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Ion chromatography of amylamine and *tert.*-butylamine in pharmaceuticals

Nirdosh K. Jagota*, Aneil J. Chetram, Jay B. Nair

Analytical R&D, Bristol-Myers Squibb, Pharmaceutical Research Institute, New Brunswick, NJ 08903-0191, USA

Abstract

Ion chromatographic (IC) methods have been developed for the assay of amylamine in BMS-181 866-02 and *tert.*-butylamine (TBA) in BMS-188 494-04. BMS-181 866-02 is the penultimate intermediate in the synthesis of a novel thromboxane antagonist, BMS-180 291-02, which is undergoing clinical trials. Amylamine may be present as a trace impurity in BMS-181 866-02. BMS-188 494-04 is the TBA salt of the prodrug ester of BMS-187 745, a novel oral hypocholesterolemic agent. Chromatographic separations were accomplished under isocratic conditions using a Dionex CS-14 column with conductivity detection. The methods differ only in the composition of the methanesulfonic acid–acetonitrile mobile phase. The detection limit and minimum quantifiable levels for amylamine were 0.01% and 0.02%, respectively. The method was linear over the range studied (1–12.5 μg/ml, $n=7$, $r=0.9993$). The method for TBA was linear from 5 to 30% (w/w) (50–300 μg/ml, $n=8$, $r=0.9993$) of working sample concentration (1 mg/ml BMS-188 494-04). The precision and accuracy of the methods are presented.

Keywords: Pharmaceutical analysis; Amylamine; Butylamine; Amines

1. Introduction

Analysis of aliphatic amines pose special challenges. Lack of an UV response and poor peak shapes on silica-based columns make conventional HPLC impractical. In this paper, simple ion chromatographic (IC) methods are described for the analysis of amylamine and *tert.*-butylamine (TBA) in pharmaceutical matrices. The separations were achieved using a Dionex CS-14 column with conductivity detection. Fig. 1 shows the structures of amylamine and TBA.

Amylamine is used in the synthesis of BMS-181 866-02 and may be present as a trace impurity. The monitoring of residual amylamine in BMS-181 866-02 is necessary for mass–balance purposes. BMS-181 866-02 is the penultimate intermediate in the synthesis of the thromboxane antagonist, BMS-180 291-02, which is undergoing clinical trials.

TBA is the counter-ion of BMS-188 494-04. BMS-188 494-04 is the TBA salt of the prodrug ester of the BMS-187 745, a new oral hypocholesterolemic agent. The analysis of TBA is necessary to assign a purity value for BMS-188 494-04 as the free acid.

Both, high-performance liquid chromatography (HPLC) and gas chromatography (GC) have been used for the analysis of aliphatic amines. HPLC analyses include detection by ultraviolet [1–4], fluorescence [5,6], electrochemical [7] and chemiluminescence excitation [8] techniques. These methods require derivatization to increase the detection sen-

*Corresponding author.

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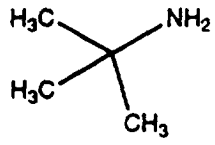
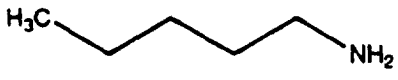


tert-Butylamine



Amylamine

Fig. 1. Structural formulae of compounds studied.

sitivity. HPLC is not specific for separating amines and interference from non-ionic species can occur when analyzing complex samples. GC analyses of underivatized primary amines result in adsorption and decomposition on the column giving tailing peaks [9–12]. Many GC methods involving derivatization have been reported [13–22]. These methods include flame ionization, electron-capture, flame thermionic, mass spectrometric, chemiluminescence and flame photometric detection. However, derivatization methods are time-consuming. In addition, the selectivity of amine derivatives cannot be guaranteed during the analysis of complex mixtures, such as pharmaceuticals.

IC of aliphatic amines has been reported by several authors [23–27]. Haddad et al. [23] reported a method using an anion-exchange column (Bio-Rad HPX-72-O, 300×7.8 mm) with a mobile phase consisting of 10 mM sodium hydroxide. Krol et al. [24] reported the IC of alkylamines and alkanolamines using a poly(butadiene–maleic acid)-coated silica column and a mobile phase of EDTA–nitric acid, containing acetonitrile or methanol. Daigle et al. [25] studied the IC of alkyl amines using a silica-based cation-exchange column with oxalic acid, dichloroacetic acid and sulfamic acid as eluents. Vialle et al. [26] reported an experimental design for the optimization of the separation of aliphatic amines using a silica-based ion-exchange column. Lacourse et al. [27] reported the separation of a mixture of linear and branched alkanolamines by reversed-phase paired-ion chromatography with pulsed amperometric detection at a gold electrode. Amylamine and *tert.*-butylamine are not included in these studies. Also, these studies do not include any quantitation and have not been applied to trace determination in pharmaceuticals. The goal of this work is to develop assays for amines present as impurities or counter-ions in pharmaceuticals.

2. Experimental

2.1. Reagents

BMS-181 866-02 and BMS-188 494-04 were obtained from the Chemical Process Technology Group, Bristol-Myers Squibb, New Brunswick, NJ, USA. Amylamine, TBA, *sec.*-butylamine, methanesulfonic acid and potassium chloride were purchased from Aldrich (Milwaukee, WI, USA). HPLC grade (B & J brand) acetonitrile was obtained from Baxter Scientific (Edison, NJ, USA) and 0.45 μm Nylon-66 filters were obtained from Schleicher and Schuell (Keene, NH, USA).

2.2. Instrumentation

A Dionex ion chromatograph composed of a gradient pump module (GPM), eluent degas module (EDM-II) and conductivity detector (CDM-II), all from Dionex (Sunnyvale, CA, USA) were used. The system was connected to a Thermo Separation Products (Fremont, CA, USA) autosampler (model 8880). Data acquisition was performed with a VG Multichrom data processor (VG Laboratory Systems, Cheshire, WA, USA).

A cation micromembrane suppressor, a CS-14 (250×4 mm) column and a CG-14 (50×4 mm) guard column, were purchased from Dionex.

2.3. Chromatographic conditions

A 5-μm CS-14 (250×4 mm) Dionex column was used for the analysis of both amylamine and TBA. The mobile phase was acetonitrile–methanesulfonic acid (50 m*M*) (5:95, v/v) for the amylamine assay.

The flow-rate was 1.0 ml/min. Acetonitrile–methanesulfonic acid (10 m*M*) (1:99, v/v) was used for the TBA assay. A self-generating cation suppressor was used before the detector to suppress the eluent conductivity. The suppressor used was a 100 m*M* solution of tetrabutyl ammonium hydroxide at a flow-rate of 8.0 ml/min. The injection volume was 20 μl.

2.4. System suitability test

A system suitability test was performed with each analysis by measuring the resolution between *sec.*-butylamine and amylamine for the amylamine assay. A resolution of 2.0 or greater between *sec.*-butylamine and amylamine was indicative of proper system performance. Fig. 2 shows the typical system suitability chromatogram. A mixture of potassium chloride and TBA was injected as the system suitability standard for the TBA assay. A resolution of 2.0 or more between potassium and TBA was determined to be acceptable. Fig. 3 illustrates a typical system suitability chromatogram.

3. Results and discussion

3.1. Amylamine

A Dionex CS-14 column was chosen for analysis because it is a solvent-compatible cation exchange column. Combinations of methanesulfonic acid and acetonitrile were used as a mobile phase for methods development. The effects of the concentration of methanesulfonic acid (20 to 100 m*M*) and acetonitrile (10 to 25%) on the retention of amylamine were studied and the best results were obtained with the mobile phase described in Section 2. It was noted that the retention time of amylamine decreased with increasing acetonitrile concentration. A major advantage of the method is that BMS-181 866-02 dissolved in water, can be directly injected into the IC

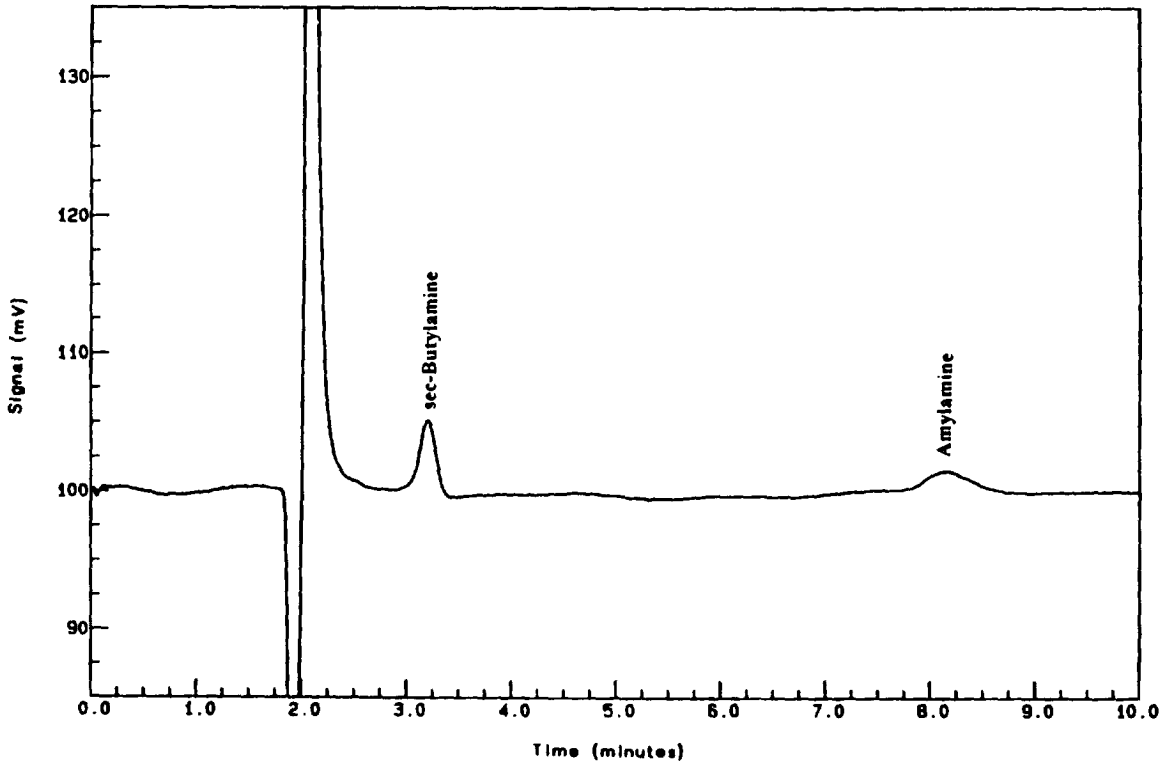


Fig. 2. Typical system suitability chromatogram of *sec.*-butylamine (1 μg/ml) and amylamine (1 μg/ml).

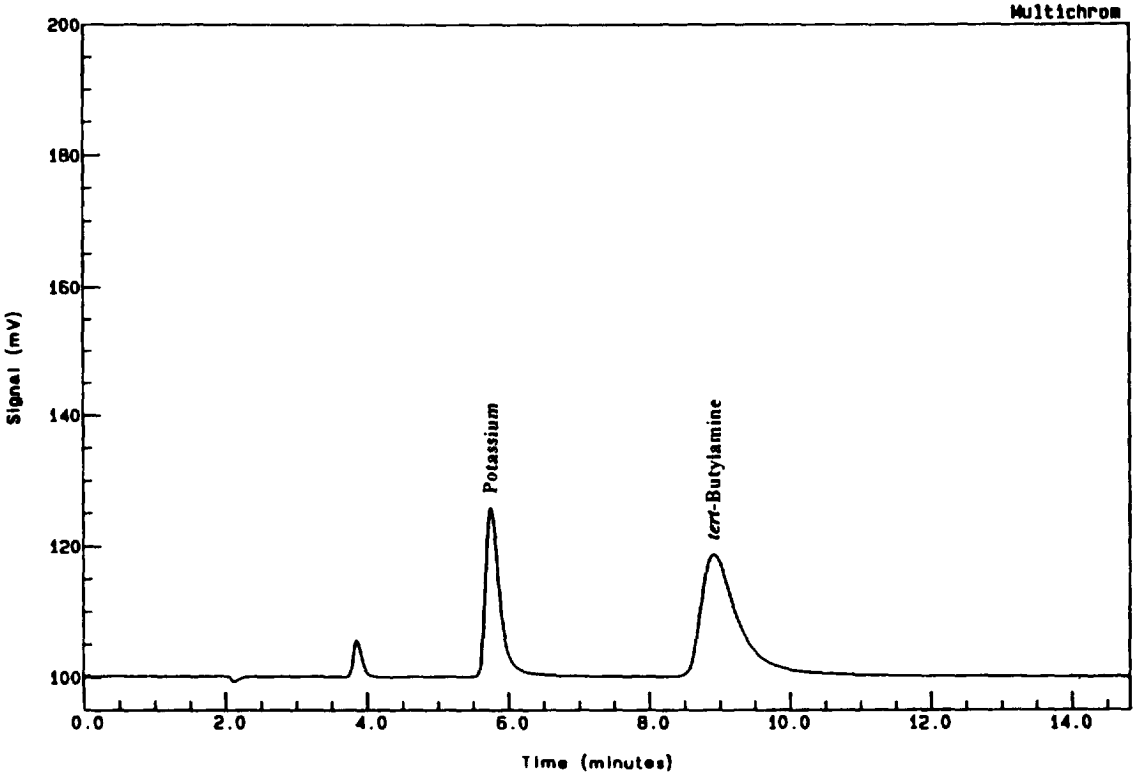


Fig. 3. Typical system suitability chromatogram of potassium (10 μg/ml) and TBA (50 μg/ml).

system. Thus, sample clean-up steps, normally associated with trace ion assays, are avoided with a concomitant saving of time.

The limit of detection for amylamine, at a signal-to-noise ratio of 3, was 0.01% (w/w) and the minimum quantifiable level, at a signal-to noise-ratio of 10, was 0.02% (w/w) in BMS-181 866-02. Fig. 4 shows a typical chromatogram of amylamine at the limit of detection (signal-to-noise ratio of 3) in the presence of BMS-181 866-02. The separation condition is selective enough that interferences from other impurities originating from the synthetic process of BMS-181 866-02 are not detectable.

The linearity of the assay was studied from 1 to 12.5 μg/ml (corresponding to 0.02–2.5% (w/w) amylamine in BMS-181 866-02). The correlation coefficient was 0.9993 for a set of seven standards. The intercept was −2851.9 and the slope was 62 126. The precision of the assay was determined by injecting, in replicate (n=8), BMS-181 866-02 at 5.0-mg/ml spiked with 0.02% amylamine. The precision obtained was 0.022±0.002 (R.S.D.=11.4%).

The accuracy of the method was determined by spiking a batch of BMS-181 866-02 at various levels with amylamine. The recovery of amylamine at levels ranging from 0.01 to 0.25% was 96–110%. Table 1 illustrates the recovery and accuracy data.

3.2. tert.-Butylamine

The amylamine method was modified to assay TBA in BMS-188 494-04. The theoretical content of TBA in this salt is 10.6%. The composition of methanesulfonic acid and acetonitrile was changed to a ratio described in Section 2. Fig. 5 shows the typical chromatogram of a batch of BMS-188 494-04 (1 mg/ml) containing 10.6% TBA. BMS-188 494-04 was dissolved in water (1 mg/ml) and directly injected into the ion chromatograph. Also, no sample clean-up was necessary here.

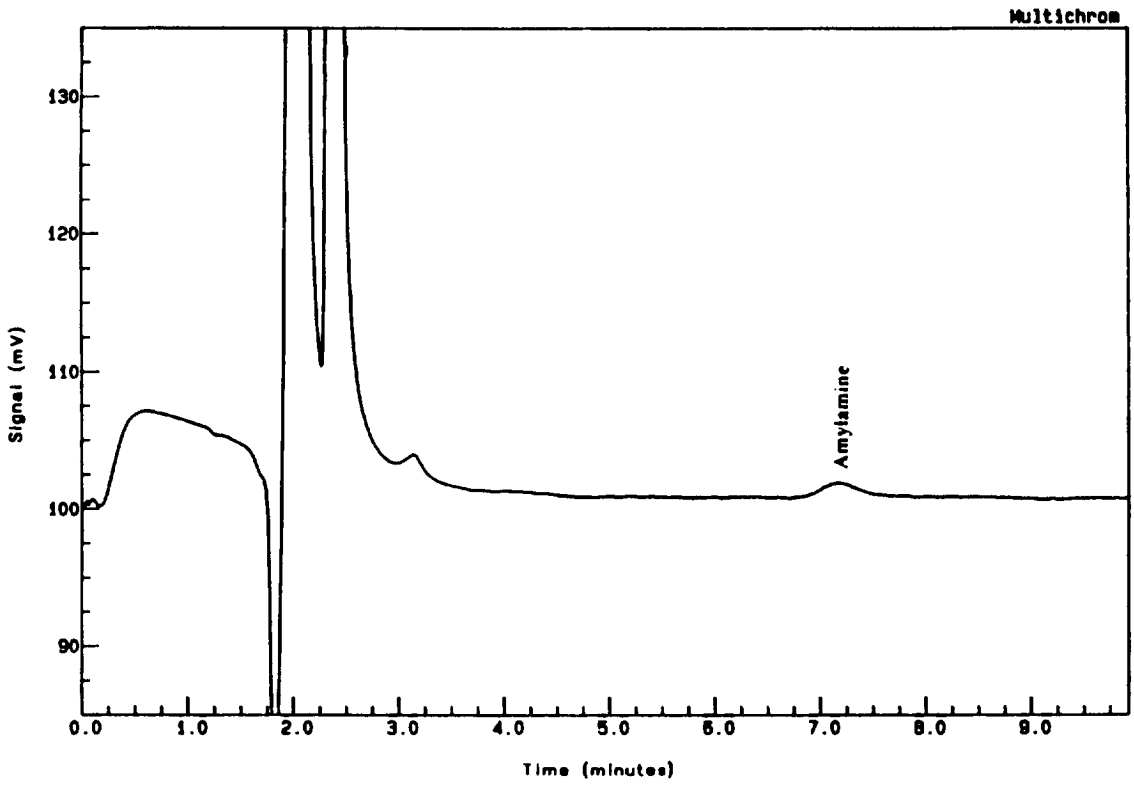


Fig. 4. Typical chromatogram of amylamine at the limit of detection (0.5 μg/ml) in the presence of BMS-181 866-02 (5.0 mg/ml).

The linearity of the response was studied from 50 to 300 μg/ml (corresponding to 5–30% (w/w) TBA in 1 mg/ml of BMS-188 494-04). The correlation coefficient was 0.9993 for a set of seven standards. The intercept was 23 719 and the slope was 369 294.

The precision of the method was determined by preparing replicate ($n=6$) working sample solutions of BMS-188 494-04. The precision for the result was 10.35±0.131 (R.S.D.=1.26%). The accuracy was determined by spiking BMS-188 494-04 with 0.5 to 1.5% (w/w) TBA, in addition to the native TBA content (Table 2). Native TBA content was determined numerous times ($n=4$).

Table 1
Recovery of amylamine from BMS-181 866-02

% Added	% Found[a]	% Recovered
0.010	0.010	100
0.020	0.022	110
0.050	0.052	104
0.080	0.077	96
0.100	0.102	102
0.160	0.158	99
0.200	0.199	100
0.251	0.262	104

[a] Based on duplicate injections.

Solution stability was determined from a 1.0 mg/ml solution of BMS-188 494-04 stored at room temperature and assayed at intervals, up to 24 h. There was no noticeable change in TBA content in the solution and the R.S.D. was 0.71% (w/w) (Table 3).

In summary, direct and rapid ion chromatographic methods have been described for the analysis of amylamine and TBA in pharmaceuticals. A wide range of analyte concentrations (0.01% to 10%) can be analyzed with the methods described. These methods may be adopted for the analysis of other cations or amines, as is evident from the separation

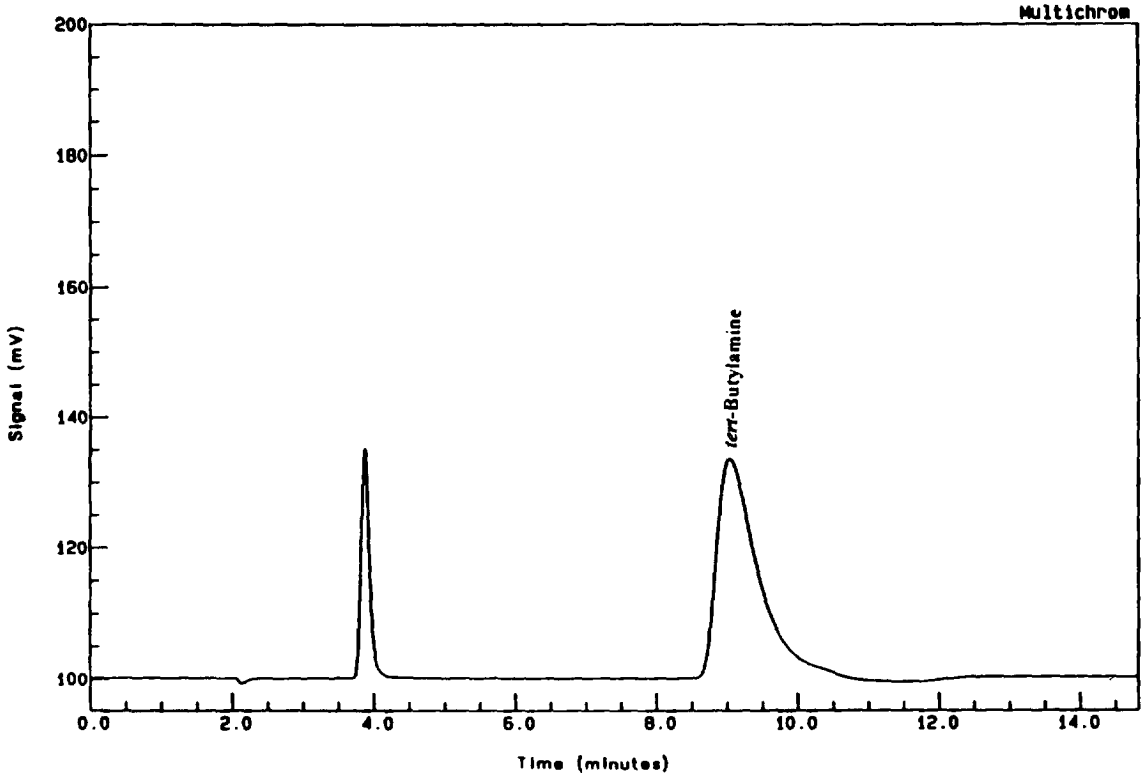


Fig. 5. Typical chromatogram of BMS-188 494-04 (1 mg/ml) containing 10.6% (w/w) TBA.

Table 2
Recovery of TBA from BMS-188 494-04

% TBA added	% TBA found[a]	% Recovery
0.00	10.62 (n=4)	–
0.50	11.01	99
1.00	11.52	99
1.50	12.01	99

[a] Based on duplicate injections.

Table 3
Solution stability of TBA in sample solvent over a 24 h time-course

Time (h)	% TBA[a]
0	10.42
5	10.31
24	10.28
Mean	10.34
S.D.	±0.074
R.S.D.(%)	0.71

[a] Based on duplicate injections.

of potassium from TBA and the separation of *sec.*-butylamine from amylamine.

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