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The separation of ipratropium bromide and its related compounds

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Abstract

Ipratropium bromide, the active component in ipratropium bromide metered dose inhalers (MDI), is used as a bronchodilator for the maintenance and treatment of bronchospasms associated with chronic obstructive pulmonary disease (COPD). The separation of ipratropium bromide, tropic acid, *N*-isopropyl-nor-atropine (NINA), 8-s ipratropium bromide, apo-ipratropium bromide and the excipients found in the formulation is important for analyzing raw materials and stability samples. We now report a reversed-phase HPLC method that can be used for separating ipratropium bromide and its related compounds, using an acetonitrile/potassium phosphate buffer (100 mM, pH 4.0) gradient mobile phase. Previous methods used for separating ipratropium bromide from its related compounds involved reversed-phase ion-pairing HPLC with UV detection. These methods exhibited less reproducibility, less ruggedness and required a high flow rate. The reported method is linear from 10 to 1000 μg ml^{-1} with a limit of detection of 60 ng ml^{-1}. In addition, analysis of samples subjected to accelerated stability conditions showed that all degradants are resolved from the active component, resulting in a stability-indicating assay. This assay also saved mobile phase and eliminated problems associated with ion-pairing reagents.

Keywords: Ipratropium bromide; HPLC; MDI; Inhalation solution; Stability indicating assay

1. Introduction

Ipratropium bromide (8-isopropyl-nor-atropine-methylbromide) is an orally active anticholinergic agent designed as an antiarhythmic therapeutic agent [1] and as a bronchodilator [2]. Its pharmacologic characteristics have previously been described [3–5]. Ipratropium bromide was developed to reduce the occurrence of side effects associated with systemic absorption from such compounds as atropine. As a result of its polar nature, ipratropium bromide is poorly absorbed across lipid membranes. Therefore, it does not easily enter the systemic circulation or the central nervous system. However, it does induce bronchodilation for long periods of time [6–8]. Ipratropium bromide appears to act primarily on the large and intermediate size airways.

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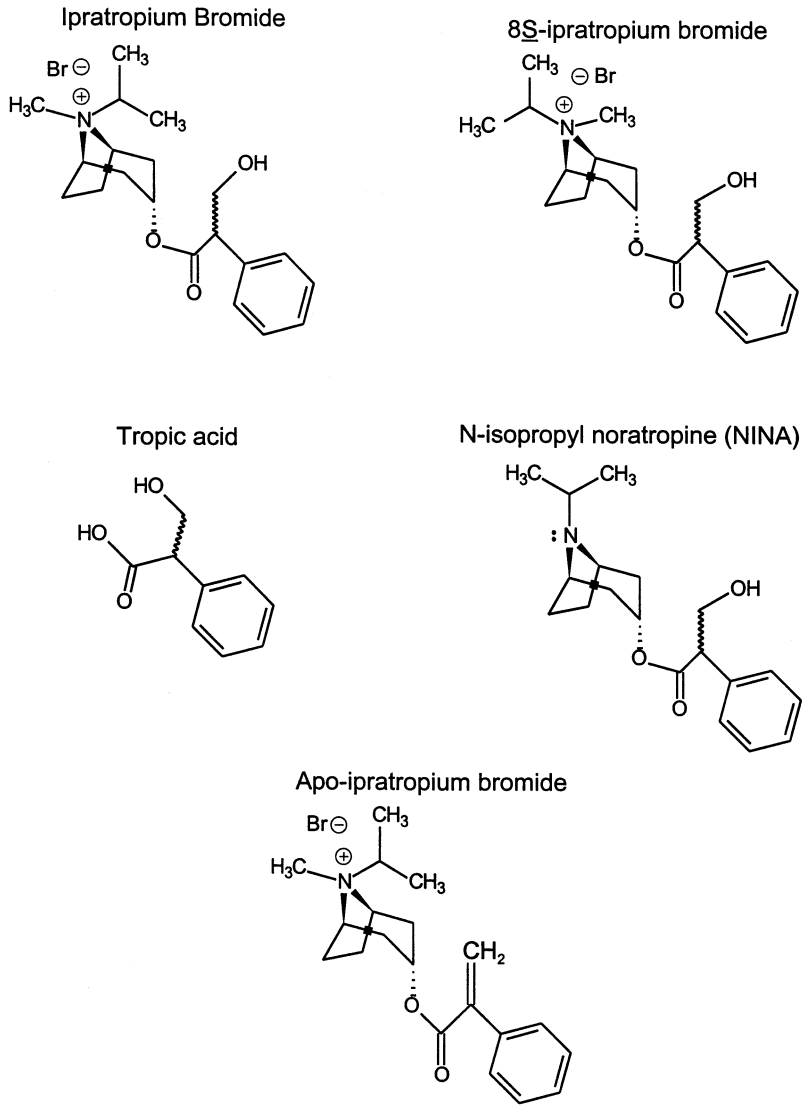

Fig. 1. The structures of ipratropium bromide, 8-s ipratropium bromide, tropic acid, NINA and apo-ipratropium bromide.

Ipratropium bromide shows a similar activity to atropine when it is delivered systemically [8]. The difference between atropine and ipratropium bromide is that ipratropium bromide does not affect the nervous system and appears to have a low gastrointestinal absorption. The only significant side effect of ipratropium bromide is a dry mouth which is usually produced with doses higher than the therapeutic dose [9].

Ipratropium bromide has been used in metered dose inhalers (MDI) and nebulized inhalation unit dose solution. The manufacture of ipratropium bromide produces several process impurities [10] that need to be separated and quantitated for release of raw materials and finished products. Presently, methods used for analyzing ipratropium bromide include reversed-phase ion-pairing HPLC [11,12] and a radio receptor assay that was used for looking at ipratropium bromide levels in biological fluids [13]. We now report on a HPLC assay for ipratropium bromide and its related compounds using an acetonitrile/potas-

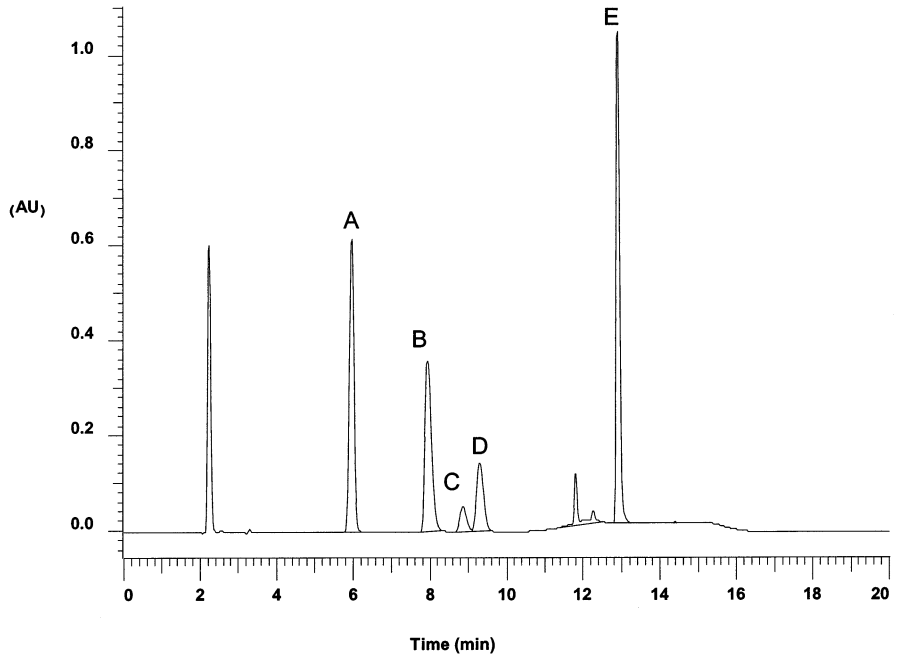

Fig. 2. Chromatogram of ipratropium bromide and its related compounds on a C18 Alltima column using an initial flow rate of 1 ml min^{-1} with 100% A for 0 to 5 min, then ramp to 100% B in 3 min. At 13 min ramp to 100% A and a flow of 2 ml min^{-1} in 1 min and the column was equilibrated for 6 min; with UV detection at 210 nm at 35°C. (A) tropic acid, (B) ipratropium bromide, (C) NINA, (D) 8-s ipratropium bromide and (E) apo-ipratropium bromide.

sium phosphate gradient mobile phase. This system showed excellent selectivity and reproducibility. In addition, there was no tailing of any peak, which is often seen in chromatograms of amine compounds on reversed-phase stationary phases when the mobile phase does not contain ion-pairing reagents. Accelerated stability studies show that the degradation components are resolved from the active compound resulting in a stability indicating assay.

2. Experimental

2.1. Materials

Ipratropium bromide, 8-s ipratropium bromide, *N*-isopropyl-nor-atropine and apo-ipratropium were provided by Sifavitor (Milan, Italy). Tropic acid was obtained from Sigma (St. Louis, MO). Ipratropium bromide MDI and ipratropium bromide inhalation solution were purchased from Hanna Pharmaceuticals (Princton, NJ). Potassium phosphate (KH_2PO_4) and acetonitrile (HPLC grade) were purchased from Fisher Scientific. Nylon syringe filters (13 mm, 0.2 μm) and the C18 column (Alltima, 25 cm × 4.6 mm, 5 μm) were obtained from Alltech Associates (Deerfield, IL).

The HPLC system used was a Hitachi liquid chromatography system, including L-7100 low pressure gradient HPLC pump, L-7200 sequential autosampler (equipped with a 100-μl sample loop), L-7300 column oven, L-7400 programmable UV detector or a L-7450 photodiode array detector, D-7000 interface module, and D-7000 Concertchrom HPLC system manager software.

2.2. Mobile phase preparation

Buffer: 100 mM (KH_2PO_4) was prepared by placing 27.2 g of KH_2PO_4 in a 2000-ml volumetric flask and dissolving it in purified water (USP).

Table 1
System suitability data

	Tropic acid	Ipratropium bromide	8-s Ipratropium bromide	NINA	Apo-ipratropium bromide
RT (min)	5.95	7.97	8.86	9.30	12.89
	5.97	7.96	8.86	9.29	12.90
	5.96	7.97	8.86	9.30	12.90
	5.95	7.98	8.89	9.30	12.91
	5.95	7.97	8.86	9.25	12.90
	5.95	7.97	8.87	9.25	12.90
Ave. %RSD	5.96	7.97	8.86	9.28	12.90
	0.14	0.08	0.83	0.27	0.63

C18 Alltima column with an initial flow rate of 1 ml min^{-1} and 100% solution A for 0 to 5 min then ramp to 100% solution B in 3 min. At 13 min ramp to 100% solution A and a flow rate of 2 ml min^{-1} and equilibrate the column for 6 min; with UV detection at 210 nm at 35°C.

The pH was adjusted to 4.0 with 85% phosphoric acid (H_3PO_4) and the resulting solution was diluted to 2000 ml with water and mixed thoroughly.

Solution A: 800 ml of buffer was filtered and mixed with 200 ml of acetonitrile to give a final volume of approximately 1000 ml.

Solution B: 550 ml of buffer was filtered and mixed with 450 ml of acetonitrile to give a final volume of approximately 1000 ml.

Table 2
Column efficiency and peak asymmetry values

Compound	Asymmetry Values		*N*	
Tropic acid	1.07[b]	1.25[a]	14 331[b]	4561[a]
Ipratropium bromide	1.60	2.16	9626	5221
8-s Ipratropium bromide	1.36	1.53	10 615	6821
NINA	1.34	1.42	11 310	5966
Apo-ipratropium bromide	1.33	1.50	120 901	7389

[a] On a C18 alltima column with solution A as the mobile phase with a flow rate of 1 ml min^{-1} and UV detection at 210 nm at 35°C.

[b] C18 Alltima column with an initial flow rate of 1 ml min^{-1} and 100% solution A for 0 to 5 min then ramp to 100% solution B in 3 min. At 13 min ramp to 100% solution A and a flow rate of 2 ml min^{-1} and equilibrate the column for 6 min; with UV detection at 210 nm at 35°C.

2.3. Chromatography conditions

Mobile phase: At an initial flow rate of 1 ml min^{-1} 100% Solution A for 0 to 5 min, then ramp to 100% B in 3 min; at 13 min ramp to 100% A and from 1 to 2 ml min^{-1} in 1 min and equilibrate for 6 min.

Detection: UV 210 nm.

Temperature: 35°C.

Injection volume: 20 μl.

2.4. Sample preparation

Standards of ipratropium bromide, 8-s ipratropium bromide, tropic acid, NINA and apo-ipratropium were prepared by dissolving approximately 18 mg, accurately weighed, of each compound in 1×10^{-4} N HCl in 100-ml volumet-

Table 3
Effect of pH on retention times

Compound	pH 3.5	pH 4.0
Tropic acid	7.98	5.78
Ipratropium bromide	7.98	7.94
8-s Ipratropium bromide	8.86	8.90
NINA	9.30	9.34
Apo-ipratropium bromide	12.81	12.92

C18 Alltima column with an initial flow rate of 1 ml min^{-1} and 100% solution A for 0 to 5 min then ramp to 100% solution B in 3 min. At 13 min ramp to 100% solution A and a flow rate of 2 ml min^{-1} and equilibrate the column for 6 min; with UV detection at 210 nm at 35°C.

Table 4
Effect of organic modifier on the retention times of ipratropium bromide and its related compounds

Compound	Isocratic condition		Gradient condition	
	A	B	A	B
Tropic acid	6.02	5.44	5.57	5.78
Ipratropium bromide	7.95	6.60	6.77	7.94
8-s Ipratropium bromide	8.87	7.25	7.46	8.90
NINA	9.34	7.52	7.76	9.34
Apo-ipratropium bromide	37.01	27.83	11.97	12.92

Isocratic conditions: (A) 20:80 acetonitrile/potassium phosphate buffer (100 mM, pH 4.0) and (B) 22:78 acetonitrile/potassium phosphate buffer (100 mM, pH 4.0) on a Alltech Alltima C18 with a flow rate of 1 ml min^{-1} at 35°C and UV detection at 210 nm. C18 Alltima column with an initial flow rate of 1 ml min^{-1} and 100% solution A for 0 to 5 min then ramp to 100% solution B in 3 min. At 13 min ramp to 100% solution A and a flow rate of 2 ml min^{-1} and equilibrate the column for 6 min; with UV detection at 210 nm at 35°C.

ric flasks, diluting to volume, and mixing thoroughly. Prior to injecting the samples onto the HPLC column, they were filtered through a 0.2-μm filter.

2.5. Preparation/analysis of samples for determination of response factors

Ipratropium bromide standards were prepared in duplicate at a final concentration of 180 μg ml^{-1}, accurately measured. Likewise, duplicate standards were prepared for 8-s ipratropium bromide, tropic acid, NINA and apo-ipratropium bromide at a final concentration of 180 μg ml^{-1} and accurately measured. Two injections were made from each weighing. The response factor was calculated by dividing the peak area of each process impurity by the peak area of ipratropium bromide.

2.6. Preparation of metered dose inhaler (MDI) formulations for recovery studies

Samples of ipratropium bromide (180 μg ml^{-1}) were prepared with soy lecithin (60 mg) and without soy lecithin in a 50-ml volumetric flask. The flask was diluted to mark with 1×10^{-4} N HCl. The samples containing soy lecithin were sonicated and an aliquot was removed and filtered through a 0.2-μm nylon filter and analyzed by HPLC. The samples without soy lecithin were filtered through a 0.2-μm nylon filter prior to analysis. Samples obtained from Hanna Pharmaceuticals were aquated into a 125-ml Erlenmeyer flask until the can was empty and 20 ml of 1×10^{-4} N HCl was added to the flask. The flask was shaken to ensure all the ipratropium bromide was dissolved into solution.

2.7. Degradation studies

Degradation studies were performed on MDI formulations and the inhalation solution at elevated temperatures and acidic pH. Samples were prepared by making ten actuations into a 125-ml Erlenmeyer flask. To the flask, 20 ml of pH 1.0 or pH 3.5 HCl was added and the mixture was shaken to collect any contents that remained on the sides of the flask. The inhalation solution pH was adjusted to 1.0 with HCl. An aliquot of both was removed for time zero, filtered and analyzed by HPLC. Approximately 8 ml of sample was put into a screw cap vial and placed on a heating block at 95°C. Samples were removed every 24 h for 5 days, filtered and analyzed by HPLC.

2.8. Validation studies

Analytical validation of this method was performed over 2 days by two different analysts to demonstrate inter-day and intra-day variability. Standard samples were analyzed over a range of 10–1000 μg ml^{-1} to demonstrate linearity. Five replicate injections were made at concentrations

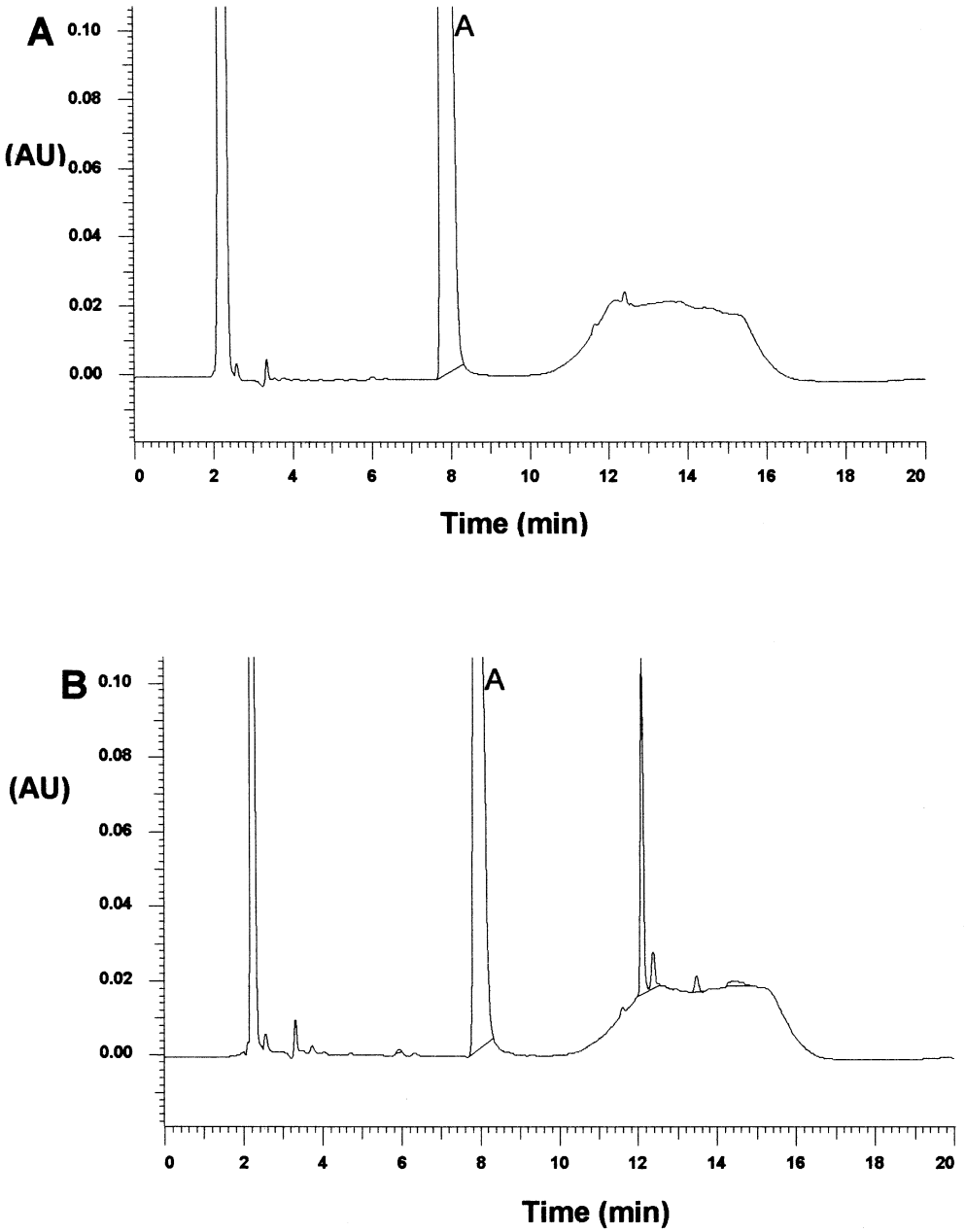

Fig. 3. Chromatogram of degradation studies of ipratropium bromide MDI at (A) time zero (pH 3.5, 95°C) and (B) time zero (pH 1.0, 95°C) using the conditions mentioned in Fig. 2. (A) Ipratropium bromide, (B) tropic acid and (C) apo-ipratropium.

of 15, 200 and 750 μg ml^{-1} to demonstrate the accuracy and precision of the method. Limit of detection was performed by injecting diluted samples of ipratropium bromide onto the HPLC column until a signal-to-noise ratio of 3:1 was reached. A blank was injected before each run to ensure that there was no carry over from the previous injection.

Table 5
Degradation studies of ipratropium bromide under acidic conditions and elevated temperature

Time (h)	%Ipratropium bromide	%Tropic acid	%Apo-ipratropium bromide
Acidic conditions			
0	100	0	0
120	0.2	99.1	0.7
Elevated temperature			
0	100	0	0
120	96	3.8	0.2

3. Results and discussion

The structures of ipratropium bromide, 8-s ipratropium bromide, tropic acid, NINA and apo-ipratropium are shown in Fig. 1. These compounds are resolved from each other in approximately 13 min using this HPLC gradient method (Fig. 2). The additional time and higher flow rate are used to equilibrate the column back to the initial conditions, making the method useful for quick routine analysis. Multiple injections of the same sample show a stable retention time for all the compounds (Table 1). This demonstrates that the equilibration time is sufficient to equilibrate the column back to the initial conditions. An investigation of peak purity using photodiode array detection showed that each peak was homogeneous and therefore the assay could be considered stability indicating. The peak that appeared at 12 min in the chromatogram when a blank injection was made. It was determined that this peak was a contaminant from the solvent.

The gradient method showed increased efficiency for all the compounds when compared with isocratic conditions capable of resolving the four compounds. In addition, there is also an improvement in the asymmetry values for ipratropium bromide and its related compounds (Table 2).

The effect of pH on the separation was examined and it was determined that only tropic acid was sensitive to changes in pH (Table 3). Decreasing the pH increased the retention time of tropic acid. Under isocratic conditions which will separate all the compounds, Apo-ipratropium bromide was very sensitive to slight changes in acetonitrile concentration. This change caused a significant fluctuation in the retention time for this compound reducing the ruggedness of this method. This phenomenon was greatly reduced when the gradient conditions were used (Table 4).

Ipratropium bromide MDI contains ipratropium bromide, soy lecithin and several chloroflouro-hydrocarbons as propellant. Upon actuation of the canister, the propellants evaporated leaving ipratropium bromide and the soy lecithin. In order to measure accurately the potency of ipratropium bromide in a MDI formulation, recovery studies were performed on solutions of ipratropium bromide with soy lecithin. The peak areas determined by HPLC-UV were compared to the peak area determined by HPLC of ipratropium bromide solutions without soy lecithin. These mixtures were sonicated to allow for maximum solubility. Aliquots were taken, filtered and assayed by HPLC. Peak area ratios (with soy lecithin:without soy lecithin) were calculated to give percent recovery of ipratropium bromide. The percent recovery was shown to be approximately 100% for ipratropium bromide from samples containing soy lecithin. This analysis was in-turn performed on canisters obtained from Hanna Pharmaceuticals showed that the recovery of ipratropium bromide was also approximately 100%.

Degradation studies of ipratropium bromide were performed at elevated temperature and under acidic conditions. Basic degradation conditions were not examined because neither product was formulated over pH 5. Therefore, there would be no basic degradation of the finished product. However, it is believed that basic degradation would lead to rapid saponification of the ester

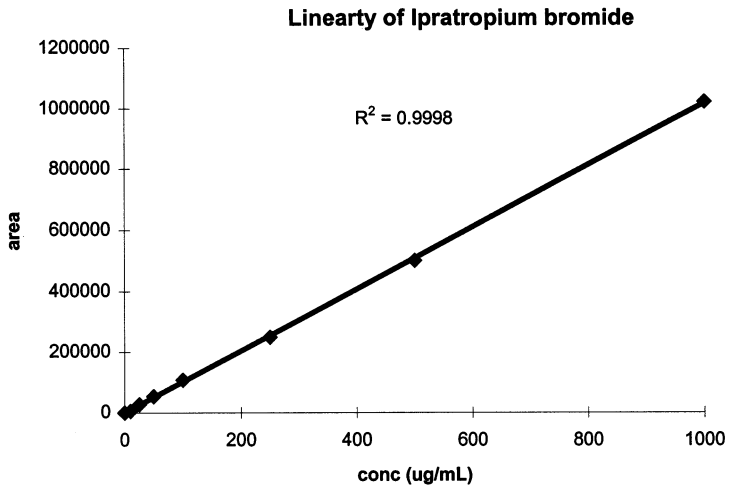

Fig. 4. Representative curve for the quantitation of ipratropium bromide in MDI formulation and inhalation solutions.

bond. Samples were taken every 24 h over a 5-day period. The degradation of ipratropium bromide was mainly effected by acidic pH rather than elevated temperature. The major degradation component is tropic acid with a small amount of apo-ipratropium forming throughout the course of the degradation (Fig. 3). At the end of the 5-day period ipratropium bromide almost completely hydrolyzed to tropic acid. MDI canisters placed on stability gave a similar degradation profile, however, the samples contained more apo-iprotropium bromide (Table 5).

The gradient method was validated with two analysts performing test for linearity, precision, accuracy, reproducibility and degradation studies. Each analyst performed the assay on different days and different instruments (one instrument used a photo diode-array detector instead of a UV variable wavelength detector) to show the ruggedness of the method. Replicate injections were made at three different levels of the standard curve to show method precision, accuracy and reproducibility.

The assay was shown to be linear over a range of 10–1000 μg ml^{-1} with a limit-of-detection of 60 ng ml^{-1} and a representative curve is shown in Fig. 4. The data collected for the replicate injections gave a RSD of less than 2.0%, which is within acceptable limits. In addition, the average correlation for linearity was approximately 0.9992 (Table 6). There was little day-to-day variability with this method, making it rugged and well-suited for use in a quality control laboratory. The degradation studies confirmed that tropic acid was the major degradation component of ipratropium bromide.

4. Conclusion

The separation of ipratropium bromide and its related compounds can be achieved in approximately 13 min using an acetonitrile/potassium phosphate buffer (100 mM, pH 4.0) gradient mobile phase. The method is reproducible and not sensitive to small changes in acetonitrile concentration when compared to an isocratic method that can separate all four compounds. The limit-of-detection for ipratropium bromide using this method is approximately 60 ng ml^{-1}, and the method is linear over a range of 10–1000 μg ml^{-1} with good reproducibility (RSD < 2.0%). In addition, all the degradation components are well resolved from the active component, ipratropium bromide, making this assay stability-indicating and practical for use in a quality control lab. Other methods required a flow rate of 2 ml min^{-1} and these methods were isocratic. Under isocratic conditions apo-ipratropium has a very long elution time and as a broad peak, decreasing the sensitivity of the assay. In addition, apo ipratropium is sensitive to

Table 6
Comparison of experimental concentrations determined by HPLC to theoretical concentrations

Analyst # 1		Analyst # 2	
Theoretical concentration ($\mu g\ ml^{-1}$)	Experimental by HPLC ($\mu g\ ml^{-1}$)	Theoretical concentration ($\mu g\ ml^{-1}$)	Experimental by HPLC ($\mu g\ ml^{-1}$)
15	14.9 (0.5)	15	14.6 (0.7)
200	208.2 (0.3)	200	201.1 (0.6)
750	746.9 (0.1)	750	744.7 (0.8)
Ave. line correlation	0.9992		0.9990

Experimental data expressed as mean, with RSD in parentheses.

small changes in acetonitrile concentration. Using the gradient method we have decreased the effect small changes in acetonitrile would have on the elution time of apo-ipratropium bromide and improved the peak shape. This allows for a much more sensitive assay. We observed superior reliability with this gradient method when the mobile phase buffers were each pre-mixed. This creates less baseline shift and fewer ghost peaks in the chromatogram.

Acknowledgements

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