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R E S E A R C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaneous Estimation of Tiotropium and Salmeterol in Bulk and Pharmaceutical Dosage Forms

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A B S T R A C T

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tiotropium and Salmeterol was done by RP-HPLC. The Phosphate buffer was $p^H3.0$ and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5μm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 253 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tiotropium and Salmeterol were found to be from 100-500 μg/ml of Tiotropium and 1-5μg/ml of Salmeterol. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Tiotropium and Salmeterol. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Methanol: Phosphate buffer, Inertsil C_{18} column, Tiotropium and Salmeterol, RP-HPLC.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Tiotropium bromide, sold under the brand name Spiriva among others, is a long-acting bronchodilator used in the management of chronic obstructive pulmonary disease (COPD) and asthma. Specifically it is used to try to prevent periods of worsening rather than for those periods themselves. It is used by inhalation through the mouth. Onset typically begins within half an hour and lasts for 24 hours.
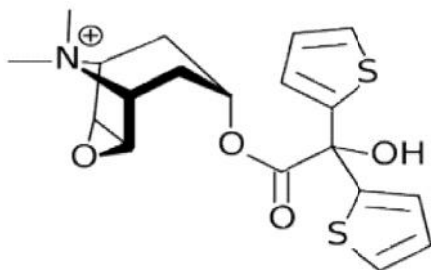
Fig 1: Structure of Tiotropium bromide

Salmeterol is a long-acting $_2$ adrenergic receptor agonist (LABA) used in the maintenance and prevention of asthma symptoms and maintenance of chronic obstructive pulmonary disease (COPD) symptoms. Symptoms of bronchospasm include shortness of breath, wheezing, coughing and chest tightness. It is also used to prevent breathing difficulties during exercise (exercise-induced bronchoconstriction.
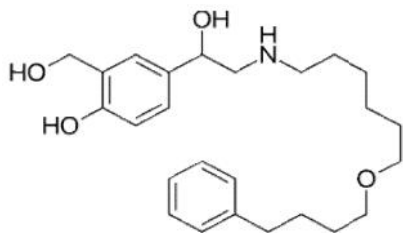
Fig 2: Structure of Salmeterol

2. Materials and Methods

Instrumentation:

SystemAllianceWaters 2690 separation module, Pump Analytical HPLC isocratic pump,DetectorPhoto diode array detector,SoftwareEmpower 2 software,ColumnAgilent (250×4.6mm, 5µ) C-18 RP-column,SonicatorAnalytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000$^+$pH meter,Weighing machine.

Chemicals:

Tiotropium andSalmeterol, KH_2PO_4 Water and Methanol for HPLC,Acetonitrile for HPLC, Ortho phosphoric acid,Tri ethyl amine.

Optimized chromatographic conditions

Mobile phase   : Phosphate buffer pH 3.0: ACN (30:70% v/v)
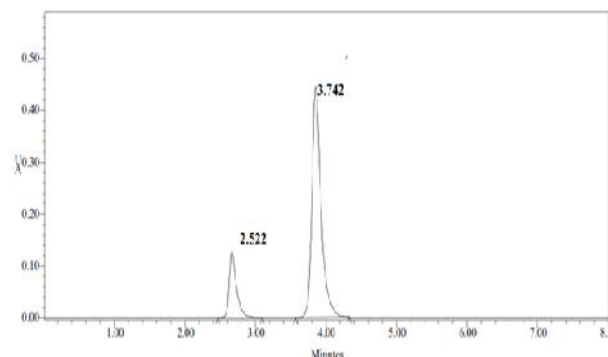Column   : Inertsil C18 5µm (4.6*250mm)
Flow rate   : 0.8 ml/min
Wavelength   : 253nm
Column temp   : Ambient

Sample Temp   : Ambient
Injection Volume: 10 µl

Fig 3: Chromatogram from Tiotropium and Salmeterol

Standard solution preparation:

Accurately weigh and transfer 200 mg of Tiotropium and Salmeterol 25mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample solution preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20.02 mg and 20.02mg of Tiotropium and Salmeterol (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 3 ml of Tiotropium and Salmeterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
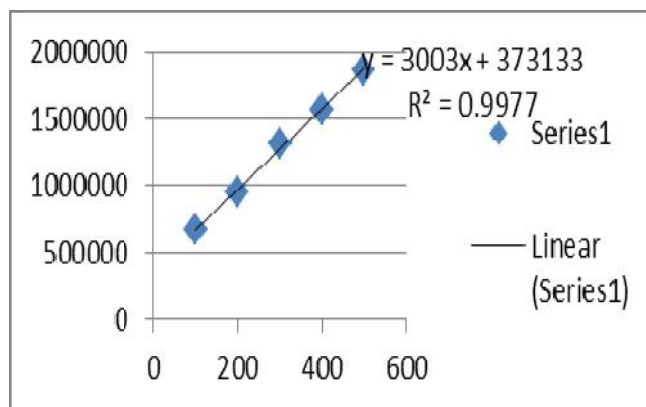
Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion
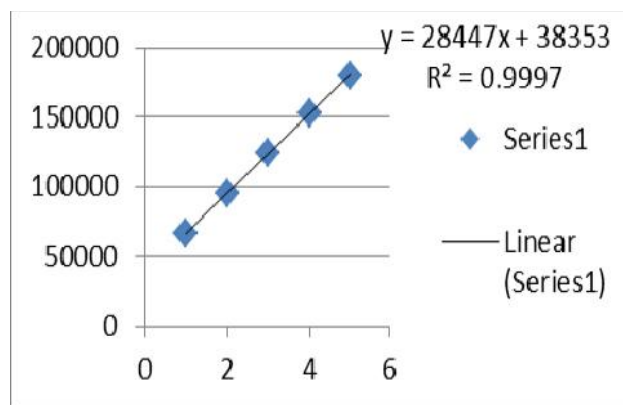
Fig 4: Calibration graph for Salmeterol

Fig 5: Calibration graph for Tiotropium

Table 1: Results of system suitability parameters forTiotropium and Salmeterol

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Tiotropium**	2.5	124505	213642		1.2	4673.4
2	**Salmeterol**	3.9	1308495	154566	6 0	1.3	6090.3

Table 2: Results of method precision for Tiotropium

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of method precession for Salmeterol

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 4: Results of Intermediate precision for Tiotropium

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5: Results of Intermediate precision for Salmeterol

Injection	Area
Injection-1	122487
Injection-2	122626

Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 6: Accuracy (recovery) data for Tiotropium

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	99.84%
150%	1854608	14.4	14.224	98.780%	

Table 7: Accuracy (recovery) data for Salmeterol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	100.51%
150%	177940	14.2	14.45	99.68%	

Table 8: Area of different concentration of Tiotropium

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.997

Table 9: Area of different concentration of Salmeterol

S.No.	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.997

Table 10: Analytical performance parameters of Tiotropium and Salmeterol

Parameters	Tiotropium	Salmeterol
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 11: Results of LOD

Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
Tiotropium	52	152	2.9
Salmeterol	52	156	3

Table 12: Showing system suitability results for Sitagliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

Table 13: Results of LOQ

Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
Tiotropium	52	522	10.03
Salmeterol	52	524	10.1

Table 14: Flow Rate (ml/min) data forTiotropium

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 15: Flow rate (ml/min) data for Salmeterol

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 16: Change in Organic Composition in the Mobile Phase for Tiotropium

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 17: Change in Organic Composition in the Mobile Phase for Salmeterol

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Tiotropium and Salmeterol was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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