Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 1191–1201
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt

Yong Guo *, Aihua Huang

Analytical Development, Drug Evaluation, Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA

Received 11 June 2002; received in revised form 13 September 2002; accepted 24 December 2002

Abstract

A hydrophilic interaction chromatography (HILIC) method using an aminopropyl stationary phase was developed and validated to determine the content of tromethamine as counter ion in an investigational drug substance. Tromethamine was a very polar compound without any chromophores, and could not be readily retained and detected by conventional reserved-phase HPLC-UV methods. Furthermore, the tromethamine salt of the drug compound also had limited solubility in aqueous solution. The method employed simple acetonitrile/water mobile phase (80/20, v/v) to provide sufficient retention for tromethamine. Meanwhile, the high acetonitrile content also helped to dissolve the drug substance sample. Refractive index (RI) was used for the detection of tromethamine. The method was found to be specific without interference from the drug compound and related impurities. The method was also validated for suitable precision, linearity and accuracy for the analysis of drug substance samples. The effects of various parameters, such as acetonitrile content, mobile phase salt concentration, and column temperature on HILIC separation were investigated.

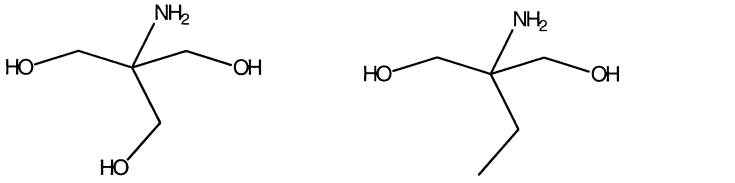
Keywords: Tromethamine; HILIC; Pharmaceutical salt

1. Introduction

2-Amino-2-hydroxymethyl-propane-1,3-diol (tromethamine) is a weak base with pK_a ~8.1 (25 °C) and is readily soluble in water [1]. It is commonly used as a buffering or emulsifying agent in pharmaceutical and cosmetic products, or as a counter ion for acidic pharmaceutical compounds to form desired salt forms. When used as an ingredient in pharmaceutical preparations, suitable analytical methods are required to establish its identity and quantity for quality control and regulatory purposes. Chromatographic methods have been developed for the determination of tromethamine in biological fluids and pharmaceutical products [2–4]. Two reverse-phase HPLC methods involved chemical derivatization to provide sufficient retention on HPLC columns as well as chromophores or fluorophores for UV or fluorescence detection [2,3]. An ion chromato-

* Corresponding author. Tel.: +1-908-704-4309; fax: +1-908-704-1612.
E-mail address: yguo2@prdus.jnj.com (Y. Guo).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00021-9



Tromethamine 2-Amino-2-ethyl-1,3-propanediol

Fig. 1. Structures for tromethamine and 2-amino-2-ethyl-1,3-propanediol (AEPD).

graphic (IC) method developed by Hall et al. avoided chemical derivatization and was used to analyze tromethamine in Alomide® ophthalmic solution (as counter ion to lodoxamide) [4]. More recently, a CE method was also developed to analyze tromethamine in a contact-lens cleaning solution (as buffering agent) [5]. The IC and CE procedures were very straightforward. These methods, however, required that pharmaceutical dosage forms or drug substance be readily soluble in aqueous solution.

An investigational agent for treating asthma is currently under development in our company, and the active pharmaceutical ingredient (API) is prepared as a tromethamine salt. An analytical method is needed to quantitate the amount of tromethamine as the counter ion in the API and also to determine the stoichiometry of the salt form. A simple and accurate analytical method is preferred for drug substance release testing. However, the IC or CE method is not readily applicable in our case due to the fact that the non-salt form (free acid) of the API under investigation is not water soluble, and even its tromethamine salt has only limited solubility in water. Therefore, an alternative chromatographic method for the analysis of tromethamine in the API is needed.

Tromethamine is a very polar compound with three hydroxyl groups as shown in Fig. 1. No retention can be attained for tromethamine on reverse-phase columns even using pure aqueous mobile phases (data not shown). An alternative approach to obtain sufficient retention in chromatographic separations for very polar compounds is hydrophilic interaction chromatography (HILIC). HILIC is often considered as a 'normal phase separation' in a reversed-phase fashion wherein separations occur on polar stationary phases (e.g. silica or aminopropyl phase) with aqueous–organic mobile phases. In contrast to reversed-phase chromatography, polar compounds have stronger retention than non-polar compounds [6]. This mode of chromatography has been used mostly in the area of sugar, nucleic acids and peptide analysis [6–8]. More recently, several literature reports demonstrate the application of HILIC for the determination of polar pharmaceutical compounds [9–11].

In this study, a HILIC method using an aminopropyl column, acetonitrile/water mobile phase, and RI detection is described. Detailed consideration for method development is discussed as well as various parameters affecting the HILIC separation. The applicability of the HILIC method is demonstrated by analyzing tromethamine salt samples of the investigational drug and determining the stoichiometry of the salt form.

2. Experimental

2.1. Apparatus

All the experiments were performed on an Agilent HP1100 HPLC system equipped with both diode array (DAD, Model G1315A) and refractive index (RI, Model G1362A) detector (Agilent Technologies, Polo Alto, CA). The HPLC system also included a quaternary pump (Model G1311A), a degasser (Model G1322A), a column heater (Model G1316A), and an auto-injector (Model G1313A). The DAD and RI detectors were connected in series. The chromatograms were recorded using Agilent Chemstation

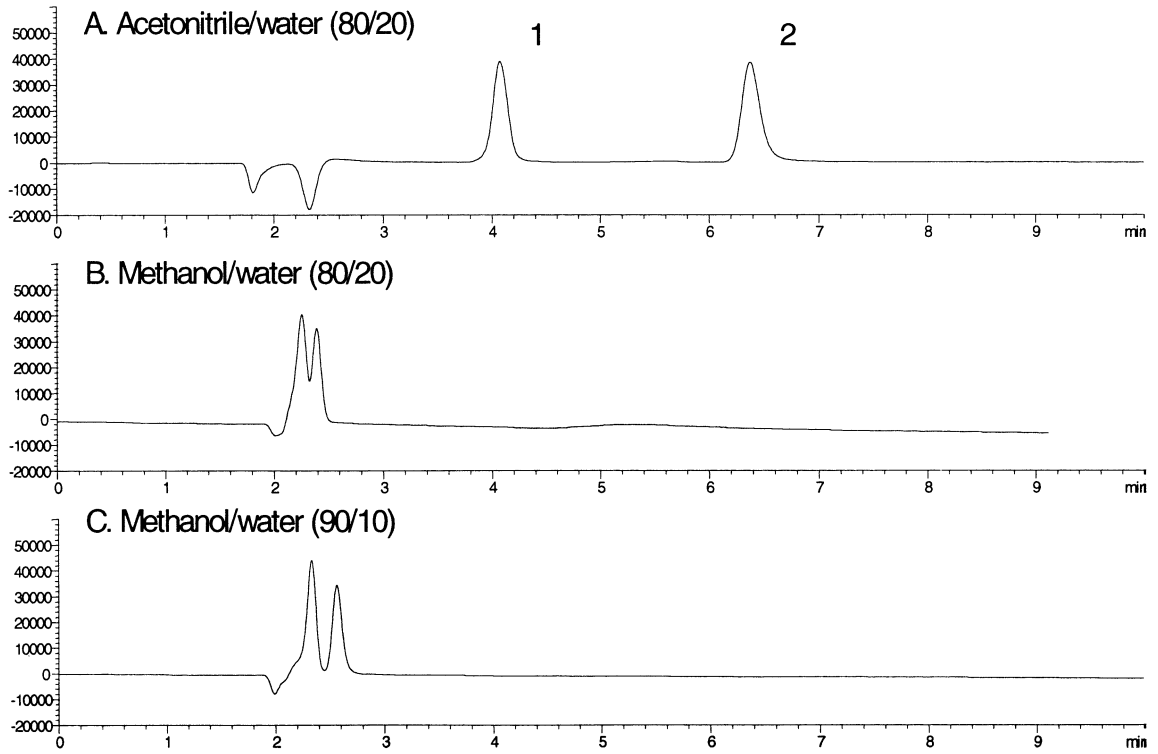


Fig. 2. Chromatograms for the separation of AEPD (Peak 1) and tromethamine (Peak 2) using the mobile phase (A) acetonitrile/water (80/20), (B) methanol/water (80/20) and (C) methanol/water (90/10). Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Column temperature: 25 °C. Flow rate: 1 ml/min. Samples: AEPD and tromethamine standards (~ 1 mg/ml) each in the mobile phase. Injection volume: 50 μl.

software (Rev. A. 09. 01). The following columns were used in the experiments: Zorbax NH_2, 70 Å pore diameter from Agilent Technologies (Wilmington DE), Nucleosil NH_2, 100 Å pore diameter from Phenomenex (Torrance, CA), YMC-Pack NH_2, 120 Å pore diameter from Waters (Milford, MA). All the columns were 150 × 4.6 mm I.D. with 5 μm particles.

2.2. Reagents

HPLC grade acetonitrile, methanol and isopropanol (IPA) were purchased from Burdick & Jackson (Muskegon, MI). Ammonium acetate (HPLC grade) was obtained from J. T. Baker (Phillipsburg, NJ). Tromethamine standard (+ 99.9%) was purchased from Aldrich (Milwaukee, WI), and 2-amino-2-ethyl-1,3-propanediol (AEPD) was from Acros (New Jersey, USA).

2.3. Chromatographic conditions

All the columns were washed with IPA for at least 30 min at 1 ml/min to remove the storage solvent (hexane), then with acetonitrile/water mixture (50/50, v/v) for about 1 h, and finally equilibrated with the mobile phase. The mobile phase was composed of only acetonitrile and water (80/20, v/v) for the final method. All reference standards and samples were dissolved in the mobile phase. The optical unit temperature of the RI detector was set at 35 °C to minimize baseline noise. The flow rate was 1.0 ml/min and injection volume was 50 μl for the method.

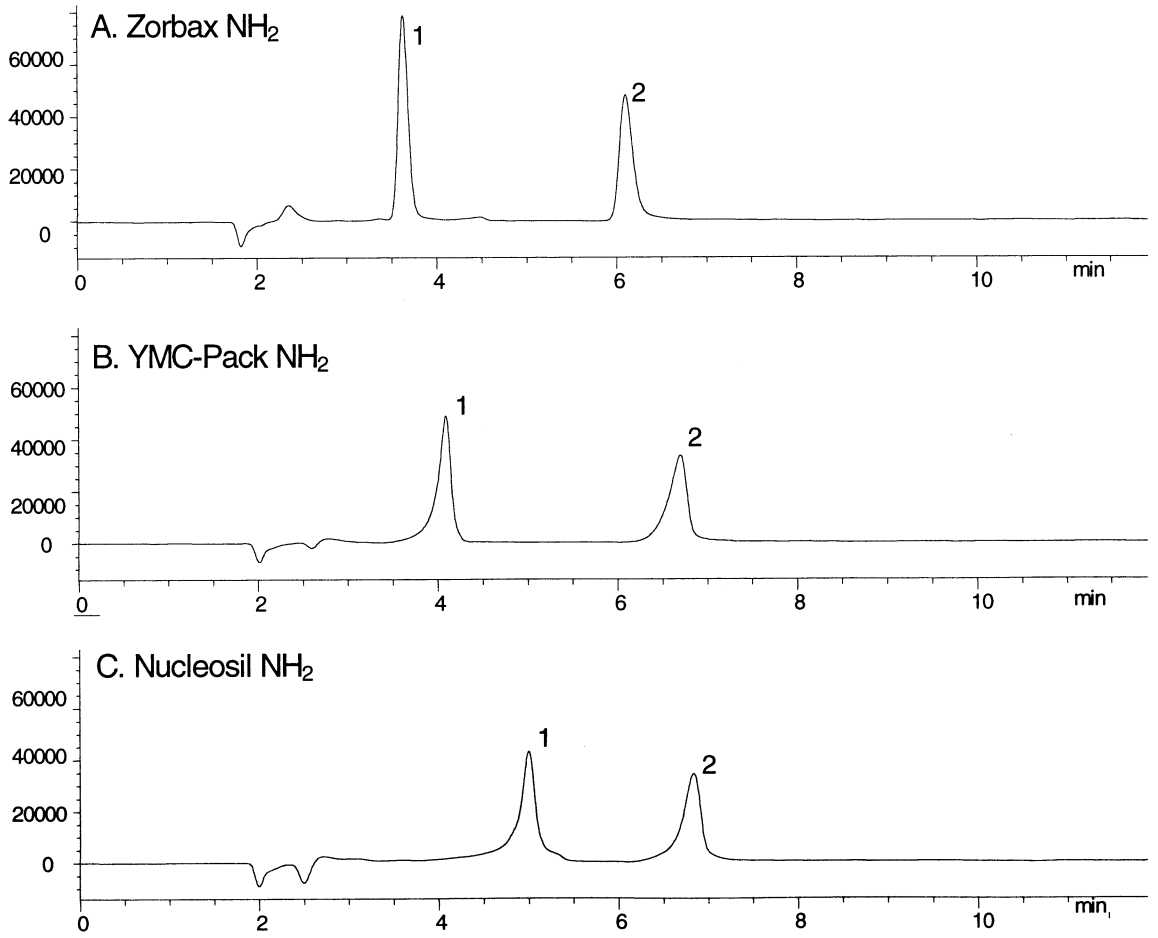


Fig. 3. Chromatograms for the separation of AEPD (Peak 1) and tromethamine (Peak 2) on three amino columns, (A) Zorbax NH_2, (B) YMC-Pack NH_2, and (C) Nucleosil NH_2 column. Column temperature: 25 °C. Mobile phase: acetonitrile/water (80/20, v/v). Flow rate: 1 ml/min. Samples: AEPD and tromethamine standards (~ 1 mg/ml) each in the mobile phase. Injection volume: 50 μl.

3. Results and discussion

3.1. Method development

The HILIC approach was employed for tromethamine separation based on the fact that it had been shown to provide sufficient retention for very polar compounds such as sugars, uracil, acetamide, etc. [6,7,9]. In addition to tromethamine, another structurally related compound, AEPD was also selected to aid method development. In this study, only amino stationary phase was used and the mobile phase was a simple mixture of water and organic solvent (i.e. acetonitrile and methanol). Fig. 2 shows the separation of tromethamine and AEPD on a Zorbax NH_2 column using acetonitrile/water or methanol/water mobile phase. Under the experimental conditions, the more hydrophilic tromethamine eluted after AEPD, which was expected for the HILIC separation. Sufficient retention was obtained using the acetonitrile/water (80/20, v/v) mobile phase ($k' \sim$ 2.3 for tromethamine); however, the retention was greatly reduced in the methanol/water (80/20, v/v) mobile phase ($k' \sim 0.2$). The retention was not significantly improved even when the methanol content was increased to 90%. Based on these results, acetonitrile/water (80/20, v/v) was chosen

Table 1
Packing properties of the three amino columns and the chromatographic data for tromethamine

Column	Packing properties			Chromatographic data		
	Carbon load (%)	Surface coverage[a]	End-capping	Capacity factor	Efficiency (plates)	Asymmetry factor
Zorbax NH_2	4.0	3.7	No	2.35	8211	1.52
YMC Pack NH_2	3.4	3.5	No	2.33	5510	0.6
Nucleosil NH_2	3.5	3.1	No	2.42	6146	0.65

[a] In the unit of μmol/m^2.

for the mobile phase. In addition, the high acetonitrile content in the mobile phase also brought extra benefit of increasing the solubility of the investigational drug and its tromethamine salt since samples had to be prepared in the mobile phase for RI detection.

Three amino columns from different vendors were tested with the aim to select the best performing column. Fig. 3 shows the separation of tromethamine and AEPD using the acetonitrile/water (80/20, v/v) mobile phase on three amino columns. The packing properties of the three columns and chromatographic data for tromethamine are presented in Table 1. Zorbax NH_2 column had slightly higher carbon load and surface coverage than YMC-Pack NH_2 and Nucleosil NH_2 columns, but Nucleosil NH_2 column produced a slightly larger retention factor for tromethamine than the other two columns. Zorbax NH_2 column yielded the highest efficiency for tromethamine. Both tromethamine and AEPD peaks showed small tailings on Zorbax NH_2 column, but significant fronting on YMC-Pack NH_2 and Nucleosil NH_2 columns. Therefore, Zorbax NH_2 column was selected for further method development.

3.2. The effect of acetonitrile content

The mobile phase used in HILIC is similar to reverse-phase HPLC, namely, aqueous–organic mixture. However, water is considered as the stronger solvent and an increase in the organic solvent content would lead to longer retention of polar analytes in HILIC separation [6]. In this study, we investigated the effect of acetonitrile content (50–95%) in the mobile phase on retention. No retention was obtained for the analytes under 50% acetonitrile. Fig. 4 shows the plot of ln k' versus acetonitrile content. The retention of both tromethamine and AEPD increased at higher acetonitrile contents, opposite to the behavior in reversed-phase HPLC. The value of ln k' increased in a linear fashion in the range from 60 to 95% acetonitrile for tromethamine and in the range of 65–95% acetonitrile for AEPD. However, the ln k' value for tromethamine at 50% acetonitrile deviated from the linear behavior, and the k' value for AEPD started to deviate from the linear fashion at 60% acetonitrile. Repeated experiments confirmed that the deviation at 50% acetonitrile was not due to the experimental error. This deviation possibly implies that the separation mechanism might not be completely based on hydrophilic interaction, and secondary interactions might be involved at lower acetonitrile content [6].

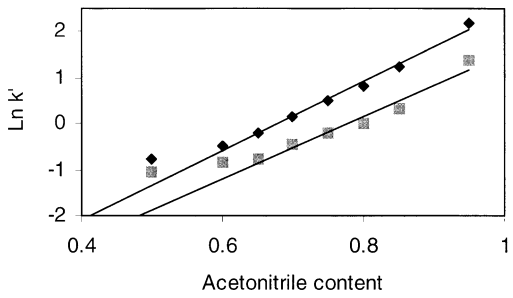


Fig. 4. Plots of ln k' vs. acetonitrile content in the mobile phase for tromethamine (◆) and AEPD (■). Column: Zorbax NH_2, and column temperature: 25 °C.

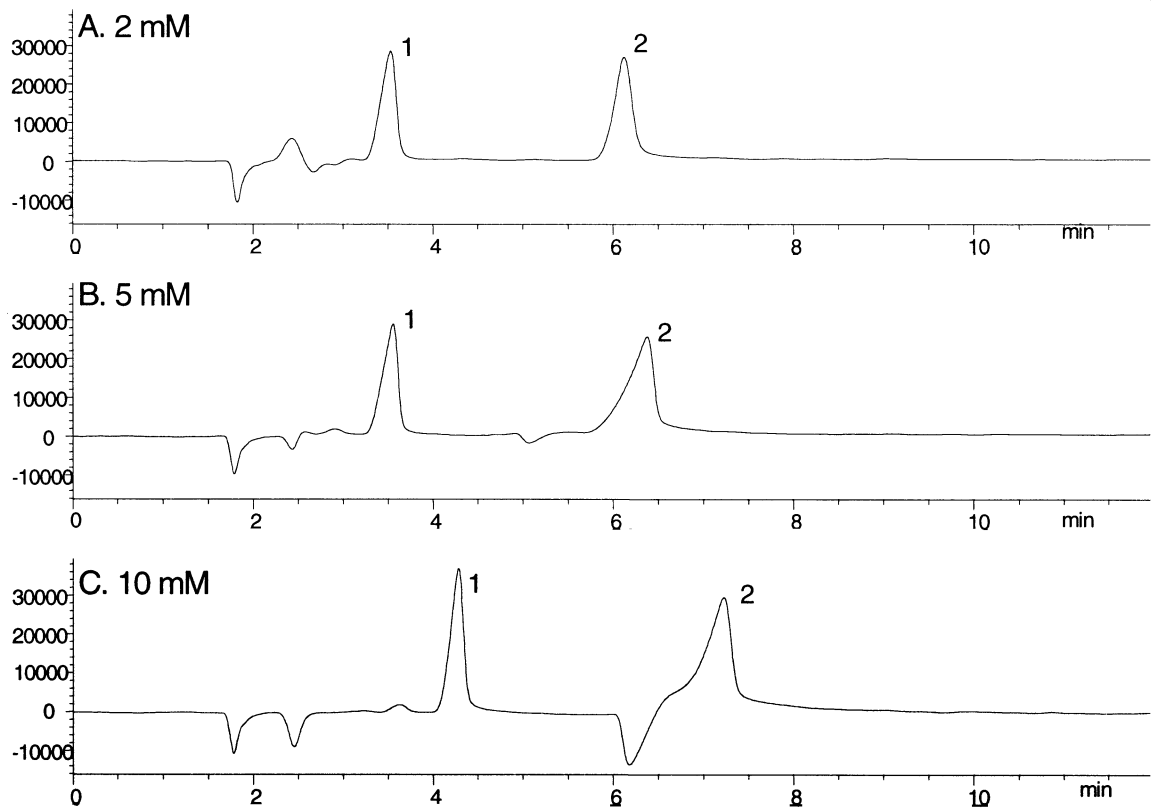


Fig. 5. Chromatograms for the separation of tromethamine and AEPD with different amounts of ammonium acetate in the mobile phase. Mobile phase: acetonitrile/water (80/20, v/v) containing (A) 2 mM, (B) 5 mM, and (C) 10 mM ammonium acetate. Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Other conditions are the same as in Fig. 3.

Table 2
Chromatographic data for AEPD and tromethamine on a Zorbax NH_2 column in the mobile phase of acetonitrile/water (80/20, v/v) containing various concentrations of ammonium acetate (NH_4Ac)

NH_4Ac (mM)	AEPD			Tromethamine		
	Capacity factor	Efficiency (plates)	Asymmetry factor	Capacity factor	Efficiency (plates)	Asymmetry factor
2	0.94	1971	0.60	2.36	4654	0.93
5	0.99	1999	0.50	2.56	2463	0.44
10	1.40	3956	0.68	3.06	3162	0.49

3.3. The effect of ammonium acetate

Ammonium acetate is often used as a buffer salt in the mobile phase for HILIC separations due to its good solubility at high organic content [8]. The effect of ammonium acetate on the separation of tromethamine and AEPD was investigated by varying ammonium acetate concentration in the acetonitrile/water mobile phase (80/20, v/v). Zorbax NH_2 column used for this study was first washed with IPA, and then with an acetonitrile/water mixture (50/50, v/v). Finally the column was equilibrated with the mobile phase containing ammonium acetate. The apparent pH of the

mobile phase containing ammonium acetate was not measured, but the pH of the aqueous solution of 10 mM ammonium acetate was about 6.6. Fig. 5 shows the chromatograms for the separation of tromethamine and AEPD using the mobile phase containing 2, 5 and 10 mM ammonium acetate, and the chromatographic data for AEPD and tromethamine are presented in Table 2. The baseline dip before the tromethamine peak in Fig. 5B and 5C was possibly due to contaminants in the ammonium salt since it was also present in the blank injection. It was noticed that the analyte peaks displayed obvious fronting as compared with the separation in the mobile phase without ammonium acetate, and the fronting became more serious at higher ammonium acetate concentration. The possibility of mismatch between the sample solvent and mobile phase causing peak fronting was ruled out since the samples were prepared in the same mobile phase containing ammonium acetate in this study as required by the RI detection. The fronting might be related to the changes in the immobile water layer on the surface of the packing material due to increasing salt concentrations. Furthermore, the capacity factors also increased for both AEPD and tromethamine as the ammonium acetate concentration in the mobile phase was raised from 2 to 10 mM. The acetate counter ions might be adsorbed onto the positively charged amino phase through electrostatic interaction. This could reduce electrostatic repulsion of the positively charged analytes from the positively charged stationary phase, thus resulting in increased retention.

3.4. Effect of column temperature

Column temperature is often evaluated as a useful variable in method development. We investigated the effect of column temperature on retention in the range 15–55 °C. The effect of column temperature on retention was studied by Van't Hoff equation, which describes the dependence of $\ln k'$ on absolute temperature (T):

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi$$

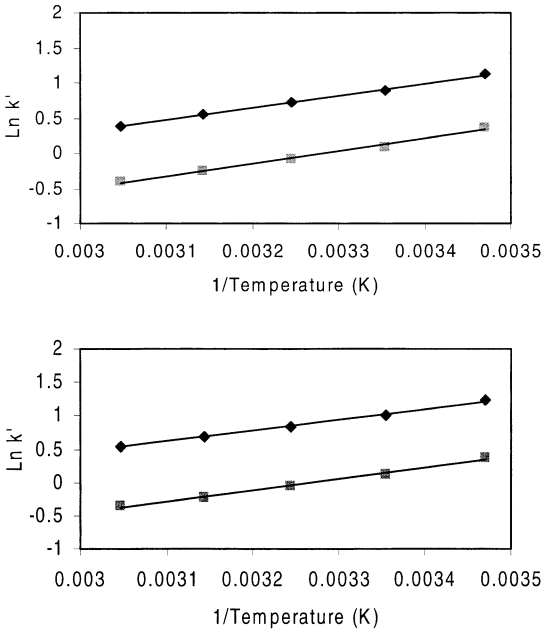


Fig. 6. Plots of $\ln k'$ vs. 1/temperature for tromethamine (◆) and AEPD (■). Top panel: mobile phase without ammonium acetate. Bottom panel: mobile phase with 5 mM ammonium acetate. Mobile phase: acetonitrile/water (80/20, v/v). Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size.

where ΔH° and ΔS° are the retention enthalpy and entropy, R is the gas constant and ϕ is the phase ratio. Fig. 6 shows the Van't Hoff plots for tromethamine and AEPD in the mobile phase of acetonitrile/water (80/20, v/v) with and without 5 mM ammonium acetate. The Van't Hoff plots for both compounds were linear and had a positive slope. The retention for both analytes decreased with increasing column temperature under both mobile phase conditions. The calculated enthalpy values ($-\Delta H^\circ$) were 14.1 ± 0.3 and 15.0 ± 0.7 kJ/mol for tromethamine and AEPD, respectively, on Zorbax NH_2 column in acetonitrile/water (80/20, v/v) mobile phase without ammonium acetate. With 5 mM ammonium acetate in the mobile phase, the enthalpy values changed to 13.4 ± 0.2 and 14.2 ± 0.5 kJ/mol for tromethamine and AEPD, respectively. There was no statistical difference in the enthalpy values for AEPD with or without the presence of 5 mM ammonium acetate in the mobile phase. However, the enthalpy

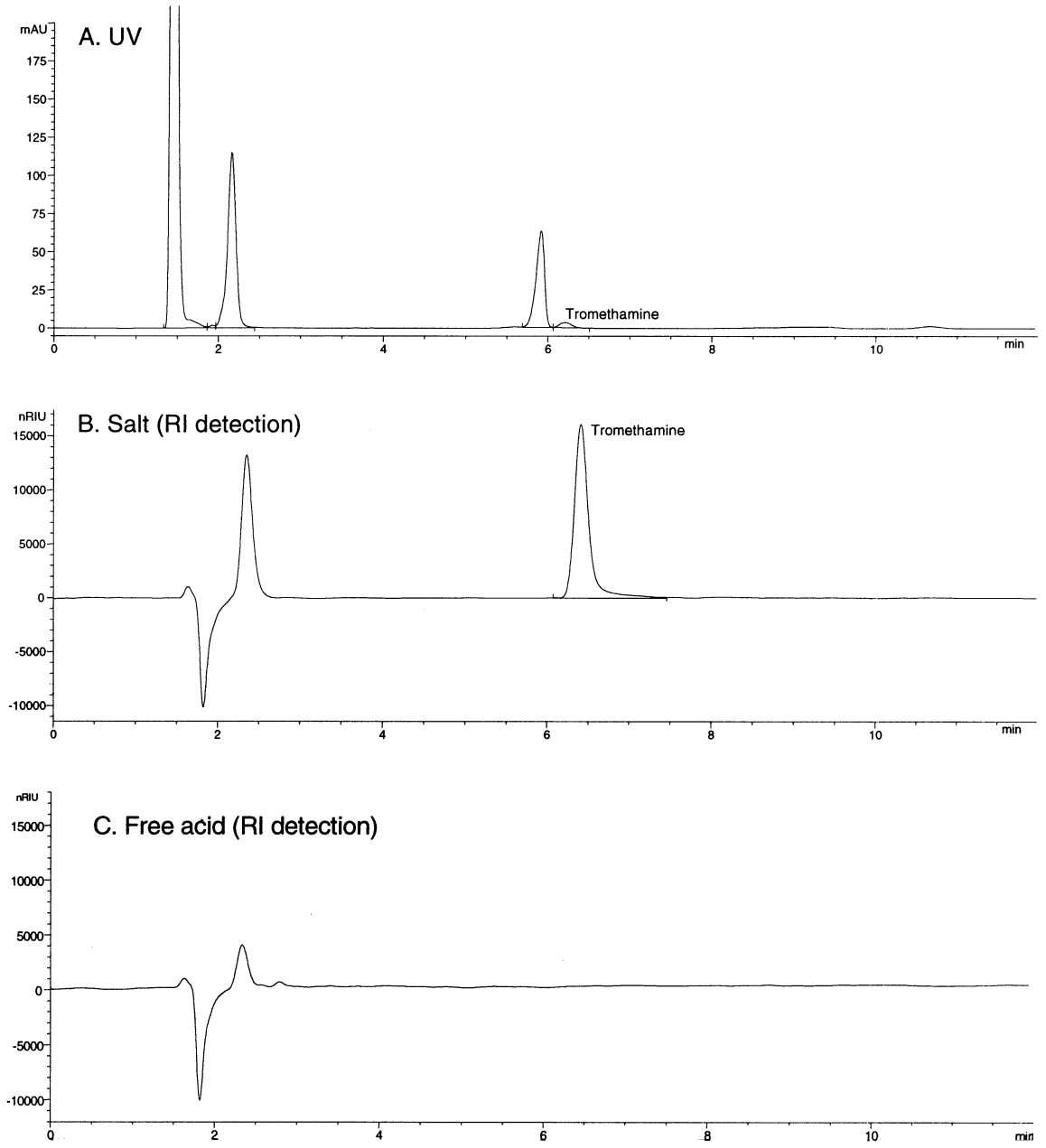


Fig. 7. Chromatograms for the salt and free acid form of the investigational drug from UV and RI detectors. Column: Zorbax NH_2, 4.6 × 150 mm, 5 μm particle size. Column temperature: 25 °C. Mobile phase: acetonitrile/water (80/20, v/v). Flow rate: 1 ml/min. Sample: the investigational API (salt form ~2 mg/ml in the mobile phase). Injection volume: 50 μl.

value for tromethamine in the mobile phase containing 5 mM ammonium acetate was slightly lower (~5%) than that in the mobile phase without ammonium acetate. The enthalpy change might be related to the retention mechanism of the HILIC separation, and may help to explain why

Table 3
Retention time of AEPD and tromethamine (Tris) under varied chromatographic conditions

	Flow rate (ml/min)			Column temperature (°C)			Acetonitrile content (%)		
	0.9	1.0	1.1	22	25	28	78	80	82
AEPD	4.52	4.07	3.72	4.26	4.07	3.96	3.99	4.07	4.58
Tris	7.08	6.37	5.80	6.65	6.37	6.14	5.99	6.37	7.69

the addition of ammonium acetate caused increased retention as discussed above.

3.5. Method validation

3.5.1. Method specificity

The HILIC method was intended for the analysis of tromethamine content in the investigational API. The method specificity was demonstrated by analyzing the free acid form and salt form of the investigational compound with online diode array (DAD) and RI detectors. Fig. 7 shows the chromatograms of the salt form from both UV (225 nm) and RI traces and the chromatogram of the free acid from the RI detector. The large peak eluting in the solvent front (1.4 min) in the UV trace was confirmed by UV spectrum to be the free acid form of the API. Other impurities in the drug substance were also observed in the UV trace, for example, the peaks at 2.2, 5.9 and 10.7 min. The small peak at 6.2 min corresponded to tromethamine. The small difference in the retention times in the UV trace and RI traces (6.2 vs. 6.4 min) was due to the fact that the DAD detector was positioned ahead of the RI detector. The RI trace of the free acid form did not show any peak around the elution time of tromethamine. The comparison of the UV and RI traces demonstrated that the free acid form of the API and related impurities presented no interference to tromethamine detection.

3.5.2. Repeatability, linearity and sensitivity

The method repeatability was validated by using tromethamine reference standard solution. Six injections of a reference standard yielded relative standard deviation (%R.S.D.) of 1.9%, and difference between the average response factors (i.e. standard weight/peak area) of three injections of two reference standards was 0.5%. The linear dynamic range of the RI detector was evaluated using six reference standards ranging from 0.1 to 2.0 mg/ml. Linear regression of peak area versus standard concentration data yielded a correlation coefficient of 0.9997.

Refractive index was chosen as the detection mode for tromethamine to overcome the lack of chromophores. The relative poor sensitivity of the RI detector was partially compensated by a large injection volume (50 μl). A limit of detection of 0.03 mg/ml was obtained based on signal to noise ratio of at least 3. The method sensitivity was not as low as that of UV detection with derivatization or conductivity detection in IC; however, it was sensitive enough for the purpose of this method.

3.5.3. Robustness

The robustness of the HILIC method was investigated by slightly changing the chromatographic conditions such as flow rate, column temperature, and acetonitrile content in the mobile phase. In robustness testing, the flow rate was changed by 10% of the normal value, and the column temperature was varied by 3 °C. The acetonitrile content was shown to have a large effect on the retention time of the analytes (Fig. 3). Therefore, only a small change in the acetonitrile content (2%) was tested. The retention time of AEPD and tromethamine obtained under different robustness test conditions is presented in Table 3. Compared with the retention time under normal running conditions, i.e. flow rate of 1 ml/min and column temperature of 25 °C, the retention time fluctuated by about 10% due to the changes in flow rate and column temperature. When the acetonitrile content was 2% lower than the normal

Table 4
Recovery of tromethamine from the investigational compound

Amount spiked (mg/ml)	Amount recovered (mg/ml)	Average recovery (%)
0.311	0.30	96.5
0.567	0.59	104.1
0.996	0.99	99.4

level (80%), the retention time changed by only 2 and 6% for AEPD and tromethamine, respectively. However, 2% upward shifting of the acetonitrile content caused 13 and 20% change in retention time for AEPD and tromethamine, respectively. This indicated that tromethamine retention time was very sensitive to the variation of the acetonitrile content in the mobile phase, especially to the upward shifting. In addition, more than three Zorbax NH_2 columns were used in this study and variation in retention time and efficiency for tromethamine was less than 10%. For example, the chromatograms in Fig. 2A and Fig. 3A showed the separation of AEPD and tromethamine on two different Zorbax NH_2 columns, and column performance was very comparable.

3.5.4. Recovery

The free acid form of the API was not soluble in the acetonitrile/water mixture (80/20, v/v). Therefore, the recovery study was not performed by spiking the solution of the free acid form with tromethamine at different levels. Instead, tromethamine solutions of different concentrations (0.3, 0.6 and 1.0 mg/ml) were used to dissolve the free acid form of the investigational API at 1.4 mg/ml. The recovery data presented in Table 4 indicated that good recovery was achieved at all the three levels. This demonstrated that the method was accurate to analyze tromethamine in the investigational drug substance.

3.6. Application

The validated method was used to analyze seven small research batches of the investigational drug substance in support of process research on salt formation. Table 5 presents the analytical results for the tromethamine content (w/w%) in the investigational API as well as the potency of the drug substance in the form of free acid (w/w%) using a separate reverse-phase HPLC method. Our results confirmed to synthetic chemists that the salt form of the drug was a bis-tromethamine salt, and at least two equivalents of tromethamine were needed in the salt formation.

4. Conclusions

We have developed a HILIC method for the analysis of tromethamine counter ion in an investigational API. The HILIC approach demonstrated distinct advantages over conventional reverse-phase HPLC for the separation of small polar compounds. In this method, the non-salt form (free acid) of the API eluted in the solvent front. This minimized interference of the active moiety (normally in larger amount) with tro-

Table 5
Tromethamine amount in the salt form of the investigational drug substance

Sample number	Tromethamine (%, w/w)	Free acid (%, w/w)	Molar ratio[a]	Tromethamine added[b]
1	24.9	73.4	1.9	2 Equivalents
2	24.8	76.5	1.9	3 Equivalents
3	27.0	76.2	2.0	4 Equivalents
4	14.8	82.4	1.0	1 Equivalents
5	25.4	77.3	1.8	2 Equivalents
6	25.4	77.2	1.8	2 Equivalents
7	26.2	77.2	1.9	2 Equivalents

[a] The ratio of moles of tromethamine to free acid.
[b] Amount of tromethamine added in the salt formation step of the synthesis.

methamine analysis, but also avoided washing steps that would be necessary if the active moiety were to elute after tromethamine. The mobile phase used in this method not only provided sufficient retention for the analyte, but also solved the solubility problem with the investigational drug. The validation proved that this HILIC method was accurate and precise for the determination of tromethamine at the level expected in the investigational drug substance.

Acknowledgements

The authors would like to recognize the support from synthetic chemists, especially Dr. Yun Qian for providing drug substance samples. We would also like to thank Dr. Ralph Ryall, Dr. Bruce Weber, Dr. Kailin Guan and Dr. Weiyong Li for reviewing the manuscript and helpful discussions.

References

[1] The Merck Index, tenth ed., Merck & Co., Inc., Rahway, NJ, 1983, p. 9576.
[2] K. Gumbhir, W.D. Mason, J. Chromatogr. B 583 (1992) 99–104.
[3] W. Naidong, S. Ghodbane, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 477–484.
[4] R.E. Hall, G.D. Havner, R. Good, D.L. Dunn, J. Chromatogr. A 718 (1995) 305–308.
[5] F.A. McArdle, C.J. Meehan, Analyst 123 (1998) 1757–1760.
[6] A.J. Alpert, J. Chromatogr. 499 (1990) 177–196.
[7] S.C. Churms, J. Chromatogr. A 720 (1996) 75–91.
[8] A.R. Oyler, B.L. Armstrong, J.Y. Cha, M.X. Zhou, Q. Yang, R.I. Robinson, R. Dunphy, D.J. Burinsky, J. Chromatogr. A 724 (1996) 378–383.
[9] P. Dellet, L. Labat, E. Kummer, J.P. Dubost, J. Chromatogr. B 742 (2000) 447–452.
[10] M.A. Strege, S. Stevenson, S.M. Lawrence, Anal. Chem. 72 (2000) 4629–4633.
[11] B.A. Olsen, J. Chromatogr. A 913 (2001) 113–122.