International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Albuterol & Ipratropium Bromide by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A reverse phase high performance liquid chromatographic method was developed for the determination of Albuterol and Ipratropium bromide in bulk and Pharmaceutical dosage form. The separation was effected on an Inertsil ODS (250x4.6mm) 5µm Column by using a mixture of 80 volumes of Methanol and 20 volumes of Water as mobile phase. The mobile phase was sonicated for 10min to remove gases and passed through column at a flow rate of 1ml/min. The detection was made at 239 nm. Calibration curve was linear over the concentration range of 10-60 µ g/ml of Albuterol and ipratropium bromide .The proposed method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug in bulk and Pharmaceutical dosage form.

Keywords: Albuterol, Ipratropium bromide, RP-HPLC.

A R T I C L E I N F O

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Article History: Received 12 September 2016, **Accepted** 27 October 2016, **Available Online** 15 November 2016

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Manuscript ID: IJCTPR3229

PAPER-QR CODE

Citation: Chatla Sunil Kumar. Analytical Method Development and Validation for the Simultaneous Estimation of Albuterol & Ipratropium Bromide by RP-HPLC Method in Bulk & Pharmaceutical Dosage Form. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(6): 369-375.

1. Introduction

Analytical methods: The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias[1]. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors.

Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs[2]. Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B). [3,4]

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behaviour[5]. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge.

The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments[6-9]. These may include forced degradation studies, which are an integral part of development of a stability-indicating method. API is typically subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients[10]

Albutero is a short-acting, selective beta2-adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart. Salbutamol is formulated as a racemic mixture of the R- and S-isomers. The R-isomer has 150 times greater affinity for the beta2-receptor than the S-isomer and the S-isomer has been associated with toxicity. This lead to the development of levalbuterol, the single R-isomer of salbutamol. However, the high cost of levalbuterol compared to salbutamol has deterred wide-spread use of this enantiomerically pure version of the drug. Salbutamol is generally used for acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic bronchopulmonary disorders such as chronic obstructive pulmonary disorder (COPD). It is also used prophylactically for exercise-induced asthma[11].

Ipratropium bromide is a muscarinic antagonist structurally related to atropine but often considered safer and more effective for inhalation use. It is used for various bronchial disorders, in rhinitis, and as an antiarrhythmic. This compound belongs to the class of organic compounds known as phenylacetic acid derivatives. These are by compounds containing a phenylacetic acid moiety, which consists of a phenyl group substituted at the second position an acetic acid.It is an anticholinergic agent. It blocks muscarinic cholinergic receptors, without specificity for subtypes, resulting in a decrease in the formation of cyclic guanosine monophosphate (cGMP). Most likely due to actions of cGMP on intracellular calcium, this results in decreased contractility of smooth muscle[12].

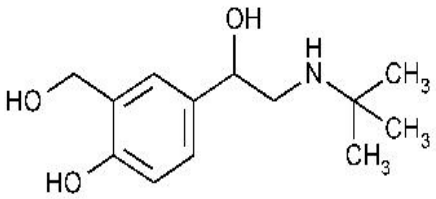

Figure 1: Albuterol

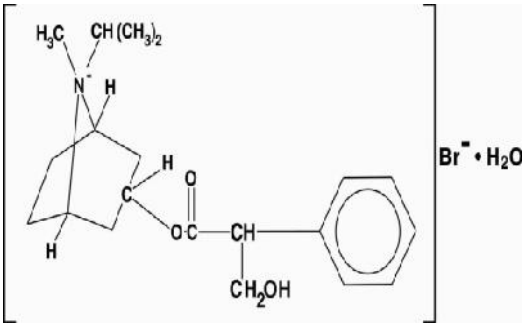

Figure 2: Ipratropium bromide

2. Materials and Methods

Apparatus: The instrument used for the study was Shimadzu (LC20ATVP) HPLC, Separation module 2695, UV detector with Spin chrome software version 2.

Reagents and Materials

The solvents used were Methanol,Acetonitrile, Potassium dihydrogen ortho phosphate, Dipotassium hydrogen phosphate, Tri Ethyl Amineof HPLC Grade and HPLC Water.

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Albuterol and Ipratropium bromide were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 239 nm was selected as the detection wavelength for the present study.

Selection of mobile phase

Initially the mobile phase tried was Methanol and water, Methanol, Buffer and water in various proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 80:20 v/v respectively.

Chromatographic trials for Simultaneous Estimation of Albuterol and Ipratropium bromide by RP- HPLC.

Trial – 1 Chromatographic condition

Mobile phase : Phosphate buffer: ACN
pH : 4.0
Ratio : 37:63
Column : Inertsil ODS 3V (250×4.6× 5µ)
Wavelength : 239 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 60 mg of Albuterol and 40 mg of Ipratropium Bromide in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Albuterol and 40 µg/ml of Ipratropium Bromide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

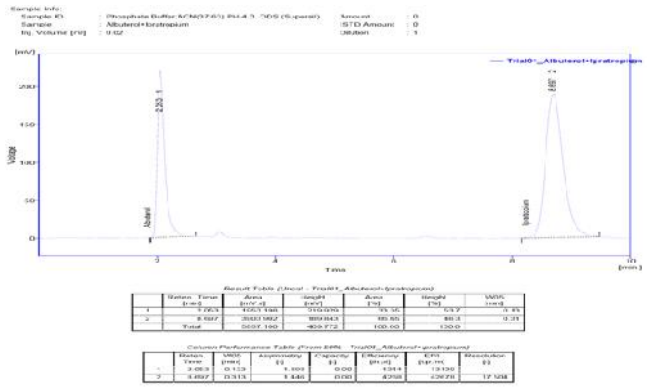

Figure 1: Chromatogram of Trail 1

Observation: The Efficiency was not satisfactory for Ipratropium Bromide and the peak response of Albuterol was very less. Hence it was not taken for optimization.

Trial- 2Chromatographic conditions

Mobile phase : KH_2PO_4: Methanol
Ph : 6.0
Ratio : 55:45
Column : Inertsil ODS 3V (250×4.6 ×5µ)
Wavelength : 239nm
Flow rate : 1ml/min

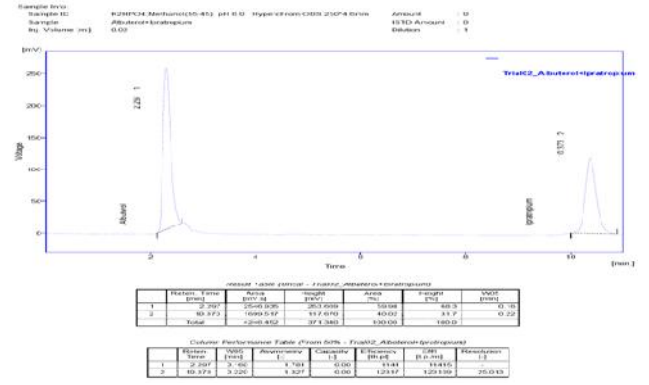

Figure 2: Chromatogram of Trail 2

Preparation of mixed standard solution

Weigh accurately 60 mg of Albuterol and 40 mg of Ipratropium Bromide in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Albuterol and 40 µg/ml of Ipratropium Bromide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

The efficiency ofboth the drugs were good but the run time is very more and the peaks of Albuterol and Ipratropium Bromide showed tailing. Hence it was not taken for optimization.

Optimization Chromatographic trial

Chromatographic conditions

Mobile phase : Methanol: Water
Ratio : 80:20
Column : Inertsil ODS 3V column, C18 (250x4.6 ID) 5µm
Wavelength : 239 nm
Flow rate : 1.0ml/min

Preparation of mixed standard solution

Weigh accurately 60 mg of Albuterol and 40 mg of Ipratropium Bromide in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Albuterol and 40 µg/ml of Ipratropium Bromide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Figure 3: Optimized Chromatogram

Observation: All the system suitability requirements were met and the peak Asymmetry factor was less than 2 for both Ipratropium Bromide and Albuterol.The efficiency was more than 2000 for both Ipratropium Bromide and Albuterol.Hence this method was optimized.

Procedure

Preparation of mixed standard solution

Weigh accurately 60 mg of Albuterol and 40 mg of Ipratropium Bromide in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Albuterol and 40 µg/ml of Ipratropium Bromide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains Ipratropium Bromide- 400 mg, Albuterol -600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Ipratropium Bromide and Albuterol were prepared by dissolving weight equivalent to 400 mg of Ipratropium Bromide and 600 mg of Albuterol

and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of Ipratropium Bromide and 60µg/ml of Albuterol was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 60 mg of Albuterol and 40 mg of Ipratropium Bromide in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Albuterol and 40 µg/ml of Ipratropium Bromide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains Ipratropium bromide-100mg and Albuterol-600mg were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Ipratropium Bromide and Albuterol (µg/ml) were prepared by dissolving weight equivalent to 10 mg of Ipratropium Bromide and 60 mg of Albuterol and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of Ipratropium Bromide and 60µg/ml of Albuterol was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Ipratropium Bromide and Albuterol present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of Ipratropium Bromide /Albuterol in mg
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation

3. Results and discussions

Method Validation Parameters

1. Specificity:

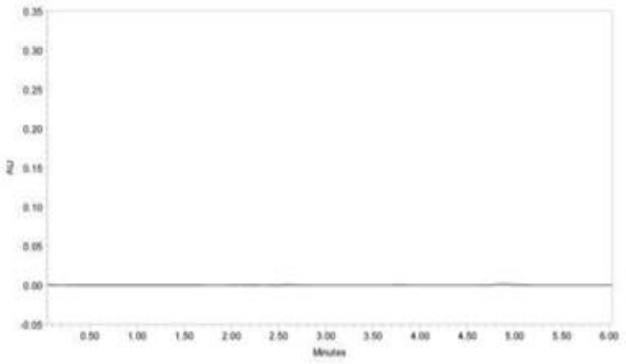

Figure 4: Chromatogram of Blank

Figure 5: Chromatogram of Sample

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank and sample

2. Linearity

Thelinearityofananalyticalmethodisitsabilitytoelicittestresult sthataredirectly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Standard stock solutions of Albuterol and Ipratropium Bromide (microgram/ml) were prepared by dissolving 60 mg of Albuterol and 40 mg of Ipratropium Bromide dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. This solution contains 36-84 µg/ml of Albuterol and 6-14 µg/ml of Ipratropium Bromide

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 36-84 ppm and 6-14 ppm for Albuterol and Ipratropium Bromide respectively

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
b) Percentage method: For these assay method samples are prepared in three concentrations of 80%, 100%, and 120% respectively.

Acceptance criteria:

The mean % recovery of the Albuterol and Ipratropium Bromideat each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

10µL of the standard and sample solutions of Albuterol and Ipratropium Bromide were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

5. Precision

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent

results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed onAlbuterol and Ipratropium Bromide formulation.

Acceptance criteria: The % RSD for the area of sample injections results should not be more than 2.

Selection of solvent

Solutions of Albuterol and Ipratropium Bromidewere prepared by dissolving in mobile phase and UV spectrum of each was recorded by scanning between 200-400 nm.

Validation of the Method

Linearity

Albuterol and Ipratropium Bromide:

Serial dilutions of Albuterol and Ipratropium Bromide (36-84 ppm and 6-14 ppm) were injected into the column and detected at a wavelength set at 239 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.9964 and 0.9973 respectively.

Table 1: Linearity of Albuterol

S.No	Conc.(µg/ml)	Area
1	36	3769.742
2	48	4743.960
3	60	5538.159
4	72	6714.107
5	84	7678.012

Table 2: Linearity of Ipratropium Bromide

S.No	Conc.(µg/ml)	Area
1	6	609.077
2	8	774.576
3	10	1007.518
4	12	1180.863
5	14	1417.216

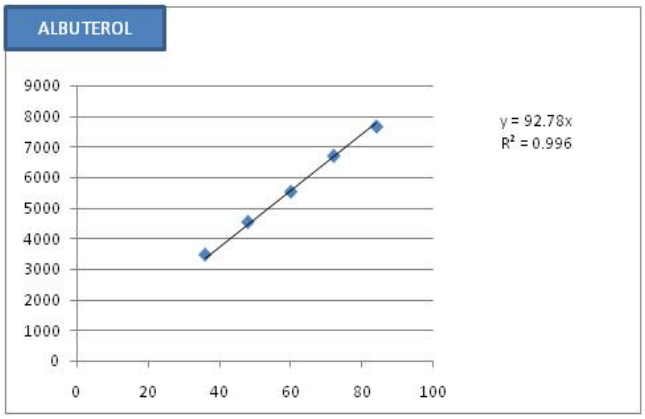

Figure 5: Linearity graph of Albuterol

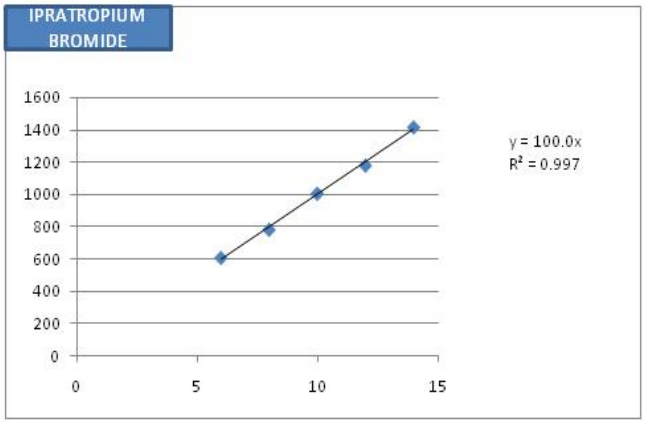

Figure 6: Linearity graph of Ipratropium Bromide

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Albuterol and Ipratropium Bromidewere added at 80%, 100% and 120% level and the contents were re-analyzed by the proposed method.

Table 3: Showing accuracy results for Albuterol and Ipratropium Bromide

Recovery level	Accuracy Albuterol					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
80%	60	4879.059	4878.164	31.78	85.95	98.33
	60	4874.809				
	60	4880.624				
100%	72	6715.130	6642.086	40.04	117.03	
	72	6416.984				
	72	6794.146				
120%	84	7889.449	7914.480	79.76	92.03	
	84	7976.993				
	84	7876.999				

Table 4: Accuracy Ipratropium Bromide

Recovery level	Accuracy Ipratropium Bromide					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
80%	10	815.841	808.663	8.7	83.55	

	10	793.602				
	10	816.547				
100%	12	1211.449	1177.796	10.02	121.69	102.45
	12	1115.460				
	12	1206.480				
120%	14	1483.271	1497.44	13.03	102.11	
	14	1515.624				
	14	1493.453				

Table 5: Result of Robustness study

Parameter	Albuterol		Ipratropium bromide	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.040	1.429	5.343	1.261
1.0 ml/min	2.420	1.448	4.270	1.297
1.2 ml/min	2.043	1.400	3.593	1.200
Wavelength				
268nm	2.450	1.379	4.293	1.263
270nm	2.453	1.414	4.300	1.231
272nm	2.420	1.448	4.270	1.225

Table 6: Results for Method precision of Albuterol and Ipratropium Bromide

Albuterol		
S.No.	Rt	Area
1	2.443	5710.568
2	2.417	5683.849
3	2.423	5662.646
4	2.423	5679.338
5	2.447	5659.977
6	2.423	5645.244
Avg	2.429333	5673.604
stdev	0.01242	22.86586
%RSD	0.005113	0.00403

Ipratropium bromide		
S.No.	Rt	Area
1	4.293	991.742
2	4.257	954.143
3	4.270	948.278
4	4.263	955.360
5	4.293	951.175
6	4.267	968.288
avg	4.273833	961.4977
stdev	0.015471	16.33345
%RSD	0.00362	0.016988

Table 7: Results for Ruggedness

Albuterol	**% Assay**	**Ipratropium Bromide**	**% Assay**
Analyst 01	100.53	**Analyst 01**	98.65
Analyst 02	100.40	**Anaylst 02**	100.41

Table 8: Results for **LOD & LOQ**

Drug name	LOD (µg)	LOQ (µg)
Albuterol	0.81	2.46
Ipratropium bromide	0.53	1.633

4. Conclusion

A new method was established for simultaneous estimation of Albuterol and Ipratropium Bromideby RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Albuterol and Ipratropium Bromideby using C18 column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (80:20 v/v) methanol: Water, detection wavelength was 239nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Albuterol and Ipratropium Bromidein pharmaceutical dosage form. The developed method was

validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Albuterol and Ipratropium Bromidein API and Pharmaceutical dosage form

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