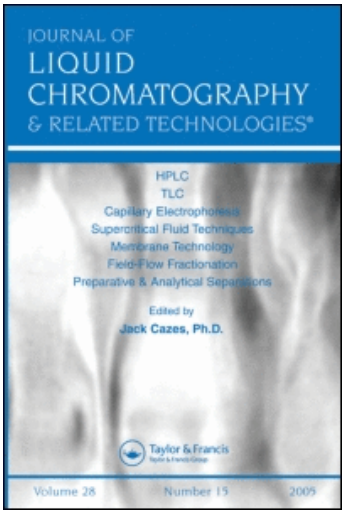

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HPLC ANALYSIS OF METHOXAMINE IN RABBIT PLASMA AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid, selective and simple high performance liquid chromatographic (HPLC) assay for methoxamine HCl has been developed. The analytical procedure involved the use of internal standardization method (l-norephedrine). It has been applied for the qualitative and quantitative determination of methoxamine HCl in rabbit plasma and in pharmaceutical formulations using an adsorption column in an isocratic mode, with resulting relative standard deviations of 1.7% and 3.3%, respectively. The applicability of the assay procedure to pharmacokinetic studies was demonstrated. Detection limits were as low as 15ng for a 30 ul injection and the determination time was less than 6 minutes.

INTRODUCTION

Methoxamine HCl is a sympathomimetic amine used for its vasopressor effect to prevent a fall in blood pressure during spinal anesthesia (1,2). Previous methods for the analysis of this drug are very scarce. Determination of methoxamine has been largely depending on long and tedious procedures (3) lacking simplicity and precision. These procedures involved long

extraction process followed by preparative TLC and final spectrophotometric determination of the eluted methoxamine HCl.

Therefore, it appeared that there was a need for a method of analysis of methoxamine HCl, in both dosage forms and biological fluids, that was reliable, accurate as well as sufficiently sensitive and simple. A method that satisfies these criteria is described herein.

The HPLC method involved the addition of an internal standard to increase precision and reproducibility; isocratic delivery of mobile phase (vs. gradient) to increase simplicity; no extensive work-up is used so as to increase simplicity and a short run time (6 min.) was required.

To our knowledge no other reported method to date utilized HPLC for quantitative analysis of methoxamine HCl. However, a qualitative HPLC was recently suggested (4) for screening 462 basic compounds (including methoxamine) employing a non-aqueous ionic eluent system.

MATERIAL AND METHODS

Instruments

The HPLC system consisted of a Waters Modular System (Waters Asocc. Inc., Milford, Mass., U.S.A.) fitted with a model M-45 solvent delivery system attached to an automated controller model 680; a model U6K injector with a 2-ml sample loop, a Lambda-Max model 481 LC spectrophotometer and a data module model 730. Chromatography was carried out on a stainless steel column (30 cm x 3.9 mm I.D.) packed with uPorasil-10 um.

Chromatographic Operating Conditions

The mobile phase consisted of a mixture of 1,2-dichloroethane, n-propanol, acetic acid, diethylamine and water

(840 : 160 : 10 : 5: 5). The flow-rate used was 3 ml/min. and the detector was set at 290 nm, which is the wavelength of maximum absorption of methoxamine HCl (5), and 0.002 (AUFS) for sensitivity. All chromatographic analyses were performed isocratically and at ambient temperature.

Chemicals and Reagents

All chemicals were of analytical grade. Solvents were HPLC or spectro-grade and were degassed and filtered through Fluoropore filters 0.5 um x 47 mm using a Waters solvent clarification kit.

Vasoxine® ampules (Wellcome Foundation Ltd., London, England) contained 20 mg/ml methoxamine HCl, citric acid anhydrous 0.3%, sodium citrate 0.3% as a buffer and 0.1% potasium metabisulfite added as an antioxident. Authentic methoxamine HCl was a gift from Wellcome Foundation Ltd., London, England.

Preparation of Internal Standard Solutions

A stock solution of the internal standard (l-norephedrine) in methanol was prepared at a concentration of 100 mg/ml.

Preparation of Standard Solutions of Methoxamine HCl

A stock solution of methoxamine HCl (1 mg/ml) was prepared by dissolving an accurately weighed amount (100 mg) of methoxamine HCl in 100 ml of methanol. This stock solution was diluted to a concentration of 10 ug/ml with methanol and was used for preparation of the standard curves as described below.

Calibration Curve of Methoxamine HCl

Six aliquots equivalent to 5, 10, 20, 40 and 60 ug of methoxamine (using stock solution previously prepared) were transfered to 10-ml volumetric flasks. A 1.0 ml aliquot of the internal standard stock solution was added. The flasks were then brought to volume with methanol and thoroughly mixed to obtain standard solutions containing 0.5, 1, 2, 4 and 6 ug/ml of methoxamine HCl and 10 mg/ml of internal standard. A volume of 30

ul of each solution was injected in triplicates onto the column to obtain a calibration graph.

Preparation of Standard Solutions in Rabbit Plasma

Standard solutions containing 0.5, 1, 2, 4 and 6 ug/ml methoxamine HCl and 10 mg/ml internal standard in rabbit plasma (obtained from freshly drawn blood) were prepared. This is done by spiking the plasma with the appropriate amount of both methoxamine HCl and the internal standard (prepared from the stock solutions described above). The prepared solutions were extracted as described below, and used for constructing a calibration curve.

Extraction of Methoxamine HCl from Rabbit Plasma

Rabbit plasma (500 ul) containing 10 mg/ml of internal standard was alkalanized with concentrated ammonia solution (30 ul) and immediately extracted with 5 ml of ethylacetate. The mixture was vortexed for one min. and centrifuged for 5 min. at 4000 rpm. The organic layer was filtered through a Millex-SR 0.5 um filter unit and a 4 ml aliquot of the filterate was evaporated to dryness at 50°C under a stream of nitrogen. The residue was reconstituted in 400 ul of methanol and an appropriate volume (usually 30 ul) was injected in triplicates into the HPLC system.

Efficiency of Extraction Method

Rabbit plasma was spiked with the appropriate amount of both methoxamine HCl and internal standard as described before to yield concentrations of 0.5, 1, 2, 4 and 6 ug/ml methoxamine HCl and 10 mg/ml internal standard. A volume of 0.5 ml of each of these solutions was extracted as described before, and 30 ul of the extract were injected into the HPLC system. The peak-area ratio (methoxamine HCl/int. standard) obtained were then compared to those obtained from similar solutions of standard methoxamine HCl in methanol, omitting the extraction step.

Assay of Methoxamine HCl in Vasoxine® Ampules

The contents of 5 Vasoxine® ampules (each containing 20 mg/ml methoxamine HCl) were pooled and a 2 ml aliquot (40 mg) was transferred to a 250 ml volumetric flask and the volume was completed to the mark using methanol. A volume of 25 ml (containing 4 mg of methoxamine HCl) of this solution was diluted to 200 ml to yield a concentration of 20 ug/ml methoxamine HCl. From this solution a working final solution containing 20 ug/ml methoxine HCl was prepared.

A volume of 1 ml of this solution was transferred to a 10 ml volumetric flask and 1 ml of the stock solution of internal standard was added and the volume was brought to 10 ml using methanol to yield a theoretical final concentration of 2 ug and 10 mg/ml of methoxamine HCl and internal standard, respectively. An appropriate volume (usually 30 ul) of this solution was injected into the HPLC system.

Construction of Calibration Cruves

Three injections of the six standard solutions containing methoxamine HCl and l-norephedrine were used to establish linearity and response ratios. The calibration curves (Fig. 2A and 2B) were constructed by plotting the ratio of the peak area of methoxamine HCl to that of the internal standard versus the concentration of methoxamine HCl.

RESULTS AND DISCUSSION

A chromatogram of a standard mixture of methoxamine HCl and the internal standard (l-norephedrine) is given in Fig. 1(A). From this chromatogram, it can be ascertained that the chromatographic working conditions were excellent for the qualitative and quantitative determination of methoxamine HCl in injectable formulations. Methoxamine HCl showed a peak (a) having a retention

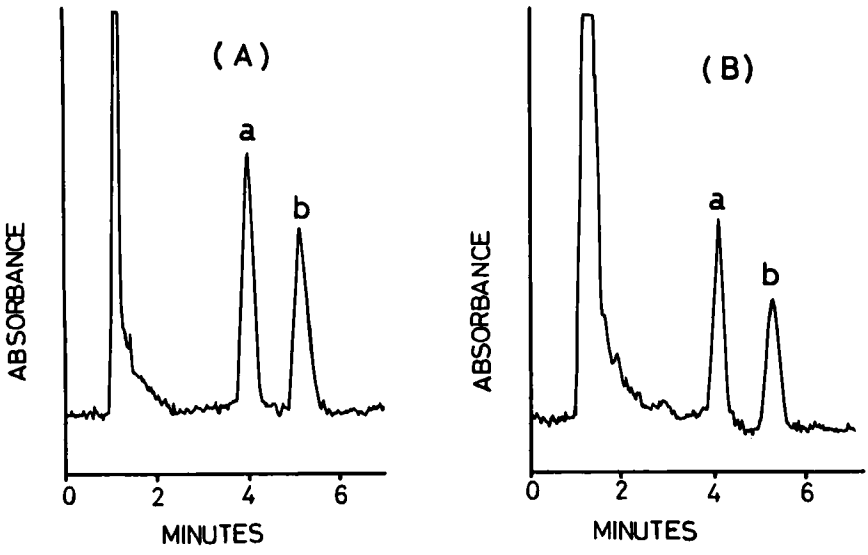

Fig.1 - HPLC chromatogram of methoxamine HCl in mobile phase (A) and in rabbit plasma (B). Peaks (a) and (b) are for methoxamine HCl and the internal standard, respectively. Conditions: uPorasil-10 um column (30 cm x 3.9 mm I.D.); mobile phase, 1,2-dichloroethane, n-propanol, acetic acid, diethylamine and water (840 : 160 : 10 : 5 : 5); flow-rate, 3 ml/min., uv monitor at 290 nm.

time (R_t) of 3.92 minutes and the internal standard showed a peak (b) with retention time (R_t) of 5.05 minutes.

Fig. 1(B) shows a chromatogram of methoxamine HCl (peak a) and the internal standard (peak b) after extraction from rabbit plasma. Both methoxamine HCl and the internal standard showed the same R_t values as those observed with standard solutions (Fig. 1A).

The selection of the internal standard was based on the fact that l-norephedrine is structurally similar to methoxamine, eluted close to it (K′ values were close) with a well resolved peak. It is also commercially available in high purity. The column capacity factors (K′) for methoxamine HCl and the internal standard were

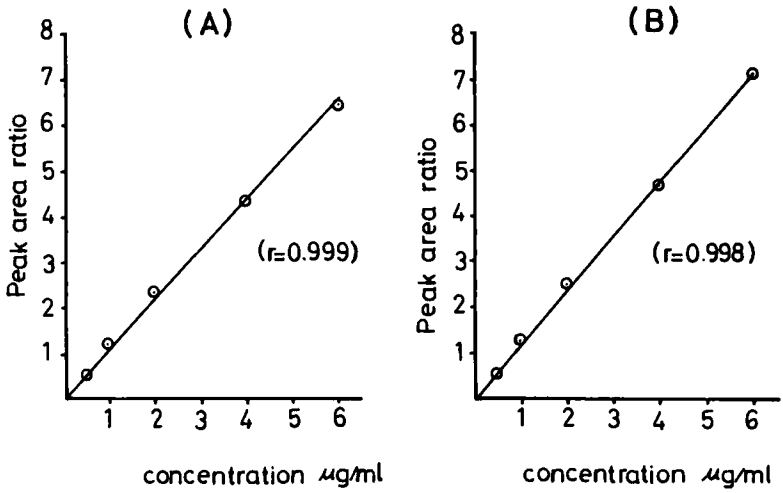

Fig.2 - Calibration graphs prepared by plotting concentration of methoxamine HCl in mobile phase (A) and in rabbit plasma (B) against the peak area ratio of methoxamine HCl/internal standard. Conditions : refer to Fig.1 and text.

2.8 and 3.9, respectively. These K' values are optimum for the quantitative determination (6).

Consequently, this HPLC technique could be employed for the analysis of methoxamine HCl both in dosage formulations and for the study of its pharmacokinetics and hence two calibration curves were constructed.

A plot of peak area ratio (methoxamine HCl/internal standard) for a series of standard solutions of methoxamine HCl in methanol is shown in Fig. 2(A). A good linearity was observed and a linear regression analysis of experimental data points showed a linear relationship with an excellent correlation coefficient (r = 0.999). This calibration curve was used for the calculation of methoxamine HCl in Vasoxine® ampules.

The HPLC analysis of Vasoxine® ampules indicated that peaks due to methoxamine HCl and the internal standard were free

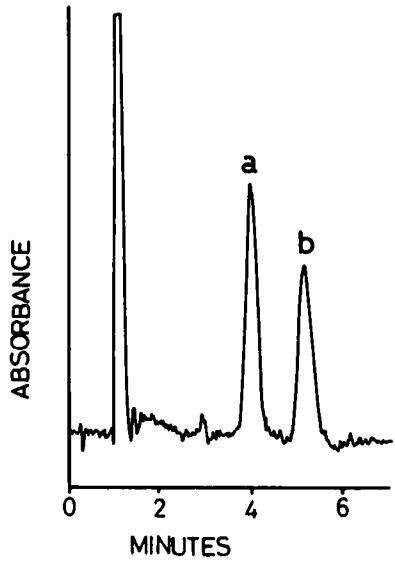

Fig.3 - HPLC of methoxamine injectable formulation (Vasoxine ®) ampules). Conditions refer to Fig. 1 and text.

from interferences from formulation excipients. The ampules were simply diluted and directly injected into the HPLC system. The chromatogram (Fig. 3) was essentially identical to that shown for the standard solutions (Fig. 1A). The assay results of six separate determinations of methoxamine HCl in Vasoxine ® ampules are summarized in Table 1. The results provided a mean potency of 99.6% with a relative standard deviation of 1.74%, indicating a high degree of precision with high sensitivity (15 ng for a 30 ul injection could be accurately quanitified).

This HPLC technique could also be employed for the determination of methoxamine HCl in plasma. A simple process was designed (see Methods) to extract methoxamine HCl from plasma and the efficiency of this extract procedure was tested. The recovery data (Table 2) showed very good efficiency with a mean and relative standard deviation of 107.2% and 3.3%, respectively.

TABLE 1

Results of the Analysis of Vasoxine® Ampules.

Number	% of label claim (20 mg/ml) methoxamine HCl
1	97.5
2	99.5
3	102.0
4	97.5
5	101.5
6	100.0
Mean (X)	99.6
*RSD,%	1.7

* Relative standard deviation.

TABLE 2

Results of The Efficiency of Extraction of Methoxamine HCl from Rabbit Plasma

Methoxamine HCl (ug/ml)	Peak Area Ratio		Recovery %
	Standard	Plasma	
0.5	0.53	0.54	103.0
1.0	1.22	1.28	104.9
2.0	2.35	2.58	110.2
4.0	4.29	4.62	107.7
6.0	6.41	7.15	111.5
Mean			107.2
*RSD ±			± 3.3

*Relative standard deviation.

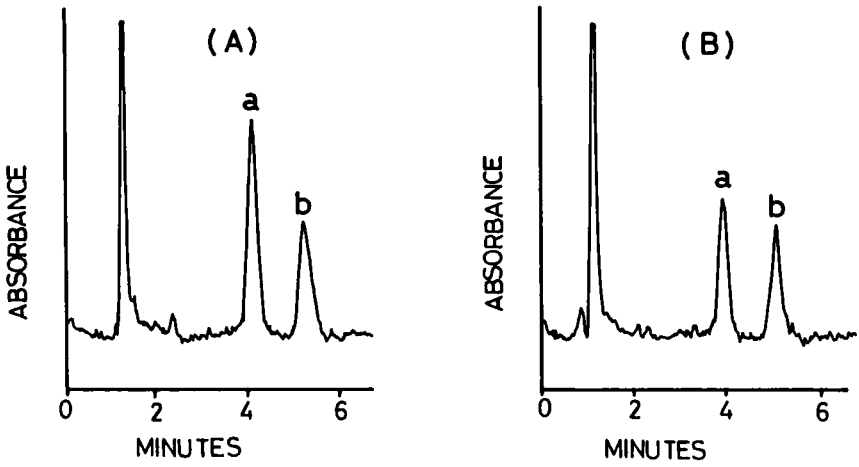

Fig.4 - HPLC chromatogram of rabbit plasma extract following the I V. administration of methoxamine HCl (10 mg) to 4.3 kg rabbit, (A) after 1 min. and (B) after 5 min. Conditions : refer to Fig. 1 and text.

Therefore, this method was used to extract methoxamine HCl from rabbit plasma and calibration curve was constructed. A plot of the peak area ratio (methoxamine HCl/int. standard) versus different concentrations of standard solutions of methoxamine HCl in rabbit plasma is shown in Fig. (2B). A straight line relationship was obtained showing excellent correlation coefficient (r=0.998). It is also observed that as low as 15 ng methoxamine HCl in plasma could be easily measured with high precision. This calibration graph could be used to monitor the methoxamine HCl level in plasma.

To test the applicability of this procedure to pharmacokinetic studies, a dose of 10 mg of methoxamine HCl was rapidly injected intravenously into a 4.3 kg male Newzealand rabbit. Blood samples were drawn at 1 and 5 min. postdose, and plasma was separated by centrifugation. The methoxamine HCl content was determined and levels of 1.4 ug/ml (Fig. 4A) and 0.8

ug/ml (Fig. 4B) were obtained for the 1 and 5 min. samples, respectively. Thus, it is possible to apply this HPLC method also, for monitoring the methoxamine HCl level in plasma.

CONCLUSION

A simple, selective and sensitive HPLC method for the analysis of methoxamine HCl in pharmaceutical formulations and in rabbit plasma is reported. Detection of lower limit was 15 ng for a 30 ul injection volume of methoxamine HCl. This HPLC technique could therefore be used in the quality control and pharmacokinetic studies of such a drug replacing the tedious, non-selective TLC and colorimetric assay procedures normally used for these purposes.

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