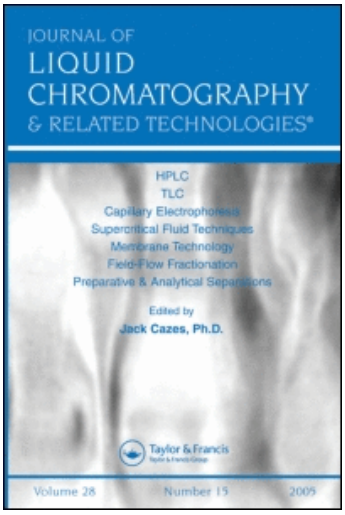

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DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR THE QUANTITATION OF TROMETHAMINE IN IOPAMIDOL INJECTION

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ABSTRACT

An HPLC method using a precolumn derivatization with 1-(1-naphthyl)-ethyl isocyanate (NEIC) was developed for the quantitation of tromethamine, an excipient in iopamidol injection. In this procedure, both tromethamine and the internal standard react with NEIC. Following this pre-column derivatization, the solution is injected onto an Inertsil ODS reverse phase HPLC column. The peaks corresponding to the two derivatives are separated from each other and from the peaks corresponding to iopamidol and its related substances. The validation studies indicate that the method is specific, accurate, precise, and rugged over the concentration range (0.50 - 1.25 mg/mL) investigated.

INTRODUCTION

Iopamidol is an injectable iodinated contrast agent for angiography, excretory urography, and myelography.[1] Tromethamine (2-amino-2-hydroxy methyl-1,3-propanediol) is used as a biological buffer in Iopamidol Injection.

Although Iopamidol Injection is manufactured at various strengths, i.e., 76% (760 mg/mL), 61% (610 mg/mL), and 41% (410 mg/mL), the tromethamine concentration is always at 1.0 mg/mL. There is a continuously increased interest to analyze excipients in the formulated pharmaceutical products. The main difficulties an analyst is faced with analyzing tromethamine in Iopamidol Injection are the lack of an ultraviolet chromophore in tromethamine and high concentration of iopamidol in the sample.

HPLC determination of tromethamine has been reported in the literature. The tromethamine counter ion in lodoxamide tromethamine has been analyzed by cation exchange HPLC with a conductivity detector.[2] This method was not suitable for the analysis of tromethamine in Iopamidol Injection because of over loading of the cation exchange column by high amounts of iopamidol, even after 50 folds of dilution of the sample with water.

Tromethamine has also been determined in biological fluids using a two-step derivatization procedure followed by fluorescence detection.[3] The derivatization method involved conversion of the amino group of tromethamine to the corresponding fluorophore using 4-fluoro-7-nitrobenzo-2-oxa-1,3,-diazole. The intact hydroxyl groups were then benzoylated with benzoyl chloride to improve the method specificity by decreasing polarity of the derivatized compounds. Iopamidol also contains several hydroxyl groups and its concentration is several hundreds fold higher than tromethamine's. Due to the competition for benzoyl chloride between iopamidol and tromethamine, the reproducibility was poor.

In this report, a novel pre-column derivatization of tromethamine in Iopamidol Injection followed by HPLC analysis with UV detection is presented. 1-(-1-Naphthyl)-ethyl isocyanate (NEIC) is used to selectively derivatize the amino group of tromethamine (Figure 1). To increase the reproducibility of the derivatization procedure, 2-amino-2-ethyl-1,3-propanediol is used as the internal standard. This compound was selected because its structure is very similar to that of the analyte. The specificity, precision, accuracy, linearity, and ruggedness of the method was evaluated in 760 mg/mL Iopamidol Injection over the concentration range of 0.5 - 1.25 mg/mL tromethamine concentration range.

EXPERIMENTAL

Materials and Reagents

The reference standards of Iopamidol and Tromethamine were received from the U.S.P. (Rockville, MD, U.S.A.). The samples of Iopamidol Injection (760 mg/mL) originated from the ESI Lederle plant (Cherry Hill, NJ, U.S.A.).

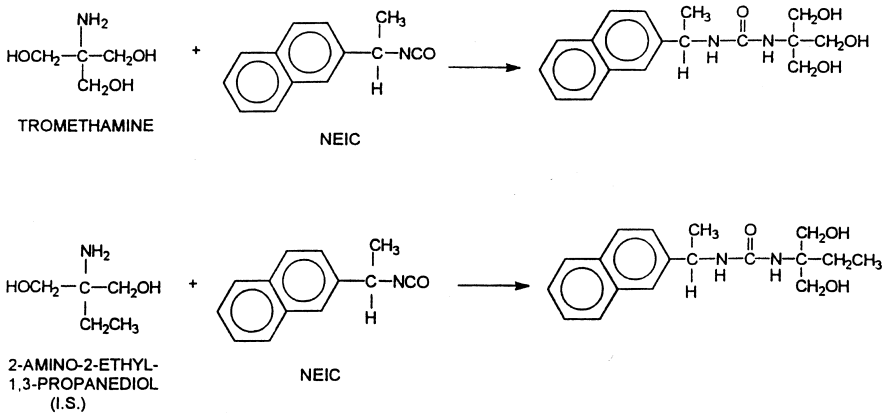


Figure 1. Derivatization of tromethamine and internal standard with 1-(1-naphthyl)ethyl-isocyanate (NEIC).

2-Amino-2-ethyl-1,3-propanediol, calcium disodium edetate, U.S.P. and 1-(1-naphthyl)-ethyl isocyanate (NEIC) were received from Aldrich Chemical Company Inc. (Milwaukee, WIS, U.S.A.). The following HPLC grade reagents: methanol, dichloromethane, and acetonitrile, were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.).

Deionized water was purified in house using a Waters MilliQ system (Milford, MA, U.S.A.).

Placebo, Standard and Sample Preparations

Placebo stock solution

A tromethamine placebo formulation was prepared. The placebo consists of all the formulation ingredients minus tromethamine (760 mg/mL of iopamidol and 0.48 mg/mL of calcium disodium edetate, adjusted to pH 7.0 with 0.01 N sodium hydroxide solution).

Blank preparation

One mL of placebo stock solution is diluted to volume with methanol in a 10.0 mL volumetric flask (Solution A). One mL of Solution A and 1.0 mL of internal standard (IS) solution (0.1 mg/mL 2-amino-2-ethyl-1,3-propanediol in methanol) were diluted to volume with methanol in a 10 mL volumetric flask.

Standard preparation

One mL of placebo stock solution and 1.0 mL of tromethamine solution (1.0 mg/mL in methanol) were diluted to volume with methanol in a 10 mL volumetric flask (Solution B). One mL of Solution B and 1.0 mL of IS solution were diluted to volume with methanol in a 10 mL volumetric flask.

Sample preparation

One mL of iopamidol injection sample is diluted to volume with methanol in a 10 mL volumetric flask (Solution C). One mL of Solution C and 1.0 mL of IS solution were diluted to volume with methanol in a 10 mL volumetric flask.

Derivatization Procedure

Each analysis includes the simultaneous derivatization of the blank, standard and sample preparations. One mL of each solution was placed in three separate test tubes. The test tubes contents were evaporated to dryness using a stream of nitrogen. The residues were reconstituted with 1.0 mL of methanol and vortexes for one minute. 0.1 mL of derivatization reagent (10 mg/mL of NEIC in dichloromethane) was added to each test tube and their contents mixed for one minute. The samples were once again evaporated to dryness using a stream of nitrogen. The residues were reconstituted with 1.0 mL of methanol and vortexes for one minute prior to HPLC analysis.

Instrument

The HPLC system consisted of a Waters 600E HPLC pump at flow rate of 1.0 mL/min, a Waters 717 autosampler, a Waters 486 UV detector set at 280 nm, and a Waters 860 VAX data system. For the ruggedness study, a Hitachi L-6200 pump and Hitachi L-4200 UV detector (Danbury, Connecticut, U.S.A.) were used.

A Metachem Inertsil ODS-2 column, 5 μm, 25 cm x 0.46 cm i.d. (Torrance, CA, U.S.A.) was used at ambient temperature. For the first 3 minutes, the eluting mobile phase consists of a mixture of acetonitrile and water (1350:1650, v/v). The later eluting peaks are washed out of the column with a mobile phase of acetonitrile and water (2700:300, v/v) for 5 minutes. The HPLC system is then re-equilibrated with initial mobile phase. The injection volume is 20 μL and the total run time is 20 minutes. The tromethamine - IS peak ratio is used for the calculation.

RESULTS AND DISCUSSION

HPLC Separation and Specificity

Figure 2 shows blank and sample preparations chromatograms. Both derivatized tromethamine and IS peaks are separated from all the interference peaks. Photo-diode array detection indicated that both tromethamine and IS peaks are homogeneous. The one-step gradient elution was used to wash out later eluting interference peaks.

Derivatization

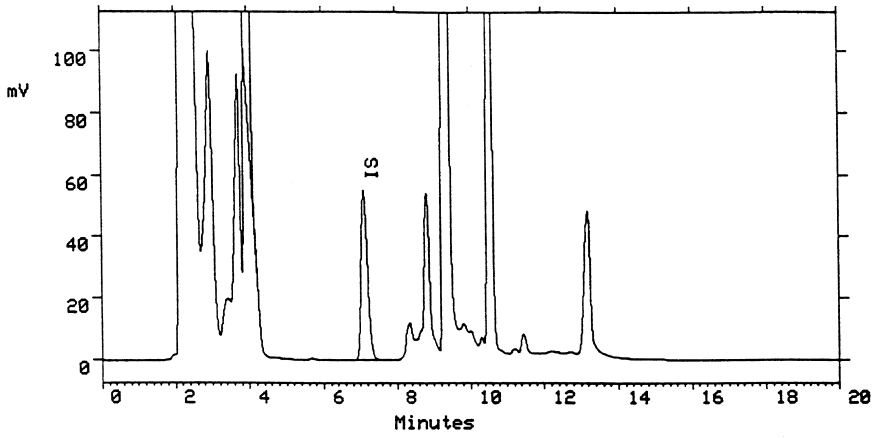
The derivatization of the amino groups in tromethamine and IS was found to be almost instantaneous, which is not surprising in light of previous studies with compounds having amino groups.[4] Longer mixing time (investigated up to 10 minutes) does not affect the reaction yield provided the residual water is removed from the sample prior to derivatization. Because NEIC degrades in water, the standard was prepared using the placebo as diluent. The reaction yield for both tromethamine and IS obtained with a standard in a pH 7.0 buffer solution was much lower than that with a standard in a pH 7.0 placebo. These results indicate a matrix effect on the derivatization.

System Precision

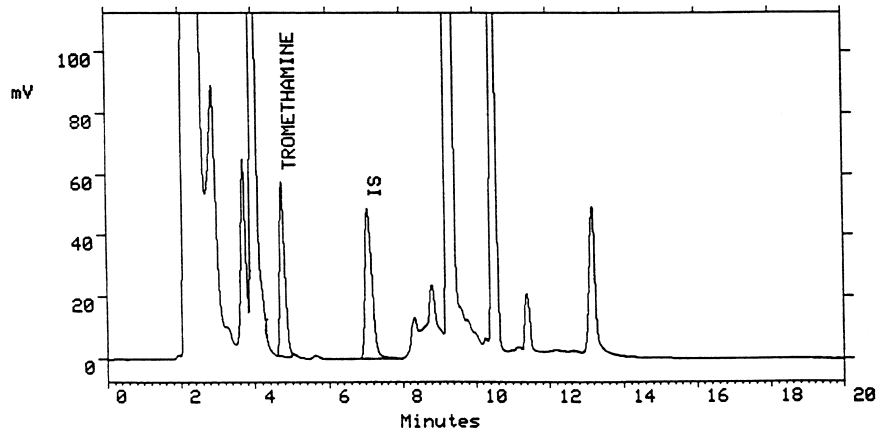
The precision of the HPLC system was evaluated by making five replicate injections of a standard solution on two different HPLC systems on different days using different reagents. Analysis of the data yielded relative standard deviations (RSD) of 0.1% and 0.4%.

Recovery and Method Precision

The placebo solutions were spiked at different levels with tromethamine. Two placebo solutions were spiked at the 1.25, 0.75 and 0.50 mg/mL levels of tromethamine in the 76% Iopamidol Injection. Five spiked solutions were prepared at the 1.00 mg/mL level. The individual recoveries ranged between 96 and 102% (Table 1). The recovery over all levels is 99% with a RSD value of 1.4%. At the 1 mg/mL level, the recovery was 99% with an RSD of 1.9%. These results indicate that the method is accurate and precise over the concentration range investigated.



Blank preparation



Sample preparation

Figure 2. Chromatograms of blank and sample preparations.

Linearity of Recovery

The linearity of recovery was evaluated by plotting the amount recovered versus the amount spiked. Linear least squares analysis of the data yielded a coefficient of correlation (R^2) value and slope of 0.9988 and 0.9809,

Table 1

Recovery of Tromethamine from Iopamidol Injection

Amount Spiked (mg/mL)	Amount Recovered (mg/mL)	Recovery (%)	Mean Recovery (%)	RSD (%)
1.25	1.21, 1.22	97, 98	98	
1.00	1.02, 0.98, 0.97, 0.99, 0.98	102, 98, 97, 99, 98	99	1.9
0.75	0.72, 0.75	96, 100	98	
0.50	0.50, 0.51	100, 102	101	

Table 2

Ruggedness Studies

	Amount Recovered (mg/mL)	
Amount Spiked (mg/mL)	**Chemist A**	**Chemist B**
1.00	1.02, 0.98, 0.97, 0.99, 0.98	1.00, 1.01, 1.00, 1.02, 1.04
Mean amount recovered (mg/mL)	0.99	1.01
RSD (%)	1.9	1.7
Overall amount recovered (mg/mL)	1.00	
Overall RSD (%)	2.2	

respectively. The R^2 value indicates that the method is linear over the concentration range investigated. The slope value is close to one which confirms the accuracy of the method over the range investigated.

Ruggedness

The ruggedness of the method was evaluated by having two chemists analyze five independent 1.0 mg/mL sample preparations of tromethamine on two different days using different HPLC systems. The results are shown in Table 2. The mean values were within 2.0% of each other. Additionally the overall RSD is 2.2%. The results obtained are excellent and indicate that the method is rugged.

CONCLUSION

An HPLC method using a one-step precolumn derivatization has been developed and validated. The data generated indicate that the method is specific, accurate, precise, and rugged over the 0.5 - 1.25 mg/mL concentration range investigated.

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REFERENCES

† Current address: 621 Rose Street, P.O. Box 80837, Lincoln, NE 68501.

1. E. Felder, M. Grandi, D. Pitre, G. Vittadini, **Iopamidol, in Analytical Profiles of Drug Substances**, K. Florey, ed., Academic Press, Inc., San Diego, CA, USA, 1988, p. 115.

2. R. E. Hall, G. D. Havner, R. Good, D. L. Dunn, "Ion Chromatographic Method for Rapid and Quantitative Determination of Tromethamine," J. Chromatogr., **718**, 305-308 (1995).

3. M. J. Morris, J. Y. K. Hsieh, "Determination of Tris (hydroxy methyl)amino methane (Tromethamine) in Human Plasma and Urine by High-Performance Liquid Chromatography with Fluorescence Detection," J. Chromatogr., **622**, 87-92 (1993).

4. W. Naidong, R. H. Pullen, R. F. Arrendale, J. J. Brennan, J. D. Hulse, J. W. Lee, "Stereospecific Determination of (±)-DU-124884 and its Metabolites (±)-KC-9048 in Human Plasma by Liquid Chromatography," J. Pharm. Biomed. Anal., **14**, 325-337 (1996).

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