INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ALBUTEROL SULPHATE AND IPRATROPIUM BROMIDE IN NASAL INHALATIONS

G. Sowjanya *[1], D. Gowri Sankar [2] and J. V. L. N. Seshagiri Rao [2]
[1]Institute of Pharmacy, GITAM Deemed to be University, Visakhapatnam, Andhra Pradesh, India
[2]College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India
*Corresponding Author Email: tatinenijishnu@gmail.com

Article Received on: 04/07/18 Approved for publication: 21/08/18

DOI: 10.7897/2230-8407.098166

ABSTRACT

A new RP-HPLC method was developed and validated for the simultaneous assay of albuterol sulphate and ipratropium bromide in nasal inhalations. The separation was performed on a non-polar peerless basic C8 column using a mixture of anhydrous potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (95:5 v/v) along with a mixture of anhydrous potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (70:30 v/v) as mobile phase in gradient elution mode. The retention time for albuterol sulphate and ipratropium bromide was at 2.927 ± 0.25 min and 10.479 ± 0.76 min. and the analyte peaks were analysed at 276 nm. and 220 nm. respectively over a run time of 22 minutes. The method obeyed linearity in the range of 0.0100 – 0.2080 mg/mL and 0.0023 – 0.0468 mg/mL for albuterol sulphate and ipratropium bromide respectively and the low coefficients of variation obtained in the intraday (0.8 % – 1.0 %) and inter day precision (1.1 % – 1.4 %) study are indicative of the precision of the method. High recovery of albuterol sulphate (98.0 – 102.0 %) and ipratropium bromide (98.1 – 101.9 %) indicate the accuracy of the method. The proposed method was also applied for the forced degradation studies on the drugs in respules and the system suitability parameters are within the acceptable limits. Therefore, the proposed method can be used for routine quality control of albuterol sulphate and ipratropium bromide in pure samples and dosage forms.

Keywords: Albuterol sulphate, Ipratropium bromide, C8 column, Gradient, Forced degradation

INTRODUCTION

Albuterol sulphate[1] chemically known as 4-[2-(tert-butyl amino)-1-hydroxyethyl]-2 hydroxymethyl) phenol sulfuric acid belongs to the class of medicines known as short acting beta2–adrenergic agonist. Albuterol sulphate (fig. 1a) is used in the treatment of bronchospasm in bronchial asthma, chronic bronchitis and emphysema, prophylaxis of exercise–induced asthma and chronic obstructive pulmonary disease. Albuterol relaxes the smooth muscles of all airways, from the trachea to the terminal bronchioles, thus making breathing easier. The world health organization recommended name for albuterol base is salbutamol. It is a white to off-white crystalline solid which is soluble in water and slightly soluble in ethanol.

Ipratropium bromide[1,2] chemically known as (1R,3R,5S,8R)-3-[(3-hydroxy-2-phenylpropanoyl)oxy]-8-methyl-8-(propan-2-yl)-8-azabicyclo[3.2.1]octan-8-ium bromide is a muscarinic antagonist structurally related to atropine but often considered safer and more effective for inhalation use **(**fig. 1b). It is used for various bronchial disorders, in rhinitis, and as an antiarrhythmic. It blocks muscarinic cholinergic receptors, without specificity for subtypes, resulting in a decrease in the formation of cyclic guanosine monophosphate (cGMP). It is freely soluble in water and methanol, sparingly soluble in ethanol, and insoluble in lipophilic solvents such as ether, chloroform and fluorocarbons. The combination preparation ipratropium bromide/salbutamol is a formulation containing ipratropium bromide and salbutamol sulphate used in the management of chronic obstructive pulmonary disease (COPD) and asthma.

An extensive literature survey revealed few RP-HPLC[3-5] methods for routine quality control analysis**,** related substances and impurity determinations[6] in dosage forms containing ipratropium bromide and salbutamol sulphate**.** An LC-MS/MS[7] method was also reported for the simultaneous determination of albuterol sulphate and ipratropium bromide in rat plasma. An attempt has been made to develop a new RP-HPLC method for simultaneous determination of ipratropium bromide and salbutamol sulphate in inhalations which could also provide the stability related information.

MATERIALS AND METHODS

Drugs and Chemicals

Reference standard samples of albuterol sulphate (purity 99.4 % w/w) and ipratropium bromide (purity 99.9 % w/w) were obtained from Lupin laboratories (Mumbai). The commercial nasal respules, "WINDEL PLUS" (2.5 mg Salbutamol (as sulphate) and 0.5 mg Ipratropium bromide /2.5 mL) were purchased from local market. Acetonitrile (HPLC grade), potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate, ortho-phosphoric acid (AR grade) were purchased from Thermo Fisher Scientific India Pvt. Ltd. HPLC grade water prepared from Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study. All other chemicals like sodium hydroxide, hydrochloric acid and hydrogen peroxide used in the study were of analytical grade.

Instrumentation

A Shimadzu HPLC (LC 2010 CHT) instrument equipped with quaternary gradient pump, UV/PDA detector, auto sampler and column heating oven was used for the study. A Peerless basic C8 (150 x 4.6 mm, 5µ) column was employed. Chromatographic analysis and data acquisition was monitored by using 'LC solutions' software. Degassing of the mobile phase was done using a PCI bath sonicator. A Sartorius SPA 225D electronic balance was used for weighing the materials. All pH measurements were made using a Metsar pH meter.

Mobile Phase

Preparation of the buffer solutions
Solution A: Accurately weighed about 6.8 g of anhydrous potassium dihydrogen orthophosphate (0.05M) and 0.5 gm of 1-pentane sulphonic acid sodium salt monohydrate was transferred into a beaker containing 1000 mL of water and mixed. The pH of the solution was adjusted to 4.0 ± 0.05 with dilute ortho-phosphoric acid and mixed well. The solution was then filtered through a 0.45µ membrane filter and degassed.

Solution B: Accurately weighed about 3.4 g of anhydrous potassium dihydrogen orthophosphate (0.025M) and 0.25 gm of 1-pentane sulphonic acid sodium salt monohydrate was transferred into a beaker containing 1000 mL of water and mixed. The pH of the solution was adjusted to 4.0 ± 0.05 with dilute ortho-phosphoric acid and mixed well. The solution was then filtered through a 0.45µ membrane filter and sonicated.

Preparation of the Mobile Phase

Mobile phase A: Degassed mixture of solution 'A' and acetonitrile in the ratio of 95:5 v/v was prepared.
Mobile phase B: Degassed mixture of solution 'B' and acetonitrile in the ratio of 70:30 v/v was prepared.

Diluent

A mixture of mobile phase 'A' and 'B' in the ratio of 75:25 was used as a diluent.

Preparation of Stock and Working Standard Solutions of Albuterol Sulphate and Ipratropium Bromide

Solutions were prepared by dissolving about 100.0 mg of albuterol sulphate and 45.0 mg of ipratropium bromide with small amount of diluent in separate 25 mL and 50 mL volumetric flasks and the volume was made up with the diluent to obtain concentrations corresponding to 4.0 mg/mL and 0.9 mg/mL respectively (primary stock). Further 5.0 mL of each of the above solutions were transferred to a 50 mL volumetric flask and diluted to volume with the diluent to get a 0.4 mg/mL solution of albuterol sulphate and 0.09 mg/mL of ipratropium bromide (working standard). Further dilutions were made from the working standard solution in the required concentration range.

Preparation of Sample Solutions of Albuterol Sulphate and Ipratropium Bromide

Sample solutions were prepared by suitably diluting the albuterol sulphate and ipratropium bromide respules. Accurately pipetted about 30.0 mg equivalent of albuterol sulphate and 5.0 mg equivalent of ipratropium bromide sample was transferred into a 50 mL volumetric flask, 20 mL of diluent was added and the flask was shaken for 10 mins. so as to completely extract all the drugs. The volume was then made up to the mark with diluent to get a solution containing 0.6 mg/mL of albuterol sulphate and 0.1mg/mL of ipratropium bromide. Further dilutions were made from the above solution in the required concentration range.

Method Optimization

Trials were carried out in a systematic approach for optimization of chromatographic conditions by varying solvent polarity in terms of various mobile phases and ratios, flow rates, injection volumes and detection wavelengths. After a series of experiments, the HPLC conditions mentioned in table 1.1 were adjusted for the assay of albuterol sulphate and ipratropium bromide in bulk samples and nasal solutions.

Method Validation

The developed method was validated in terms of linearity, specificity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability testing as per the ICH guidelines[8].

Mixed standard solution of albuterol sulphate and ipratropium bromide was prepared and injected five times before starting each validation parameter to check the system suitability.

Linearity and Range

Linearity of the method was determined by preparing six mixed standard solutions of albuterol sulphate and ipratropium bromide, each injected twice. Standard solutions of albuterol sulphate and ipratropium bromide in the working range of 0.0100 – 0.2080 mg/mL (albuterol sulphate) and 0.0023 – 0.0468 mg/mL (ipratropium bromide) were prepared in 10 mL volumetric flasks by taking suitable aliquots from the stock solution and diluted up to the mark with the diluent. Twenty microliters of each dilution were injected in replicate into the column and the drugs in the eluent were monitored at 276 nm and 220 nm. From the chromatograms obtained the mean peak area was noted and a plot of concentration vs. peak area was constructed. The regression of the plot was computed by least squares method.

Precision

The precision of the method was studied in terms of repeatability and intermediate precision by injecting six preparations of albuterol sulphate and ipratropium bromide sample, each injected twice on the same day (intra-day assay) and on a different day (interday assay). The % RSD was calculated for assay in repeatability and intermediate precision study.

Accuracy

The accuracy of the method was determined by suitably diluting the sample (inhalation) solution to obtain concentrations corresponding to 50 %, 100 % and 150 % levels of albuterol sulphate and ipratropium bromide respectively. Three preparations were made at each level, each preparation injected twice and analysed. The percent recovery was calculated from the amount recovered by comparing the average peak areas obtained for standard and formulation solutions.

Robustness

A study was conducted to determine the effect of deliberate variations in the optimized chromatographic conditions like flow rate (0.8 & 1.2 mL/min.) and column temperature (25 & 35 ^{o}C). Albuterol sulphate and ipratropium bromide standard and sample solutions were evaluated at the altered conditions and the effect

of these changes on the system suitability parameters like tailing factor and theoretical plates was studied.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated using residual standard deviation of the response and the slope of the regression line.

Analysis of Albuterol Sulphate and Ipratropium Bromide from Respules

Sample solutions were prepared by suitably diluting the respules (WINDEL PLUS-2.5 mg Salbutamol (as sulphate) and 0.5 mg Ipratropium bromide/2.5 mL). Accurately pipetted about 30.0 mg equivalent of albuterol sulphate and 5.0 mg equivalent of ipratropium bromide sample was transferred into a 50 mL volumetric flask, 20 mL of diluent was added, and the flask was shaken for 10 mins. so as to completely extract all the drugs. The volume was then made up to the mark with diluent to get a solution containing 0.6 mg/mL of albuterol sulphate and 0.1mg/mL of ipratropium bromide. Further dilutions were made from the above solution in the required concentration range. 20 µL of the above solution was then injected twice into the column. The mean peak area of the drugs was calculated and the drug content in the formulation was calculated by the regression equation of the method.

Specificity

Specificity of the method can be studied in the presence of excipients, degradation products and impurities.

a. Interference from Excipients in Nasal Inhalation
A placebo mixture of the commonly used ingredients in inhalations was prepared and injected into the column. No peaks were eluted for the excipients. Standard drug solutions of albuterol sulphate and ipratropium bromide were injected separately to check for any starting materials or impurities. The resulting specificity chromatogram at 220 nm (fig. 5c) showed clear separation of ipratropium bromide from the additional peaks.

b. Forced Degradation Study on Albuterol Sulphate and Ipratropium Bromide Nasal Inhalations [9, 10]
The proposed method was applied on albuterol sulphate and ipratropium bromide respules to observe the effective separation of both the drugs and the forced degradation products at the retention times. The forced degradation study was conducted by subjecting the samples of albuterol sulphate and ipratropium bromide to acid/base hydrolysis, oxidative, photolytic and thermal stress conditions as per ICH guidelines. All sample solutions used in forced degradation studies were employed at an initial concentration of 1.0 mg/mL for albuterol sulphate and 0.2 mg/mL for ipratropium bromide approximately. The stressed samples were then diluted to give a final concentration of 0.1 mg/mL and 0.02 mg/mL of albuterol sulphate and ipratropium bromide respectively. 20 µL each of the diluted solution was injected in duplicate and analysed. Mixed standard solution of the drugs was prepared and injected five times before starting the forced degradation study to check the system suitability.

Control Sample

A 20 µL of albuterol sulphate (0.1 mg/mL) and ipratropium bromide (0.02 mg/mL) sample solution was injected into the chromatographic system and the obtained chromatogram was used as a control for the study of degradants in the further study.

Acidic Degradation

1.6 mL of albuterol sulphate and ipratropium bromide sample solution was pipetted out into a 10 mL volumetric flask, 1.0 mL of 1M hydrochloric acid was added, heated the solution to 60 ºC for 3 hr., cooled and immediately neutralized the solution using 1M sodium hydroxide solution. The stressed sample was made up to the volume with the diluent, 20 µL was injected in duplicate and analysed.

Alkaline Degradation

1.6 mL of albuterol sulphate and ipratropium bromide sample solution was pipetted out into a 10 mL volumetric flask, 1.0 mL of 1M sodium hydroxide was added, heated the solution to 60 ºC for 3 hr., cooled and immediately neutralized the solution using 1M hydrochloric acid. The stressed sample was made up to the volume with the diluent, 20 µL was injected in duplicate and analysed.

Oxidative Degradation

Oxidative stress studies were conducted by treating 1.6 mL of albuterol sulphate and ipratropium bromide sample solution with 2.4 mL of 6% hydrogen peroxide in a 10 mL volumetric flask. The solution was kept at room temperature for 2 hr., made up to the volume with diluent and injected in duplicate into the chromatograph.

Photolytic Degradation

Dark Control
Dark control studies were carried out by transferring 1.6 mL sample solution of albuterol sulphate and ipratropium bromide into a 10 mL volumetric flask, stoppered with a lid and wrapped into an aluminium foil. The flask was subjected to an illumination of 1.2 million Lux hours of cool fluorescent light and an integrated near UV energy exposure of 200 watt hours / m^2 simultaneously in a photo stability chamber maintained at 25 °C. The stressed sample was made up to the volume with diluent and injected in duplicate.

Exposure to Light
1.6 mL of albuterol sulphate and ipratropium bromide sample solution was transferred into a 10 mL stoppered volumetric flask. The solution was subjected to an illumination of 1.2 million Lux hours of cool fluorescent light and an integrated near UV energy exposure of 200 watt hours / m^2 simultaneously in a photo stability chamber maintained at 25 °C. The stressed sample was made up to the volume with diluent and injected in duplicate into the chromatograph.

Dry Heat
Thermal stress was carried out by heating 1.6 mL of albuterol sulphate and ipratropium bromide sample solution in a controlled temperature oven at 60 ºC for 7 days. The stressed sample mixture was cooled, made up to the volume with diluent and injected in duplicate into the chromatograph.

Table 1.1: Optimized chromatographic conditions

Parameter	Value	
Column	Peerless basic C8 (150 X 4.6 mm, 5 µm) column	
Mobile phase	**Mobile phase A**: Mixture of anhydrous potassium dihydrogen orthophosphate (0.05M) and 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 ± 0.05 (Sol. A), acetonitrile (95:5 v/v) **Mobile phase B**: Mixture of anhydrous potassium dihydrogen orthophosphate (0.25M) and 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 ± 0.05 (Sol. B), acetonitrile (70:30 v/v)	
Elution mode	Gradient	
Flow rate	1.0 mL/min.	
Detection wave length	Albuterol sulphate	276 nm
	Ipratropium bromide	220 nm
Column temperature	30 ºC	
Volume of injection	20 µL	
Run time	22.0 min.	
Retention time obtained	Albuterol sulphate	2.927 ± 0.16 min.
	Ipratropium bromide	10.479 ± 0.51 min.

Table 1.2: Gradient Program

Time (min.)	Mobile phase 'A' (%)	Mobile phase 'B' (%)
0.01	75	25
4	75	25
7	40	60
9	25	75
12	15	85
12.1	0	100
15	0	100
15.1	75	25
22	75	25

Table 2: Linearity data

Preparation (%)	Albuterol sulphate		Ipratropium bromide	
	Conc. (mg/mL)	* Peak area ± SD, % RSD	Conc. (mg/mL)	* Peak area ± SD, % RSD
10	0.0100	71716 ± 318.91, 0.44	0.0023	21149 ± 149.2, 0.71
25	0.0260	183859 ± 531.04, 0.29	0.0059	54466 ± 501.3, 0.92
50	0.0520	364653 ± 391.74, 0.11	0.0117	114424 ± 993.4, 0.87
100	0.1040	745849 ± 310.42, 0.04	0.0234	225406 ± 255.9, 0.11
150	0.1560	1112929 ± 388.20, 0.03	0.0351	340323 ± 555.7, 0.16
200	0.2080	1488488 ± 1190.06, 0.08	0.0468	454106 ± 726.2, 0.16

* Mean of two replicates

Table 3: Precision study

Drug	Repeatability		Intermediate precision	
	*Sample peak area	*Assay ± SD, % RSD	*Sample peak area	*Assay ± SD, % RSD
Albuterol sulphate	963400	100.1 ± 0.971, 1.0	981563	99.9 ± 1.097, 1.1
Ipratropium bromide	211508	100.4 ± 0.789, 0.8	210928	100.1 ± 1.384, 1.4

* Mean of six preparations, two replicates each

Table 4: Accuracy data

Drug	Level (%)	Standard peak area	*Sample peak area	*Amount added (mg)	*Amount found (mg)	*Recovery ± SD, % RSD
Albuterol sulphate	50	783686	489305	0.0540	0.0548	101.7 ± 0.5, 0.46
	100	783686	967442	0.1079	0.1083	100.4 ± 1.2, 1.17
	150	783686	1420808	0.1619	0.1590	98.4 ± 0.3, 0.29
Ipratropium bromide	50	194242	100972	0.0111	0.0110	100.3 ± 1.4, 1.42
	100	194242	211503	0.0231	0.0232	100.4 ± 0.9, 0.91
	150	194242	328866	0.0357	0.0361	101.1 ± 0.8, 0.74
	* Mean of two preparations, two replicates each					

Table 5: Robustness study

Albuterol sulphate	Parameter	Condition	*Assay (%)	Tailing factor	Theoretical plates	Mean assay ± SD, % RSD
	Flow rate (± 0.2 mL/min.)	0.8	101.8	1.282	2016	101.9 ± 0.1, 0.09
		1.0	**101.9**	**1.095**	**2958**	
		1.2	102.0	1.177	2100	
	Column oven temperature (± 5 °C)	25	101.9	1.190	2206	101.8 ± 0.2, 0.17
		30	**101.9**	**1.095**	**2958**	
		35	101.6	1.184	2437	
Ipratropium bromide	Flow rate (± 0.2 mL/min.)	0.8	101.2	1.244	46842	101.4 ± 0.3, 0.25
		1.0	**101.4**	**0.985**	**27689**	
		1.2	101.7	1.131	32673	
	Column oven temperature (± 5 °C)	25	101.5	1.153	28292	101.6 ± 0.2, 0.21
		30	**101.4**	**0.985**	**27689**	
		35	101.8	1.120	37247	

* Mean of two injections

Table 6: Assay of albuterol sulphate and ipratropium bromide from respules

Brand name	Drug	Labeled amount (mg/mL)	Amount found ± S.D.	*Assay (%) ± S. D.
LIORESAL	Albuterol sulphate	2.5	2.51 ± 0.02	100.6 ± 0.9
	Ipratropium bromide	0.5	0.49 ± 0.01	100.67 ± 1.07

*Mean of two injections

Table 7a: Forced degradation data (albuterol sulphate)

Stress condition	Standard average area	*Sample average area	Assay (%)
Control sample	722510	955286	101.9
Dark control		946644	100.9
Exposure to light		940054	100.2
Dry Heat		939555	100.2
Acid stress		935651	99.8
Alkaline stress		932453	99.4
Peroxide stress		945485	100.8

* Mean of two injections

Table 7b: Forced degradation data (ipratropium bromide)

Stress condition	Standard average area	*Sample average area	Assay (%)
Control sample	208681	205718	99.8
Dark control		193906	94.0
Exposure to light		206138	99.9
Dry Heat		214123	99.3
Acid stress		213824	99.5
Alkaline stress		Not detected	-
Peroxide stress		205324	99.6

* Mean of two injections

Table 8: Comparison chart of the published HPLC methods for simultaneous determination of albuterol sulphate and ipratropium bromide

Method	Column	Mobile phase	Flow rate (mL/min)	Wave length (nm)	Retention time (min.)		Linearity range		Remarks
					ALB	IPT	ALB	IPT	
Proposed method	Peerless basic C8 column (150 X 4.6mm, 5µ)	Mixture of anhydrous potassium dihydrogen orthophosphate,1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 and acetonitrile (95:5 v/v, mobile phase 'A'). Mixture of anhydrous potassium dihydrogen orthophosphate, 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 and acetonitrile (70:30 v/v, mobile phase 'B'), Gradient mode	1.0	276 & 220	2.927	10.479	0.01 – 0.20 mg/mL	0.002 – 0.046 mg/mL	Stability indicating within a short run time of 22.0 min.
N. Jyothi et al[3]	Symmetry C18 column (150 X 4.67 mm, 5µ)	0.05M phosphate buffer and methanol, pH 3.5, (40:60, v/v)	0.6	226	2.995	3.437	0.03 – 90 µg/mL	0.19 – 16 µg/mL	Peaks eluted very close, Not stability indicating

V. Ravi et al[4]	Intersil ODS 3V-RP C18 column, (250 x 4.6mm, 5µ)	0.03M di-potassium hydrogen phosphate buffer and acetonitrile, pH 3.2, (70:30, v/v)	0.8	242	7.016	5.206	5 - 15 µg/mL	2 – 6 µg/mL	Not stability indicating
P. Nagaraju et al[5]	Enable C18 column (250 X 4.6 mm, 5µ)	0.01M potassium dihydrogen phosphate and methanol, pH 3.0, (50:50, v/v)	1.0	245	2.11	5.19	2.5 - 15 µg/mL	1 – 6 µg/mL	Not stability indicating
G. B. Kasawar et al[8]	Inertsil C8-3 column (250 X 4.6 mm, 5µ)	Potassium dihydrogen phosphate and heptane-1-sulfonic acid sodium salt, pH 4.0 (solvent 'A') and acetonitrile (solvent 'B'), Gradient mode	1.0	220	16.45	53.02	0.03 - 7.47 µg/mL	0.01 – 1.24 µg/mL	Method for impurities and related substances, Very long run time of 95.0 minutes

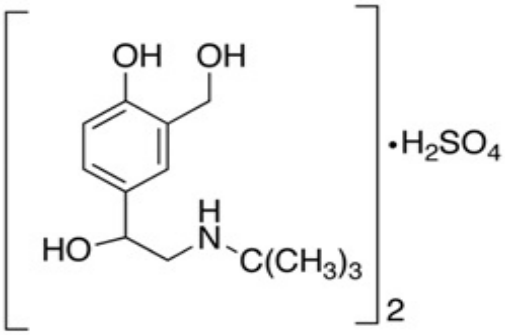

Fig. 1a: Structure of albuterol sulphate

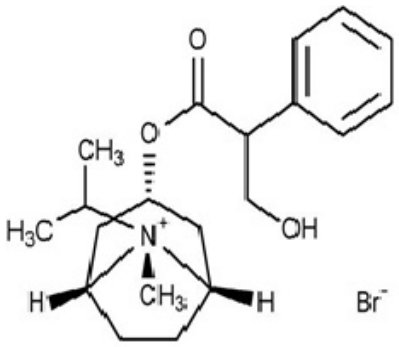

Fig. 1b: Structure of ipratropium bromide

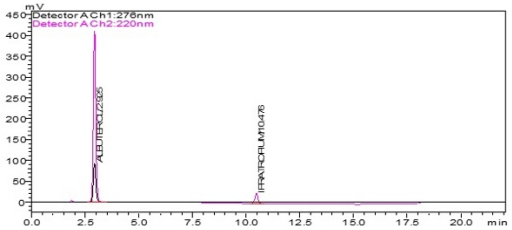

Fig. 2: A model chromatogram showing the separation of standard albuterol sulphate (276 nm) and ipratropium bromide (220 nm)

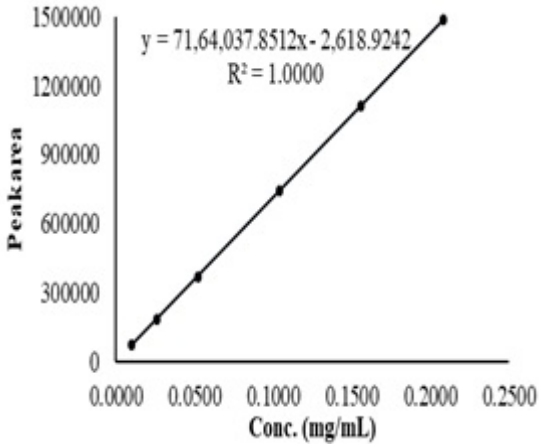

Fig 3a: Linearity curve for albuterol sulphate

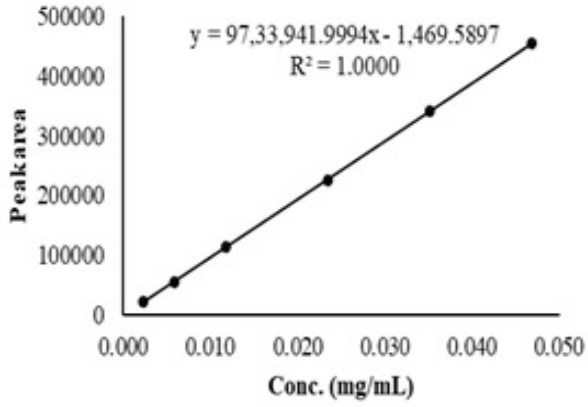

Fig 3b: Linearity curve for ipratropium bromide

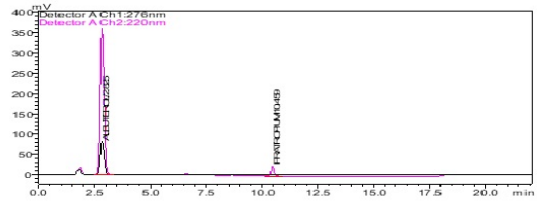

Fig. 4: Chromatogram for assay of albuterol sulphate and ipratropium bromide from respules

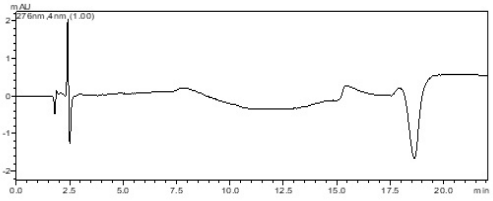

Fig. 5a: Placebo chromatogram for specificity of albuterol sulphate and ipratropium bromide (276 nm)

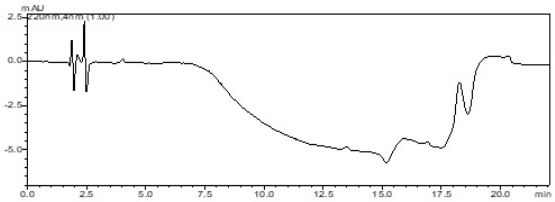

Fig. 5b: Placebo chromatogram for specificity of albuterol sulphate and ipratropium bromide (220 nm)

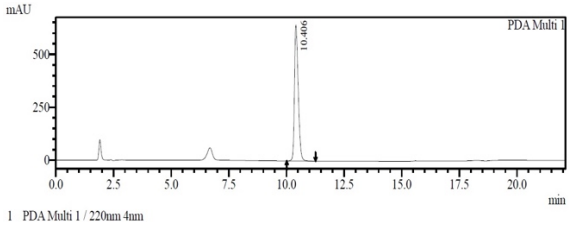

Fig. 5c: Specificity chromatogram for ipratropium bromide (220 nm)

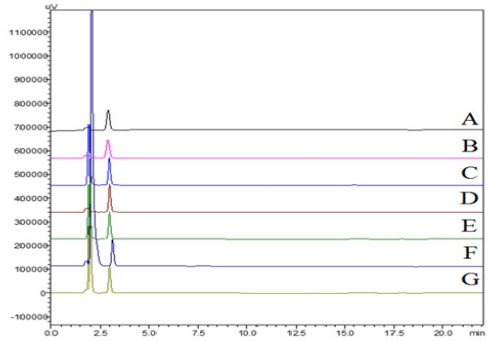

Fig. 5d: Overlain chromatograms for forced degradation study of albuterol sulphate and ipratropium bromide at 276 nm (A: Control, B: Dark control, C: Exposure to light, D: Dry heat, E: Acid, F: Base and G: Oxidation)

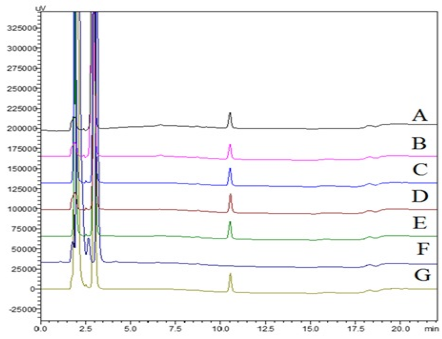

Fig. 5e: Overlain chromatograms for forced degradation study of albuterol sulphate and ipratropium bromide at 220 nm (A: Control, B: Dark control, C: Exposure to light, D: Dry heat, E: Acid, F: Base and G: Oxidation)

RESULTS AND DISCUSSION

A simple and specific liquid chromatographic method has been developed for the simultaneous assay of albuterol sulphate and ipratropium bromide after a series of trials with various stationary and mobile phase conditions. A mixture of anhydrous potassium dihydrogen orthophosphate and 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 ± 0.05 (Sol. A) with acetonitrile in different molar concentrations suited best for the separation process. Albuterol sulphate showed good absorption both at 220 nm and 276 nm but ipratropium bromide showed absorption only at 220 nm and was not detected at 276 nm. Hence 276 nm was selected for albuterol sulphate and 220 nm was selected for ipratropium bromide as the detection wavelengths. The optimized gradient elution is given in table 1.2. The Beer's law was obeyed in the working range of 10-200 %. The corresponding linearity data and curves obtained for albuterol sulphate and ipratropium bromide are given in table 2 and fig.3a and 3b.

The repeatability (intra-day assay) and intermediate precision (inter-day assay) studies for albuterol sulphate and ipratropium bromide revealed minor variations in the repetitive assay values (% RSD < 1.5) as given in table 3 indicating the precision of the method. Accuracy of the method was established from the percentage recovery as calculated from the amount recovered by comparing the average peak areas obtained for standard and formulation solutions and was found to be in the range of 98.0 – 102.0 % for albuterol sulphate and 98.1 – 101.9 % for ipratropium bromide as given in table 4. The LOD of albuterol sulphate and ipratropium bromide were found to be 5.73×10^{-5} mg/mL and 1.35×10^{-5} mg/mL. The corresponding LOQ values were found to be 1.73×10^{-4} mg/mL and 4.09×10^{-5} mg/mL.

The robustness of the method was proved by bringing minor variations in optimized flow rate and column oven temperature which did not majorly affect the assay (% RSD < 0.5). The related system suitability parameters are given in table 5. The developed assay method was applied for the determination of albuterol sulphate and ipratropium bromide in dosage forms and the results obtained are given in table 6 along with the corresponding chromatogram in Fig 4.

The method proved to be highly specific for both the drugs even in the presence of excipients as observed from the placebo and standard chromatograms given in fig. 5a, 5b and 5c.

The sample solutions of the drugs were also subjected to stress conditions to account for specificity in the presence of degradants if any. A moderate degradation of albuterol sulphate was observed in acid stress (2.1 %) and alkaline stress (2.5 %) conditions while ipratropium bromide did not show any degradation in acid stress but was very sensitive to alkaline stress

conditions as the peak was not detected. Mild degradations were observed in light, heat and peroxide stress for albuterol sulphate (< 2.0 %). Ipratropium bromide showed a sensible degradation (5.8 %) in dark control studies while it was stable to light, heat and peroxide stress conditions. There was no interference of additional peaks with the analyte peaks in the optimized run time and all the system suitability parameters are within the limits. The overlain chromatograms are given fig. 5d and 5e while the forced degradation data is given in table 7a and 7b. A comparison data of the performance characteristics of the present method with the published methods is given in table 8.

CONCLUSION

An economical RP-HPLC method which is specific, sensitive, precise and accurate has been developed for quantification of albuterol sulphate and ipratropium bromide in bulk drugs and pharmaceutical dosage forms without any interference from excipients. The proposed method could also be applied to study the interference of degradants that are likely to be formed during various stages of formulation development, storage or transportation and hence can be applied as a stability indicating HPLC method for routine quality control analysis of albuterol sulphate and ipratropium bromide.

ACKNOWLEDGEMENTS

The authors are grateful to M/s GITAM University for providing necessary facilities.

REFERENCES

1. Neil MJO: The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. 14th ed. Merck Research Laboratories, Merck & Co; 2006. 882-83.
2. Abdine HH, Belal F, Al-Badr AA. Ipratropium Bromide: Physical Properties. Profiles of Drug Substances, Excipients, and Related Methodology 2003; 30: 59-83. https://doi.org/10.1016/S0099-5428(03)30003-6
3. Jyothi N, Venu Gopal K, Seshagiri Rao JVLN. Development and Validation of a HPLC Method for the Simultaneous Estimation of the Salbutamol Sulphate and Ipratropium in Inhalation Dosage Forms. International Journal of Pharma Sciences 2012; 2: 79-83.
4. Ravi V, Ravi PP, Umasankar B, Sai Sneha G, Swaroopa Ch, Aparna A. RP-HPLC Method for Simultaneous Estimation of Ipratropium Bromide and Levosalbutamol in Pharmaceutical Metered Dose Inhalers. International Journal of Research in Pharmacy and Chemistry 2013; 3: 112-20.
5. Nagaraju P, Appaji SCHVSS. Development and Validation of Novel RP-HPLC Method for Simultaneous Estimation of Levosalbutamol and Ipratropium Bromide in Pharmaceutical Dosage Forms. International Journal of Research in Pharmacy and Chemistry 2014; 4: 628-35.
6. Kasawar GB, Farooqui. Development and Validation of a Stability Indicating RP-HPLC Method for the Simultaneous Determination of Related Substances of Albuterol Sulphate and Ipratropium Bromide in Nasal Solution. Journal of Pharmaceutical and Biomedical Analysis 2010; 52: 19–29. https://doi.org/10.1016/j.jpba.2009.11.026
7. Wu J, Ding C, Ge Q, Li Z, Zhou Z, Zhi X. Simultaneous Determination of Ipratropium and Salbutamol in Rat Plasma by LC–MS/MS and its Application to a Pharmacokinetic Study. Journal of Chromatography B 2011; 879: 3475–83. https://doi.org/10.1016/j.jchromb.2011.09.027
8. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use: Validation of Analytical procedures, Text and Methodology, Q2 (R1), 2005.
9. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use: Stability Testing of New Drug Substances and Products Q1A (R2), 2005.
10. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use: Stability Testing: Photostability Testing of New Drug Substances and Products Q1B, 2005.

Cite this article as:

G. Sowjanya *et al*. Development and validation of a new RP-HPLC method for the simultaneous determination of albuterol sulphate and ipratropium bromide in nasal inhalations. Int. Res. J. Pharm. 2018;9(8):63-70 http://dx.doi.org/10.7897/2230-8407.098166

Source of support: Nil, Conflict of interest: None Declared