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SIMULTANEOUS RP-HPLC METHOD FOR DETERMINATION OF IMPURITIES IN FORMOTEROL FUMARATE AND ACLIDINIUM BROMIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, cost effective, sensitive, accurate and precise reverse phase high performance liquid chromatographic method is developed for the simultaneous determination of impurities in formoterol fumarate and aclidinium bromide in Duaklir Genuair (powder for inhalation) dosage form. Ascentis express C8, 15cm x 4.6mm, 2.7 µ i.d in gradient mode, with mobile phase containing 2% trimethylamine pH adjusted to 3 Orthophosphoric acid, acetonitrile (85:15 v/v) was used. A flow rate of 1.0mL/min and detection was carried out with 250 nm. The retention times of formoterol fumarate and aclidinium bromide were the 4.3 and 6.6min, respectively. The method is validated by determining its sensitivity, precision, linearity, accuracy. 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 aclidinium bromide in Duaklir Genuair (powder for inhalation) dosage forms.

Keywords *– RP-HPLC,Formoterol fumarate, Aclidinium bromide, Impurities*

1. INTRODUCTION

Aclidinium bromide (INN) is a long-acting, inhaled muscarinic antagonist approved in the US on July 24, 2012[1] as a maintenance treatment for chronic obstructive pulmonary disease (COPD).[2]Evidence shows that it can improve quality of life and prevent hospitalization in those with COPD.[3] However, it does not appear to affect the risk of death or the frequency steroids are needed.[3] It is unclear if it differs from the similar medication tiotropium or another commonly used medication class of LAMAs.[3]Aclidinium is delivered via a multidosedry powder inhaler, the Genuair inhaler. Formoterol fumarate1 is chemically N-[2-hydroxy-5-[(1R)-1-hydroxy-2-[[(2R)-(4-methoxyphenyl)-1-methylethyl]-amino] ethyl] phenyl]formamide [1-3]. Structure of Aclidinium bromide and its impurity are shown in Fig. 1 & 2.

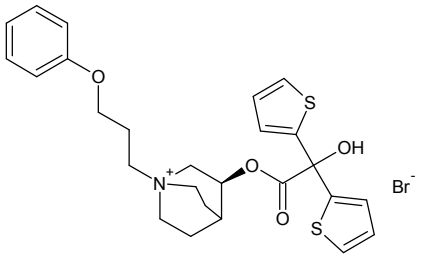

Fig 1. Aclidinium bromide

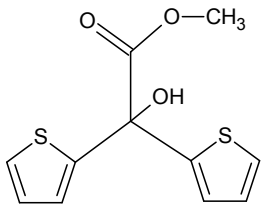

Fig 2. Aclidinium bromide Impurity 1

Formoterol is a long-acting b2-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 assay methods in biologicalfluids[4-7] and one in formulation[8] and one spectrophotometric method[9] for formoterol and two separate HPLC methods for tiotropium[10,11] were reported. No HPLC method reported for combined dosage form. The combination dosage form of Aclidinium bromide and formoterol fumarate are available in the market for the treatment for asthma. Present study involves development and validation of RPHPLC method for the estimation of impurities in Aclidinium bromide and formoterol fumarate in combination dosage form. Structure of Formoterol fumarate and its impurities shown in Fig. 3, 4 & 5.

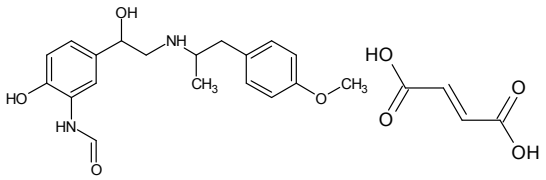

Fig 3: Formoterol fumarate

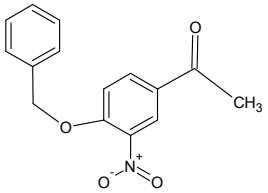

Fig 4: Formoterol fumarate impurity 1

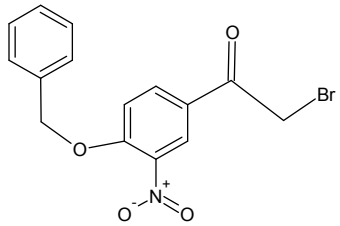

Fig 5: Formoterol fumarate impurity 2

2. EXPERIMENTAL DETAILS

A HPLC instrument (Agilent HPLC with PDA detector) was used with Chromeleon software. HPLC grade acetonitrile (Rankem Ltd., Ranbaxy India) and HPLC grade water (Milli-Q water) were used in this study. AR grade trifluoro-aceticacid and trimethylamine were obtained from Merck, India. The fixed dose of powder for inhalation formulation containing Acidinium bromide 400µg and formoterol fumarate 12 µg (Duaklir Genuair) were procured from local market. Mobile phase A comprised of 2% Triethylamine pH adjusted to 3.0 with trifluoroacetic acid and mobile phase B comprised of acetonitrile. Chromatographic separations were achieved by using Ascentis C8 (150 mm× 4.6 mm, 2.7µm) analytical column with flow rate of 1.0 mL/min with detection at250 nm as per the gradient tabulated in table 1.The injection volume was kept as 5µL. The column temperature was kept at 45ºC.Diluent used for the sample and standard preparation was water: acetonitrile (1:1) v/v

Table 1: Gradient Programme

Time (mins)	Mobile phase A (mL)	Mobile phase B (mL)
0.0	85	15
6.0	85	15
30.0	40	60
35.0	20	80
40.0	20	80
41.0	85	15
55.0	85	15

2.1 Preparation of standard stock solution of Aclidinium Bromide (Stock A): Accurately about 100 mg of Aclidinium Bromide standard was weighed and transferred to 100 mL of volumetric flask. Added about 75 mL of diluent and sonicated to dissolve. The flask was cooled and diluted upto the mark with diluent.

2.2 Preparation of standard stock solution of Aclidinium Bromide Impurity 1(Stock B): Accurately about 100 mg of Aclidinium Bromide Impurity 1 standard was weighed and transferred to 100 mL of volumetric flask. Added about 75 mL of diluent and sonicated to dissolve. The flask was cooled and diluted upto the mark with diluent.

2.3 Preparation of standard stock solution of Formetrol Fumarate(Stock C): Accurately about 10 mg of Formetrol Fumarate standard was weighed and transferred to 100 mL of volumetric flask. Added about 75 mL of diluent and sonicated to dissolve. The flask was cooled and diluted upto the mark with diluent.

2.4 Preparation of standard stock solution of Formetrol Fumarate Impurity 1(Stock D): Accurately about 10 mg of Formetrol Fumarate Impurity 1 standard was weighed and transferred to 100 mL of volumetric flask. Added about 75 mL of diluent and sonicated to dissolve. The flask was cooled and diluted upto the mark with diluent.

2.5 Preparation of standard stock solution of Formetrol Fumarate Impurity 2(Stock E): Accurately about 10 mg of Formetrol Fumarate Impurity 2standard was weighed and transferred to 100 mL of volumetric flask. Added about 75 mL of diluent and sonicated to dissolve. The flask was cooled and diluted upto the mark with diluent.

2.6 Preparation of standard level solution: Pipetted out 4.0 mL each of Stock A, Stock B and 1.2 mL of each of Stock C, Stock D and Stock E in 100 mL of volumetric flask and dilute upto the mark with diluent. (Fig. 6)

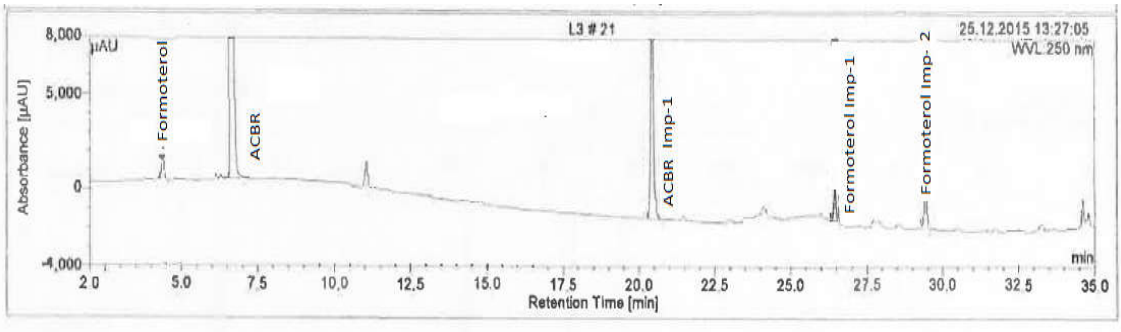

Fig. 6: Standard chromatogram

2.7 Preparation of LOQ level solution: Pipetted out 0.4 mL each of Stock A, Stock B, Stock C, Stock D and Stock E in 100 mL of volumetric flask and dilute upto the mark with diluent. The S/N Ratio for LOQ was observed to be more than 10.

2.8 Preparation of LOD level solution: Pipetted out 3.0 mL LOQ level solution in 10 mL of volumetric flask and dilute upto the mark with diluent. The S/N Ratio for LOD was observed to be more than 3.

2.9 Preparation of Linearity level solutions: The linearity solutions were prepared as per the table below Table-2.

Table 2: Linearity level and Concentration

Levels	Vol of Stock A (mL)	Vol of Stock B (mL)	Vol of Stock C (mL)	Vol of Stock D (mL)	Vol of Stock E (mL)	Diluted to (mL)
Lin 1	0.4	0.4	0.4	0.4	0.4	100.0
Lin 2	2.5	2.5	0.8	0.8	0.8	100.0
Lin 3	4.0	4.0	1.2	1.2	1.2	100.0
Lin 4	5.0	5.0	1.5	1.5	1.5	100.0
Lin 5	6.0	6.0	1.8	1.8	1.8	100.0

The linearity levels for Aclidinium bromide and acilidinium bromide impurity 1 are 4 ppm, 25 ppm, 40 ppm, 50 ppm and 60ppm. The linearity levels for formetrol fumarate, formetrol fumarate impurity 1 and formetrol fumarate impurity 2 are 0.4 ppm, 0.8 ppm, 1.2 ppm, 1.5 ppm and 1.8ppm. The linearity solution were run on to the stabilised chromatographic systems pre settled as per the parameters mentioned above. Peak areas are recorded and a calibration graph is plotted against peak areas *versus* concentration of respective standards (Table 2A,Fig. 1A) for Acildinium bromide, (Table 2 B,Fig. 1B) for aclidinium bromide impurity 1, (Table 2C,Fig. 1C) for formetrol fumarate, (Table 2D,Fig. 1D) for formetrol fumarate impurity 1 and (Table 2E,Fig. 1E) for formetrol fumarate impurity 2.

Levels	Conc (ppm)	Area ACBR		
		Inj 1	Inj 2	Mean
L1	4	24731.5	24582.2	24656.9
L2	25	150402.0	149756.6	150079.3
L3	40	247827.3	246983.7	247405.5
L4	50	310054.1	311243.4	310648.8
L5	60	369987.2	370145.4	370066.3

Table 2A: Linearity Area Aclidinium bromide

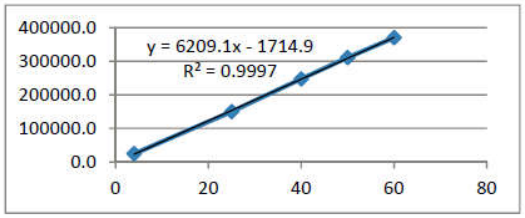

Fig 1A: Linearity graph Acilidinium bromide

Levels	Conc (ppm)	Area ACBR IMP1		
		Inj 1	Inj 2	Mean
L1	4	54879.4	55002.1	54940.8
L2	25	317204.5	319857.8	318531.2
L3	40	537106.7	536895.1	537000.9
L4	50	671648.2	671400.7	671524.5
L5	60	805634.6	805245.0	805439.8

Table 2B: Linearity Area Aclidinium bromide

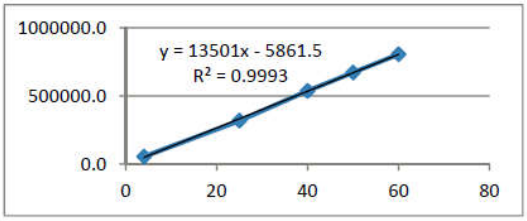

Fig 2B: Linearity graph Acilidinium bromide

Levels	Conc (ppm)	Area Formetrol		
		Inj 1	Inj 2	Mean
L1	0.4	2097.5	2104.5	2101.0
L2	0.8	4371.1	4306.7	4338.9
L3	1.2	6310.3	6315.8	6313.1
L4	1.5	7890.6	7875.9	7883.3
L5	1.8	9489.5	9488.8	9489.2

Table 2C: Linearity Area Formetrol fumarate

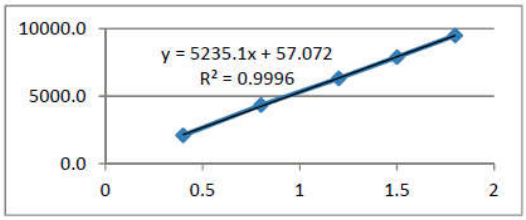

Fig 2C: Linearity graph Formetrol fumarate

Levels	Conc (ppm)	Area FOR IMP 1		
		Inj 1	Inj 2	Mean
L1	0.4	7794.1	7780.9	7787.5
L2	0.8	15997.4	16025.6	16011.5
L3	1.2	23678.1	23664.3	23671.2
L4	1.5	29684.8	29703.7	29694.3
L5	1.8	35474.3	35497.6	35486.0

Table 2D: Linearity Area Formetrol fumarate

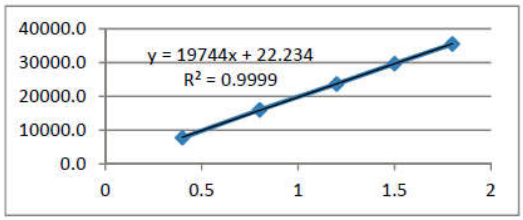

Fig 2D: Linearity graph Formetrol fumarate

Levels	Conc (ppm)	Area FOR IMP 2		
		Inj 1	Inj 2	Mean
L1	0.4	4075.9	4038.2	4057.1
L2	0.8	8295.7	8351.4	8323.6
L3	1.2	12378.1	12454.6	12416.4
L4	1.5	15386.1	15310.2	15348.2
L5	1.8	18505.2	18478.3	18491.8

Table 2E: Linearity Area Formetrol fumarate Impurity 2

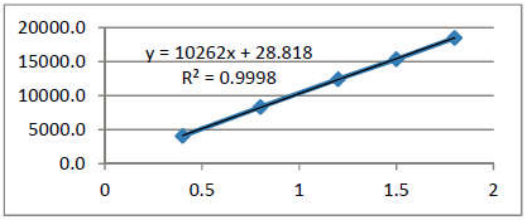

Fig 2E: Linearity graph Formetrol fumarate Impurity 2

2.10 Preparation of Sample solution: Opened dry power inhalation device and weighed and transferred the contents equivalent to 400000 µg of acildinium bromide 12000 µg of formetrol fumarate into 10 mL of volumetric flask. About 7.5 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 40000 and 1200µg/mL for the aclidinium bromide and formoterol fumarate respectively.

2.11 Sample preparation for precision study: Sample solution was prepared in 6 replicates and working level solution was prepared in duplicate from two different stock solutions of respective impurity and API standards. These solutions were injected into stabilised chromatographic system and results for System suitability and precision are tabulated in table 3 and 4 respectively.

System Suitability parameter	Aclidinium Bromide	Aclidinium Bromide Impurity 1	Formetrol Fumarate	Formetrol Fumarate Impurity 1	Formetrol Fumarate Impurity 2
Theoritical Plates	26338	308517	16528	554080	687031
Assymetry factor	1.2	1.0	1.2	1.0	1.0
Similarity Factor	0.98	0.99	1.01	1.01	0.99

Table 3: System Suitability

Sample No.	% Content Formetrol Fumarate Imp 1	% Content Formetrol Fumarate Imp 2	% Content Aclidinium Bromide Imp 1
Sample 1	Not detected	Not detected	Not detected
Sample 2	Not detected	Not detected	Not detected
Sample 3	Not detected	Not detected	Not detected
Sample 4	Not detected	Not detected	Not detected
Sample 5	Not detected	Not detected	Not detected
Sample 6	Not detected	Not detected	Not detected

Table 4: Results for Precision Samples

2.12 Preparation of Accuracy samples: The Accuracy for Formetrol Fumarate Impurity 1, Formetrol Fumarate Impuity 2 and Aclidinium bromide Impurity 1 was performed by spiking the reapecstive volumes of impurity stock solutions into the sample solution. The volume of spiked standard solution and results for accuracy are tabulated below in table 5 and 6A, 6B, 6C respectively.

Level	No of Cannister of Formulation	Vol of Stock B (mL)	Vol of Stock D (mL)	Vol of Stock E (mL)	Final Volume
L1	10	0.4	0.4	0.4	100
L3	10	4.0	1.2	1.2	100
L5	10	6.0	1.8	1.8	100

Table 5: Prpearation of Accuracy Levels

Levels	Conc(ppm)	Area of ACBR IMP 1 as such Sample	Mean Area of ACBR IMP 1 in Respective Levels	Area of ACBR IMP 1 in Accuracy Sample ACBR IMP1		Accuracy ACBR IMP 1	
				Inj 1	Inj 2	Acc Inj 1	Acc Inj 2
L1	4	4884.273	54940.8	59458.5	58984.2	99.3	98.5
L3	40	4884.273	537000.9	540153.1	539136.9	99.7	99.5
L5	60	4884.273	805439.8	803156.3	800287.4	99.1	98.8

Accuracy table 6A: Aclidinium bromide impurity 1

Levels	Conc(ppm)	Area of formoterol IMP 1 as such Sample	Mean Area of FOR IMP 1 in Respective Levels	Area of Formoterol IMP 1 in Accuracy Sample		Accuracy formoterol IMP 1	
				Inj 1	Inj 2	Acc Inj 1	Acc Inj 2
L1	4	0	7787.5	7656.3	7726.7	98.3	99.2
L3	40	0	23671.2	23837.1	23469.5	100.7	99.1
L5	60	0	35486.0	35012.6	35192.4	98.7	99.2

Accuracy table 6B: Formoterol fumarate impurity 1

Levels	Conc(ppm)	Area of formoterol IMP 2 as such Sample	Mean Area of FOR IMP 2 in Respective Levels	Area of Formoterol IMP 2 in Accuracy Sample		Accuracy formoterol IMP 2	
				Inj 1	Inj 2	Acc Inj 1	Acc Inj 2
L1	4	0	4057.1	4119.3	4104.8	101.5	101.2
L3	40	0	12416.4	12241.1	12305.2	98.6	99.1
L5	60	0	18491.8	18252.9	18294.5	98.7	98.9

Accuracy table 6C: Formoterol fumarate impurity 2

3. CONCLUSION

From above results for LOD, LOQ, System Suitability, Precision and Accuracy it is demonstated that the developed method stands validated and can be used for routine quality control of the Duaklir Genuair formulation for content of Formetrol fumarate impurity 1, Formetrol fumarate impurity 2 and aclidinium bromide impurity 1.

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