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

Simultaneous Estimation of UV Spectroscopy and Method Development and Validation for Tiotropium and Salmeterol by RP-HPLC

Sathukumati Yaswanth Kumar*[1], V. Haribaskar[2]

[1]*PG Research Scholar, Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore, A.P.*
[2]*Professor, Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Nellore, A.P.*

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^H 3.0 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 (4.6x150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 225nm. 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 100-500µg/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 C18 column, Tiotropium and Salmeterol, RP-HPLC.

A R T I C L E I N F O

Corresponding Author
Sathukumati Yaswanth Kumar
PG Research Scholar,
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy, Nellore, A.P.
MS-ID: JPBMAL4422

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C O N T E N T S

1. Introduction

Tiotropium is a muscarinic receptor antagonist, often referred to as an antimuscarinic oranticholinergic agent. Although it does not display selectivity for specific muscarinic receptors, when topically applied it acts mainly

on M3 muscarinic receptors.

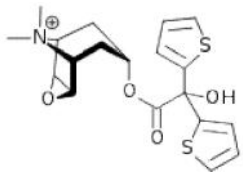

Figure 1 Tiotropium

IUPAC Name: (1α,2β,4β,7β)- 7-[(hydroxidi-2-thienylacetyl) oxy]-9,9-dimethyl- 3-oxa-9-azoniatricyclo [3.3.1.02,4] nonane bromide
Chemical formula : C19H22BrNO4S2
Molecular weight : 472.416 g/mol

Salmeterol's long, lipophilic side chain binds to exosites near beta(2)-receptors in the lungs and on bronchiolar smooth muscle, allowing the active portion of the molecule to remain at the receptor site, continually binding and releasing.

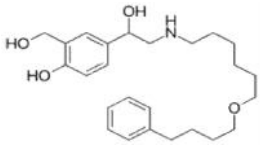

Figure 2 Salmeterol

IUPAC Name: 4-(1-hydroxy-2-{[6-(4-phenylbutoxy) hexyl] amino} ethyl)-2-(hydroxymethyl)phenol
Chemical formula: $C_{25}H_{37}NO_4$
Molecular weight: 415.5656 g/mol
pKa: 2.86

2. Methodology

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Tiotropium	Boehringer
2	Salmeterol	Cipla
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

Optimized chromatographic conditions
Instrument used: Waters HPLC with auto sampler and PAD or detector.
Temperature: Ambient
Column : Inertsil ODS (4.6 x 150mm, 5µm)
Buffer: 6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with ortho phaosparic acid.
pH: 3.0
Mobile phase: 30% buffer 70% Methanol
Flow rate: 1 ml per min
Wavelength: 260 nm
Injection volume : 10 µl
Run time: 10min

Preparation of Phosphate buffer:
Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with orthophosphoric acid.

Preparation of mobile phase:
Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Standard Solution Preparation:
Accurately weigh and transfer 10 mg of Tiotropium and Salmeterol 10mg 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 10 mg 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.

Procedure:
Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Tiotropium and Salmeterol peaks and calculate the %Assay by using the formulae.

3. Results and Discussion

Trial 1:
Mobile phase : Water: Methanol (50:50%v/v)
Column : Xterra C18 (4.6*250mm) 5µm
Flow rate: 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient

Injection Volume : 10 µl

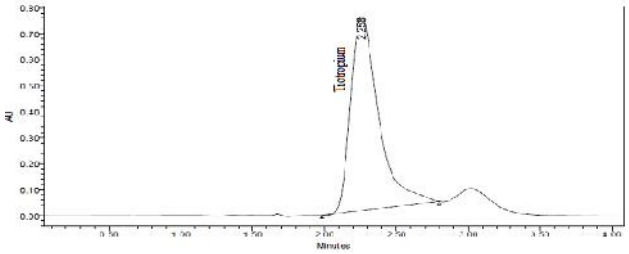

Figure 3: Chromatogram for Tiotropium and Salmeterol

Trial 2:

Mobile phase: Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v)
Column : Make; Xterra C18 (4.6*250mm) 5µm
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume: 10 µl

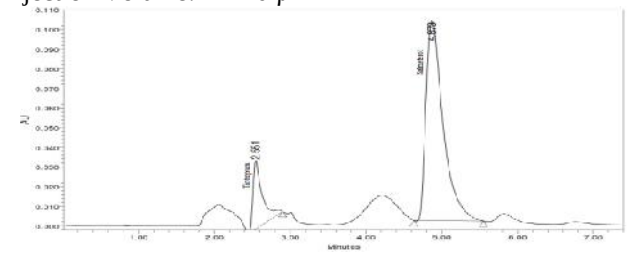

Figure 4: Chromatogram for Tiotropium and Salmeterol

Trail for optimized chromatogram

Mobile phase: Phosphate buffer pH 3.0: Methanol (30:70%v/v)
Column: Inertsil C18 5µm (4.6*250mm)
Flow rate: 0.8 ml/min
Wavelength: 260 nm
Column temp: Ambient
Sample Temp : Ambient
Injection Volume: 10 µl

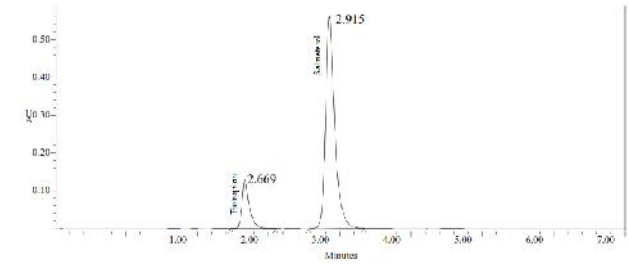

Figure 5: Trial chromatogram for Tiotropium and Salmeterol

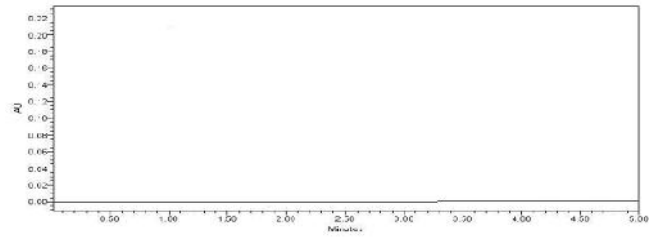

Figure 6: chromatogram for blank

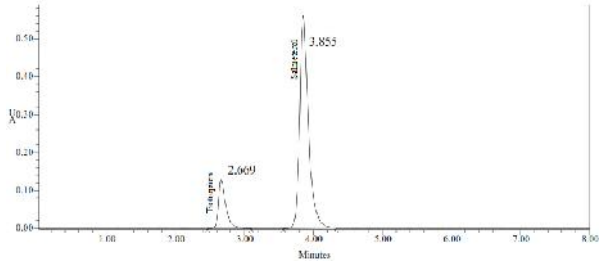

Figure 7: Chromatogram for Tiotropium and Salmeterol sample Preparation

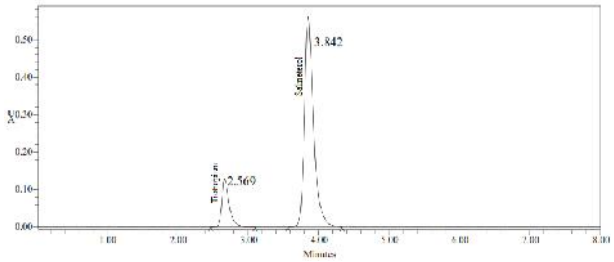

Figure 8: Chromatogram for Tiotropium and Salmeterol Standard Preparation

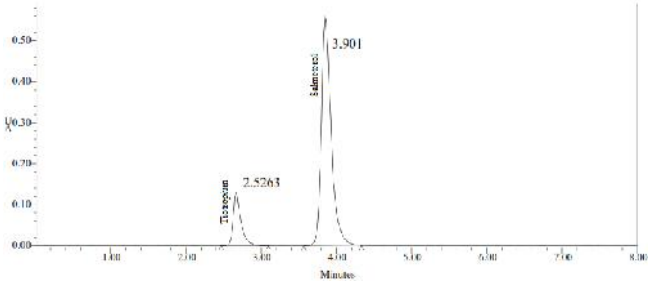

Figure 9: Chromatogram for system suitability

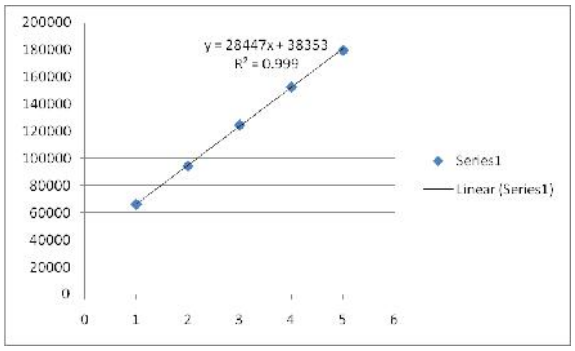

Figure 10: Calibration graph for Tiotropium

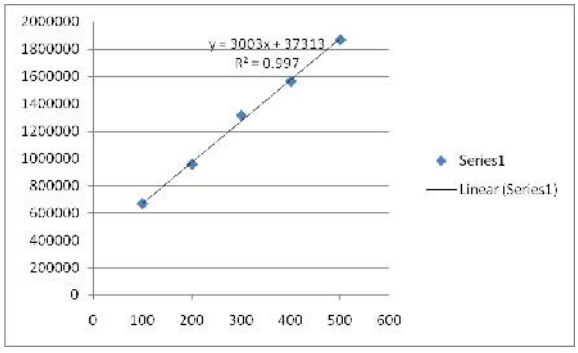

Figure 11: Calibration graph for Salmeterol

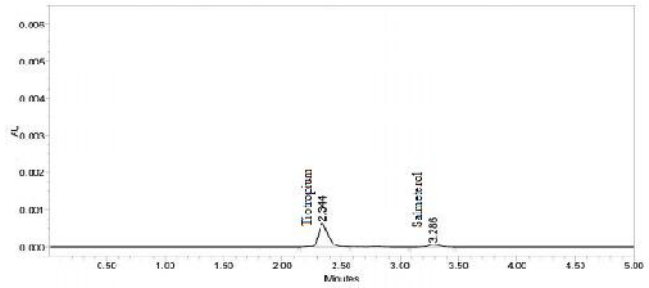

Figure 12: Chromatogram of Tiotropium and Salmeterol showing LOD

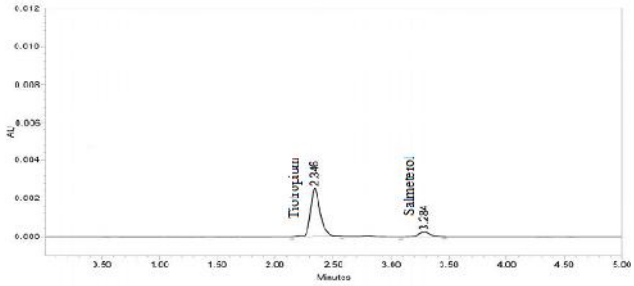

Figure 13: Chromatogram of Tiotropium and Salmeterol showing LOQ

4. Conclusion

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^H 3.0 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 x150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 225 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 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.

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