Research Article

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Stability indicating rp-hplc method for simultaneous estimation of tiotropium bromide and formotetrol fumerate in bulk and pharmaceutical dosage form

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ABSTRACT

Scientific discipline used to study the chemical composition, structure and behavior of matter. The purposes of chemical analysis are together and interpret chemical information. Quality control and support of fundamental and applied research are the principal applications and involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information. Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Tiotropium Bromide and Formoterol Fumarate. The developed method will be validated according to ICH guidelines

Keywords: RP-HPLC, Mobile Phase, Tiotropium bromide Formotetrol fumerate.

INTRODUCTION

The chromatography was discovered by Russian Chemist and botanist Micheal Tswett (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments.“ Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system".

“In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system Chromatography has progressed considerably from Tswett’s time and now includes a number of variations on the basic separation process”. Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase.

Method validation can be defined as per ICH “Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics”.chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

Drug profile

Drug name : Tiotropium bromide

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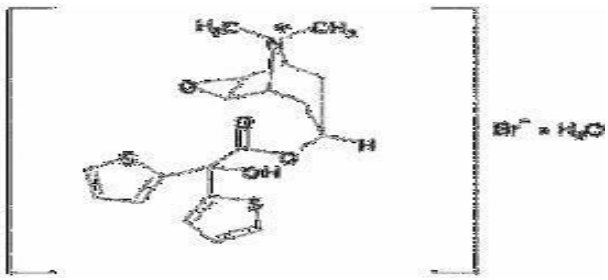


Molecular formula: C19H22NO4S2, Mol. Weight: 392.512, Bioavailability:2-3%, Half Life: 5-6 days, Protein binding: 72%, Category: Anti Asthmatic agents, Bronchodilator agents.

Tiotropium is a long-acting, 24 hour, Anticholinergic bronchodilator used in themanagement of chronic obstructive pulmonary disease (COPD). Tiotropium is a muscarinic receptor antagonist, on topical application it acts mainly on M3 muscarinic receptors located in the airways to produce smooth muscle relaxation, thus producing a bronchodilatory effect.

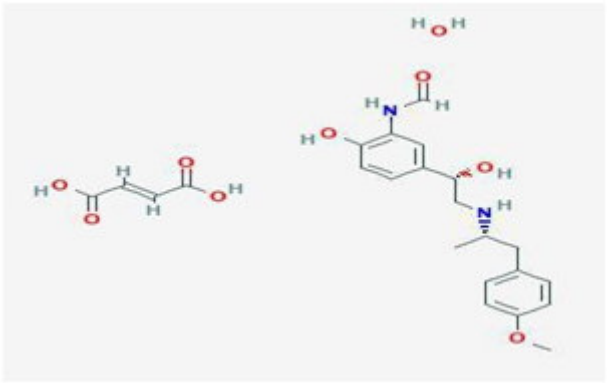
Solubility:It is freely soluble in dimethyl sulphoxide, soluble in methanol, sparingly soluble in water and practically insoluble in methylene chloride. The solubility in aqueous solutions at room temperature is approx. 2.5%, independent of pH.

Melting point : 218-2200C

CAS NO : 186691-13-4

Formoterol fumerate

Description : Formoterol is a long-acting (12 hours) beta2-agonist used in the management of asthma and/or chronic obstructive



pulmonary disease (COPD). Inhaled formoterol works like other beta2-agonists, causing bronchodilatation through relaxation of the smooth muscle in the airway so as to treat the exacerbation of asthma.

Melting point : 138-140°C

CAS NO : 73573-87-2

Molecular formula: $C_{19}H_{24}N_2O_4$

Molecular weight : 344.4049

Bioavailability : 24%

Half-life : 10hrs

Category: Anti-asthmatic agents, peripheral nervous system agents.

Experimental methodology

Instruments and Chemicals used: HPLC, UV/VS spectrophotometer, pH meter, Weighing machine, Pipettes and Burettes, Beakers, Tiotropium Bromide,Formetorol Fumarate, KH_2PO_4 Water and Methanol for HPLC Acetonitrile for HPLC, Ortho phosphoric acid.

SL.No	Instrument	Model
1	HPLC	WATERS, software: 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

SL.No	Chemical	
1	TIOTROPIUM BROMIDE	Mylon
2	FORMETOROL FUMARATE	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

HPLC method development

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 20: 80 v/v respectively.

Wave length selection: 200nm to 400nm.

Optimization of Column: The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1.2 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used:Waters HPLC with auto sampler and PAD or detector.

Temperature: Ambient

Column: kromosil C_{18} Column (250mm x 4.6mm)5µg.

Buffer : 6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted to 3 with ortho phaosparic acid.

pH : 3.0

Mobile phase : 20% buffer: 80% Methanol Flow rate: 1.2 ml per min

Wavelength: 254 nm Injection volume:20µl Run time:10min.

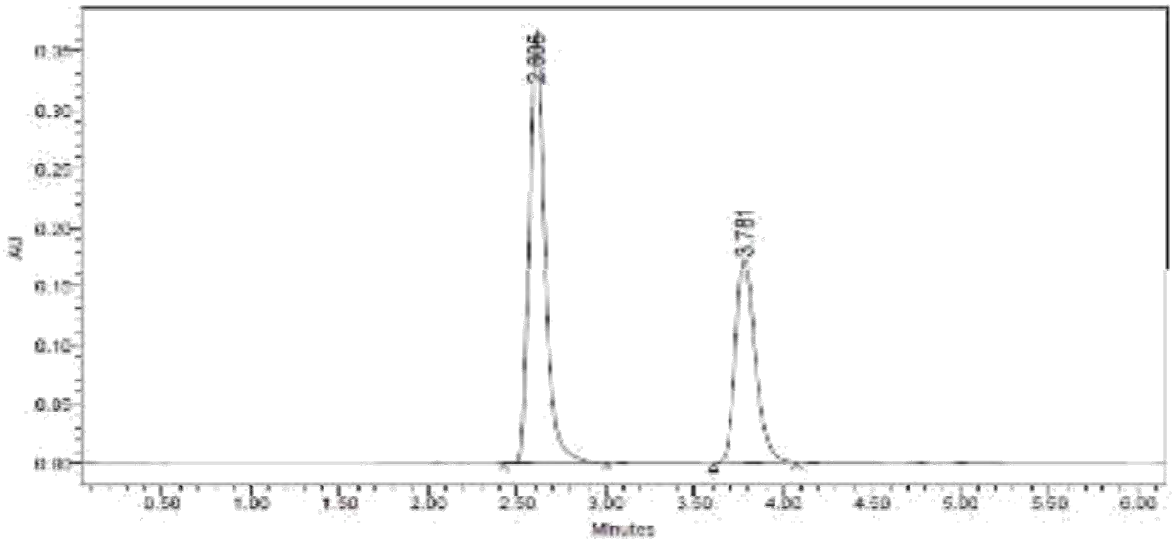
RESULTS AND DISCUSSION

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1.2 ml/min flow.

Optimized chromatogram is obtained by following conditions:

Parameters	Description
Flow rate	1.2 ml min^{-1}
Column	kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer:Methanol P^H 3(20:80 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 3 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min



Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Chromatogram for Tiotropium bromide and Formetorol fumarate

Colum:Inertsil C18 (4.6 x250mm,5□m)

Buffer pH: 3.0.

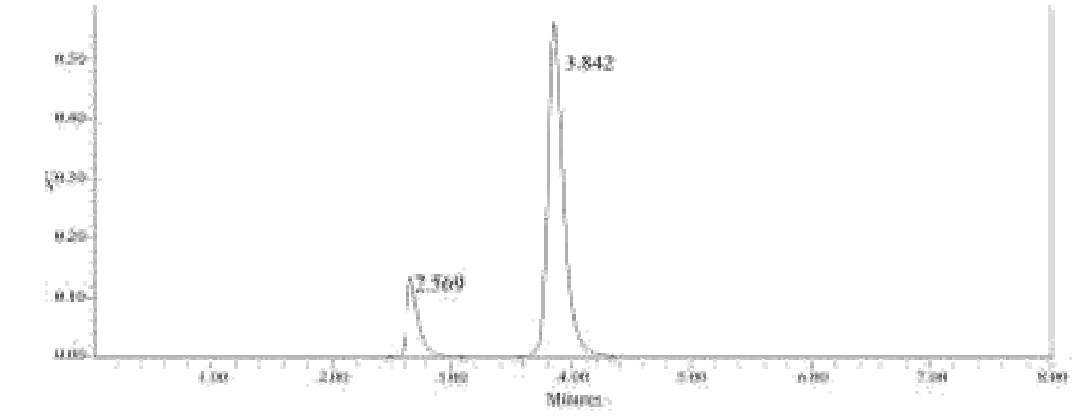
Mobile phase: Buffer: Methanol 30:70 Flow rat 1.0ml per min

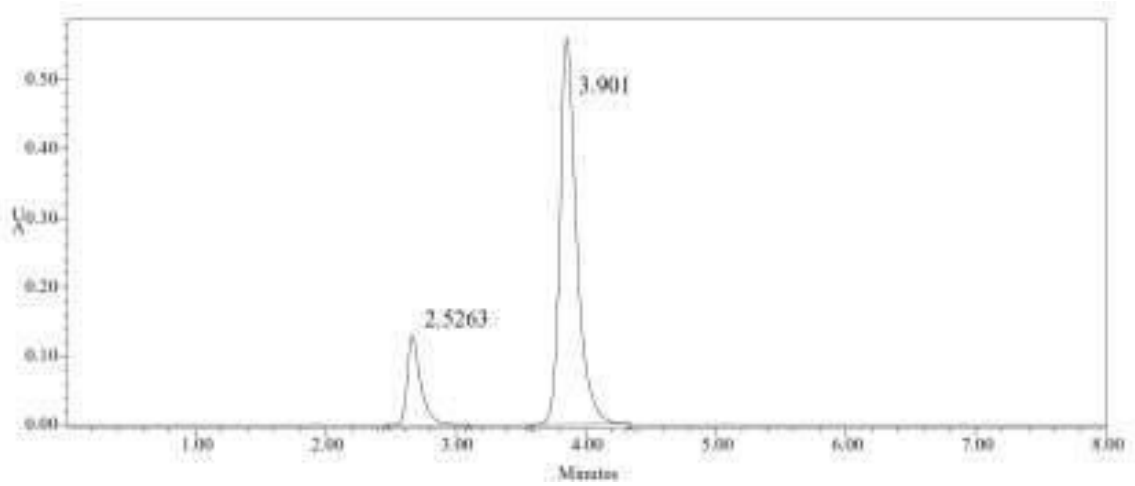
Wavelength : 260 nm

Temperature : ambient.

Run time : 10min.

Chromatogram for Tiotropium bromide and formetrol fumarate Retention time 2.469 and 3.842





chromatogram for system suitability

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

Acceptance criteria: Resolution between two drugs must be not less than 2, Theoretical plates must be not less than 2000, Tailing factor must be not less than 0.9 and not more than 2.

Validation parameters

Precision, Accuracy, Linearity, Intermediate Precession (Ruggedness), Limit of Detection, Limit of Quantification, and Robustness are found within the range of limits. The results obtained on the validation parameters met ICH and USP requirements.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tiotropium bromide and Formetorol fumarate 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 80:20 % v/ v. kromosil C_{18} column C18 (4.6 x 150mm, 5□m) or equivalent chemically bonded to porous silica particles was

used as stationaryphase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographic at a constant flow rate of 1.2 ml/min. the linearity range of Tiotropium bromide and Formetorol fumarate were found to be from 100-500 g/ml of Tiotropium bromide and 1-5g/ml of Formetorol fumarate. 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 bromide and Formetorol Fumarate. 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.

REFERENCES

1. http://www.wikipedia.com/venlafaxine.
2. www.drugbank.ca/drugs/DB00285.
3. Rajasekaran A, Arulkumaran M and Kannanraj S. Spectrophotometric estimation of Venlafaxine with Folin - Ciocalteu reagent. Indian J Pharm Sci 2004; 66(1): 101-03.
4. Pillai S and Singhvi I. Spectrophotometric methods for estimation of Venlafaxine from tablet formulation. Indian Pharmacist 2006; 5(48): 75-76.
5. Armagan O, Kepekci SE, Mugecetin S and Erturk S. Spectrophotometric determination of certain anti-depressant in pharmaceutical preparations. J of AOAC Int 2006; 89(4): 966-71.
6. Vimal D. Shirvi, Vijaya Kumar G. and Channabasavaraj K.P. Second order derivative spectrophotometric estimation of venlafaxine hydrochloride in bulk and pharmaceutical formulations. IJCRGG Vol.2, No.1, pp 572-75.
7. Baldania SL, Bhatt KK, Mehta RS, Shah DA and Gandhi TR. RP-HPLC method of Venlafaxine hydrochloride in tablet dosage form. Indian J of Pharm sci 2008; 70(1): 124-28.
8. Makhija SN and Vavia PR. Stability indicating LC method for the estimation of Venlafaxine in pharmaceutical formulation. J Pharm Biomed Anal 2002; 28(6):1055-9.
9. www.drugs.com/monograph/tramadol-hydrochloride.html.
10. www.pharmamanufacturerindia.com/doctors pdf/Tramadol.pdf.
11. Srinivasan KK, Alex J, Shir waikar AA, Jacob S, Sunil Kumar MR and Prabu SL. Simultaneous derivative spectrophotometric estimation of Aceclofenac and Tramadol with Paracetamol in combination solid dosage forms. IJPER 2007; 69(4): 540-5.
12. Manisha Puranik, Hirudkar A, Wadher SJ and Yeole PG. Development and validation of spectrophotometric methods for simultaneous estimation of Tramadol hydrochloride and Chlorzoxazone in tablet dosage form. Ind J Phar ma Sci 2006; 68(6):737-739.
13. Aysel Kucuk and Yucel Kadioglu. Determination of Tramadol hydrochloride in ampoule dosage forms by using UV spectrophotometric and HPLC-DAD methods in methanol and water media. Sci Direct 2005; 60(2):163-69.
14. Salmeron- Gar cia A, Navas N, Martin A, Roman E, Cabeza J and Capitan-Vallvey LF. Determination of Tramadol, Metamizole, Ropivacaine, and Bupivacaine in analgesic mixture samples by HPLC with DAD detection. J Chrom Sci 2009; 47(3):231-37.
15. Over beck P and Blaschke G. Direct determination of Tramadol glucuronides in human urine by High-Performance Liquid Chromatography with Fluorescence detection. J Chromatogr B Biomed Sci Appl 1999; 732(1):185-92.
16. Qu L, Feng S, Wu Y and Wu Y. HPLC method for determination of Tramadol hydrochloride in human plasma. Sichuan Da Xue Xue Bao Yi Xue Ban 2003; 34(3):574-5.
17. Milan Nobilis, Jii Pastera, Pavel Anzenbacher, Dalibor Svoboda, Jii Kopecky and Frantis ek Perlik. High- Performance Liquid Chromatographic method for determination of Tramadol in human plasma. J Chromatogr B Biomed Sci Appl 1996; 681(1):177- 83.
18. Elsing B and Blaschke G. Achiral and chiral High-Performance Liquid Chromatographic determination of Tramadol and its major metabolites in urine after oral administration of racemic Tramadol. J Chromatogr B Biomed Sci Appl 1993; 612(2,26):223- 30.
19. Gan SH, Ismail R, Wan Adnan WA and Wan Z.
20. J Chromatogr B Analy Tech Biomed Life Sci 2002; 772(1):123-29.