Ion Chromatography

Assay of Tromethamine in Pharmaceutical Formulations

Sachin Patil and Jeff Rohrer
Thermo Fisher Scientific, Sunnyvale, CA, USA 94085

Abstract

Purpose: To develop an accurate method of determining tromethamine in pharmaceutical formulations using high-performance ion chromatography with suppressed conductivity detection

Methods: A cation exchange column was used to separate tromethamine, which was then detected by suppressed conductivity using the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Results: The proposed method was validated using criteria prescribed in USP <1225>.

Introduction

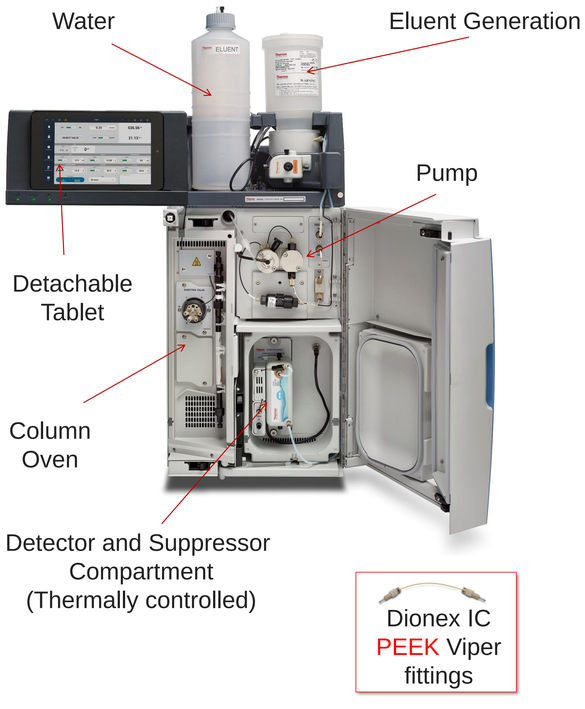
Current methods to determine tromethamine are not suitable for those seeking specificity and labor savings. Some methods such as the United States Pharmacopeia (USP) tromethamine monograph titrimetric assay [1] and a flow injection pseudo titration [2] do not provide specificity. Derivatization with various reagents has been used to add chromophores for high-performance liquid chromatography [3-5] or spectrophotometry [6] and to increase volatility for gas chromatography [7-8]. Ion chromatography (IC) offers a significant improvement to the existing assays because it can simultaneously determine sodium, ammonium, and other common cations in a single injection [9]. There is a published IC method uses manually prepared eluents and non-suppressed conductivity detection [10], but there is a need for an improved IC method for the determination of tromethamine that takes advantage of modern technology.

Materials and methods

Instrument

The Dionex Integrion HPIC system, Reagent-Free™ IC (RFIC™) model (Figure 1) was configured for conductivity detection, which included automated eluent generation and Dionex IC Viper fittings (Figure 1 bottom right), for increased reproducibility and optimum performance.

Figure 1.The Dionex ICS 5000+ HPIC system flow diagram configured for ED detection.



Conditions:

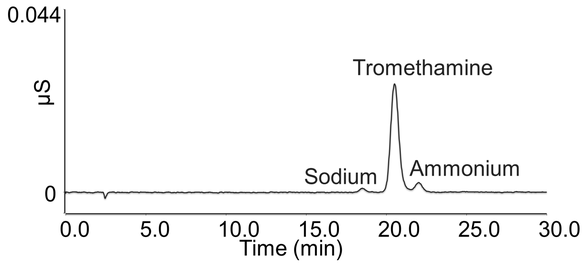
Columns: Thermo Scientific Dionex IonPac CS20 2x250 mm, IonPac CG20 Guard 2x50 mm
Eluent flow rate: 0.3 mL/min
Column temperature: 40 ° C
Run time: 30 min
Injection Volume: 2.5 µL (Full loop)
Eluent: 2 mM MSA isocratic
Eluent Source: EGC 500 MSA
Detection: Suppressed Conductivity, With CDRS 600 Suppressor

Results

Separation

Separation of tromethamine was achieved using a Dionex IonPac CS20, 2 x 250 mm column under isocratic elution conditions. Figure 2 shows separation of a 2 ppm tromethamine solution. In order to achieve good separation from the nearest cations i.e. sodium and ammonium, a low eluent concentration (2 mM) was required throughout the run. Sodium and ammonium elute before and after the tromethamine peak respectively. The resolution between sodium and tromethamine is 2.53, and 1.67 between tromethamine and sodium. Total method run time is 30 min. This run time is sufficiently long to ensure that any cations that elute after ammonium ~~peak~~ are removed. It also enables handling an increase in retention time encountered during some of the conditions of the robustness studies.

Figure 2. Separation of 2 ppm tromethamine on an IonPac CS20 column

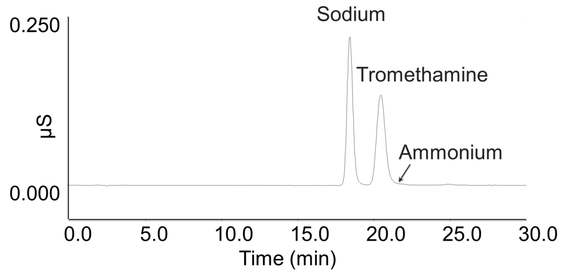


A simulated matrix sample was also prepared based on a COVID-19 vaccine excipient composition listed by John Hopkins's Institute of Vaccine Safety. [12] The composition is listed in Table 1. This composition does not include the active ingredient, which is mRNA encapsulated in a lipid nanoparticle. Figure 2 shows a chromatogram obtained by analyzing a 50-fold dilution of this composition. Ammonium, which is an environmental contaminant, elutes after the tromethamine peak.

Table 1. Composition of tromethamine matrix sample

Component	mg in 0.5 mL
Sucrose	43.5
Acetic acid	0.043
Sodium acetate	0.12
Tromethamine	0.31

Figure 3. Separation of a 50-fold dilute blank matrix sample on an IonPac CS20 column



Linearity and Precision

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay [11]. For a drug substance or finished product, the minimum specified range is from 80 to 120% of the test concentration. Method linearity was studied using tromethamine standards at seven concentration levels ranging from 1 to 50 mg/L (ppm). The coefficient of determination value determined was 1 for a quadratic fit.

Assay precision was evaluated by injecting three replicates at three tromethamine concentration levels, 2, 5, and 10 ppm and expressed as the RSDs of retention time and peak area from the series of measurements. The RT RSDs were ≤0.83% and the peak area RSDs were ≤0.85% (Table 2).

Table 2. Method precision determined at three tromethamine concentrations (n=3).

	RSD	
Conc (ppm)	RT	Peak Area
2	0.17	0.22
5	0.83	0.85
10	0.48	0.73

Linearity and Precision

Though method sensitivity is not important when assaying tromethamine as a major component of a formulation, it will be important if tromethamine is to be measured as a related substance or as the analyte in a limit test. Method sensitivity was determined by analyzing tromethamine standards and adjusting concentrations until S/N ratios of ~3 (LOD) and ~10 (LOQ) were obtained. To determine the LODs and LOQs, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1-min segment of the baseline where no peaks elute but close to the peaks of interest. The signal was determined from the average peak height of three injections of tromethamine. The LOD and LOQ for tromethamine were 0.05 ppm and 0.015 ppm respectively (Table 3).

Table 3. Method sensitivity (n=5)

Conc (ppm)	S/N
0.05	3.4
0.15	9.5

Accuracy

Accuracy studies were conducted by spiking a 50x-fold diluted tromethamine matrix sample at 3 different levels as shown in Table 4. The three spike levels were 5, 10, and 25 ppm. All three levels yielded good tromethamine recoveries indicating good method accuracy.

Table 4. Method accuracy (n=3)

Spike level (ppm)	Average (ppm)	%Recovery
0	11.9	-
5	17.5	112.0
10	21.4	95.4
25	36.9	100.0

Robustness

Assay robustness was determined on two columns. The robustness was studied by introducing ±10% variations in common chromatographic parameters. The parameters varied in this study were: eluent concentration, column temperature, and flow rate. Method performance under these conditions was evaluated by injecting a standard containing 2 mg/L tromethamine and calculating percent difference in three key chromatographic parameters: retention time, peak asymmetry, and resolution.

Robustness-continued

Tables 4 and 5 contains representative data from columns 1 and 2 respectively, which show minimal disruption in chromatographic parameters.

Table 4. Method robustness on column 1 (n=3)

	% Difference					
	10% Higher Flow	10% Lower Flow	10% Higher Eluent Conc	10% Lower Eluent Conc	10% Higher Temp	10% Lower Temp
Retention Time	-8.0	11.0	-8.9	11.1	-0.6	1.0
Asymmetry	0.6	1.2	2.0	-0.8	0.6	0.6
Resolution	-3.1	2.0	-5.5	9.4	-19.0	20.1

Table 5. Method robustness on column 2 (n=3)

	% Difference					
	10% Higher Flow	10% Lower Flow	10% Higher Eluent Conc	10% Lower Eluent Conc	10% Higher Temp	10% Lower Temp
Retention Time	-7.9	11.2	-8.7	11.2	-0.6	1.7
Asymmetry	1.1	0.8	1.9	2.8	1.4	1.4
Resolution	-2.4	10.6	-11.4	3.3	-24.3	20.8

Conclusions

- A 30 min method was developed to separate tromethamine.
- The method accuracy was determined by measuring the tromethamine content in a simulated sample.
- The method was validated as per analytical performance characteristics outlined in USP General Chapter <1225>.

References

1. Tromethamine. U.S. Pharmacopeial Convention (USP); Rockville, MD, USP43-NF38; 4538.
2. Analyst, **1988** 113, 755.
3. J. Chromatogr. **1992,** 583, 99.
4. J. Anal. Toxicol. **1984**, 8, 231.
5. J Pharm Biomed Anal. **2003**, 31(6), 1191-201
6. J. Pharm. Belg. **1981, 36,** 203.
7. J. Chromatogr. **1978**, 145, 155.
8. J. Chromatogr. **1984**, 295, 248.
9. Anal. Chem. **1980**, 52, 1519–1522.
10. J. Chromatogr. A. **1995**, 718 (2), 305-308.
11. Validation of Compendial Methods, General Chapter <1225>. U.S. Pharmacopeia/National Formulary: Rockville, MD, USP36-NF31; p 983.
12. Johns Hopkins Institute of Vaccine Safety. [Online] https://www.vaccinesafety.edu/components-Excipients.htm (Accessed May 16, 2022]

Trademarks/licensing

Learn more at thermofisher.com/FURL