Quantitative Determination of Hexamidine Isethionate in Pharmaceutical Preparations by High-Performance Liquid Chromatography

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Abstract □ Hexamidine isethionate in various pharmaceutical formulations was analyzed quantitatively by high-performance liquid chromatography. The method is rapid, accurate, and precise. Excellent results were obtained from four commercial bases.

Keyphrases □ Hexamidine isethionate—quantitative determination in pharmaceutical preparations, high-performance liquid chromatography □ High-performance liquid chromatography—quantitative determination of hexamidine isethionate, pharmaceutical preparations □ Preparations, pharmaceutical—quantitative determination of hexamidine isethionate by high-performance liquid chromatography

Hexamidine isethionate [4,4′-(hexamethylenedioxy)-dibenzamidine bis(2-hydroxyethanesulfonate)], used as a topical antiseptic in pharmaceutical products, belongs to a group of compounds (aromatic diamidines) which have good antibacterial and antifungal properties (1). Diamidines can be identified in their pure form microscopically (2) and quantified colorimetrically in pharmaceutical preparations using glyoxal sodium bisulfite solution (3). The latter method is nonspecific and subject to interferences. This fact has prompted more recent workers to investigate the use of spectrophotometric (4), GLC (5, 6), and high-performance liquid chromatographic (HPLC) (7) techniques.

The purpose of this study was to develop a rapid HPLC method which would be suitable for the analysis of hexamidine isethionate (I) in pharmaceutical preparations. Validation data are presented for the analysis of a topical cream, and the methodology was found to be applicable to ointments and eyewash solutions.

EXPERIMENTAL

Reagents and Chemicals—The methanol[1] and water[1] used were HPLC grade and the glacial acetic acid[1], chloroform[1], and ether[1] were analytical reagent grade. Hexamidine isethionate[2] was used as received.

Apparatus—The high-pressure solvent pump[3] was connected to a fixed-wavelength detector[4], a fixed-volume injection valve[5], and a recording integrator[6]. The polar column[7] (30 cm × 3.9-mm i.d.) consisted of a monomolecular layer of cyanotrichlorosilane permanently bonded by silicone–carbon bonds to microparticulate silica.

The mobile phase consisted of water–methanol–glacial acetic acid (4:2:1, v/v/v) which was deaerated by vacuum filtration before use. The temperature was ambient, and the flow rate was 1.0 ml/min. The detector sensitivity was 0.02 AUFS (254 nm), the recorder/integrator attenuation was 8, and the chart speed was 0.5 cm/min.

[1] B. D. H. Chemicals, Poole, Dorset, England.
[2] Rhône-Poulenc, Paris, France.
[3] Waters 6000A pump, Waters Associates, Hartford, Cheshire, England.
[4] Waters 440 UV detector, Waters Associates, Hartford, Cheshire, England.
[5] Rheodyne 7125 injection valve, Rheodyne Inc., Berkeley, Calif.
[6] Model 3380A recorder/integrator, Hewlett-Packard, Winnersh, Berkshire, England.
[7] μBondapak-CN, Waters Associates, Hartford, Cheshire, England.

Hexamidine Isethionate Standard Solutions—Standard samples of I in the range of 0–75 mg were accurately weighed into 1000-ml volumetric flasks and diluted to volume with mobile phase. After mixing to dissolve the standards, 10.0-ml aliquots were diluted to 100.0 ml in volumetric flasks using the mobile phase. Twenty microliters of each solution was injected into the HPLC.

Preparation of Samples—*Creams*—Samples of cream (0.5 g) equivalent to 0.5 mg of I were accurately weighed into a 50-ml centrifuge tube and shaken with 40 ml of mobile phase. After centrifugation the supernatant liquid was transferred to a 250-ml separatory funnel, and the process was repeated with a second 40-ml aliquot of mobile phase. The combined extracts were then washed with two 40-ml portions of chloroform, transferred to a 100-ml volumetric flask, and diluted to volume with mobile phase.

Ointments—Samples (0.5 g) equivalent to 0.5 mg of I were first dissolved in 50 ml of hexane and then extracted with two 40-ml aliquots of mobile phase. Treatment was as described above for creams.

Eyewash Solutions—Eyewash solutions were diluted to 200 ml (20-fold) with water, and 20.0 ml of this solution (equivalent to 1 mg of I) was then extracted with three 20-ml aliquots of ether. The ether extracts were discarded, and the aqueous phase was transferred to a 200-ml volumetric flask and diluted to volume with mobile phase. Twenty-microliter aliquots of each sample solution were injected into the HPLC.

Calculations—The peak heights from the injections of the standard hexamidine isethionate solutions were measured manually and plotted as a function of concentration. Since the calibration curve indicated that peak heights were directly related to concentrations (0–0.0075, mg/ml) of I, sample results were calculated by:

$$\frac{(Ph)_a}{(Ph)_s} \times \frac{C}{1000} \times D \times 100 = \%\ \text{w/w (or w/v) of I}$$

where $(Ph)_a$ and $(Ph)_s$ are the peak heights of I in the assay and standard solution chromatograms, respectively, C is the concentration of standard solution of I in mg/ml, and D is the dilution factor.

Reproducibility and Accuracy—Replicate analyses (n = 6) were carried out on a single batch of cream formulated to contain 0.1% w/w of I to test reproducibility of the assay. Known weights of hexamidine isethionate representing levels between 50 and 150% of label claim were added to five 0.5-g samples of placebo cream to check the assay accuracy. After mixing, samples were prepared as described above and injected into the HPLC.

RESULTS AND DISCUSSION

The described method was validated using a topical cream developed in-house. A typical cream sample chromatogram is shown in Fig. 1. Hexamidine isethionate elutes as a sharp peak in <8 min. The calibration curve of the concentration of I *versus* peak height was found to be linear over 0–0.0075 mg/ml and passed very close to the origin (slope = 1.694×10^5, intecept = −1.53, and r = 0.9996). Recovery data from placebo samples with added I indicate that the procedure is quantitative for I over the range 0.25–0.75 mg/0.5 g (Table I). This range corresponds to 50–150% of label for the typical (0.1%, w/w) cream formulations (0.5 mg/0.5 g). The reproducibility of the method was found to be good at 0.9% *RSD* (Table II).

A placebo sample without I when carried through the procedure showed the absence of interferences from formulation excipients, although a small peak which elutes immediately after I could *potentially* interfere should column efficiency deteriorate. However, in this event the positive bias introduced would be small (<2%).

During sample preparation of creams and ointments, it was necessary to extract with chloroform to overcome severe chromatographic inter-

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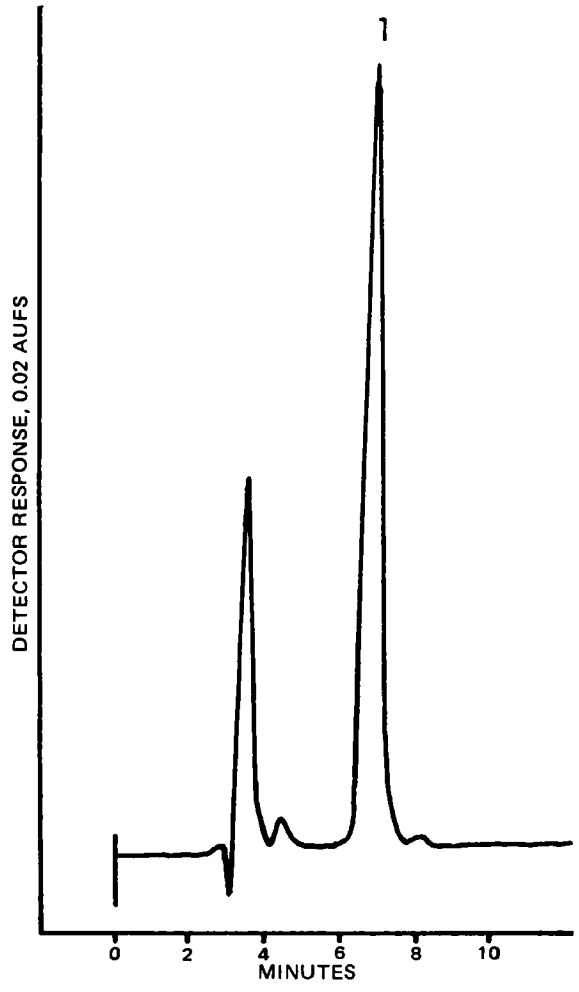

Figure 1—*High-performance liquid chromatogram of a topical oil-in-water cream preparation. Peak 1 is hexamidine isethionate. Other peaks are unidentified components of the cream base.*

ferences. Eyewash solutions required extraction with ether to remove the methylparaben and propylparaben which were included in these formulations. The relatively high concentration of acetic acid used in the mobile phase was essential for adequate resolution and rapid analysis of I. Column stability was satisfactory throughout the experiments, although it was necessary to thoroughly cleanse the chromatograph and column of the corrosive mobile phase between analyses.

The results for the analyses of I in three different dosage forms is given in Table III. In general, results were very close to the label claim. The low result found with ointment 2 could have been due to incomplete extraction from the base.

Table I—Accuracy of the HPLC Assay of Hexamidine Isethionate in a Topical Cream

Added, mg	Found, mg	Recovery, %
0.2525	0.2579	102.1
0.3788	0.3739	98.7
0.5050	0.5043	99.9
0.6313	0.6318	100.1
0.7575	0.7477	98.7

Table II—Reproducibility of the HPLC Assay of Hexamidine Isethionate in a Topical Cream

Sample	Percent w/w
1	0.101
2	0.099
3	0.100
4	0.101
5	0.100
6	0.099
Mean	0.100
SD	8.94×10^{-4}
RSD[a], %	0.894

[a] Relative standard deviation derived from 100 × *SD*/Mean.

Table III—Assay Results for Hexamidine Isethionate in Commercial Dosage Forms

Sample	Claim per Dosage Form, mg/100 g	Hexamidine Isethionate, % of Label Claim
Cream	100	99.9
Ointment 1	100	101.5
Ointment 2	100	83.0
Eyewash solution	100	100.5

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