ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 751–755

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

A high-performance liquid-chromatographic assay for amphotericin B in a hydrophilic colloidal paste base

J.M. Wilkinson *, C. McDonald, J.E. Parkin, V.B. Sunderland

School of Pharmacy, Curtin University of Technology, GPO Box U 1987, Perth, Western Australia 6845, Australia

Received 29 July 1997; received in revised form 3 November 1997; accepted 10 November 1997

Abstract

A stability-indicating high-performance liquid-chromatographic (HPLC) assay has been developed for amphotericin B (AmB) in a paste, containing AmB, tobramycin (or gentamicin) sulphate, colistin sulphate, liquid paraffin and Orabase®. Extraction of AmB was performed by partitioning the antibiotic between *N*,*N*-dimethylformamide (DMF) and cyclohexane, which led to precipitation of the polymeric materials and extraction of the liquid paraffin into the cyclohexane and AmB into the DMF. Analysis by HPLC of the latter layer gave a linear relationship between concentration and peak area response for the AmB over the range 5.0×10^{-4} to 7.5×10^{-3}% (w/v) ($r = 0.9995$) with a relative standard deviation of $\pm 1.46\%$ ($n = 8$). The efficiency of extraction was $100.6 \pm 2.4\%$ ($n = 5$). © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Amphotericin B analysis; Hydrophilic colloidal paste; Two-phase organic solvent extractive partitioning

1. Introduction

A mixture of amphotericin B (AmB) together with colistin sulphate and either tobramycin or gentamicin sulphate has been proposed for use as part of a regimen for the selective decontamination of the digestive tract of patients in intensive care units [1–6]. The recommended base for this antibiotic mixture is Orabase®, which consists of a 56.7% (w/w) dispersion of liquid paraffin in a matrix of pectin, gelatin, carboxymethylcellulose and polyethylene glycol. As AmB is chemically unstable [7,8], it was considered desirable to submit the paste to a stability trial to ascertain a suitable shelf-life and storage conditions based on the stability of the antibiotic. AmB is a heptaene macrolide antifungal antibiotic and is available in two grades: Type I, which is for parenteral use and contains 5% or less of amphotericin A (AmA), a co-fermented tetraene that is less active than AmB; and Type II, which is restricted to topical use and contains 15% or less of AmA [9]. The grade used for this formulation is Type II. The precise route of the decomposition of this antibiotic has never been fully elucidated [7,8,10]. However, a number of methods utilising high-performance liquid chromatography (HPLC) have been reported for AmB which separate the AmB from its decomposition products and the minor component AmA [9–13].

* Corresponding author. Fax: +61 8 92662769; e-mail: iwilkin1@info.curtin.edu.au

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PII S0731-7085(97)00250-1

A prerequisite for the assessment of the stability of AmB in the Orabase® formulation was to devise a method for selective quantitative extraction of the antibiotic from the paste matrix. This paper reports a novel extractive procedure utilising partitioning of the AmB between dimethylformamide (DMF) and cyclohexane such that the polymeric materials are precipitated, liquid paraffin is extracted into the cyclohexane layer and the AmB is made available as a clean solution in the DMF layer for analysis by an established chromatographic method [14].

2. Experimental

2.1. Materials

Orabase® protective paste (E.R. Squibb, New Zealand) consists of 16.7% (w/w) each of gelatin, pectin, and carmellose sodium in Plastibase®, a plasticised hydrocarbon gel base consisting of polyethylene glycol and liquid paraffin. AmB (E.R. Squibb, Australia), gentamicin sulphate (Roussel UCLAF, Australia), tobramycin sulphate (Roussel UCLAF, France) and colistin sulphate (Parke Davis, Australia) were used as supplied. Disodium ethylenediaminetetraacetic acid (EDTA; Ajax Chemicals, Australia) was analytical reagent grade, DMF (Ajax Chemicals) and cyclohexane (Ajax Chemicals) were LR grade, and the methanol and acetonitrile (BDH Chemicals Australia, Australia) were HPLC grade.

2.2. Preparation of paste

The paste was prepared by triturating AmB, tobramycin sulphate (or gentamicin sulphate) and colistin sulphate (2.0% (w/w) of each) with 10% (w/w) liquid paraffin and incorporating this material into 84.0% (w/w) Orabase®. The resulting product nominally contains 57.6% (w/w) liquid paraffin, 14.0% (w/w) gelatin, pectin and carmellose sodium and 2.8% (w/w) polyethylene glycol.

2.3. Chromatographic equipment and conditions

The liquid chromatograph consisted of a Model 501 pump (Waters Associates, Milford, MA, USA), Rheodyne Model 7125 loop injector (Cotati, CA, USA), Model 484 variable-wavelength absorbance detector (Waters Associates) and Model 3396A integrating recorder (Hewlett-Packard, Palo Alto, CA, USA), together with a C_{18} guard column (Direct Connect®; Alltech Associates, (Australia) and a μBondapak C_{18} column 10 µm particle size, 25 cm $\times$ 4.6 mm i.d. (Waters Associates). The mobile phase was methanol–acetonitrile–0.0025 M EDTA in water (50:35:20) at a flow rate of 1.6 ml min^{-1}. The injection volume was 20 µl and the monitoring wavelength 405 nm.

2.4. Extraction procedure

The extraction of the AmB was performed in 50 ml glass tubes with plastic caps and pertetrafluoroethylene wads by the following method.

The contents of a container were mixed to ensure homogeneity, and approximately 1 g of the antibiotic formulation, accurately weighed, was mixed with DMF (20 ml) and cyclohexane (15 ml) and shaken thoroughly until the AmB was dissolved. Following centrifugation the upper cyclohexane–liquid paraffin layer was removed by aspiration and discarded and a sample of the DMF layer (1 ml) was diluted to 20 ml with mobile phase and subjected to analysis by HPLC.

2.5. Validation of analytical methods

A stock solution of AmB (2.0×10^{-1}%, w/v) was prepared in and diluted with DMF to produce a series of solutions in the range of 1.0×10^{-2} to 1.5×10^{-1}% (w/v) of the AmB. These solutions (20 ml) were added to glass tubes together with Orabase® (1 g) to produce solutions calculated to contain the equivalent of 0.2–3.0% (w/w) of AmB in the Orabase® formulation. Cyclohexane (15 ml) was then added and the resulting solutions were then submitted to the extraction procedure. The relative standard deviation (R.S.D.) of the assay was assessed by eight replicates at a concentration of 5.0×10^{-3}% (w/

v), equivalent to 2.0% (w/w) of AmB in the original formulation. The specificity of the assay was evaluated by preparing a sample of the antibiotic paste with 2.0% (w/w) colistin sulphate and 2.0% (w/w) of either tobramycin or gentamicin sulphate in Orabase®, but from which the AmB was omitted, and submitting this to analysis.

To confirm that the degradation products did not interfere, a sample containing 1×10^{-1}% (w/v) of AmB in DMF was irradiated in the presence of air in a Chromato-Vue® light cabinet at 302 nm for 4 weeks and the sample was then submitted to analysis.

2.6. Efficiency of extraction

The efficiency of the extractive procedure was evaluated by adding a solution of AmB (1×10^{-1}%, w/v) in DMF (20 ml) to a tube containing 0.96 g of the formulation base (containing Orabase®–liquid paraffin) and submitting this to analysis. The other antibiotics were not included in the formulation. Upon shaking and centrifugation, the levels of the meniscus of the DMF and the cyclohexane–liquid paraffin layers were marked by scoring and the liquid phases were then carefully discarded by decantation, taking care to retain the precipitate. The volumes of the two organic phases were then determined by titration with water to the marks on the tube.

3. Results and discussion

Due to the poor solubility characteristics of AmB and the complexity of the formulation of Orabase®, containing AmB, colistin sulphate and tobramycin (or gentamicin) sulphate, a novel extraction procedure has been developed using two organic solvents. The use of DMF and cyclohexane enabled the AmB to be solubilised into the DMF, with the hydrocolloid components and other antibiotics being precipitated and the liquid paraffin being extracted into the upper cyclohexane layer. Possibly this is the first time that such a solvent system has been employed. The resulting solution in DMF, upon dilution with mobile phase, afforded a chromatographic peak equivalent to a simple solution of AmB in DMF (Fig. 1A).

Because of the complexity of the extractive procedure, no suitable internal standard could be found with similar extractive characteristics to that of AmB, and the analysis was performed using AmB as an external standard in the DMF extraction solvent and performing the assay on blank paste. The method was validated by submitting to the extraction procedure, followed by HPLC analysis, solutions of AmB in DMF to which were added the appropriate amounts of the other antibiotics, liquid paraffin and Orabase®. The assay was found to give a linear relationship between final concentration and peak area over the range 5.00×10^{-4} to 7.5×10^{-3}% (w/v) (equivalent to 0.2–3.0%, w/w, AmB in the original formulation). The relationship of AmB versus peak area was linear and passed through the origin ($r = 0.9995$, for $n = 6$), the equation for the relationship being:

$$\text{Peak area response} = 3.89 \times 10^{9} \times \text{AmB concentration } (\%, \text{w/v}) - 0.27 \times 10^{6}$$

The R.S.D. was $\pm 1.46\%$ ($n = 8$), at a concentration of 5.00×10^{-3}% (w/v) of AmB (equivalent to a concentration of 2.0%, w/w, of AmB in the paste). The assay showed no interference from the paste base or other antibiotics present in the formulation (Fig. 1B–D) and a photodegraded sample of the drug displayed no peaks at the retention time of the AmB.

The efficiency of extraction was determined by analysis of AmB in the DMF layer with appropriate adjustment in phase volumes. As expected with a two-phase organic system, the phase volumes changed and were found to be 23.73 ml (initially 20 ml) for DMF and 10.73 ml (initially 15 ml) for the cyclohexane at equilibrium. From the concentration of AmB in the DMF layer it could be calculated that the efficiency of extraction of the antibiotic was $100.6 \pm 2.4\%$ ($n = 5$). When the assay was applied to the formulation in which the AmB was incorporated with the other antibiotics, it was found that the assay afforded $103.4 \pm 2.7\%$ ($n = 3$) of the stated content of

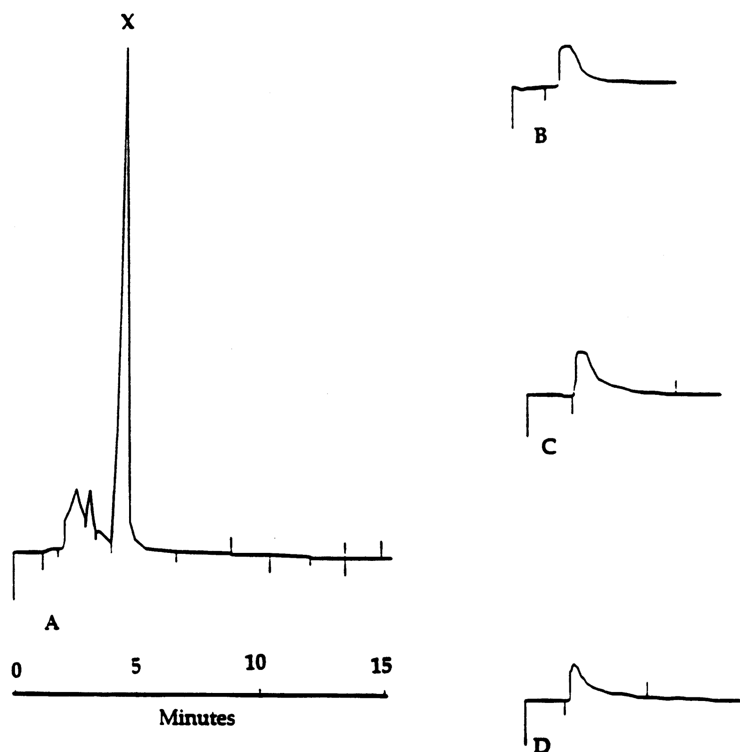

Fig. 1. Representative chromatograms of extracts of the paste. X indicates AmB. Detection wavelength 405 nm, chart speed 0.5 cm min^{-1}, 0.256 AUFS. (A) Paste containing 2% (w/w) AmB; (B) paste containing gentamicin sulphate and colistin sulphate 2% (w/w) but containing no AmB; (C) paste containing tobramycin sulphate and colistin sulphate 2% (w/w) but containing no AmB; (D) blank paste containing no antibiotics.

AmB. This confirms that there was no loss of AmB upon analytical work-up and that the approach of preparing standards of AmB in DMF was valid for the assay of the antibiotic in the paste formulation.

It was therefore concluded that the AmB was quantitatively extracted into the DMF layer, free of all other interfering substances and that there were no losses into either the cyclohexane layer or by adsorption to the precipitated components. The liquid paraffin was quantitatively extracted into the cyclohexane layer and discarded, preventing contamination of the HPLC column from this source.

The AmB in the DMF layer was sufficiently clean such that no column deterioration was encountered upon repeated injection of samples. This analytical method has been applied to a stability trial of AmB in this formulation, the results of which will be reported elsewhere.

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