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Analytical Method Development and Validation of Uv Spectrophotometric Method For Simultaneous Estimation Of Ipratropium Bromide And Levosalbutamol In Combined Respule Formulation

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ABSTRACT:
The present study describes simple, rapid, accurate, precise UV spectrophotometric method for the simultaneous estimation of Ipratropium Bromide and Levosalbutamol in combined repsule formulation. In this method absorbance is measured at two wavelengths 221 nm and 242 nm λmax of zIpratropium Bromide and Levosalbutamol respectively. Linearity was observed in the range of 12µg/ml to 18µg/ml (r 2 =0.996) for Ipratropium Bromide and 36µg/ml to 54µg/ml (r 2 =0.998) for Levosalbutamol. The percentange mean recovery was found to be 100.19% for Ipratropium Bromide and 99.58 % for Levosalbutamol. In recovery study the percentage RSD was found to be less than 2. The methods were validated as per ICH guidelines.
Keywords: Ipratropium Bromide (IPRA), Levosalbutamol (LEVO), Simultaneous equation, validation, UV spectrophotometer.

I. INTRODUCTION:

Ipratropium Bromide is chemically [8-methyl-8-(1-methylethyl)-8-azoniabicyclo [3.2.1] oct-3-yl] 3-hydroxy-2-phenyl-propanoate. Ipratropium is mainly used in Chronic Obstructive Lung Disease. Trade name for Ipratropium Bromide is Atrovent. Ipratropium is mainly a type of anticholinergic drug which opens the medium and large airways in the lungs. Ipratropium is chemically quaternary ammonium compound obtained from atropine and isopropyl bromide. Anticholinergic drug binds with acetylcholine receptor and prevent acetylcholine binding which results in bronchodilation.
It helps to improve lung function. It relaxes the muscles along the airway passage easing the breathing difficulty. It reduces phlegm production improving cough symptoms.

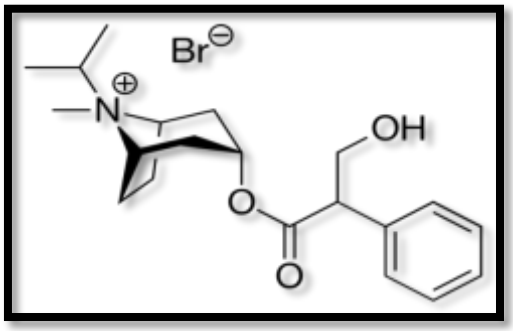

Figure1:StructureofIpratropium Bromide

The chemical name for Levosalbutamol is 4-[(1R)-2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol.Levosalbutamol is primarily used as ashort acting β_2 adrenergic receptor agonist. It is also known as levalbuterol. It is used in treatment of asthma and chronic obstructive pulmonary disease. Levosalbutamol binds with β_2 adrenergic receptor and causes activation of adenylatecyclase which results in increase in intracellular concentration of 3', 5'-cyclic adenosine monophosphate (cyclic AMP). Then the increased concentration of cyclic AMP activates protein kinase A which inhibits phosphorylation of myosin which decreases intracellular calcium concentration which results in muscle relaxation. Levosalbutamol causes relaxation of airways from trachea to terminal bronchioles.

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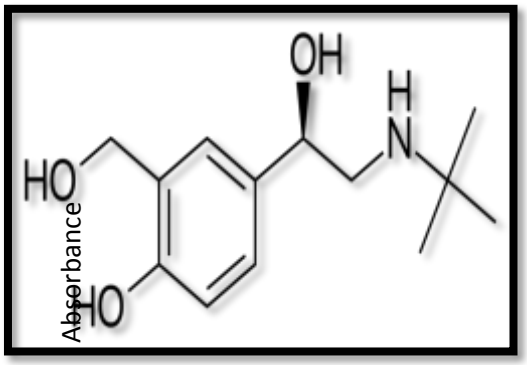

Figure2:StructureofLevosalbutamol

From literature survey it was found that no any UV method has been reported for simultaneous estimation of Ipratropium Bromide and Levosalbutamolrespectively.Inthispresentresearchwork,itwasproposedthat thedeveloped UV Spectroscopic method is simple, precise, specificandaccurate and which is also validatedfor simultaneous estimation of Ipratropium Bromide and Levosalbutamolinmarketed dosageformulations.

The present work the UV spectroscopic method for simultaneous estimation of Ipratropium Bromide and Levosalbutamolincombineddosageform is developed andvalidatedas perICHguidelines.

II. MATERIAL AND METHOD:

Chemicalsand Reagents:

Analytical pure sample of Ipratropium Bromide and Levosalbutamolwere received as a giftsamplefromCiplaPrivate Limitedwereusedinthestudy.Thepharmaceuticaldosageformusedinthisstudy was DEOLIN RESPULES labeled to contain Ipratropium Bromide and Levosalbutamol. The labeled formulation contains Ipratropium Bromide and Levosalbutamol 500mcg/1.25 mg in 2.5 ml repsules. The diluents used were 0.1% Perchloric acid and Distilled water used in preparation ofmobilephase.

Selectionofwavelength:

10 µg/ml of IPRA Working Standard and 10 µg/ml of LEVO Working Standard were scanned in the UV range of 190-400 nm. The overlay of both the spectrum was recorded. From the overlain spectra wavelengths 221 nm (λmax of IPRA) and 242 nm (λmax of LEVO) were selected for analysis of both drugs using simultaneous method. (λ1-221 nm and λ2-242 nm). The Isobestic wavelength was found to be 233 nm.

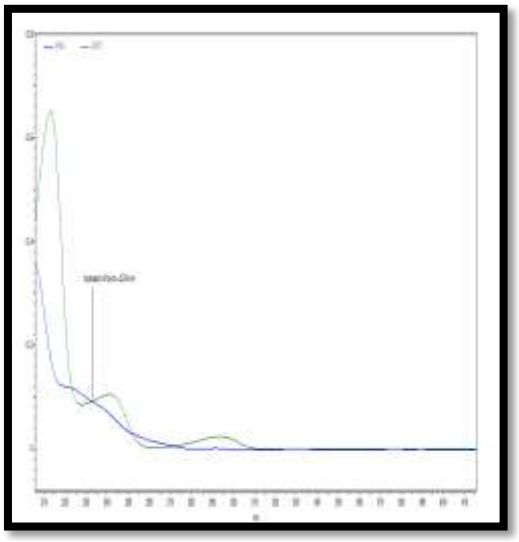

Figure3:Overlay UV SpectraofIpratropium Bromide &Levosalbutamol

Instrumentation:

A shimadzu 1800UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used forallspectral measurements.

Preparationof Mobilephase:

Preparation of 0.1% Perchloric acid:

Add 0.1 ml of Perchloric acid in 100 ml of Water, Mix and filtered.

Preparation of Standard Solution of Ipratropium Bromide and Levosalbutamol:

a. Initially Prepare a Standard Stock Solution (SSS-I) of Ipratropium by adding 15 mg in 10 ml volumetric flask & add 5 ml diluent and Mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (Conc. = 1500 µg/ml)

b. Prepare a Standard Stock Solution (SSS-II) of Levosalbutamol by adding 45 mg in 5 ml volumetric flask & add 5 ml diluent and Mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (Conc. = 4500 µg/ml)

c. Pipette out 1.0 ml of SSS-I and 1.0 ml of SSS-II in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (Conc. of IPRA = 150 µg/ml and LEVO = 450 µg/ml)

d. Pipette out 1.0 ml of above solution and transfer it to 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (Conc. of IPRA = 15 µg/ml and LEVO = 45 µg/ml)

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Determination of absorptivity value of Ipratropium Bromide and Levosalbutamol:

The required dilutions of the standard stock solution were done to get concentration 15 µg/ml of Ipratropium Bromide and 45 µg/ml of Levosalbutamol. The absorbances were measured for Ipratropium Bromide and Levosalbutamol at 221nm (λmax of IPRA), 242nm (λmax of LEVO). The absorptivity values of the drugs were determined at the selectedwavelengths. These absorptivity values are mean of six determinations.

SimultaneousestimationofIpratropium Bromide andLevosalbutamol:

In simultaneous method we used Absorbances at two selected wavelengths. To determine the λmax ofboththedrugswescanintherangeof190-400nm.Standardsolutionsofdifferentconcentrations ofbothdrugs were prepared in mobile phase. Absorbance of Ipratropium Bromide (15µg/ml)and Levosalbutamol(45µg/ml) were recorded at two wavelenghts 221nm and 242nm by usingsimultaneous equationmethod.

$$Cx = A2ay1 - A1ay2 / ax2ay1 - ax1ay2$$

$$Cy = A1ax2 - A2ax1 / ax2ay1 - ax1ay1$$

Cx =concentrationofIpratropium Bromide

Cy= concentrationofLevosalbutamol

ax1andax2=absorptivityvalueof Ipratropium Bromideat 221nmand242nm

ay1anday2=absorptivityvalueofatLevosalbutamol221nmand 242nm

A1 =absorbanceof standard mixtureat 221nm

A2 =absorbanceof standard mixtureat 242 nm

Analysisofmarketedformulation:

10 repsules content were accurately weighed to calculate the average weight, and mixed in the mortar and pestle and the powder equivalent to 1.5 mg Ipratropium and 4.5 mg Levosalbutamol was weighed accurately and transferred to 10 ml volumetric flask and 5-6 ml of diluent was added and sonicated for 2 minutes and made up to the mark with diluent. (Conc. of IPRA = 150 µg/ml and LEVO = 450 µg/ml). Further 1 ml of the above solution was pipetted out in 10 ml volumetric flask and mix with 5 ml diluent and made up to the mark with diluent. (Conc. of IPRA = 15 µg/ml and LEVO = 45 µg/ml)

The above solution was measured for absorbance at wavelengths 221nm and 242nm and the concentration was calculated using simultaneous equation method.

Table1:Analysisofmarketedformulation

Sr. No.	Ipratropium Bromide			Levosalbutamol		
	Absorbance	Amount Recovered	% Recovery	Absorbance	Amount Recovered	% Recovery
1	0.177	14.93	99.53	0.477	45.02	100.04
2	0.174	14.88	99.20	0.473	44.98	99.96
3	0.176	14.92	99.47	0.465	44.43	98.73
4	0.175	14.85	99.00	0.472	44.96	99.91
5	0.177	14.95	99.67	0.469	44.82	99.60

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AVG	99.37	**AVG**	99.65
STDEV	0.27	**STDEV**	0.54
RSD	0.27	**RSD**	0.54

Methodvalidation:
Validation of an analytical method is the process to develop documentary proof about the performance characteristics of developed method meet the requirements of the intended analytical application. The UV method wasvalidatedin terms oflinearity,accuracy, precision,LOD andLOQ.

Linearity:
Linearity was studied by plotting a graph of absorbance verses concentration. The absorbance is directly proportional to concentration of analyte. For linearity study we measure absorbances of aseriesofstandardsolutionofIpratropium Bromideintheconcentrationrangeof about 12µg/mlto18µg/mland Levosalbutamol in the concentrationrangeof about36µg/mlto54µg/mlisshowninbelowtables(table no. 2 & 3). LinearitygraphofIpratropium Bromide andLevosalbutamol is shownin fig.no.4&5.

Table2:LinearitystudyofIpratropium Bromide

Ipratropium Bromide		
% Level	**Concentration (µg/ml)**	**Absorbance**
80	12	0.138
90	13.5	0.161
100	15	0.177
110	16.5	0.195
120	18	0.212

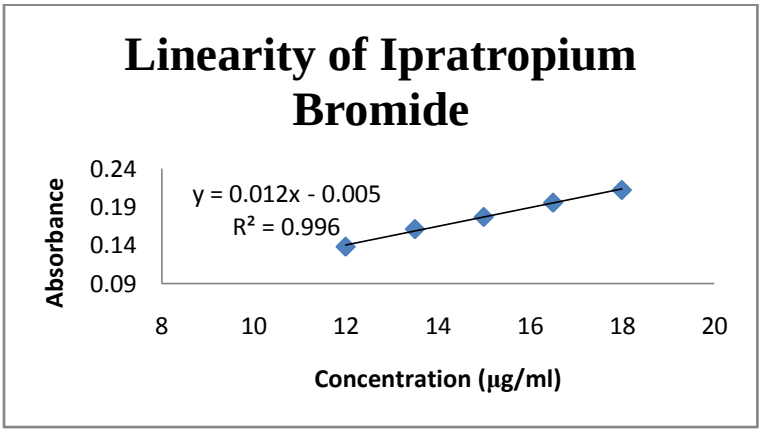

Fig4:linearitygraph ofIpratropium Bromide

Table3:LinearitystudyofLevosalbutamol

Levosalbutamol		
% Level	**Concentration (µg/ml)**	**Absorbance**
80	36.0	0.372
90	40.5	0.429

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100	45.0	0.477
110	49.5	0.525
120	54.0	0.572

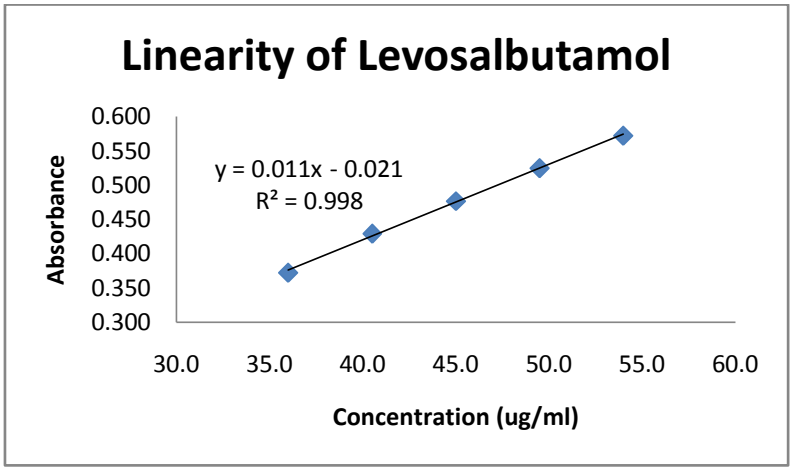

Fig5:linearitygraphofLevosalbutamol

Precision:

Precision study were carried out by analyzing 6 different solutions of Ipratropium Bromide and Levosalbutamol.The percentage RSD were found to be 0.91 for Ipratropium Bromide and 0.39 for Levosalbutamol so the method is precise.

The working standard and drug product samples were freshly prepared and analysed in morning and evening for Intra-day precision.The same working standard and drug product were used for analysis on 2nd day for inter-day precision.

Assay was calculated for the confirmation of precision.

Resultof %RSD was found to be below2 shownin below tables (4,5)

Table4:Precision Dataof Ipratropium Bromide and Levosalbutamol

Sample ID	IPRA ABS	LEVO ABS
100% Rep 1	0.177	0.477
100% Rep 2	0.174	0.479
100% Rep 3	0.176	0.478
100% Rep 4	0.178	0.477
100% Rep 5	0.174	0.479
100% Rep 6	0.176	0.474
AVG	0.176	0.48
STDEV	0.002	0.00
RSD	0.91	0.39

Table5:Intra-dayand Inter-day Precisionof Ipratropium Bromide and Levosalbutamol

Condition	Sample ID	Interval	Ipratropium Bromide		Levosalbutamol	
			Conc (ug/ml)	% Assay	Conc (ug/ml)	% Assay

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Intraday	**WS**	**Mrng**	15.00	-	45.00	-
	DP	**Mrng**	14.88	99.20	44.36	98.58
	WS	**Evng**	15.00	-	45.00	-
	DP	**Evng**	14.81	98.73	44.33	98.51
Interday	**WS**	**Day 2**	15.00	-	45.00	-
	DP	**Day 2**	14.72	98.13	44.28	98.40

Accuracy:

The Accuracy parameteris performed todeterminethecloseness ofthetestresultswiththatofth etruevaluewhichis expressed as % recovery. These studies were performed at three different levels (80%, 100% and120%) and the % recovery of Ipratropium Bromide and Levosalbutamol was calculated belowtable(6&7).

Table6:Recoverystudyof Ipratropium Bromide

Ipratropium Bromide								
% Level	**Reps**	**Spiked Conc (µg/ml)**	**Abs**	**Amount Recovered (µg/ml)**	**% Recovery**	**AVG**	**STDEV**	**RSD**
80	Rep 1	12.00	0.144	12.07	100.56	99.63	1.07	1.07
	Rep 2	12.00	0.141	11.82	98.46			
	Rep 3	12.00	0.143	11.98	99.86			
100	Rep 1	15.00	0.178	14.92	99.44	100.19	0.85	0.85
	Rep 2	15.00	0.179	15.00	100.00			
	Rep 3	15.00	0.181	15.17	101.12			
120	Rep 1	18.00	0.212	17.77	98.70	99.78	0.97	0.97
	Rep 2	18.00	0.216	18.10	100.56			
	Rep 3	18.00	0.215	18.02	100.09			

Table7:Recoverystudyof Levosalbutamol

Levosalbutamol								
% Level	**Reps**	**Spiked Conc (µg/ml)**	**Abs**	**Amount Recovered (µg/ml)**	**% Recovery**	**AVG**	**STDEV**	**RSD**
80	Rep 1	36.00	0.379	35.83	99.53	99.18	0.40	0.40
	Rep 2	36.00	0.378	35.74	99.26			
	Rep 3	36.00	0.376	35.55	98.74			
100	Rep 1	45.00	0.477	45.09	100.21	99.58	0.56	0.56
	Rep 2	45.00	0.472	44.62	99.16			
	Rep 3	45.00	0.473	44.72	99.37			
120	Rep 1	54.00	0.572	54.08	100.14	99.85	0.27	0.27
	Rep 2	54.00	0.569	53.79	99.61			
	Rep 3	54.00	0.57	53.89	99.79			

Sensitivity:

For study sensitivity parameter the limit of detection and limit of quantification were calculated. Thelimitofdetection[LOD]andlimitofquantitation[L

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OQ]parameterswerecalculatedusingfollowingequations
LOD=3.3σ/S andLOQ=10 σ/S
Where,
σ = standard deviation of y-intercept of regression line.
S=slope ofthe calibration curve.

Limitof Detection(LOD)andLimitof Quantitation(LOQ)Determination:
Limit of quantitation is 3 times more than the limit of detection resp. The LOD value of Ipratropium BromideandLevosalbutamolis1.79µg/mland3.35µg/mlrespectivelyandtheLOQvaluewerefoundtobe5.44 µg/mland10.15µg/mlfor Ipratropium Bromide andLevosalbutamol respectively.

Table8:ResultofLODAND LOQ

Srno.	Nameof drugs	LOD (µg/ml)	LOQ (µg/ml)
1	Ipratropium Bromide	1.79	5.44
2	Levosalbutamol	3.35	10.15

III. RESULT AND DISCUSSION:

Theproposedmethodisbasedonspectrophotometricsimultaneousestimationof IpratropiumBromide and Levosalbutamolin this method diluents used are 0.1% perchloric acid and water.For thecalibrationcurvethe linearityrange selected was 12 to 18 µg/mlfor Ipratropium Bromide and 36 to 54 µg/ml for Levosalbutamol. In this method wavelength selected was 221nm ($\lambda_{max}$for Ipratropium Bromide) and 242nm ($\lambda_{max}$for Levosalbutamol). For determining concentration of Ipratropium Bromide and Levosalbutamol in marketed formulation the absorptivities were calculated for both drugs based on selected wavelenghs and that values are substituted in simultaneous equation. The % assay was found to be 99.37% for Ipratropium Bromide and 99.65% for Levosalbutamol.TheCorrelation coefficients(r^2)was found to be 0.996and0.998forIpratropium Bromide andLevosalbutamolrespectively.The sensitivity of method was indicated by low value of LOD and LOQ. The LOD and LOQ were found to be 1.79 µg/ml and 5.44µg/ml for Ipratropium Bromide and 3.35µg/ml microgram/ml and 10.15µg/ml for Levosalbutamolrespectively.The Proposed method was foundtobepreciseandasthe%RSDvaluesforintra-dayand inter-day were found to be less than 2% for Ipratropium Bromide andLevosalbutamolrespectively.
Thepercentangemeanrecoverywasfoundtobe 100.19 %forIpratropium Bromide and 99.58 % forLevosalbutamol. The proposed methodwasalso successfully used for routine quality control method for simultaneous estimation of Ipratropium Bromide and Levosalbutamol.

IV. CONCLUSION:

The results of our study indicate that the proposed UV spectroscopic method for simultaneous estimation of Ipratropium Bromide and Levosalbutamol is simple, rapid, precise,and accurate. The developed UV spectroscopic methods is suitable for determination ofIpratropium Bromide andLevosalbutamol in combined dosage formulation withoutanyinterferencefromtheexcipients.The Proposed method was validated as per ICH guidelines. Statisticalanalysisprovesthat,thesemethodsarerepeatable \and selectiveforthe analysis ofIpratropium Bromide andLevosalbutamol.

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