Pharmaceutical Development of a Lyophilised Dosage Form for the Investigational Anticancer Agent Imexon Using Dimethyl Sulfoxide as Solubilising and Stabilising Agent

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ABSTRACT: Imexon is a member of the class of 2-cyanoaziridine derivatives, which have been of interest as immunomodulators and anticancer agents since the late 1970s. For the scheduled phase I clinical trials a stable, sterile, injectable pharmaceutical dosage form containing 100 mg Imexon was required. Despite adequate solubility, its instability in aqueous media seriously hampered the pharmaceutical development of Imexon. In this study we describe the successful use of the organic solvent dimethyl sulfoxide (DMSO) as a formulation vehicle for Imexon. DMSO is shown to provide the stability required for Imexon during manufacturing and to be a suitable vehicle for lyophilisation, which was employed to gain sufficient shelf-life for the final product. The relatively low vapour pressure of DMSO, which would theoretically result in extremely slow sublimation during lyophilisation, was shown not to limit the successful lyophilisation of Imexon from DMSO at a concentration of 25 mg/mL. The lyophilisation cycle developed for Imexon resulted in residual DMSO contents of $4.6 \pm 0.6\%$ in the lyophilised product, limiting the amount of DMSO administered to the patient to well below the 50 mg/day acceptable in pharmaceutical products as stated in ICH guidelines. Imexon 100 mg/vial lyophilised product was shown stable for at least 12 months of storage at $-20^{\circ}C$ and $+5 \pm 3^{\circ}C$ in the dark. J Pharm Sci 94:1101–1114, 2005

Keywords: Imexon; chemical stability; DMSO; lyophilisation; calorimetry (DSC); solubility; formulation

Imexon (4-imino-1,3-diazabicyclo[3,1,0]-hexan-2-one; Fig. 1A) is a member of the class of 2-cyanoaziridine derivatives, which have been of interest as immunomodulators and anticancer agents since the late 1970s.[1] Recent *in vitro* research demonstrated high activity against lymphoid malignancies such as multiple myeloma.[2] The proposed mechanism of action for Imexon-induced cytotoxicity suggests a sequential pathway initiated by binding of the aziridine moiety of Imexon to sulfhydryl groups of cysteine residues resulting in decreased levels of cellular thiols in myeloma cells, which compromises the antioxidant defence systems leading to oxidative stress and the induction of apoptosis.[3,4]

For the scheduled phase I clinical trials a stable, sterile, injectable pharmaceutical dosage form containing 100 mg Imexon was required. Despite adequate solubility, its instability in aqueous media seriously hampered the pharmaceutical development of Imexon. In this study we describe

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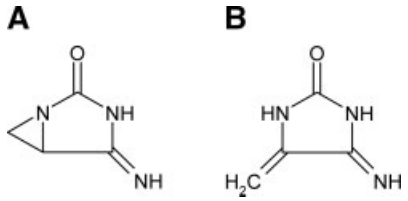

Figure 1. Chemical structures of Imexon ($C_4H_5N_3O$, Mw = 111) (A) and BM41.209 ($C_4H_5N_3O$, Mw = 111) (B).

the successful use of the organic solvent dimethyl sulfoxide (DMSO) as formulation vehicle for Imexon. DMSO is shown to provide the stability required for Imexon during manufacturing and to be a suitable vehicle for lyophilisation, which was employed to gain sufficient shelf-life for the final product. We further describe the theoretical and practical aspects of lyophilisation from DMSO, whose unfavourable low vapour pressure did not appear to limit effective sublimation.

MATERIALS AND METHODS

Chemicals

The Imexon (Mw = 111) active drug substance and its main degradation product BM41.209 (Mw = 111) were provided by Heidelberg Pharma GmbH (Ladenburg, Germany). Imexon final product was manufactured in-house (Department of Pharmacy & Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands) by freeze-drying a DMSO (BUFA, Uitgeest, The Netherlands) solution containing Imexon, polysorbate 80 (BUFA) and polyvinylpyrrolidone (PVP; Kollidon 12 PF; Brunschwig Chemie, Amsterdam, The Netherlands). Sterile Water for Injection (WfI) was obtained from B. Braun Medical (Melsungen, Germany). Hydroxypropyl-β-cyclodextrin (HPβCD) with a molar degree of substitution of 0.65 was purchased from Roquette (Kleptose HB, Lestrem, France). TRIS, *tert*-butanol, ethanol, water for chromatography, disodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany) and were of analytical grade.

All pharmaceutical excipients and primary packaging materials used in the manufacturing of Imexon final product were of European Pharmacopoeia (Ph.Eur.)[5] or United States Pharmacopoeia (USP)[6] grade and provided by the suppliers with a Certificate of Analysis. Throughout the experiments as well as manufacturing, 8 mL colourless glass vials (hydrolytic class 1 Type Fiolax-clear, Aluglas, Uithoorn, The Netherlands) and grey siliconised butyl rubber lyophilisation stoppers (Type FM157/1, Helvoet Pharma N.V., Alken, Belgium) were used. Substances and materials used in the manufacturing of Imexon final product were approved on the basis of in-house quality controls carried out according to monographs in the mentioned pharmacopoeias.

High Performance Liquid Chromatography (HPLC)

Imexon was analysed using a linear, precise, accurate, selective and stability-indicating HPLC method. The HPLC system consisted of an 1100 Series binary HPLC pump, Model G1312A (Agilent Technologies, Amstelveen, The Netherlands), a Model SpectraSERIES AS3000 automatic sample injection device, equipped with a 100 μL sample loop (Thermo Separation Products, Breda, The Netherlands), and a photodiode array detector (PDA) Model Waters™ 996 (Waters Chromatography B.V., Etten-Leur, The Netherlands). Chromatograms were processed with Chromeleon software (Dionex Corporation, Sunnyvale, CA). Separation was achieved with a Zorbax Bonus RP analytical column (150 mm × 4.6 mm I.D., particle size 5 μm, Rockland Technologies, Inc., Newport, DE), which was protected by a guard column packed with reversed-phase material (3 × 10 mm) (Chrompack, Middelburg, The Netherlands). A phosphate buffer (pH 6, 50 mM), pumped with a flow rate of 0.8 mL/min, was used. Sample volumes of 10 μL were injected and a run time of 20 min was employed. UV-detection was performed at 230 nm.

Under these conditions the chromatogram of Imexon consisted of a single peak eluting at approximately 3.5 min. Samples were diluted with phosphate buffer (pH 7.4, 20 mM) to a final concentration of 20 μg/mL and analysed immediately to prevent degradation.

Liquid Chromatography/Mass Spectrometry

The liquid chromatography (LC) system consisted of an HP1100 liquid chromatograph (Agilent Technologies) with a binary pump, autosampler and degasser. The mobile phase used for the LC/MS experiments was pure water. Other LC conditions were as described above. The eluate was led into the interface of an API 2000 triple quadrupole MS equipped with an electrospray ionisation (ESI) source (Sciex, Thornhill, ON,