Reversed-phase Liquid Chromatographic Determination of Ipratropium Bromide, Methylparaben and Propylparaben in Pharmaceutical Dosage Forms

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A reversed-phase HPLC (RP/HPLC) method using Lichrosphere RP select B, C8 column has been developed for the simultaneous determination of ipratropium bromide, methylparaben and propylparaben in formulations. The mobile phase consists of an aqueous solution containing heptane sulphonic acid sodium salt (pH 3.2) and 345 ml of acetonitrile, with detection at 210 nm. Recovery study values of 98-102% and a relative standard deviation of less than 1% for the assay showed that the method is accurate and precise.

Chemically, ipratropium bromide, is a quaternary ammonium compound. It is an antimuscarinic agent used as bronchodilator in inhalation therapy of obstructive airway disease and allergic rhinitis[1]. Methylparaben and propylparaben are used as preservatives in aqueous preparations of ipratropium bromide meant for nebulising. The BP[2,3] describes a titrimetric method for ipratropium bromide and RP/HPLC method for ipratropoim bromide pressurised inhalation.

Other methods reported in the literature are UV spectroscopy[4] and gradient RP/HPLC[5]. In this present work, efforts have been made to develop an isocratic method for the simultaneous determination of ipratropium bromide and parabens in nebulising and nasal spray solution. As the preservatives used form part of the label claim, there is a need for a suitable method for the simultaneous quanitification of the above ingredients.

A high performance liquid chromatographic system from Hewlett Packard 1100 series comprising of Rheodyne injector, UV detector and a computer with software was used for controlling the instrumentation as well as processing the data generated. Analysis was carried out using a Lichrospher RP select B column (Merck, C_8, 5, µ, 3.9 x 250 mm), with a flow rate of 1.2 ml/min. A Rheodyne 7725i injector with 20 µl loop was used for injecting the samples. Detection was carried out at 210 nm.

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Acetonitrile of HPLC grade, ortho phosphoric acid of AR grade were obtained from Merck (India), sodium heptane sulphonate of HPLC grade procured from Lancaster (England) and water collected from a Millipore milli Q system were used. Working reference standards of methylparaben, propylparaben obtained from M/s Rasula Pharmaceuticals & Chemicals, Hyderabad and ipratropium bromide procured from M/s. Mediolast SPA Italy, were used for the analysis.

For mobile phase preparation, 1.82 g of sodium heptane sulphonate was dissolved in 750 ml of water. The pH was adjusted to 3.2 with 5% v/v ortho phosphoric acid solution. The solution was filtered through 0.45 µ membrane filter. To this solution 345 ml of acetonitrile was added, mixed and sonicated for 10 min.

About 25 mg of ipratropium, bromide, 60 mg of methylparaben and 27 mg of propylparaben were accurately weighed and each one was transferred into the same 100 ml volumetric flask. About 50 ml of the mobile phase was added, mixed thoroughly on a cyclomixer to dissolve the contents and finally diluted to volume with mobile phase (S_1).

One millilitre of (S_1) was transferred into a 10 ml volumetric flask and diluted to volume with mobile phase and mixed well (S_2). One millilitre of the sample (equivalent to 0.250, 0.610 and 0.270 mg/ml of ipratropium bromide, methylparaben and propylparaben respectively) was

TABLE 1: ESTIMATION OF IPRATROPIUM BROMIDE, METHYLPARABEN AND PROPYLPARABEN IN PHARMACEUTICAL PREPARATIONS

Contents	Sample A*			Sample B*		
	Label claim (mg/ml)	Found (mg/ml)	% (label) claim)	Label claim (mg/ml)	Found (mg/ml)	% (label) claim)
Ipratropium bromide	0.250	0.254	101.6	0.250	0.255	102.0
Methylparaben	0.610	0.614	100.7	0.610	0.610	100.0
Propylparaben	0.270	0.266	100.74	0.270	0.266	98.5

* Sample A and B are in-house samples from Production Batches

transferred into a 10 ml volumetric flask and diluted to volume with mobile phase (E_1).

An amount of 20 µl each of standard preparation (S_2) and sample preparation (E_1) was injected into the liquid chromatographic system. The approximate retention times were found to be 5.8, 6.6 and 14.0 min for methylparaben, ipratropium bromide and propylparaben respectively. From the respective areas obtained from the standard and sample chromatograms, the contents were calculated.

Assay data by the proposed method is given in Table 1. The calibration curve for IBR, MP and PP were linear in the range of 12-38, 30-90, and 13-40 µcg/ml respectively. Accuracy of the method was determined by analysis of spiked placebo sample at five levels. Percentage recoveries were found to be between 98.5 to 101.5. Precision of the method was established by five replicate analysis of the sample. Percentage RSD of 0.89, 0.38 and 0.58 for IBR, MP and PP resepectively show very good reproducibility.

The proposed method is simple, accurate and precise and can be used for routine quality control analysis.

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