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Simultaneous RP-HPLC Method for the Estimation of Formoterol Fumarate and Tiotropium Bromide in Pharmaceutical Dosage Forms

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A simple, fast, sensitive, accurate and precise reverse phase high performance liquid chromatographic method is developed for the simultaneous determination of formoterol fumarate and tiotropium bromide in rotacaps (powder for inhalation) dosage form. A develosil C_{18}-5, 5 μ column having 250 mm × 4.6 mm i.d. in isocratic mode, with mobile phase containing 20 mM KH_2PO_4:acetonitrile (65:35; pH 3.0) was used. A flow rate of 1.2 mL/min and detection was carried out with 240 nm. The retention times of formoterol fumarate and tiotropium bromide were the 3.6 and 4.7 min, respectively. The method is validated by determining its sensitivity, precision, linearity, accuracy and LOD and LOQ. The proposed method is simple, rapid, sensitive, accurate and precise and so that it can be applied for routine quality control analysis of formoterol fumarate and tiotropium bromide in rotacaps dosage forms.

Key Words: Formoterol fumarate and tiotropium bromide, Waters HPLC with PDA detector, Develosil C_{18}-5, KH_2PO_4 and tiomate rotacaps (powder for inhalation).

INTRODUCTION

Tiotropium bromide[1] is chemically (1α, 2β, 4β, 7β)-7-[(hydroxide-2-thienyl acetyl)-oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.0] nonane bromide. Tiotropium administered as tiotropium bromide is a long-acting anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease (COPD). Tiotropium is a muscarinic receptor antagonist, often referred to as an antimuscarinic or anticholinergic agent. Formoterol fumarate[1] is chemically N-[2-hydroxy-5-[(1R)-1-hydroxy-2-[[(2R)-(4-methoxyphenyl)-1-methylethyl]-amino] ethyl] phenyl] formamide. Formoterol is a long-acting β_2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). It is available in four forms: a dry powder inhaler (DPI), metered dose inhaler (MDI), an oral tablet and as an inhalation solution. Literature survey reveals few chromatographic methods in biological fluids[2-5] and one in formulation[6] and one spectrophotometric method[7] for

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formoterol and two separate HPLC methods for tiotropium[8,9] were reported. No HPLC method reported for combined dosage form. The combination dosage form of tiotropium bromide and formoterol fumarate are available in the market for the treatment for asthma. Present study involves development and validation of RP-HPLC method for the estimation of tiotropium bromide and formoterol fumarate in combination dosage form.

EXPERIMENTAL

A HPLC instrument (Waters HPLC with PDA detector) was used with Empower software. HPLC grade acetonitrile, methanol (Rankem Ltd., Ranbaxy India) and HPLC grade water (Milli-Q water) were used in this investigation. AR grade perchloric acid and KH_2PO_4 obtained from Merck, India. The fixed dose of rotacaps formulation containing tiotropium 18 µg and formoterol fumarate 12 µg (Tiomate®, Lupin Ltd.) were procured from local market.

Mobile phase is prepared by mixing the 350 mL of 20 mM of KH_2PO_4 of pH 3.0 with perchloric acid and 650 mL of acetonitrile and filtered through 0.45 µ membrane filters. Chromatographic separations were achieved by using develosil C_{18} (250 mm × 4.6 mm, 5 µ) analytical column with flow rate of 1.2 mL/min with detection at 240 nm. A rheodyne injector with a 50 µL loop is used for injection of sample preparation. The column and the HPLC system is kept in ambient temperature.

Preparation of standard stock solution: Standard stock solution is prepared with pure formoterol fumarate dihydrate (25 mg) and tiotropium bromide (44 mg) standards in 100 mL volumetric flask dissolved by adding diluent (prepared in a ratio of 70:30 v/v water and methanol) and diluted to 100 mL with diluent.

Preparation of working standard solution: 2 mL of the above standard stock solution is diluted to 20 mL volumetric flask with diluent and made up to volume with diluent to get a concentration of 24 and 36 µg/mL for formoterol fumarate and tiotropium, respectively.

Linearity: Calibration solutions prepared by taking appropriate aliquots of standard stock solution in different 100 mL volumetric flask and diluted to the mark with diluent to obtain concentration about 2, 5, 12, 18, 24, 30 and 37 µg/mL of formoterol fumarate and 4, 7, 18, 27, 36, 46 and 57 µg/mL of tiotropium bromide. Evaluation was performed with PDA at 240 nm and peak areas are recorded and a calibration graph is plotted against peak areas *versus* concentration of formoterol fumarate (Fig. 1A) and tiotropium bromide (Fig. 1B).

Preparation of sample solution: Twenty rotacaps accurately weighed and opened and transferred carefully the total contents equivalent to 240 µg of formoterol fumarate and 360 µg of tiotropium bromide into 10 mL of volumetric flask. About 8 mL diluent was added and sonicated for 10 min with intermediate shaking. Then the volume was finally made up to the mark to obtain the concentration of 24 and 36 µg/mL for the formoterol fumarate and tiotropium, respectively.

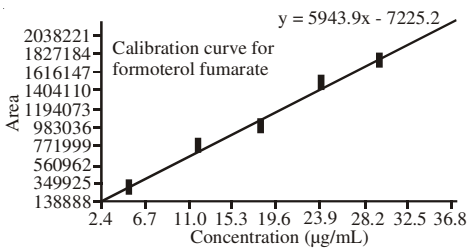

Fig. 1A. Calibration curve of formoterol fumarate by HPLC

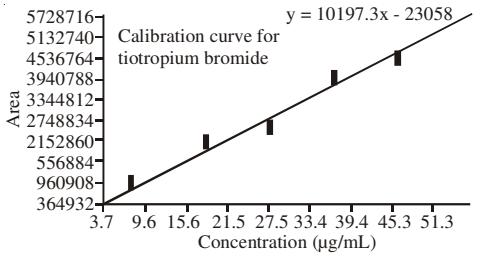

Fig. 1B. Calibration curve of tiotropium bromide by HPLC

Assay: Sample solution was injected (n = 6) into liquid chromatograph. The retention time was found at 3.645 min for formoterol fumarate and 4.714 min for tiotropium bromide (Fig. 2). The amount of drug present in rotacaps was calculated by using calibration curve (Table-1).

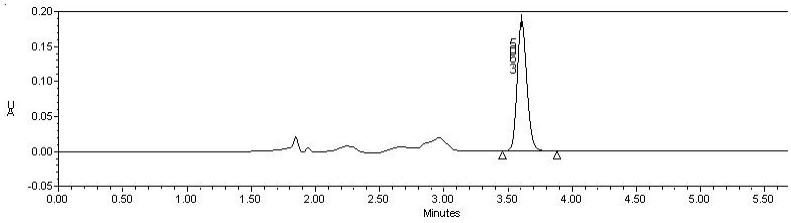

Fig. 2A. Typical chromatogram of formoterol fumarate 24 µg/mL

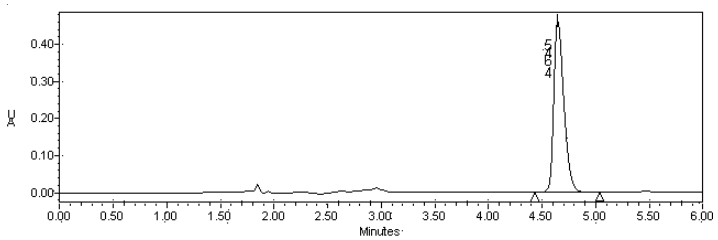

Fig. 2B. Tiotropium bromide 36 µg/mL by HPLC

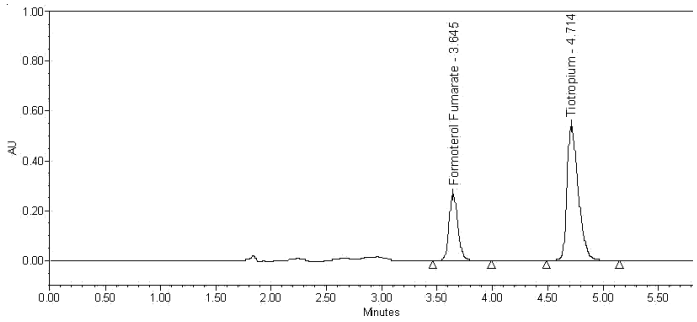

Fig. 2C. Typical chromatogram of formoterol fumarate (24 µg/mL) and tiotropium bromide (36 µg/mL) by HPLC

TABLE-1
ASSAY OF FORMOTEROL FUMARATE AND TIOTROPIUM BROMIDE IN ROTACAPS (POWDER FOR INHALATION)

Labeled amount (μg)		Amount found (μg)	
Formoterol fumarate	Tiotropium bromide	Formoterol fumarate	Tiotropium bromide
12.0	18.0	12.26	17.93
		12.27	17.98
		12.24	17.93
		12.27	18.01
		12.27	17.98
		12.26	17.97
		12.26	17.97
SD		± 0.01	± 0.03
RSD (%)		0.09	0.18

Accuracy/recovery studies: Accuracy was checked for formoterol fumarate and tiotropium bromide in the samples by spiking the known amount of drug substance analyzed by proposed HPLC method. Results of recovery studies are shown in Table-2. The study is made at three different concentration levels.

TABLE-2
RECOVERY STUDIES OF FORMOTEROL FUMARATE AND TIOTROPIUM IN ROTACAPS

Formoterol fumarate		Tiotropium bromide	
Amount added (μg)	Amount found (μg)	Amount added (μg)	Amount found (μg)
10.24	10.21	19.94	19.8
160.24	159.17	270.00	269.71

*:Average of three different concentration levels.

RESULTS AND DISCUSSION

Active pharmaceutical ingredients of formoterol fumarate and tiotropium bromide in bulk form are soluble in water and methanol. Different mobile phase compositions were tried to separate the formoterol fumarate and tiotropium bromide in mixed standard. Different mobile phases like water:acetonitrile (50:50), ammonium acetate:methanol (60:40), ammonium acetate:sodium acetate:methanol (50:0.1:50), KH_2PO_4 with orthophosphoric acid:methanol (60:40) and KH_2PO_4 with perchloric acid:acetonitrile (60:40) and (65:35) are tried in the method development with different columns like C_{18}, phenyl and C_8. Adequate resolution is achieved with KH_2PO_4 with perchloric acid:acetonitrile (65:35, pH 3.0) with C_8 column and this solvent system was found to be most suitable for method development and validation.

Formoterol fumarate shows the maximum absorbance [λ_{max}] at three different wavelengths 214, 240 and 284 nm; while tiotropium bromide has the maximum absorbance [λ_{max}] at 240 nm. The detection was carried out at 240 nm in the proposed method, at this wavelength both the molecules shows maximum absorbance. From

the typical chromatogram of formoterol fumarate and tiotropium bromide shown in Figs. 1A, B and C. It was found that the retention times were 3.645 min for formoterol and 4.714 min for tiotropium bromide.

In the present developed HPLC method, the standard and sample preparation involves simple and rapid extraction procedure and requires less time. A good linear relationship was observed for formoterol fumarate and tiotropium bromide in the concentration range of 2.4-37 and 3.6-57 μg/mL and linear regression equation being y = 59439x - 7225 and y = 101973x - 23058, respectively. Linear regression least square fit, slope (m), intercept (b), standard deviation, residual sum of squares and correlation coefficient data obtained from the measurements are given in Table-3. The assay results and recovery studies shows good extraction and recovery from 50 to 150 % of test concentration.

TABLE-3
LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Drug	Formoterol fumarate	Tiotropium bromide
Concentration range (μg/mL)	2-37	4-57
Slope (m)	59439	101973
Intercept (b)	-7225	-23058
Intercept (%)	-0.5	-0.6000
Residual sum of squares	30205	79494
Correlation coefficient	0.9994	0.9993
RSQ (r^2)	0.9990	0.9990
RSD (%)	0.1000	0.1800

Thus, the developed HPLC method is simple, precise, linear, accurate, sensitive and reproducible and can be easily used for the routine quality control analysis in a short analysis period of time. As per the USP-XXVI system suitability tests were carried out on freshly prepared standard stock solution and summery is given in Table-4. These parameters indicate good sensitivity and selectivity of the method.

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) for formoterol and tiotropium was found to be 0.028 and 0.026 μg/mL and 0.095 and 0.092 μg/mL, respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

TABLE-4
VALIDATION SUMMARY:SYSTEM SUITABILITY AS PER USP

Drug	Formoterol fumarate	Tiotropium bromide
Retention time (min)	3.645	4.714
Theoretical plates (N)	10380	10307
Tailing factor	1.190	1.490
Resolution	–	6.380
Relative retention time	1.000	1.290
Limit of detection (μg/mL)	0.028	0.026
Limit of quantification (μg/mL)	0.095	0.092

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