

Original Article

UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IPRATROPIUM BROMIDE IN API AND PHARMACEUTICAL DOSAGE FORM

ANJALI P. KOKANE*, VARSHA S. TEGELI, BHAGYASHRI S. SHINDE

Department of Quality Assurance D. S. T. S. Mandal's College of Pharmacy, Solapur, Maharashtra, India
Email: anjalikokane333@gmail.com

Received: 26 Jan 2020, Revised and Accepted: 24 Mar 2020

ABSTRACT

Objective: The current work intended towards the developed and validated by using Simple, rapid, sensitive, precise and specific method UV Spectrophotometric method for the estimation of Ipratropium bromide in API and pharmaceutical formulation.

Methods: Water used as a solvent and the absorbance of the drug was measured at the absorbance's maxima of Ipratropium bromide λ_{max} is 214 nm.

Result: Calibration curve plotted in concentration range 20-120 μ g/ml exhibit the linearity relationship with line equation $y=0.0062x+0.3161$ and $r^2=0.995$. The Accuracy was found to be 99.5-100.1%, the precision % RSD= 0.12888-0.30533, and the LOD and LOQ is 8.78266-28.5881. The method was found to comply with all the validation parameters as per ICH guidelines indicating the sensitivity of the method towards analyte.

Conclusion: The method can be used satisfactory for the routine analysis of Ipratropium Bromide present in API and Pharmaceutical dosage form.

Keywords: Ipratropium bromide, UV spectrophotometer, Method validation

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ijcpr.2020v12i3.38309> Journal homepage: <https://innovareacademics.in/journals/index.php/ijcpr>

INTRODUCTION

Ipratropium bromide is a muscarinic cholinergic antagonist and is used in asthma. Ipratropium bromide has bronchial smooth muscle relaxant properties due to its action on muscarinic receptor. Ipratropium bromide is a bromide salt form of Ipratropium, synthetic derivative of alkaloid atropine with anticholinergic properties. It is used in treating symptoms of asthma, cold, chronic obstructive pulmonary disease due to chronic bronchitis. Ipratropium bromide is short-acting anticholinergic drug in asthma.

Chemical name is (1R,3R,5S,8R)-3-[(3-hydroxy-2-phenyl propanoyl)oxy]-8-methyl-8 (propan-2-yl) 8-azabicyclo [3.2.1] octan-8-ium-bromide.

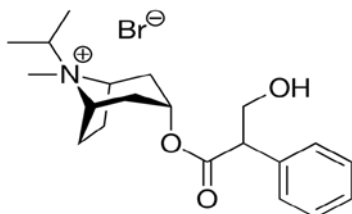


Fig. 1: Chemical structure of Ipratropium bromide

Literature survey reveals that the Ipratropium bromide has been estimated by RP-HPLC¹, LC/MS, HPTLC with fluorescence detection.

The aim of work is to develop UV spectrophotometry and RP-HPLC [2-6] method for the estimation of Ipratropium bromide in pharmaceutical formulation.

MATERIALS AND METHODS

Instrument

For weighing a calibrated weighing balance (Shimadzu AY220) of 1 mg sensitivity was used. A systronics UV visible double beam

spectrophotometer 2201 was used with 1 cm matched quartz cell. All the glassware were made of borosilicate and were calibrated.

Chemicals

API-Ipratropium bromide pure API was gifted by Vamsi Pharmaceutical Ltd. Solapur, Maharashtra.

UV spectroscopic method

Solvent selection

Ipratropium bromide is soluble in water so the water was used as the solvent.

Preparation of standard stock solution

The standard stock solution of Ipratropium bromide was prepared by transferring accurately weighed 10 mg of Ipratropium bromide into 10 ml volumetric flask containing 5 ml of water, dissolve properly. The volume was made up to the mark by using water to give a concentration of 1000 μ g/ml, from this 4 ml of the solution was transferred to 20 ml of volumetric flask and made up the volume with water to give a concentration of 200 μ g/ml which is a standard solution and it is further diluted with water to get concentration range 20-120 μ g/ml.

Determination of absorption maxima

The standard stock solution of 200 μ g/ml was scanned in the range of 200-400 nm to determine the wavelength of maximum absorption. The drug showed maximum absorption at 214 nm.

Preparation of calibration curve

For the preparation of calibration curve solutions of concentration 20-120 μ g/ml were prepared by pipetting out 1, 2, 3, 4, 5, 6, ml of 200 μ g/ml solution into 10 ml volumetric flask and made up the volume up to the mark with water. The absorption of each solution was measured at 214 nm against water as blank. Calibration curve of the Ipratropium bromide was plotted by taking the absorption obtained on the Y-axis and concentration of the solution on the X-axis. The curve showed linearity in the range of 20-120 μ g/ml with a correlation coefficient of 0.9954.

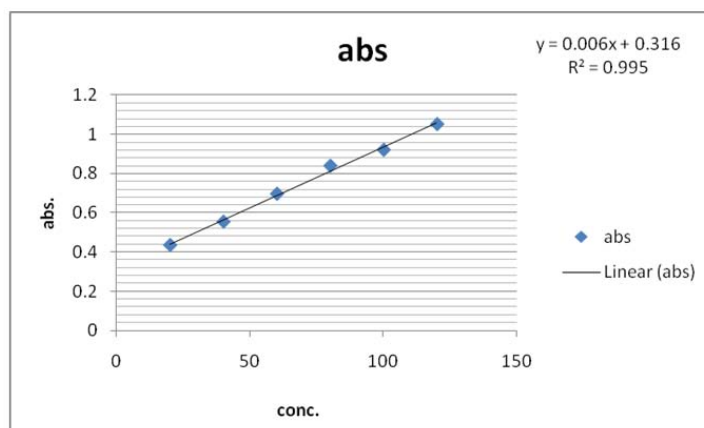


Fig. 2: Calibration curve of ipratropium bromide (IPRA)

Method validation

The developed method was validated as per ICH guidelines for the following parameters.

Linearity

1, 2, 3, 4, 5, 6 ml of standard Ipratropium bromide solution was transferred into a series of 10 ml volumetric flask. The volume was made up to the mark with water to obtain the concentration of 20, 40, 60, 80, 100, 120 µg/ml. The absorption of these solutions was recorded and the graph of absorption against concentration was plotted. The correlation coefficient (r^2) of the least squares linear regression of Ipratropium bromide was calculated.

Range

The range of the analytical method was decided from the interval between the upper and lower level of the calibration curve by plotting the curve.

Accuracy

The recovery study was carried out by the standard addition method by adding the known amount of Ipratropium bromide to the pre-analyzed sample at three different concentration levels that are 80%, 100%, 120% of assay concentration and percent recovery were calculated.

$$\% \text{ Recovery} = \frac{\text{observed value}}{\text{true value}} \times 100$$

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same sample under the prescribed condition. The precision of the method was determined in term of repeatability, intra-day precision and inter-day precision (Intermediate precision). Intra-day precision was determined by analyzing the drug at the concentration (60µg/ml) and each concentration for these two times on the same day. Inter-day precision was determined similarly, but the analysis is carried out daily for the two consecutive days.

Repeatability: Repeatability of the method was determined by analyzing six samples of the same concentration of the drug 60µg/ml. The absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small changes in altered concentration. To

determine the robustness of the method, the wavelength was studied at ± 5 nm.

Ruggedness

Ruggedness was determined by carrying out the analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of detection

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard stock solution of Ipratropium bromide (60 µg/ml) and LOD was calculated by $\text{LOD} = 3.3(\text{SD}/S)$.

Where, SD-standard deviation; S-slope of the curve

Limit of quantification

Quantification limit was determined based on the standard deviation of the peak area of same concentration that is a standard solution of Ipratropium bromide (60µg/ml) was prepared six times and $\text{LOQ} = 10(\text{SD}/S)$.

Where, SD-standard deviation; S-slope of the deviation.

RESULTS

Preliminary analysis of drug

Observation and Results of Preliminary Analysis of Ipratropium bromide.

Linearity

The linearity of this method was determined at range from 20-120 µg/ml for the Ipratropium Bromide the regression equation was found to be $0.0062x + 0.3161$, $R^2 = 0.995$

Accuracy

The accuracy of the analytical method for Ipratropium Bromide was determined at 80, 100, 120% level of the standard solution. Absorbance was measured at 214 nm and the results were expressed in the term of % recovery.

Precision

The precision (measurement of intra-day, inter-day and repeatability) result showed good reproducibility with the relative standard deviation (%RSD) below 0.12888-0.30533 and this indicates that the method was highly precise.

Table 1: Preliminary analysis of drug

Test	Observation	Result
Description	White powder	Complied
Solubility	Soluble in water, methanol	Complied

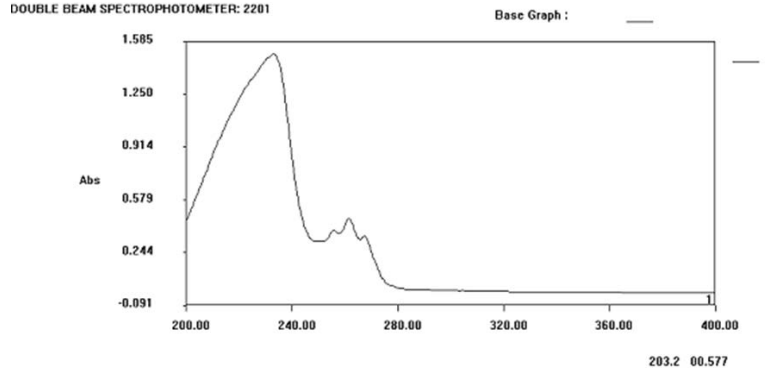


Fig. 3: Determination of wavelength of maximum absorption was found to be 214 nm

Table 2: Linearity

S. No.	Conc. (µg/ml)	Absorbance
1	20	0.434
2	40	0.553
3	60	0.695
4	80	0.838
5	100	0.919
6	120	1.105

The linearity for Ipratropium bromide was found to be linear in the range of 20-120 µg/ml with R²=0.995 and the straight line equation as y=0.0062x+0.3161

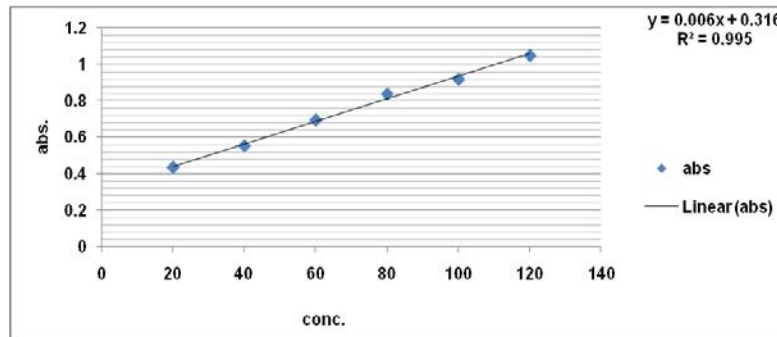


Fig. 4: The accuracy of the analytical method for Ipratropium Bromide was determined at 80, 100, 120% level of the standard solution. Absorbance was measured at 214 nm and the results were expressed in the term of % recovery

Table 3: Accuracy

S. No.	% level	Spike amount (µg/ml)	Spiked amount (wrt. sample)	Abs.	Amount recovered	% RSD of % recovery
1	80	79.840	79.8	0.735	99.5	1.0
2	100	99.800	99.8	0.919	99.5	0.3
3	120	119.760	119.8	1.105	100.1	0.4

Table 4: Intra-day morning precision

S. No.	Concentration (µg/ml)	Absorbance	SD	% RSD
1.	60	0.694	0.00089443	0.12888
2.	60	0.695		
3.	60	0.694		
4.	60	0.693		
5.	60	0.693		
6.	60	0.695		

Table 5: Inter-day precision

S. No.	Concentration	Absorbance	%SD	%RSD
1.	60	0.683	0.00209762	0.30533009
2.	60	0.689		
3.	60	0.688		
4.	60	0.687		
5.	60	0.687		
6.	60	0.688		

Limit of detection

For the limit of detection

Limit of quantification

For Limit of Quantification

Robustness

For Robustness

Ruggedness

Ruggedness was determined by carrying out the analysis by two different analyst and the respective absorbance was noted and the results were indicated as %RSD.

DISCUSSION

Preliminary analysis of Ipratropium bromide such as description, solubility was performed.

UV spectrophotometry for Ipratropium bromide**Selection of wavelength**

Ipratropium bromide showed the maximum absorbance at 214 nm. Hence, analysis is carried out at 214 nm. Ipratropium bromide showed absorption maxima at 214 nm and obeyed Beer's law in the concentration range of 20-120 µg/ml with the correlation coefficient 0.995 which is within acceptance criteria. The accuracy was from 99.5 and 100.1% at 214max 200-400 nm, respectively. The %RSD less than showed the method is precise. The limit of detection (LOD) was found to be 8.78266 and the limit of qualification (LOQ) to be 28.5881, respectively.

Summary and conclusion

A simple, precise, rapid and accurate UV method for determination of Ipratropium bromide from pure and its tablet formulation has been developed and validated. The proposed method can be used for the routine determination of Ipratropium bromide in bulk and pharmaceutical dosage formulation.

Table 6: Limit of detection

LOD(µg/ml)	8.78266
------------	---------

Table: 7 Limit of quantification

LOQ(µg/ml)	28.5881
------------	---------

Table 8: For robustness

S. No.	Wavelength	Absorbance	SD	%RSD
1.	214	0.434	0.002	0.4587
		0.436		
		0.438		
		Avg.=0.436		
2.	216	0.432	0.004	0.9262
		0.437		
		0.440		
		Avg.=0.436		

Table 9: Ruggedness**Analyst 1**

Concentration	Absorbance	Statistical analysis
60	0.683	Avg.=0.684
60	0.684	SD =0.001
60	0.685	%RSD =0.1461

Analyst 2

Concentration	Absorbance	Statistical analysis
60	0.684	Avg.=0.685
60	0.685	SD =0.001
60	0.686	%RSD =0.1459

Table 10: Summary of UV spectrophotometry method of Ipratropium bromide

S. No.	Parameter	Values(214 nm)	
1.	Beers law limit (µg/ml)	20-120	
2.	Absorption maxima (nm)	214 nm	
3.	Correlation coefficient (r ²)	0.995	
4.	Standard regression equation	0.0062x+0.3161	
5.	Accuracy (%recovery)	99.5-100.1%	
6.	Precision (%RSD)	0.12888-0.30533	
		Inter-day	0.12888
		Intra-day	0.30533
7.	LOD	8.78266	
8.	LOQ	28.5881	
9.	Robustness (%RSD)	0.4587 and 0.9262	
10.	Ruggedness (%RSD)	0.1461 and 0.1459	

The development of a validated method for the determination of Ipratropium bromide at λ_{max} has shown a similar result; therefore, analysis can be done either at 214 nm with the same accuracy and precision.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. R Vankudoth, RP Pulla, B Umasankar, G Saisneha, S Chawan, A Aparna. RP-HPLC method for simultaneous estimation of Ipratropium bromide and levalbutamol in pharmaceutical metered-dose inhalers. *Int J Res Pharm Chem* 2013;3:2231-781.
2. GB Kasawar, M Faoouqi. Development and validation of stability indicating RP-HPLC for the simultaneous determination of the related substance of albuterol sulphate and ipratropium bromide in nasal solution. *J Pharm Biomed Anal* 2010;52:19-20.
3. A Rasheed, O Ahmed. Analytical separation and characterization of degradation product and the development and validation of stability indicating method for the estimation of impurities in Ipratropium bromide respules formulation. *Int J Appl Pharm Sci Res* 2017;2:55-63.
4. P Nagarju, SCHVSS Appaji. Development and validation of novel RP-HPLC method for simultaneous estimation of levosalbutamol and Ipratropium bromide in pharmaceutical dosage form. *Int J Res Pharm Chem* 2014;4:635.
5. Gummadi Sowjanya D, Gowri Sankar JVLN, Seshagiri Rao. Development and validation of a new RP-HPLC method for the simultaneous determination of albuterol sulphate and ipratropium bromide in nasal inhalation. *Int Res J Pharm* 2018;9:63-70.
6. K Kranthi Kiran. A new analytical method development and validation for the simultaneous estimation of albuterol and Ipratropium bromide using RP-HPLC. *Int J Pharm Investigation Res* 2017;4:63-70.