Ternary ASD | SOLUPLUS, SDS | 1:6:1 | 10 | – | – | 60 | 10 | 80 |
| X | Ternary ASD | SOLUPLUS, SDS | 1:3:1 | 10 | – | – | 30 | 10 | 50 |
| Y | Crystalline API | – | – | – | – | – | – | – | – |
| Z | Freeze dried API | – | – | 10 | – | – | – | – | – |
| PM1 | Physical mixture crystalline API | PVPK30, SDS | 1:6:1 | – | – | – | – | – | – |
| PM2 | Physical mixture freeze dried API | PVPK30, SDS | 1:6:1 | 10 | – | – | – | – | – |

ASD: amorphous solid dispersion; PVPK30: povidone K30; PVPVA64: polyvinylpyrrolidone vinylacetate 60/40; SDS: sodium dodecyl sulfate; API: active pharmaceutical ingredient (in this case elacridar hydrochloride); PM: physical mixture.

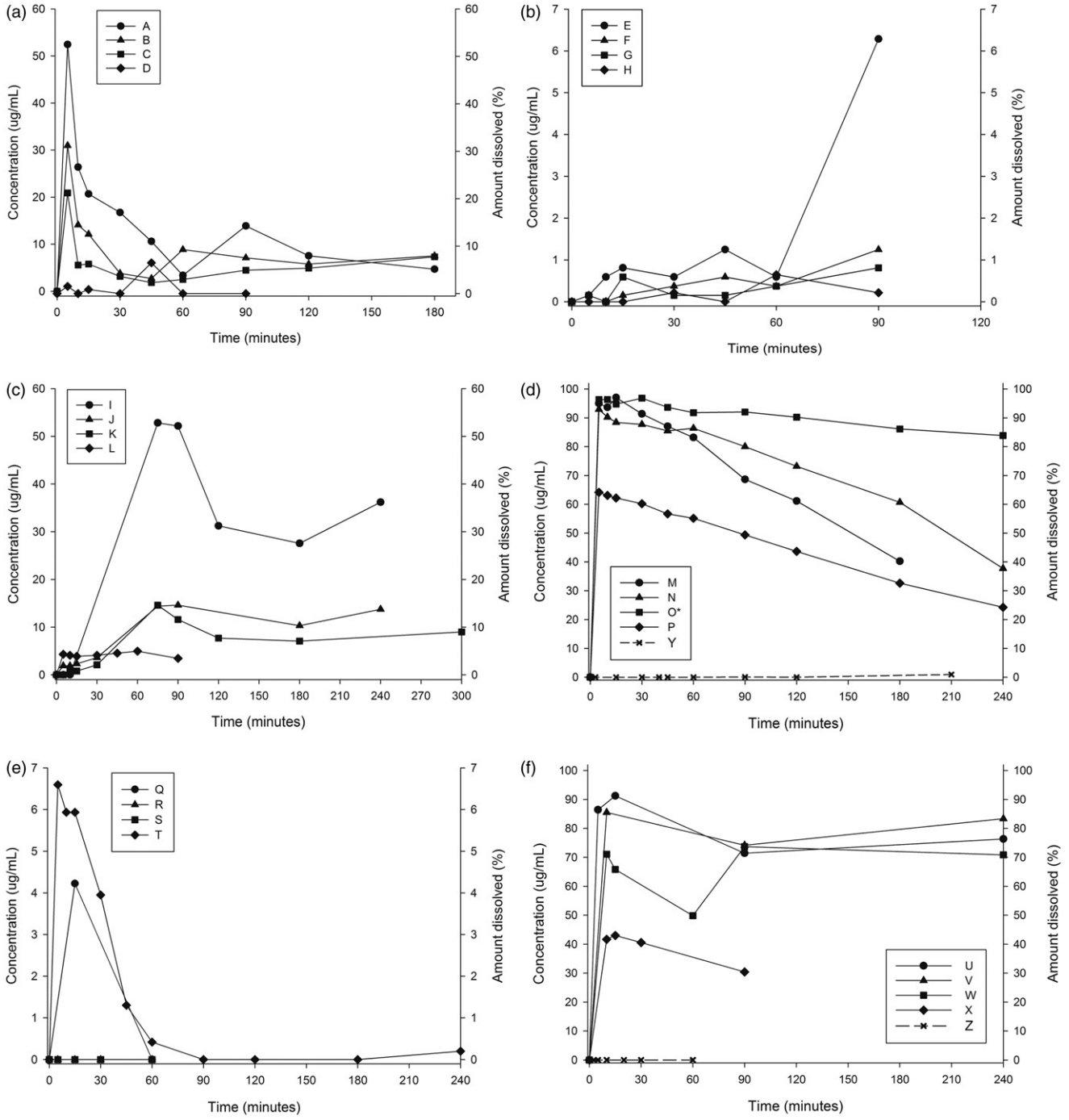


Figure 4. Dissolution screening of freeze dried ASDs (formulations A–X, Table 1) in comparison to crystalline elacridar hydrochloride and freeze dried elacridar hydrochloride (formulations Y and Z respectively, Table 1). Each line represents one formulation ($n = 1$). Each figure has two *Y*-axes and one *x*-axis. The left *y*-axis displays the absolute concentration of elacridar hydrochloride in μg/mL and the title of this axis is shortened to "concentration (μg/mL)". The right *y*-axis shows the concentration of elacridar hydrochloride relative in percent to the theoretical maximum concentration of 100 μg/mL and is shortened to "amount dissolved (%)". The *x*-axis shows the time (*x*-axis) of the dissolution experiment. Binary ASDs with PVPK30, PVPVA64 or Soluplus are shown in (a)–(c), respectively. Ternary ASDs with PVPK30-SDS, PVPVA64-SDS or Soluplus-SDS are shown in (d)–(f), respectively. *Formulation chosen for further development.

### *Step 3: dissolution and characterization*

#### *Dissolution*

The dissolution of elacridar hydrochloride from freeze dried ASDs was compared to crystalline elacridar hydrochloride and freeze dried elacridar hydrochloride. The results are shown in Figure 4(a)–(f).

Dissolution from binary ASDs is shown in Figure 4(a)–(c). For ASDs with PVPK30 (Figure 4(a), formulations A–D), increasing the excipient content resulted in higher dissolution, which demonstrates the importance of PVPK30 in dissolution enhancement. The highest dissolution was 52% (formulation A). However, formulations A–D already precipitated after 5 min. Binary ASDs with PVPVA64 (Figure 4(b), formulations E–H) resulted in poor dissolution (<10%) and this can be explained by the fact that PVPVA64 is less hydrophilic than PVPK30[35]. Binary ASDs with Soluplus® (Figure 4(c), formulations I–L) resulted in moderate dissolution and among them formulation I resulted in the highest dissolution (53%). The dissolution after 4 h was still higher than

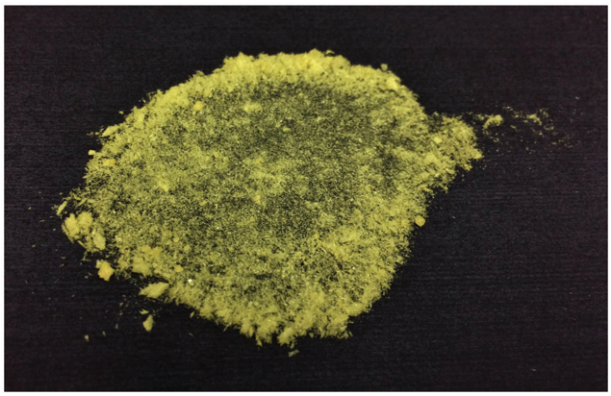

Figure 5. Photographic image of freeze dried ASD powder containing elacridar hydrochloride–PVPK30–SDS 1:6:1 (w/w/w).

that of crystalline elacridar hydrochloride (Figure 4(d), formulation Y). It is likely that long dissolution enhancement was caused by micellar formation with Soluplus®. This effect was previously also observed by Lim et al. who developed a docetaxel-Soluplus® ASD[36].

Figure 4(d)–(f) shows dissolution from ternary ASDs. Formulations with PVPK30-SDS (Figure 4(d), formulations M–P) resulted in a higher dissolution and slower precipitation compared to binary ASDs with PVPK30 (Figure 4(a), formulations A–D). This shows that SDS plays an important role in super-saturation and in reducing precipitation. Formulation O resulted in complete dissolution (>90%). Formulations M and N resulted in similar super-saturation but in faster precipitation than formulation O, despite the fact that these two formulations contained higher amounts of PVPK30. When placed in the dissolution medium, formulations M and N appeared as larger powder agglomerates than formulation O, therefore the disintegration process of these ASDs could be less homogeneous, affecting the dissolution. Ternary ASDs with PVPVA64-SDS (Figure 4(e), formulations Q–T) resulted in poor dissolution (<10%) which was related to the less hydrophilic nature of PVPVA64[35]. ASDs with Soluplus®-SDS (Figure 4(f), formulations U–X) resulted in good dissolution (40–90%) and no precipitation, again suggesting micelle formation. Increasing the amount of Soluplus® resulted in higher dissolution, showing the importance of Soluplus® in the dissolution process of elacridar hydrochloride. It is likely that SDS made finer micelles, with that less agglomeration, explaining the higher dissolution than from binary ASDs with Soluplus®.

The dissolution of crystalline elacridar hydrochloride (Figure 4(d), formulation Y) and freeze dried elacridar hydrochloride (Figure 4(f), formulation Z) was 1% and only 1 μg/mL, showing the drug belongs to the category "practically insoluble in water"[24].

Formulation O was selected for further development because with this formulation highest dissolution enhancement was achieved (90 times higher than dissolution from crystalline elacridar hydrochloride). Another advantage of formulation O is that the polymeric carrier (PVPK30) is a generally regarded safe excipient, and it is already widely used in pharmaceutical and commercial development of ASDs[20].

### *Characterization*

The physical characterization of formulation O (further referred to as ASD) is shown in Figures 5 and 6. The ASD appeared as a yellow dry powder (Figure 5). The XRD examination is shown in Figure 6(a). The absence of diffraction peaks in the spectrum of the ASD confirmed the amorphous state. Freeze dried elacridar hydrochloride was not amorphous, although the number and intensity of diffraction peaks were less compared to unprocessed elacridar hydrochloride. Amorphous elacridar hydrochloride is physically unstable because of strong crystal bonding between drug molecules, a common feature of drugs with a high melting temperature[37]. Therefore, elacridar hydrochloride requires a polymeric excipient to remain amorphous. Regarding IR (Figure 6(b)), elacridar hydrochloride contained a sharp peak at 1660 $cm^{-1}$ which corresponded to carbonyl (C–O), C–N and/or aromatic rings (depicted in Figure 6(b) with a gray rectangle). In the case of PVPK30, the large and broad peak at 1660 $cm^{-1}$ was caused by the carbonyl group. Peak 1660 $cm^{-1}$ in physical mixtures appeared sharp, but was blunt in the ASD and this suggests the establishment of extra dispersion, polar or hydrogen bond interactions between elacridar hydrochloride and PVPK30. In the MDSC of the ASD (Figure 6(c)), a $T_g$ was detected (29.6 °C). Higher temperatures for MDSC were not studied because elacridar hydrochloride decomposes at 200 °C (see Figure 3) which disrupts heat flow signals. Therefore, MDSC could not be used to identify crystallinity of elacridar hydrochloride. Instead, MDSC was only used to study thermal events occurring in the ASD around ambient conditions.

The chemical purity and content of elacridar hydrochloride were 100% and 88.1 ± 0.5% (110.2 ± 0.6 mg/g), respectively. Residual DMSO was 9.1 ± 0.3% and residual water was 4.2 ± 0.1% (Table 3) despite the fact that no water was used during the production method. This was caused by residual DMSO which is hygroscopic[25].

DMSO is a solvent with low toxic potential (Class 3), and amounts higher than 50 mg/0.5% w/w may be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice[38]. To administer a dose equivalent to 1000 mg, elacridar requires 9.1 g ASD powder which contains 826 mg DMSO. This is far below the $LD_{50,oral}$ of DMSO (14.5 g/kg). DMSO is considerably less toxic compared to other solvents commonly used in pharmaceutical productions (e.g. ethanol, also a Class 3 solvent with $LD_{50,oral}$ of 7.1 g/kg). In fact, DMSO is used parentally to patients receiving autologous bone marrow transplantation up to 50 mL (~55 g) DMSO per dose[25]. Therefore, the residual DMSO content in the ASD powder can be considered nontoxic in this context.

To conclude, a freeze dried ASD powder containing elacridar hydrochloride–PVPK30–SDS (1:6:1, w/w/w) was developed, was fully amorphous and had a 90 times increased dissolution compared to physical mixtures of crystalline elacridar hydrochloride.

### *Production method optimization*

The residual solvent content in the ASD was a limitation because DMSO and water worked as plasticizers and this explained the $T_g$ around room temperature. We investigated whether lowering the concentration resulted in less residual DMSO/water and a higher $T_g$. Results are shown in Table 2. Decreasing the freeze drying concentration did not lower the total residual solvent content. In formulations, 40 mg/mL and 20 mg/mL residual DMSO decreased but residual water increased and all formulations had a $T_g$ close to room temperature. The inability to decrease total residual solvents by decreasing freeze drying concentration might have been caused by the fact that the freeze drying solution was still too viscous, even at the lowest studied concentration, and that this induced sublimation resistance[39].

Then, an attempt was made to develop a spray drying method. DMSO, however, was unsuitable because of its high boiling

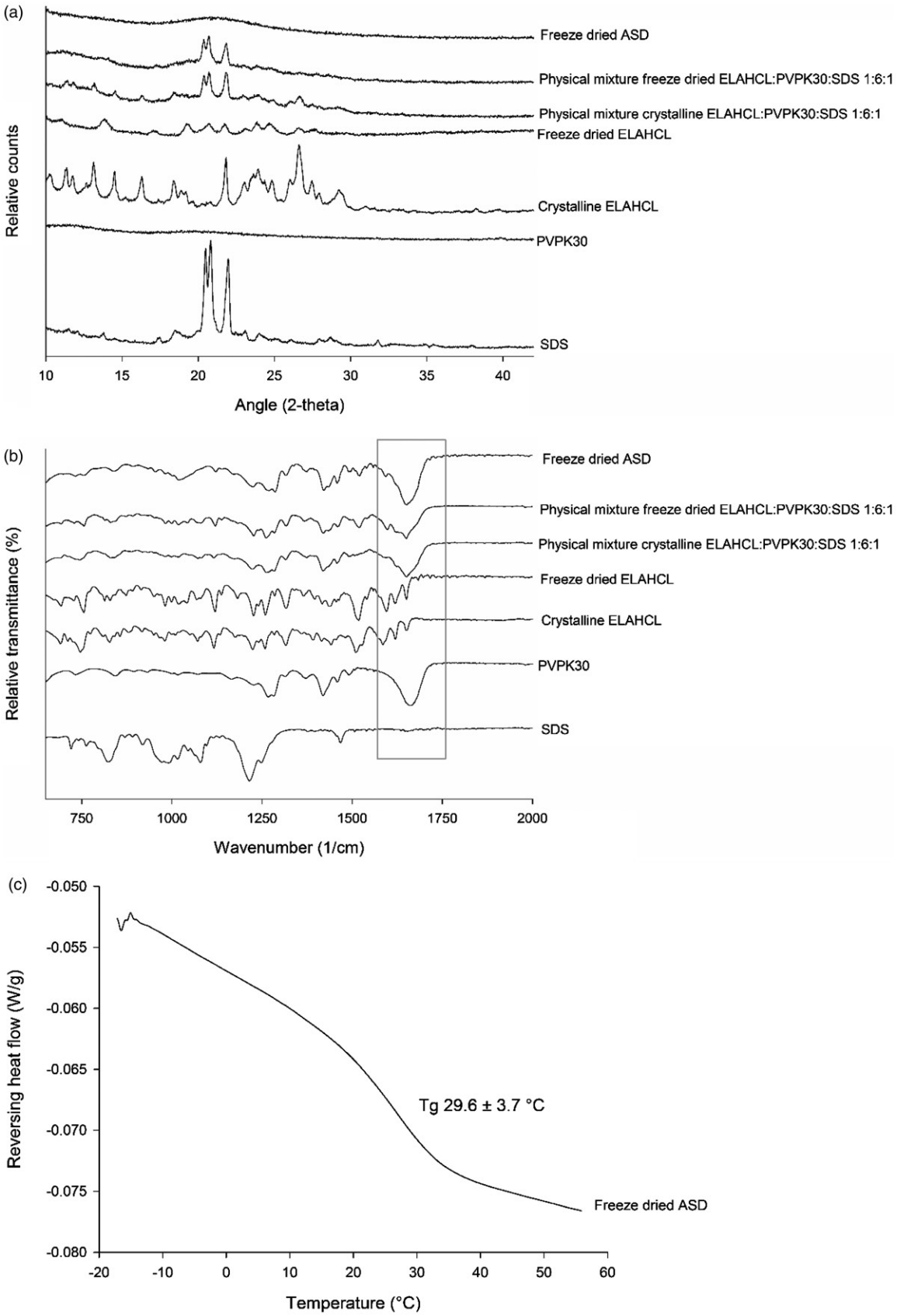


Figure 6. Characterization of elacridar hydrochloride ASD by X-ray diffractometry (a), infrared absorption spectroscopy (b) and MDSC (c). ELAHCL = elacridar hydrochloride. The gray rectangle in (b) at 1660 $cm^{-1}$ indicates the carbonyl, C=N and aromatic ring peak.

Table 2. Optimization of the freeze drying process in order to reduce residual DMSO and residual water and to increase $T_g$ by modifying the total excipient concentration in the freeze drying solution in DMSO (elacridar hydrochloride–PVPK30–SDS 1:6:1, w/w/w).

| Total excipient concentration (mg/mL) | Elacridar hydrochloride concentration (mg/mL) | Content of active ingredient (%)[a] | Chemical purity (%)[a] | Residual DMSO (%, w/w)[a] | Residual Water (%, w/w)[a] | Total residual solvents (%)[a] | $T_g$ (°C)[b] |
|---|---|---|---|---|---|---|---|
| 80 | 10.0 | 90.2 ± 0.8 | 100.0 | 8.5 ± 0.4 | 3.5 ± 0.3 | 12.0 ± 0.4 | 34.5 |
| 60 | 7.5 | 89.7 ± 0.4 | 100.0 | 8.6 ± 0.1 | 4.0 ± 0.2 | 12.6 ± 0.2 | 31.6 |
| 40 | 5.0 | 91.0 ± 0.4 | 100.0 | 6.0 ± 0.2 | 4.7 ± 0.2 | 10.7 ± 0.2 | 33.9 |
| 20 | 2.5 | 90.5 ± 0.5 | 100.0 | 6.0 ± 0.2 | 6.6 ± 0.1 | 12.6 ± 0.2 | 29.8 |

[a] $n = 3$.
[b] $n = 1$.

Table 3. Quality control results of six batches freeze dried ASD containing elacridar hydrochloride–PVPK30–SDS (1:6:1, w/w/w).

| Batch | Content (%)[a] | Content (mg/g)[a] | Chemical purity (%)[a] | Residual DMSO (%, w/w)[a] | Residual water (%, w/w)[a] | Amorphous (XRD)[b] | $T_g$ (°C)[b] | Absolute yield (g)[b] | Yield efficiency (%)[b] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 88.1 ± 0.5 | 110.2 ± 0.6 | 100.0 | 9.1 ± 0.3 | 4.2 ± 0.1 | Yes | 25.9 | 33.1 | 103.1 |
| 2 | 89.2 ± 0.3 | 111.5 ± 0.4 | 100.0 | 7.9 ± 0.2 | 4.6 ± 0.1 | Yes | 33.3 | 32.9 | 102.8 |
| 3 | 88.9 ± 0.3 | 111.2 ± 0.4 | 100.0 | 8.6 ± 0.3 | 4.0 ± 0.1 | Yes | 29.7 | 31.6 | 98.4 |
| 4 | 93.8 ± 0.5 | 117.2 ± 0.6 | 100.0 | 8.3 ± 0.1 | 3.5 ± 0.0 | Yes | ND | 32.4 | 100.7 |
| 5 | 93.9 ± 0.4 | 117.4 ± 0.5 | 100.0 | 8.6 ± 0.3 | 3.4 ± 0.1 | Yes | ND | 32.6 | 101.4 |
| 6 | 91.9 ± 2.8 | 114.9 ± 3.5 | 100.0 | 8.8 ± 0.2 | 3.3 ± 0.1 | Yes | ND | 32.9 | 102.2 |

ND: not determined.
[a] $n = 3$.
[b] $n = 1$.

temperature (~190 °C) and could not be dried. Elacridar hydrochloride was practically insoluble in methanol, ethanol, acetone, isopropanol, ethylacetate and very slightly soluble in dichloromethane. Spray dried ASD containing elacridar hydrochloride–PVPK30–SDS (1:6:1, w/w/w) from dichloromethane was not fully amorphous and resulted in only 53% dissolution and precipitated already after 10 min. This shows that dissolution enhancement from spray dried ASD was considerably worse than that of freeze dried ASD. Besides, spray drying was unpractical because 10 times more solvent was required to produce the same amount of ASD as with freeze drying. Dichloromethane is a far more toxic solvent than DMSO (dichloromethane belongs to Class 2, not more than 6.0 mg/day and <600 ppm/day and $LD_{50,oral}$ is 1.6 g/kg)[38].

To conclude, modifying the production process did not result in improved pharmaceutical features of the ASD, thus the freeze drying method was retained at a total solid concentration of 80 mg/mL (elacridar hydrochloride 10 mg/mL). As the ASD was hygroscopic with a low $T_g$ it required storage in an environment where further water adsorption was minimized in order to avoid further decrease of the $T_g$. Therefore, the powder was stored in airtight-sealed primary package material in a desiccator.

### Evaluation

Results of six batches freeze dried ASD are shown in Table 3. The average content was 91.0 ± 2.6% (113.7 ± 3.2 mg/g elacridar hydrochloride), the average chemical purity was 100.0 ± 0.0%, the average residual DMSO content was 8.6 ± 0.4%, the average residual water content was 3.8 ± 0.5%, all were amorphous and the average $T_g$ was 29.6 ± 3.7 °C. These results were similar to the results discussed in section "Step 3: Characterization", so the production method was reproducible. The average yield efficiency was 101.4 ± 1.7%. Yield efficiency exceeded 100% because of residual DMSO/water. The average absolute yield was 32.6 ± 0.5 g. Knowing that the average content in the ASD is 113.7 mg/g (Table 3) means that one batch of 32.6 g contains 3706.2 mg elacridar hydrochloride. Therefore, one batch supplies three doses of 1000 mg elacridar as hydrochloride salt. For a proof-of-concept study involving single dose administration of 1000 mg to 6–12 volunteers, it means that 2–4 production batches are required.

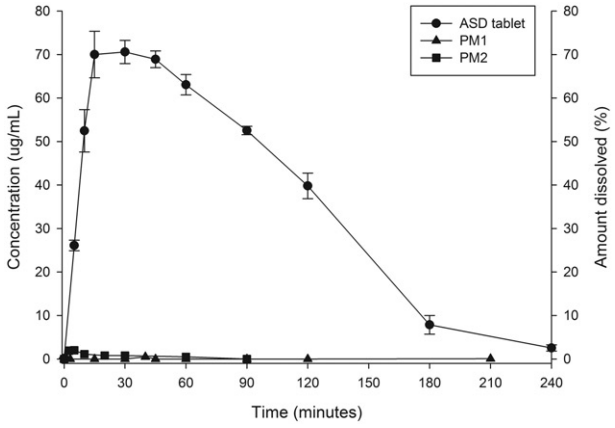


Figure 7. The dissolution of ASD tablets compared to physical mixture formulations (PM1 and PM2, Table 1).

### Step 4: dosage form development and evaluation

The 90 times increased dissolution from ASD compared to crystalline elacridar hydrochloride implied the ASD might considerably increase the oral bioavailability. Based on this, the target dose strength of the final drug product was 10–100 mg. The ASD powder was highly porous and therefore it was not possible to fill one capsule with ASD powder equivalent to 25 mg elacridar hydrochloride. Compaction of ASD powder resulted in vitreous tablets, thus a diluent was required. By diluting the ASD powder with lactose (≥60%), it was possible to make tablets of 25 mg elacridar hydrochloride with a resistance to crushing 60–150 N, a mass ~750 mg and dimensions of 16 × 8.5 × 6.9 mm (length, width and thickness respectively). The tablet formulation contained granulated lactose–ASD–croscarmellose–colloidal silicon dioxide–magnesium stearate (63:30:5:1:1, w/w/w/w/w). Tablets with higher doses with this powder mixture were not considered because they were unacceptably large. Figure 7 shows that the dissolution from the crystalline physical mixture (PM1) was 1% and the dissolution of a physical mixture formulation containing freeze dried elacridar hydrochloride (PM2) was 2%. The dissolution from the ASD tablet was ~70%. These results show ASD tablets considerably increase the dissolution of elacridar hydrochloride.

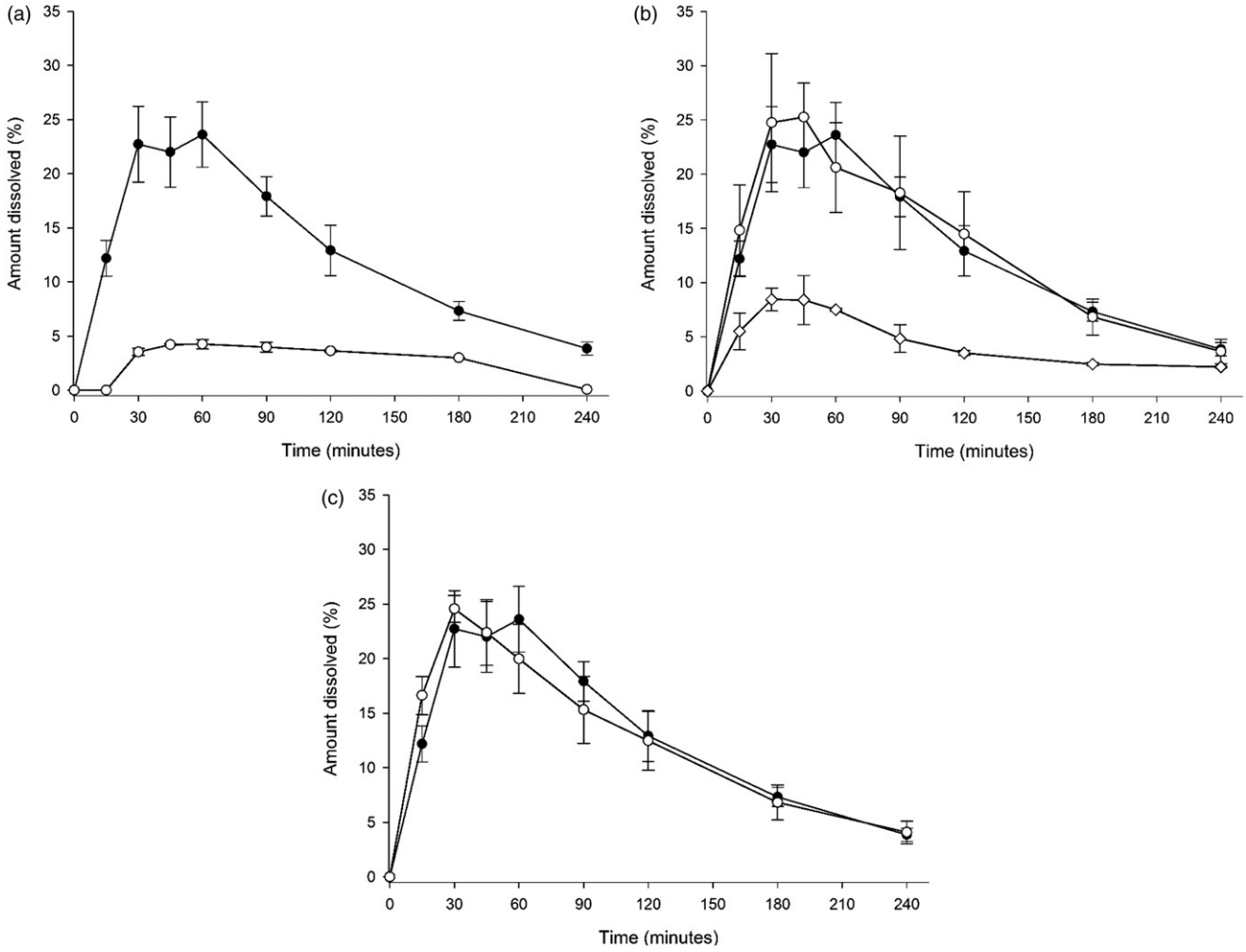


Figure 8. Dissolution in the USP type II paddle apparatus of two batches ASD tablets stored at (a) 15–25 °C 60% RH immediately after production (●●●) and after three months (○○○). (b) 2–8 °C dark immediately after production (●●●), after three months (○○○) and after six months (◇◇◇) (c) − 20 °C immediately after production (●●●) and after 12 months (○○○).

Table 4. The stability of ASD tablets containing 25 mg elacridar hydrochloride.

| Time (months) | Storage condition | Appearance | Mass increase (%) | Resistance to crushing (N) | Content relative to label claim (%) | Difference factor ($f_1$) | Similarity factor ($f_2$) |
|---|---|---|---|---|---|---|---|
| 0 | −20 °C | Intact | – | 101 ± 19 | 99.2 ± 0.4 | – | – |
| 3 | +15–25 °C/60%RH | Elastic | +5.6 | 487 ± 1 | 98.8 ± 7.1 | 85 | 38 |
| 3 | +2–8 °C | Elastic | +5.1 | 487 ± 1 | 101.9 ± 5.0 | 14 | 77 |
| 6 | +2–8 °C | Elastic | +8.4 | 487 ± 1 | 98.6 ± 4.7 | 63 | 44 |
| 3 | −20 °C | Intact | +0.9 | 109 ± 29 | 99.4 ± 4.1 | 10 | 79 |
| 6 | −20 °C | Intact | +1.4 | 117 ± 25 | 99.4 ± 4.4 | 8 | 85 |
| 9 | −20 °C | Intact | +2.6 | 89 ± 11 | 100.3 ± 0.9 | 12 | 76 |
| 12 | −20 °C | Intact | +2.5 | 110 ± 41 | 101.2 ± 5.5 | 13 | 75 |

Dissolution of ASD tablets immediately after production in the USP type II dissolution tester was 23.6 ± 3.0% (see Figure 8(a)–(c), ●●●). The dissolution from crystalline physical mixture (formulation PM1) was 1.4 ± 0.1% and the dissolution of pure drug powder was 0% (data not shown). The ASD tablet thus resulted in significantly enhanced dissolution compared to a crystalline physical mixture which means the ASD tablet is feasible for dissolution enhancement and provides a time window for increased *in vivo* absorption.

### *Step 5: stability*

The critical quality attributes (CQAs) for the chemical stability were drug content 90–110% relative to label claim and the chemical purity ≥98%. CQAs for the physical stability of ASD tablets during storage were appearance, mass increase <3%, resistance to crushing 60–150 N, and dissolution similarity relative to tablets at 0 months expressed as $f_1$<15% and $f_2$>50%[27].

Results regarding the appearance, mass increase, resistance to crushing, content and dissolution similarity ($f_1$ and $f_2$) are shown in Table 4. The dissolution profiles of ASD tablets stored for one year at +15 to 25 °C/60% RH, +2 to 8 °C or −20 °C are shown in Figures 8(a)–(c), respectively. The content and chemical purity were compliant with CQAs at all storage conditions during the entire study period, thus the ASD tablets containing elacridar hydrochloride were chemically stable.

Tablets stored at +15 to 25 °C/60% RH or +2 to 8 °C appeared elastic, were not resistant to crushing, their mass was considerably

increased with a reduced dissolution. Tablets stored for 12 months at −20 °C appeared intact, resistant to crushing, mass increase <3% and equivalent dissolution. The poor stability at +2 to 8 °C and +15 to 25 °C/60% RH was a consequence of the $T_g$ of the ASD (29.6 °C). $T_g$ was close to these two storage conditions and therefore kinetic energy in the ASD was sufficient to induce glass transition from the hard ("intact") state into the rubbery ("elastic") state. The consequence of this was that tablets did not crush anymore, instead they were distorted ("chewing gum-like") during the tensile strength measurement. The mass increase at +15 to 25 °C/60% RH and +2 to 8 °C was caused by moist adsorption. Tablets stored at −20 °C remained intact during storage and were crushed with the tensile strength tester at similar forces as tablets initially after production (Table 4). This is because ASD tablets at −20 °C did not have sufficient kinetic energy to undergo glass transition and therefore remained intact. Also, at −20 °C there was considerably less moist adsorption and therefore tablet mass did not increase significantly.

To conclude, ASD tablets were physically stable for at least 12 months when stored at −20 °C. For proof-of-concept studies involving a single dose administration, this was considered manageable as according to our knowledge there is currently no other GMP-compliant formulation with elacridar available. For clinical applications with daily oral dosing further research for a new formulation is required.

## Conclusions

This paper discussed the pharmaceutical development of an ASD tablet containing 25 mg elacridar hydrochloride for proof-of-concept clinical trials involving a single dose administration up to 1000 mg. The dissolution from 24 different ASDs (produced by freeze drying) was compared to that of crystalline elacridar hydrochloride. Freeze dried ASD containing elacridar hydrochloride–PVPK30–SDS (1:6:1, w/w/w) resulted in a 90 times increased dissolution and was fully amorphous. Subsequently, tablets with the ASD were developed and resulted in a considerably increased dissolution compared to a crystalline physical powder mixture. Content, chemical purity and dissolution were stable for at least 12 months when stored at −20 °C. This makes the ASD tablet feasible for proof-of-concept clinical trials and tablets were handled according to conclusions of this paper (EudraCT, registration number 2013-001131-47).

## Novelty statement

Elacridar is a promising drug for increasing the brain penetration and bioavailability of many anticancer agents. A tablet containing elacridar hydrochloride was previously used in clinical trials but the absorption was poor and unpredictable because of the drug's low solubility in water. This research paper describes the pharmaceutical development of a novel tablet formulation of elacridar hydrochloride suitable for proof-of-concept clinical studies, in which an amorphous solid dispersion was used to increase the aqueous solubility.

## Disclosure statement

The authors report no conflicts of interest.

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