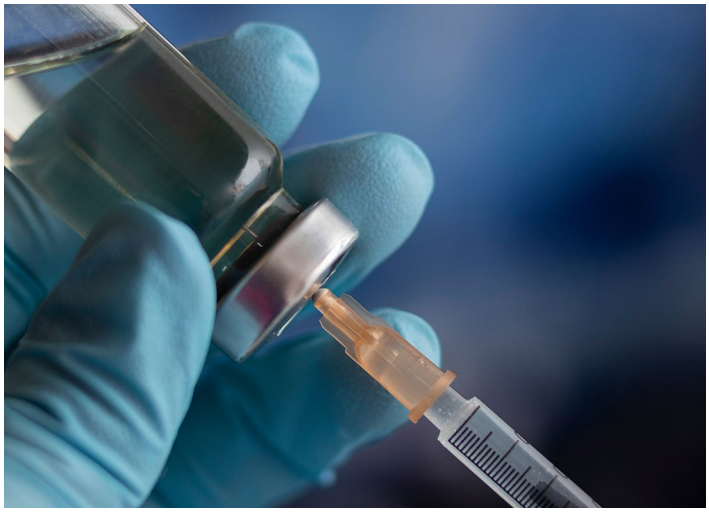
Assay of tromethamine in pharmaceutical formulations

Authors: Sachin Patil and Jeffrey Rohrer

Thermo Fisher Scientific, Sunnyvale, CA

Keywords: Dionex IonPac CS20 column, suppressed conductivity detection, RFIC, CDRS, COVID-19 vaccine, CDRS suppressor



Goal

To develop an IC method for the determination of tromethamine in pharmaceutical formulations using an RFIC system with suppressed conductivity detection

Introduction

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. 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]. 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.

The goal of this work is to design an IC method that uses a Thermo Scientific™ Dionex™ IonPac™ CS20 cation-exchange column, electrolytically generated MSA eluent, and suppressed conductivity detection to determine tromethamine in pharmaceutical formulations. The Dionex IonPac CS20 column provides a large separation window between monovalent and divalent cations. This allows most amines to elute after the alkali metals but before the alkaline earth metals, thus moving the amines away from matrix ions that would potentially make quantitation difficult. The selectivity makes the Dionex IonPac CS20 column particularly useful for determining a variety of alkyl and alkanol amines.

ThermoFisher SCIENTIFIC

The eluent used in this separation is generated using a Thermo Scientific™ Dionex™ EGC 500 MSA eluent generator cartridge and purified online using a Thermo Scientific™ Dionex™ CR-CTC 500 continuously regenerated cation trap column. The Thermo Scientific™ Dionex™ CDRS 600 (Cation Electrolytically Regenerated Suppressor) produces the regenerant ions necessary for eluent suppression and allows continuous operation with minimum maintenance. Because the RFIC system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. The method also requires no time-, resource-, and money-consuming analyte derivatization.

The method proposed in this application note was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures[11] to meet the requirements specified for tromethamine quantification prescribed in the USP tromethamine monograph.

Experimental

Equipment

- A Thermo Scientific™ Dionex™ ICS-5000+ Reagent-Free™ Ion Chromatography system* including
  - DP Dual Pump with degas option
  - DC detector compartment with single temperature zone
- Thermo Scientific™ Dionex™ AS-AP autosampler (P/N 074926) with cooling tray option (recommended)
- Dionex Eluent Generator Cartridge EGC 500 MSA (P/N 075779)
- Dionex CR-CTC 500 continuously regenerated cation trap column (P/N 075551)
- 10 µL sample loop
- 10 mL polypropylene autosampler vials, with caps (P/N 074228)
- Thermo Scientific™ Nalgene™ Rapid-Flow 0.2 µm filter units, 1,000 mL, nylon membrane, 90 mm diameter (P/N 164-0020)

* Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-6000 system.

Method conditions

Parameter	Value
Columns	Dionex IonPac CS20 2 × 250 mm analytical (P/N 302606), Dionex IonPac CG20 guard 2 × 50 mm (P/N 302607)
Eluent flow rate	0.3 mL/min
Column temperature	40 °C
Run time	30 min
Injection volume	2.5 µL (Full loop)
Back pressure	~2,700 psi (100 psi = 0.6894 MPa)
Eluent	2 mM MSA isocratic
Eluent source	Dionex EGC 500 MSA (P/N 075779)
Detection	Suppressed Conductivity Detection using a Dionex CDRS 600, 2 mm suppressor (P/N 088670)
Suppressor current	3 mA
Compartment temperature	25 °C

Reagents and chemicals

- Tromethamine (Sigma P/N T6687)

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography[12], the robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and resolution after imposing variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature). A standard containing 2 ppm tromethamine was injected. The same procedure was applied to another column set and the following variations were tested:

- Flow rate at 0.27 mL/min, **0.3 mL/min,** 0.33 mL/min
- Column temperature at 36 °C, **40 °C**, 44 °C
- MSA eluent initial concentrations at 1.8 mM, **2 mM,** 2.2 mM

The bold parameters belong to the main method and the other two are the tested variations.

Results and discussion

Separation

Separation of tromethamine was achieved using a Dionex IonPac CS20 column under isocratic elution conditions. Figure 1 shows a separation of a 2 ppm tromethamine solution. To achieve good separation from the nearest cations, i.e., sodium and ammonium, a low eluent concentration (2 mM) was required. Sodium and ammonium elute before and after the tromethamine peak, respectively. Both peaks are well separated from the tromethamine peak. The resolution between sodium and tromethamine is 2.53, and 1.67 between tromethamine and sodium. The total method run time is 30 min, which is sufficiently long to ensure that any cations that elute after the ammonium peak are removed. It also enables handling an increase in retention time encountered during some of the conditions of the robustness studies.

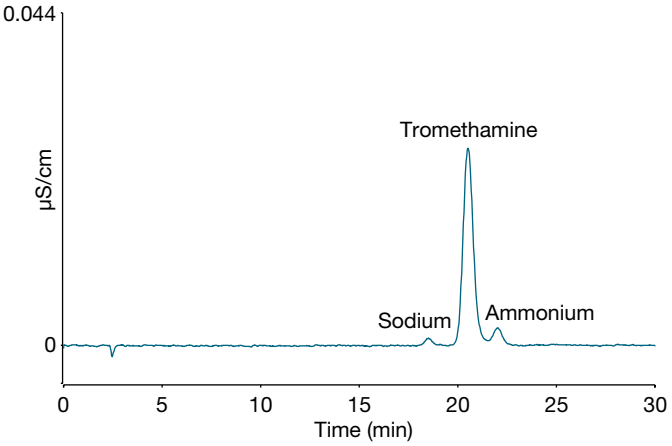


Figure 1. Separation of 2 ppm tromethamine on a Dionex IonPac CS20 column

A simulated matrix sample was also prepared based on a COVID-19 vaccine excipient composition listed by John Hopkins's Institute of Vaccine Safety.[13] 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. The tromethamine peak is well resolved from the sodium peak, which is the largest component of the composition. Ammonium, which is an environmental contaminant, elutes after the tromethamine peak.

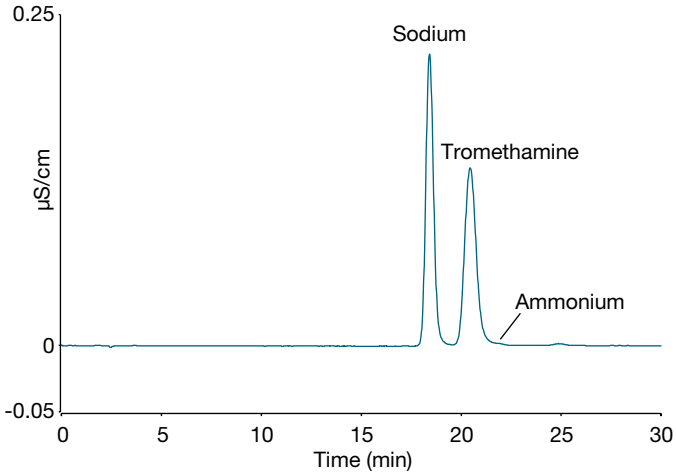


Figure 2. Separation of a 50-fold dilution of the matrix sample on a Dionex IonPac CS20 column

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

Method 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.[13] 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 (Figure 3).

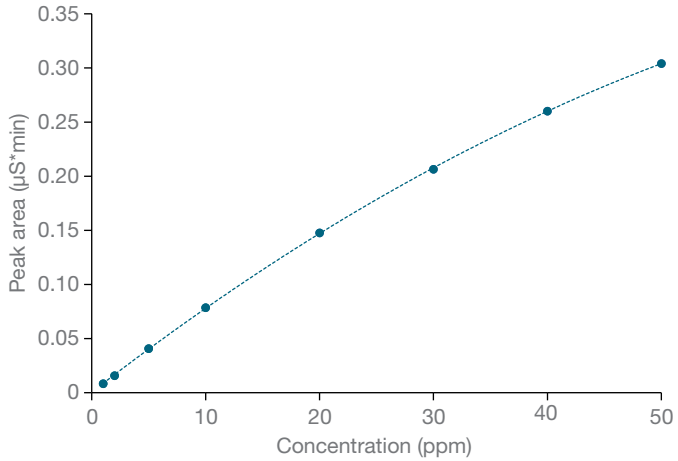


Figure 3. Calibration plot for tromethamine (n=3) for each concentration

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. Tromethamine assay precision (n=3)

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

Method sensitivity

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 0.15 ppm, respectively (Table 3). Figure 4 shows chromatograms obtained using 0.05 and 0.15 ppm injections of tromethamine.

Table 3. Method sensitivity (n=5)

Conc. (ppm)	S/N
0.05	3.4
0.15	9.5

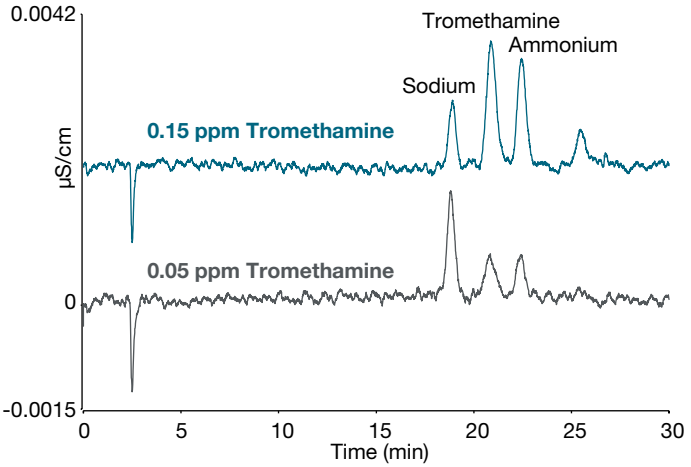


Figure 4. Sensitivity of the tromethamine assay

Method accuracy

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

Table 4. Method accuracy (n=3)

Spike level (ppm)	Average	% Recovery
0	11.9	-
5	17.5	112
10	21.4	95.4
25	36.9	100

Method robustness

Method robustness was studied by introducing ±10% changes to method conditions and monitoring changes to key chromatographical parameters—retention time, asymmetry, and resolution (to ammonium). Robustness studies were performed on two different columns. The peak asymmetry was measured using the USP formula.[12] A standard (2 mg/L tromethamine) was injected three times (n=3) at each chromatographic condition.

Table 5. Method robustness studied on column 1 using 2 ppm tromethamine (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 6. Method robustness studied on column 2 using 2 ppm tromethamine (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

As shown in Tables 5 and 6, the only factor that has a significant effect on tromethamine chromatography is temperature. At a higher temperature (44 °C), resolution between sodium and tromethamine is reduced (Figure 5, blue trace). Whereas at a lower temperature (36 °C), resolution between tromethamine and ammonium is reduced (Figure 5, red trace). The method temperature used here (40° C) represents a good compromise (Figure 5, gray trace).

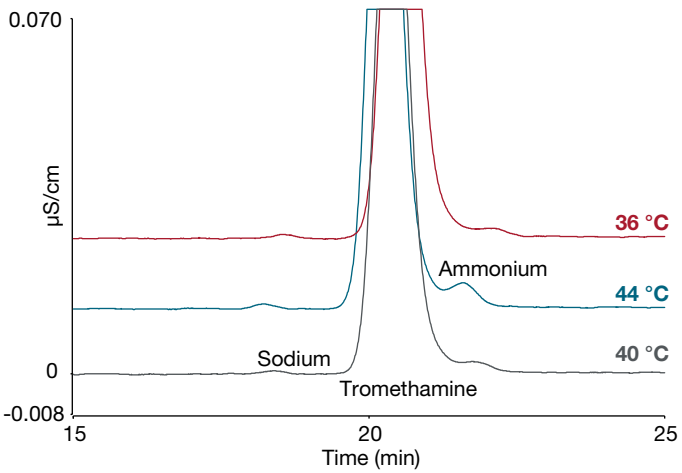


Figure 5. Effect of temperature on tromethamine and ammonium separation

Conclusions

This study describes an IC-based assay for the determination of tromethamine in a simulated pharmaceutical formulation. Tromethamine was separated on a cation-exchange column and detected by suppressed conductivity in 30 min. This method allows the concentration of tromethamine to be determined in an automated way, circumventing the need to perform a cumbersome titration. This assay for tromethamine was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and was shown to measure accurately the content of tromethamine in a simulated pharmaceutical formulation. This assay offers a simple, accurate, and robust measurement approach to determine tromethamine. It should be applicable to other pharmaceutical formulations.

thermo scientific

References

1. Tromethamine. *U.S. Pharmacopeial Convention (USP);* Rockville, MD, USP43-NF38; 4538.
2. Constantinos, C. A. and Koupparis, M. A. Automated flow injection spectrophotometric non-aqueous pseudotitrations of amines and their hydrohalide salts. ***Analyst*** **1988,** *113,* 755.
3. Gumbhir K. and Mason. W.D. High-performance liquid chromatographic method for the determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma. *J. Chromatogr.* **1992,** *583,* 99.
4. Blanke, S. R. and Blanke, R. V. The Schotten-Baumann reaction as an aid to the analysis of polar compounds: application to the determination of tris(hydroxymethyl) aminomethane (THAM). *J. Anal. Toxicol.* **1984,** *8,* 231.
5. Guo, Y. and Huang, A. A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt. *J. Pharm. Biomed. Anal..* **2003,** *31*(6), 1191–201.
6. Bubois, P.; Lacroix, J.; Lacroix, R.; Levillain, E.; Viel. C. Application of Z α,β-dinitrostilbene to aminic drugs assay. *J. Pharm. Belg.* **1981,** *36,* 203.
7. Hulshoff, A. and Kostenbauder, H.B. Gas chromatographic method for the quantitative determination of tris(hydroxymethyl)aminomethane in plasma *J. Chromatogr.* **1978,** 1*45,* 155.
8. Vincent, G.; Desage, M.; Comet, F.; Brazier, J.; and Lecompte, D. Gas chromatographic determination of tris(hydroxymethyl)amino-methane in pharmaceutical preparations after silylation. *J. Chromatogr.* **1984,** *295,* 248.
9. Fritz, J.S.; Gjerde, D.T.; Becker, R.M. Cation Chromatography with a Conductivity Detector. *Anal. Chem.* **1980,** *52,* 1519–1522.
10. Hall, R.E.; Havner, G.D.; Good, R.; Dunn, D. Ion chromatographic method for rapid and quantitative determination of tromethamine, *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. Physical Tests, *<621> Chromatography.* U.S. Pharmacopeia/National Formulary (USP): Rockville, MD, USP36-NF21; p 268.
13. Johns Hopkins Institute of Vaccine Safety. [Online] https://www.vaccinesafety.edu/components-Excipients.htm (Accessed May 29, 2021]

Find out more at **thermofisher.com/ic**

ThermoFisher SCIENTIFIC