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ESTIMATION OF IPRATROPIUM BROMIDE FROM AEROSOLS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ipratropium bromide, a derivative of N-isopropyl noratropine, was estimated from the samples of aerosols. An isocratic, reversed-phase liquid chromatographic separation method was developed by using an ODS, 5 µm column with the mobile phase acetonitrile-sodium dihydrogen orthophosphate (0.05M)-diethylamine (50 : 50 : 0.1, v/v) pH adjusted to 4.5 with phosphoric acid. Recoveries obtained were in the range 98% to 102% of ipratropium bromide from aerosol. Fluoxetine hydrochloride was used as an internal standard for quantitation.

INTRODUCTION

The development in recent years of an effective anticholinergic agent which produces bronchodilation has proved advantageous to the asthamatic patients. Ipratropium bromide, one such bronchodilator has been administered in the treatment of acute asthama (1,2). Generally this is used in the form of an aerosol, turned to a suitable alternative to adrenoceptor agonist drugs which otherwise not fully respond to the threapy. In the event of its popularity of use in the western continent, has produced a substantial need for the parallel development of sensitive and convenient method for detection and quantitation of this drug.

A non-aqueous titration method for ipratropium bromide has already been reported (3). The resulting need for direct trace-level determination of ipratropium bromide in biological fluids has been partially solved by the development of radioreceptor assays (4-6). The principal difficulty encountered in this approach is that the drug undergoes tedious extraction process and thereby compromises both the selectivity and the detection limits afforded by the detector.

In the present work, a reversed-phase liquid chromatographic method has been developed for the estimation of ipratropium bromide from aerosols. Provision was made for incorporating fluoxetine hydrochloride as an internal standard for accurate quantitation. No interference from the excipients such as fluorohydrocarbons contained in areosols was noted.

EXPERIMENTAL

Apparatus : A liquid chromatographic system (BRUKER Instruments, Bremen, F.R.G.) consisting of LC-21A solvent delivery system, LC-313 UV-visible detector and 7125 Rheodyne valve injector fitted with a 20 µl loop. The column used was ECONOSPHERE, ODS, 150 mm X 4.6 mm, 5 µm from Alltech Assoc., Illinois, U.S.A. Data acquisition was accomplished by using an integrator, ORACLE-2 (INDTECH Systems, Andheri, Bombay, India).

Reagents and Chemicals : Analytical grade sodium dihydrogen phosphate, orthophosphoric acid (85%) and diethyl amine (E.Merck India Ltd., Bombay, India), HPLC grade acetonitrile (S.D. Fine Chemicals, Tarapur, India) and distilled deionised water prepared in our laboratory was used to prepare mobile phase.

Chromatographic conditions : The mobile phase consisted of acetonitrile: sodium dihydrogen phosphate(0.05M):diethylamine (pH 4.4 adjusted with phosphoric acid, 50 : 50 : 0.1, v/v). A flow rate of 1.0 ml/min was maintained throughout the analysis, UV detector being set at 219 nm. Mobile phase was filtered through a 0.45 µm Millipore filter and then degassed before use.

Reference standards : Standards of ipratropium bromide (purity 99.5 %, 0.1 mg/ml), fluoxetine hydrochloride (purity 99.5%, 0.1 mg/ml) were prepared in acetonitrile. The calibration curve for ipratropium bromide was prepared in the range 5 to 20 µg/ml, with internal standard 0.1 mg/ml fluoxetine hydrochloride.

Sample Preparation : (Ipratropium bromide areosol spray)
In a 100 ml beaker containing 50 ml acetonitrile, 50 sprays of ipratropium bromide were actuated. The contents were then transferred to a erlenmayer flask and warmed it under vacuum at 50 $^{\circ}$ C for 5 min. The final solution was cooled to ambient temperature, diluted to 50 ml with acetonitrile and directly used for analysis. The container was shaken after actuation of every 10 sprays.

Recovery Experiments : The recovery of the added standard of ipratropium bromide was studied at two different levels. Known amounts of the drug at concentrations 10 and 20 µg/spray were added to the preanalysed samples and analysed by the present method. Each level was repeated at least three times. These experiments were conducted in two different laboratories and performed on identical batches wherein one container was taken from which 50 sprays in 3 different beakers actuated. The results were calculated by using the following equation :

$$\% \text{ Recovery} = N \times \text{Slope}$$

$$= N \frac{(\lessgtr XY) - (\lessgtr X)(\lessgtr Y)}{(\lessgtr X^2) - (\lessgtr X)^2}$$

where, X = Amount of standard ipratropium bromide added

Y = Amount of ipratropium bromide found by the present method

N = Number of determinations.

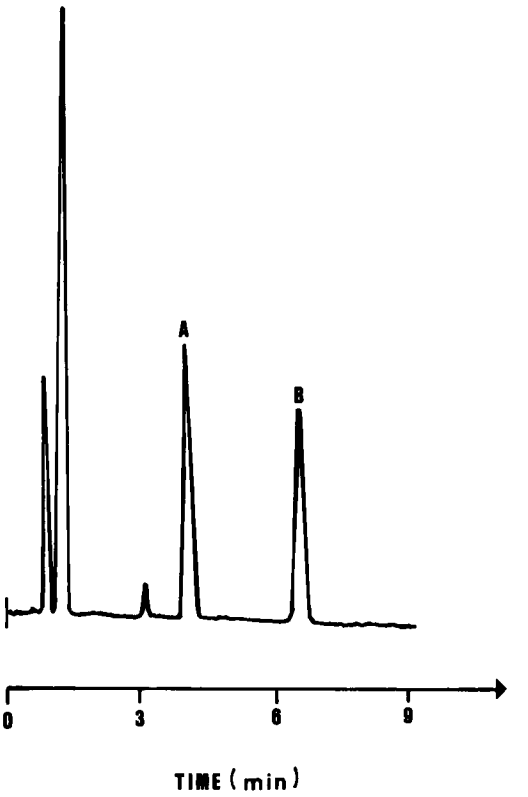

FIGURE 1

A chromatogram of an aerosol sample containing 100 µg/ml Ipratropium bromide (A) and 500 µg/ml Fluoxetine hydrochloride (B).

For Chromatographic conditions see text.

TABLE I : PRECISION DATA FOR IPRATROPIUM BROMIDE ASSAY FROM LABORATORY A

Conc. Added (µg/spray)	Conc. Found (µg/spray)	% Recovery (Mean ± S.D. n = 3)	Coeff. of variation (%)	Standard error of estimation (%)
WITHIN-DAY VARIATION				
10.16	9.96	98.03 ± 0.072	0.073	0.041
20.32	19.96	98.23 ± 0.088	0.089	0.051
DAY-TO-DAY VARIATION				
10.16	9.89	97.35 ± 0.059	0.061	0.034
20.32	20.02	98.53 ± 0.115	0.115	0.066

RESULTS AND DISCUSSION

The estimation of ipratropium bromide from aerosol evolved problems of finding a suitable mobile phase that would separate the drug from the excipients. However, addition of buffer, sodium phosphate, to the mobile phase gave sufficient selectivity to achieve the separation of ipratropium bromide from fluoxetine hydrochloride (internal standard) and the aerosol excipients. A representative

TABLE II : PRECISION DATA FOR IPRATROPIUM BROMIDE ASSAY FROM LABORATORY B

Conc. Added (µg/spray)	Conc. Found (µg/spray)	% Recovery (Mean ± S.D. n = 3)	Coeff. of variation (%)	Standard error of estimation (%)
	WITHIN-DAY VARIATION			
10.00	10.03	100.3 ± 0.165	0.16	0.095
20.00	19.98	99.9 ± 1.051	1.05	0.606
	DAY-TO-DAY VARIATION			
10.00	9.98	99.8 ± 0.121	0.12	0.070
20.00	20.01	99.5 ± 0.149	0.15	0.086

chromatogram obtained from an aerosol sample is shown in Figure 1.

Quantitation was accomplished using internal standard method expressed in terms of a plot of peak area ratio (peak area of ipratropium bromide / peak area of internal standard) versus the concentration of the drug in the range 5 to 20 µg/ml. The response of the detector was found to be linear with a regression equation y = 1.3125 x + 0.1235,

correlation coefficient being 0.999. Detection levels of ipratropium bromide from aerosols were estimated to be 5 µg/ml monitored at 219 nm, 0.08 A.U.F.S.

The recovery of ipratropium bromide from aerosol sprays was assessed by comparing peak area ratios from the standard stock solutions of the drug added to the preanalysed samples. To assess the precision of this analytical procedure, reproducibilities for within-day and day-to-day variations were determined. Also, these experiments were performed in two different laboratories A and B using different liquid chromatographs and by different personnels. Results are summarised in Tables I and II respectively. The recoveries from both laboratories averaged 98%. As shown in Table I, the coefficients of variation for ipratropium bromide from aerosol sprays ranged from 0.07 to 0.11 for within-day and day-to-day analysis while 0.12 to 1.05 for laboratory B (Table II). Each recovery level was repeated at least three times.

The method described for the estimation of ipratropium bromide from an aerosol is rapid and precise. The use of an internal standard method was to correct possible errors in handling pipettes and syringes.

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