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Original Article

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

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ABSTRACT

Objective: To developed and validated UV spectrophotometric method for the estimation of ipratropium bromide in API and pharmaceutical formulation.

Methods: Methanol is used as a solvent and the absorbance of the drug was measured at absorbance's maxima of ipratropium bromide max is 214 nm.

Results: Maximum absorbance obtained in 214 nm. Calibration curve plotted in concentration range 20-120 µm/ml exhibit the linearity relationship with line equation y=.0.0091x+0.1503 The Accuracy was found to be 99.7-100.2%, the precision %RSD= 0.08613-0.2668, and the LOD and LOQ is 6.33, 19.19. The method was found to comply all the validation parameters as per the ICH guideline indicating the sensitivity of the method analyte.

Conclusion: This method is used as satisfactory for the routine analysis of ipratropium bromide in API and pharmaceutical dosage forms.

Keywords: Ipratropium bromide, UV Spectrophotometer, Methanol, Validation

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INTRODUCTION

Ipratropium Bromide chemically is [8-methyl-8-(1-methyl ethyl)-8-azoniabicyclo [3.2.1] oct-3-yl] 3-hydroxyl-2-phenyl-propanoate (fig. 1). Ipratropium bromide was used for various bronchial disorders in rhinitis and as an antiarrhythmic [1, 2]. It is opens up the medium and large airways in the lungs. Ipratropium bromide is stable and it affects the safety and efficacy of the finished drug product. Ipratropium bromide is freely soluble in water, methanol and sparingly soluble in ethanol. In this method, methanol is used as a solvent. Various methods were developed by using HPLC, IR, MS, UV and others [3, 4].

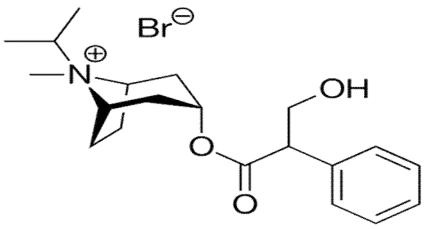

Fig. 1: Chemical structure of ipratropium bromide [5]

MATERIALS AND METHODS

Selection of solvent and instruments

Ipratropium bromide is dissolving in various solvents for trails of selecting the ideal solvent. Various solvents i.e. water, methanol, ethanol, acetonitrile. Ipratropium bromide is soluble in methanol and better absorption was found to be at 214 nm. UV-Visible Spectrophotometer (shimadzu AY220) is used for this study.

API-Ipratropium Bromide was obtained as a gift sample from the vamsi Pharmaceutical Ltd. Solapur, Maharashtra. Capsule of 40 mg is purchased from the local pharmacy in Solapur. Brand name is Ipravent (Cipla) methanol was used for this study.

Experimental work

Method development

Selection of detection wavelength

Dilution of Ipratropium bromide was prepared from the stock solution (50µg). Ipratropium bromide was scanned over a range of 200-400 nm. Drug showed maximum absorbance at 214 nm was selected as the wavelength for detection.

Preparation of standard drug solution

10 mg of Ipratropium bromide was weighed and transferred into a 10 ml volumetric flask containing methanol. Concentration of stock solution is (1000µg/ml). Then pipette out 2 ml form the stock solution and adjust to volume.

RESULTS AND DISCUSSION

Method validation [6]

The method was validated for several parameters i.e. Accuracy, Linearity, Precision, Robustness, Ruggedness, Limit of Detection, Limit of Quantification [6].

Accuracy

Accuracy is defined as, analytical procedure it expresses the closeness of an agreement between the value that is accepted and either as a true conventional value. This study was carried out at three different levels that are 80%, 100%, 120%, by the standard addition method. Analyzed samples by triplicate by according to the method. Known amount of standard ipratropium bromide was a spike on the capsule sample. Check the absorbance and calculated (table 1)

%Recovery= observed value/true value×100

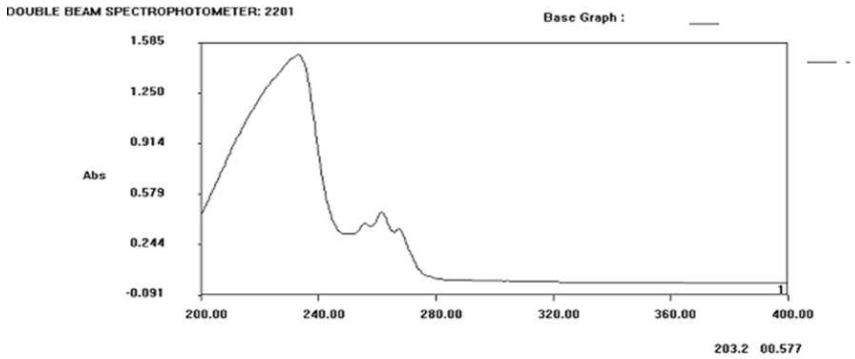

Fig. 2: UV visible spectrophotometer graph

Table 1: Result of accuracy

S. No.	% level	Spike amount (µg/ml)	Spiked amount (wrt sample)	Abs.	Amount recovered	% RSD % recovery
1	80	79.911	79.9	0.861	101.7	0.7
2	100	99.889	99.9	1.038	99.7	0.9
3	120	119.867	119.9	1.253	100.2	1.1

Based on the % recovery data, it was concluded that the developed method is used for the estimation of ipratropium bromide and is adequate for routine analysis.

Linearity and range

Linearity is defined as an ability of the analytical procedure to obtain test results, which is directly proportional to the concentration of the analyte in the sample. Pipette out 1,2,3,4, 5,6 ml of standard ipratropium bromide solution in a 10 ml calibrated volumetric flask. Adjust the volume up to the mark with methanol. Concentration of this solution is 20,40,60,80,100, 120 µg/ml. Taking the absorbance at 214 nm and calculating the correlation coefficient. Range is (20-120µg/ml).

Table 2: Result of linearity

S. No.	Concentration(µg/ml)	Absorbance
1	20	0.352
2	40	0.489
3	60	0.698
4	80	0.87
5	100	1.05
6	120	1.25

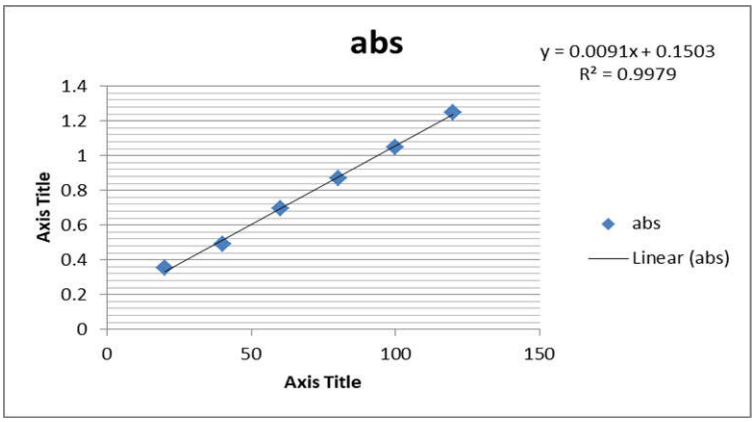

Fig. 3: Calibration curve of ipratropium bromide

By taking six different concentrations for linearity, the regression coefficient was found to be 0.997 i.e. in the limit of standard. Hence the linearity parameter was found to be validated (table 2, fig. 3).

Precision

Precision is defined as an analytical procedure is to define the closeness of agreement between a sample of measurements

obtained from multiple sampling of the same homogenous sampling in specific conditions. It is determined by an inter-day, intra-day study. Reading is taking 3times on the same day. The percent relative standard deviation (%RSD) was calculated. Precision results were given in the table 3 and 4 for intra and inter-day precision respectively.

Table 4: Result of Intra-day morning precision

S. No.	Concentration (µg/ml)	Absorbance	SD	%RSD
1	80	0.874		
2	80	0.875		
3	80	0.873		
4	80	0.874	0.000753	0.08613
5	80	0.875		
6	80	0.874		

Table 5: Result of inter-day precision

S. No.	Concentration (µg/ml)	Absorbance	%SD	%RSD
1	80	0.873		
2	80	0.879		
3	80	0.877		
4	80	0.878	0.002338	0.198804
5	80	0.877		
6	80	0.874		

For Intra-day and inter-day precision relative standard deviation is in limit i.e. less than 2% hence parameter is validated (table 4, 5)

Limit of detection

Detection limit is defined as the lowest amount of analyte in a sample can be detected. It is calculated based on the standard deviation of the absorbance of the same concentration that is a standard stock solution of 80µg/ml of ipratropium bromide.

LOD= 3.3(SD/S) SD= Standard deviation, S= slope of the curve

Table 6: Result of limit of detection

LOD (µg/ml)	6.33

The LOD was found to be 6.33 (µg/ml). Hence the parameter was found to be validated (table 6).

Limit of quantification

Limit of quantification is defined as, it is based on the standard deviation of the peak area of the same concentration that is standard solution prepared and calculated by LOQ

LOQ= 10(SD/S) SD= Standard deviation, S= slope of the curve

Robustness

Robustness is defined as, the capacity of analytical procedure to remain unaffected by small changes or these method deliberate small variation in the method parameters. Main aim of this test is create a method that allows for some variations in the parameters. In this study wavelength was change at±5 nm that is 214 nm and 216 nm. Then robustness is calculated (table 8)

Ruggedness

Ruggedness is defined as, reproducibility of the results when the defined method was performed under different analysts, laboratories, columns, chemicals, solvents, instruments, sources of reagents and etc.

Table 7: Result of limit of quantification

LOQ(µg/ml)	19.19

The LOQ was found to be19.19 (µg/ml. Hence the parameter was found to be validated (table 7).

Table 8: Result of Robustness of ipratropium bromide

S. No.	Wavelength	Absorbance	SD	%RSD
1	214	0.874		
		0.876	0.001528	0.174
		0.873		
		Avg= 0.874		
2	216	0.872		
		0.873	0.001528	0.174
		0.875		
		Avg= 0.873		

The change in the wavelength i.e. 216 nm. The robustness was found to be within limit i.e. relative standard deviation is less than 2%. Hence the parameter was found to be validated (table 8)

Table 9: Result of the ruggedness of ipratropium bromide

Analyst 1

Concentration	Absorbance	Statistical analysis
80	0.874	Avg =0.874
80	0.875	SD =0.0015
80	0.873	% RSD =0.114

Table 10: Result of ruggedness of ipratropium bromide

Analyst 2

Concentration	Absorbance	Statistical analysis
80	0.877	Avg =0.875
80	0.875	SD =0.0015
80	0.874	% RSD =0.114

Ruggedness was calculated by taking analyte at two different analyte and the respective absorbance was noted and obtained results shown that does not affected by it (table 9, 10)

CONCLUSION

UV spectrophotometric method of ipratropium was developing method is simple, precise, rapid, and accurate. Method is developing and validated by pure API and its capsule formulation. These method used for the routine determination of ipratropium bromide in bulk and pharmaceutical dosage formulation

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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