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Ion chromatographic method for rapid and quantitative determination of tromethamine

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Abstract

Tromethamine is commonly used as a buffering agent, alkalizer, and emulsifying agent in pharmaceutical and cosmetic preparations and as a counterion for acidic drug substances.

Methods reported in the literature for the determination of tromethamine have typically been non-specific or required derivatization and are not suitable for quantitative analysis in a dosage form or drug substance matrix. An ion chromatographic method with conductivity detection was employed to provide rapid and quantitative determination of tromethamine in these matrices. The method was found to be specific with regard to drug substance and dosage form components. The method was validated and found to provide suitable linearity, accuracy and precision for use as a pharmaceutical assay method.

1. Introduction

Global regulatory agencies are more frequently requiring assay and identity methods for all ingredients in pharmaceutical dosage forms and for active counterions such as tromethamine. What is required is a method which is straightforward enough to be used on a routine basis and which will provide a high level of precision, accuracy and specificity.

A survey of literature assays for tromethamine did not identify any that were satisfactory for our purpose. Some methods such as the USP monograph titrimetric assay and a flow injection pseudotitration [1] do not provide specificity. Derivatization with various reagents has been used to add chromophores for high-performance liquid chromatography [2,3] or spectrophotometry [4–6] and to increase volatility for gas chromatography [7,8]. We preferred to use high-performance liquid chromatography, if possible, since these methods have routinely proven to provide performance suitable for pharmaceutical assays and because we had an aqueous matrix. We also preferred to use direct detection rather than derivatization because derivatization methods necessarily are less straightforward and provide somewhat diminished performance characteristics. Since tromethamine is an ionic species without an ultraviolet chromophore, we investigated and subsequently validated ion-exchange chromatography with conductometric detection for this application.

In this work, we employed a commercially available high-performance liquid chromatographic cation-exchange column to separate tromethamine under acidic conditions followed by conductivity detection. The method was

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evaluated for USP system suitability, linearity by analyzing standard curves and for precision by analyzing replicates prepared using the drug lodoxamide tromethamine. The linearity, precision and accuracy of the method was also evaluated in Alomide® ophthalmic solution, a pharmaceutical dosage form of lodoxamide tromethamine.

2. Experimental

2.1. Chemicals

Tromethamine was a USP (Rockville, MD, USA) reference standard (RS). Lodoxamide tromethamine, lodoxamide (free acid) and Alomide were from Alcon Laboratories (Fort Worth, TX, USA). The mobile phase was acidified with ACS reagent grade hydrochloric acid from J.T. Baker (Phillipsburg, NJ, USA). The column was a Dionex IonPac CS5 (250 × 4 mm I.D.) available from Dionex Corporation (Sunnyvale, CA, USA).

2.2. Equipment

Two chromatographic systems were used for analysis. The first was a Shimadzu LC-600 liquid chromatograph with a Shimadzu SIL-9A autosampler, a Waters 431 conductivity detector and a PE Nelson Turbochrome data acquisition system. The second consisted of a Waters liquid chromatograph with a Waters WISP autosampler, a Waters 431 conductivity detector and a Spectra-Physics ChromJet integrator.

2.3. Method

An aliquot of USP RS tromethamine was diluted in water to obtain a 0.260 mg/ml standard. For lodoxamide tromethamine drug substance samples, approximately 30 mg was accurately weighed and diluted to 50.0 ml in water to give a nominal tromethamine concentration of 0.26 mg/ml. Alomide contains 0.178% lodoxamide tromethamine and samples were diluted in water to obtain a solution with a 0.26 mg/ml of tromethamine.

The mobile phase consisted of 10 m*M* HCl which was filtered through a 0.45-μm membrane prior to use. A flow-rate of 1.5 ml/min and an injection volume of 20 μl were used. The retention time for tromethamine was about 5 to 8 min under these conditions. Fig. 1 shows typical chromatograms.

3. Results and discussion

3.1. System suitability

USP system suitability tests were performed using three different columns on two different systems and the relative standard deviations were 0.4, 0.5 and 0.3%, respectively. The number of theoretical plates were 1800, 1500 and 1900, and the tailing factors were 0.9, 1.2 and 1.1 for the tromethamine peak.

3.2. Linearity

Aqueous tromethamine standard curves over the range 0.195–0.325 mg/ml (75–125% of the target sample concentration) were analyzed in duplicate on two different days. The curves were found to be linear over this range; *R*-squared values were 0.9989 and 0.9998, intercepts were 0.8 and 3.2% relative to the target concentration, and relative standard deviations of response factors were 0.8 and 0.7%. We consider this linearity suitable to allow single-point standardization for this range.

3.3. Precision

Sets of eight replicate aqueous tromethamine standards with a concentration of 0.268 mg/ml were prepared and assayed on two different days. The method was suitably precise and the

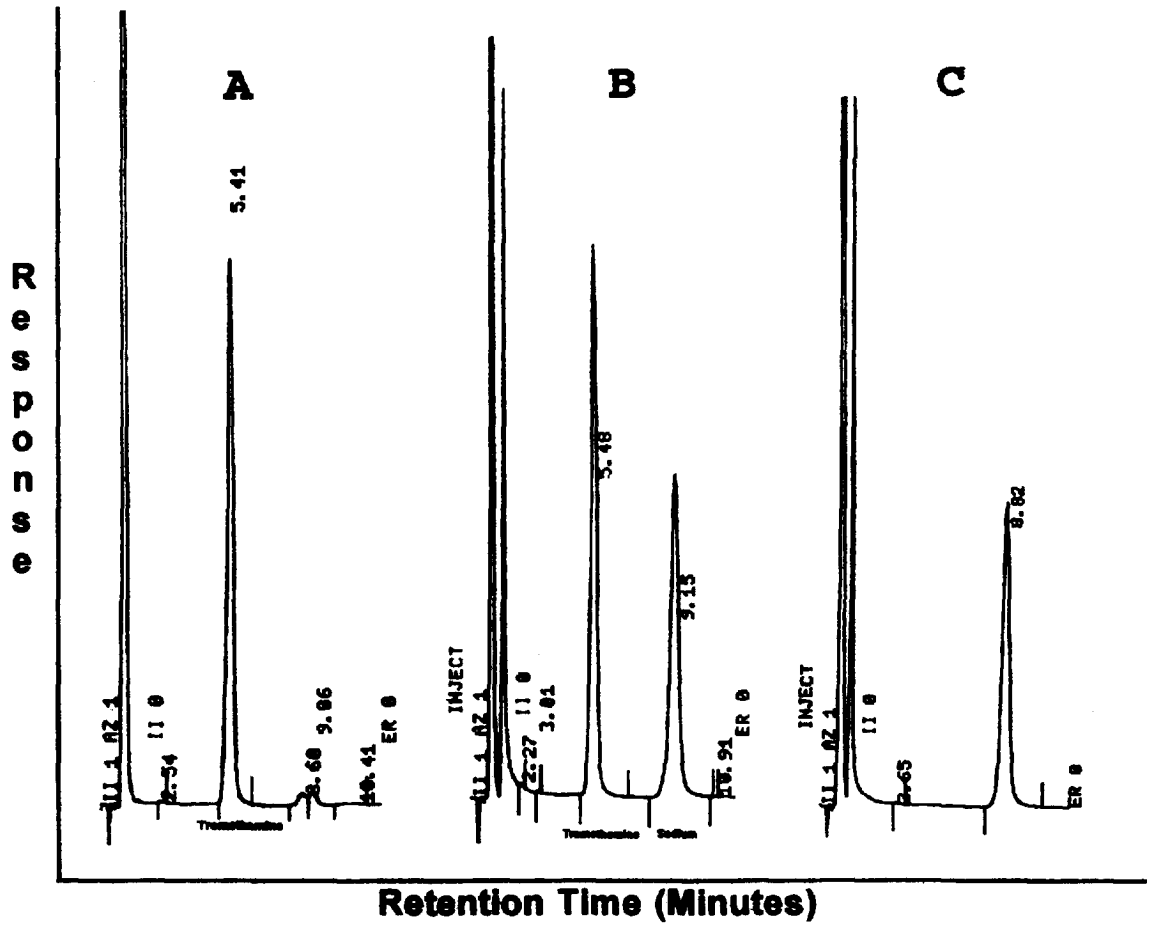

Fig. 1. (A) Chromatogram of a 0.268 mg/ml aqueous standard of tromethamine. Conditions: column, Dionex IonPac CS5; mobile phase, 10 m*M* HCl; flow rate, 1.5 ml/min; injection volume, 20 μl; detector, conductivity. (B) Chromatogram of a 0.277 mg/ml Alomide vehicle standard. (C) Chromatogram of an Alomide vehicle blank.

relative standard deviations were 0.3% and 0.5%.

3.4. Validation in Alomide formulation

A vehicle was prepared which contained all of the ingredients of Alomide except for tromethamine at their normal concentrations (lodoxamide free acid was used in place of lodoxamide tromethamine). Aliquots of this vehicle were then spiked with varying amounts of a concentrated tromethamine standard to provide vehicle standards containing the appropriate concentrations of vehicle and tromethamine. The concentrations of these vehicle standards ranged from 0.208 to 0.347 mg/ml of tromethamine. A vehicle standard curve over this range was analyzed in duplicate and recoveries were calculated against a 0.260 mg/ml aqueous tromethamine standard. A set of eight replicates with a tromethamine concentration of 0.277 mg/ml was also analyzed. The method was linear and precise for analysis of tromethamine in Alomide. The curve had an *R*-squared value of 0.9998, relative intercept of 0.4% and a relative standard

deviation of 0.3%. The replicate set had a relative standard deviation of 0.3% ($n = 8$).

3.5. Accuracy

The recovery of Alomide vehicle spiked with tromethamine ranged from 99.7 to 101.5% and demonstrates suitable accuracy of the method. The mean recovery for the 14 vehicle standards from the curve and replicate set was 100.5% with a relative standard deviation of 0.5%.

3.6. Specificity

The method was found to be selective with regard to lodoxamide and to the formulation excipients in Alomide. Sodium ion was found to elute near the tromethamine peak under the initial chromatographic conditions used. However, by decreasing the strength of the mobile phase, the retention of tromethamine was increased from 3–4 min to 5–7 min and this allowed complete separation from sodium. Water and Alomide vehicle blanks were analyzed and showed no interference in the region where tromethamine would have eluted (Fig. 1).

3.7. Ruggedness

An interlaboratory method transfer was conducted between our R&D laboratory and the Alcon-Fort Worth Quality Assurance laboratory for analysis of lodoxamide tromethamine. The differences between mean assay results for three lots were 0.9, 2.4 and 0.0%, respectively.

3.8. Column regeneration

One problem that was encountered with this method was the gradual loss of column efficiency during analysis, possibly due to buildup of the cationic drug on the column. It was found that the column could normally be regenerated by washing for 30 min at 1 ml/min with a solution of 5% acetonitrile in water (concentrations greater than 5% will damage the column) and then for 60 min at 1 ml/min with 1 *M* HCl.

4. Conclusion

An ion chromatographic method which is rapid, specific and does not require derivatization has been developed for routine assay of tromethamine in drug substances and dosage forms.

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