EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 10
298-307
Year: 2019

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FORMOTEROL AND GLYCOPYRROLATEIN PURE AND DOSAGE FORMS BY USING RP-HPLC

R. Mallikarjuna*, L. Ramachandra Reddy, D. Dhachinamoorthi

Department of Pharm. Analysis and Quality Assurance, QIS College of Pharmacy, Ongole-523272.

***Corresponding Author: R. Mallikarjuna**

Department of Pharm. Analysis and Quality Assurance, QIS College of Pharmacy, Ongole-523272.

Article Received on 17/07/2019 **Article Revised on 06/08/2019** **Article Accepted on 27/08/2019**

ABSTRACT

A Simple, precise, accurate method was developed by simultaneous estimation of Formoterol and Glycopyrrolate in dosage form, an easy, accurate, linear technique was optimized. Chromatogram runs through BDS C8 150 x 4.6 mm, 5µm.Mobile phase comprising Buffer 0.1 % OPA: Acetonitrile taken in the 50:50 ratios was pumped through the column at a flow rate of 0.8 ml / min. The selected optimized wavelength was 223 nm.Formoterol and glycopyrrolate retention time were obtained to be 2.202min and 2.962. The %of RSD of Formoterol and Glycopyrrolate was 0.4 and 0.4 respectively. The % recovery for Formoterol and Glycopyrrolate was 99.10 % and 99.40 %alone. LOD, LOQ values acquired from Formoterol and Glycopyrrolate regression equations were 0.03, 0.10 and 0.35, 1.06 in that order. Formoterol regression equation is y = 83345x + 10897, y = 24261x + 59983 glycopyrrolate. Retention times have been reduced and runtime has been reduced, so the technique created has been easy and economical that can be used in periodic quality control tests in industries.

KEY WORDS: Formoterol, Glycopyrrolate, RP-HPLC.

INTRODUCTION

A Drugs' nature assumes imperative role in ensuring drug's well-being and viability. Confirmation of quality and control of pharmaceutical and synthetic definitions is essential to ensure that sheltered and strong drug plans are accessible to buyers. Hence forth Analysis of unadulterated drug substances and their pharmaceutical measurement frames has a crucial role to play in assessing patient suitability for use.

CHROMATOGRAPHY

Chromatography(Chromo means 'color' and graph in methods to 'write ') is the aggregate term for an accumulation of blend partition research facility techniques.

Different types of chromatographic techniques were summarized in

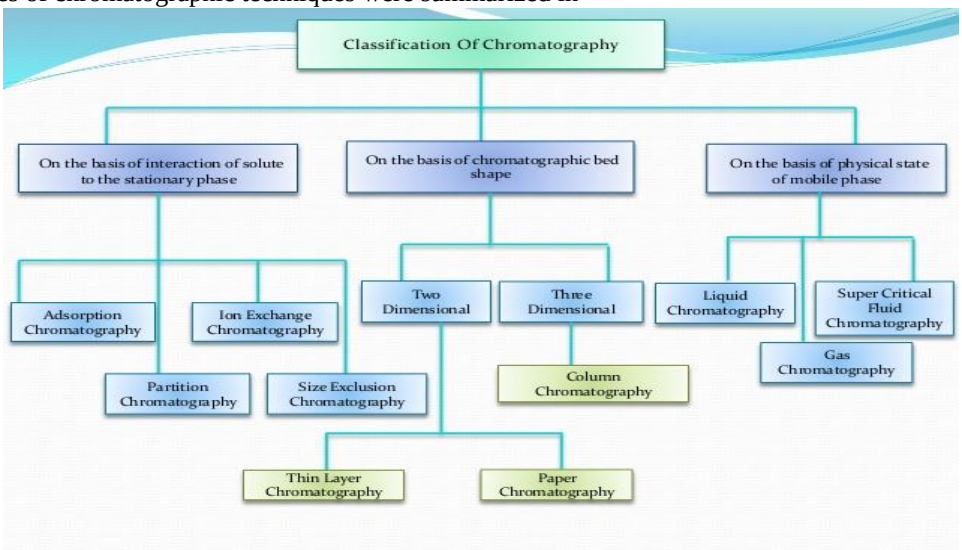

Fig: Classification of Chromatography.

1.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is a procedure in analytical chemistry utilized to isolate, distinguish, and evaluate every element in a blend.

CLASSIFICATION OF HPLC[1-5]

	Normal Phase	RP-HPLC
Mobile phase	**Non polar**	**polar**
Stationary phase	**polar**	**Non polar**

Adsorption chromatography	partition chromatography
based on adsorption	separation on the stationary phase occurs by partition
Solid -liquid phases	Liquid- Liquid phases

DRUG PROFILE[5-6]

GLYCOPYRROLATE PROPIONATE

It is an anti-cholinergic synthetic agent with a quaternary ammonium framework. A competitive muscular antagonist used as an antispasmodic in certain gastrointestinal disorders and with certain anesthetics to decrease salivation.

Structure

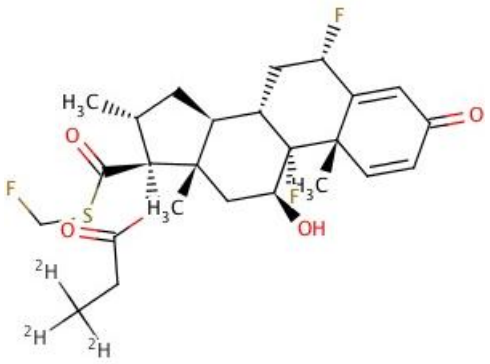

Fig: Structure Of Glycopyrrolate Propionate.

FORMOTEROL

Formoterol is a long-acting beta2 agonist (12 hours) used in asthma management and/or chronic obstructive pulmonary disease (COPD). Inhaled formoterol works like other beta2-agonists, which causes broncho dilation to treat exacerbation of asthma by relaxing the smooth muscle in the airway.

Structure

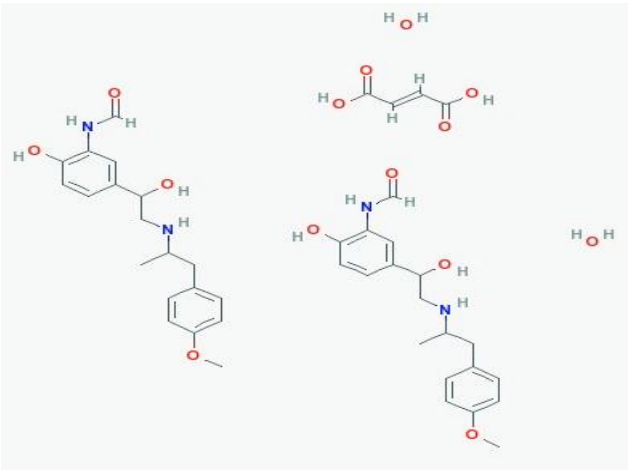

Fig: Structure Of Formoterol.

MATERIALS AND METHODS

Materials

Formoterol and Glycopyrrolate pure drugs (API), Combination Formoterol and Glycopyrrolate tablets (**BEVESPI AEROSPHERE**) (**Formoterol 4.8 mcg / Glycopyrrolate-9 mcg**)), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments

Instrument	Manufacturer
Electronics Balance	Denver
p^H meter	BVK enterprises, India
Ultrasonicator	Lab man India
HPLC	WatersHPLC 2695 SYSTEM
UV-VIS spectrophotometer	PG Instruments T60

Methods

Preparation of Standard stock solutions: Exactly weighed 1.5mg of Formoterol, 25mg of Glycopyrrolate and moved to individual volumetric flasks of 25ml and added 3/4th of diluents to the flask and sonicated for 10 minutes. Flask was composed of diluents and marked as a standard stock solution. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample stock solutions: Combimation of 1 RotacapInhaler(6&100 µg each) transferred into a 100ml volumetric flask, 25ml of Acetonitrile was added, and sonicated for 25 min, further the volume was made up with diluent, andIt was centrifuged for 20 min.Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF).1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.

Preparation of buffer

Buffer0.1%OPA: Accurately 1ml of OPA in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Formoterol (6ppm) and Glycopyrrolate (100ppm) and the solutions were injected six times and the Variables like peak tailing, resolution and USP plate count were resolute. The % RSD for the area of six standard injections results should not be > 2.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Precision

Six individual preparations of the same concentration as the sample preparation are prepared from the formulation

Linearity

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm(Formoterol)	Concentration in ppm(Glycopyrrolate)	%Linearity Level
1	0.25	10	1.5	25	25
2	0.5	10	3	50	50
3	0.75	10	4.5	75	75
4	1	10	6	100	100
5	0.25	10	7.5	125	125
6	0.50	10	9	150	150

Accuracy

This is achieved by spiking the standard optimized concentration to three levels of sample preparing.

Accuracy Level	Add concentration (from sample stock solution)	Standard concentration (from standard stock solution)
50%	0.5ml	1ml
100%	1ml	1ml
150%	1.5ml	1ml

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.

Robustness

Small deliberate changes are made in methods such as fl ow rate, mobile phase ratio, and temperature, but the resu lt has not been recognized and are within the range of IC H Guide lines.

LOD and LOQ sample Preparation

Linearity calibration method LOD and LOQ samples were prepared.

Degradation studies

Oxidation

Add 1 ml of 20 % hydrogen peroxide (H2O2) to 1 ml of sam ple stock solution. The alternatives were held at 60^0c for 30 m inute.

The resulting solution was diluted to achieve a solution of 6µ g / ml&100µg / ml.

AcidDegradationStudies

To 1 ml of sample stock ssolution add 1 ml of 2N Hydrochloricacidand refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 6µg/ml & 100µg/ml solution.

AlkaliDegradationStudies

To 1 ml of sample stock solution add 1 ml of 2N sodium hydroxideand refluxed for 30mins at 60^0c. The result ant solution was diluted to obtain 6µg/ml&100µg/ml solution.

Dry Heat Degradation Studies

The standard drug solution was placedinovenat105°C for1h after that theresultant solution was diluted to 6µg/ml&100µg/ml solution**.**

Photo Stability studies

Sample stock solution that exposes to UV light by upholding the beaker in the UV chamber for 1days or 200-Watt hours / m2 in the UV stabilization chamber. The resulting solution was diluted after 1 day to achieve 6µg / ml&100µg / ml alternatives.

Neutral Degradation Studies

Sample stock solution refluxingthedruginwaterfor1hrs atatemperature of 60ºC. The resultant solution was diluted to 6µg/ml&100µg/ml solution**.**

RESULTS AND DISCUSSION

Determination of λ_{max} and Optimized wavelength

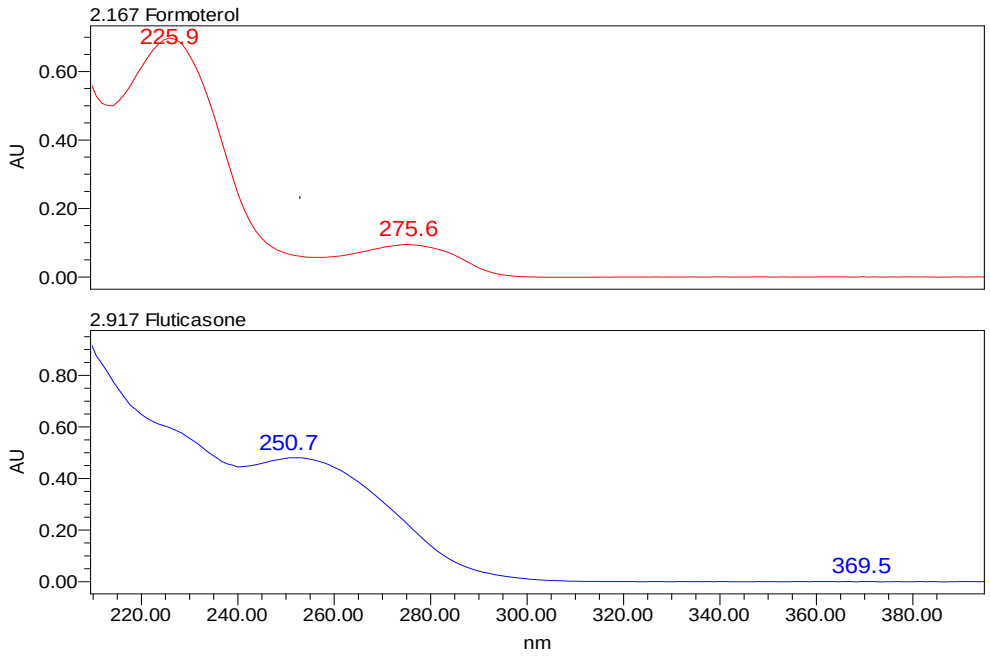

Fig 6.1 Individual UV spectra of Formoterol and Glycopyrrolate.

λ_{max} of Formoterol andGlycopyrrolate was 225nm and 250.7nm singly.
Overlay spectra gave the optimized wavelength for these two drugs.

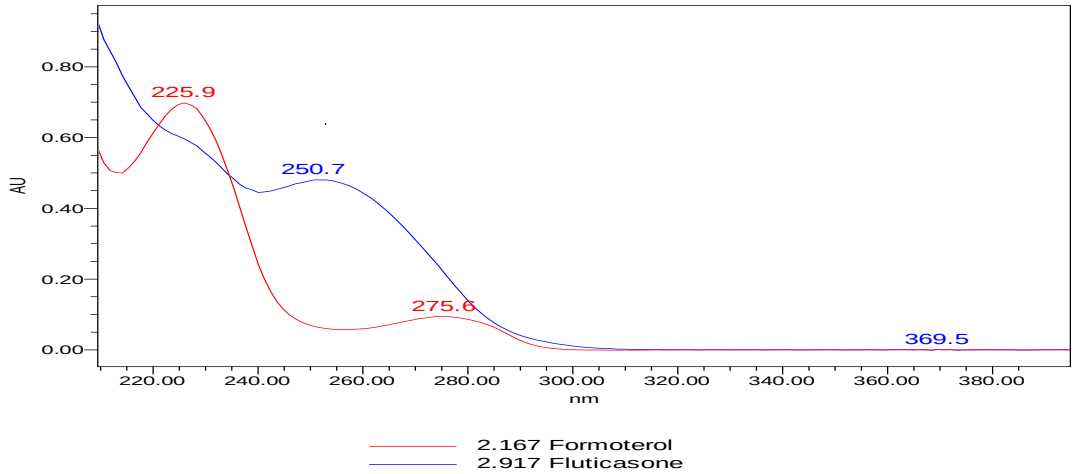

Fig 6.2 Overlay UV spectra of Formoterol and Glycopyrrolate.

Optimized wavelength selected was 223nm.

Optimized method
Chromatographic conditions
Mobile phase **:** 50% 0.1% OPA: 50% Acetonitrile
Flow rate **:** 0.8ml/min
Column **:** BDS C8 (4.6 x 150mm, 5µm)
Column temperature **:** 30°C
Injection volume **:** 10µL

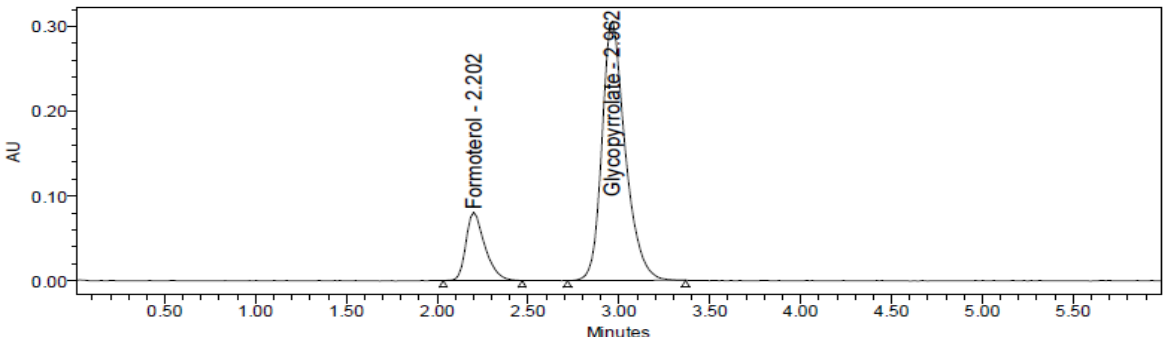

Fig. Optimized Chromatogram.

Table: system suitability of Formoterol and Glycopyrrolate

S no	Formoterol			Glycopyrrolate			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.161	3414	1.22	2.907	3779	1.11	4.4
2	2.169	3188	1.23	2.924	3714	1.10	4.3
3	2.182	2337	1.33	2.936	2880	1.25	3.6
4	2.183	2387	1.35	2.938	2796	1.26	3.6
5	2.187	2158	1.38	2.946	2696	1.25	3.6
6	2.202	2119	1.36	2.962	2644	1.28	3.5

Validation
Specificity

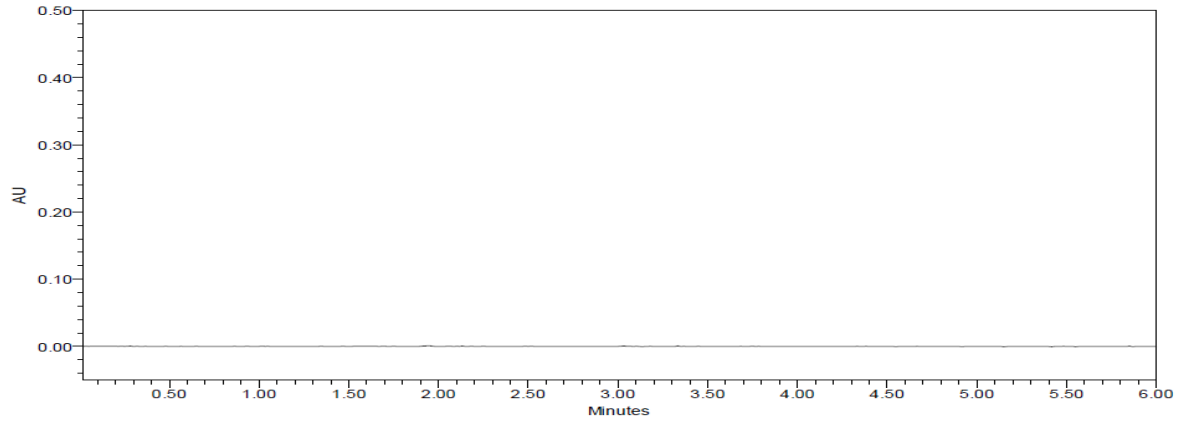

Figure No. 6.10.Chromatogramof blank.

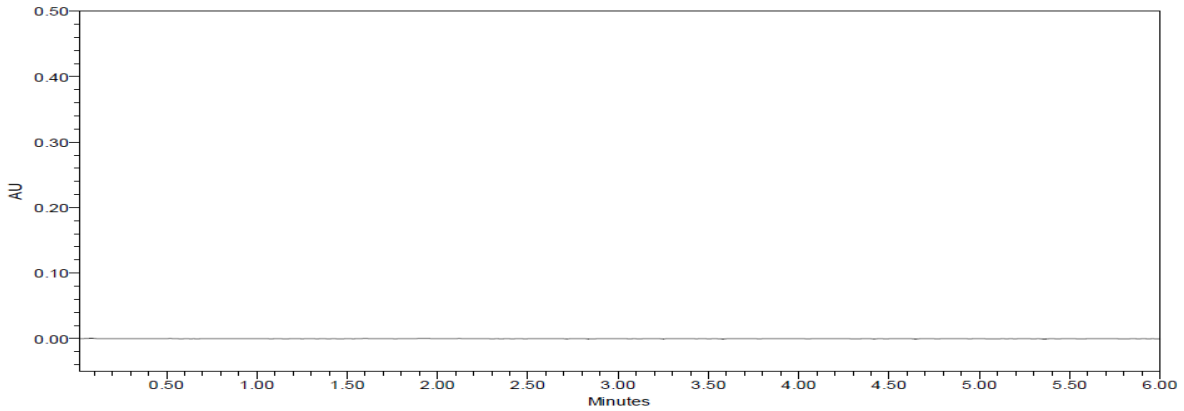

Figure No. 6.11Chromatogramof placebo.

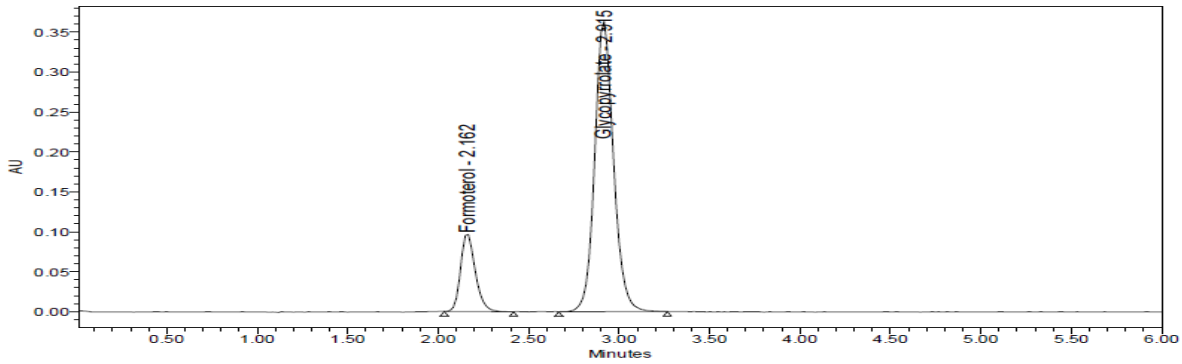

Fig 6.12 Typical Chromatogram.

Linearity

Table 6.2 Linearity table forFormoterol and Glycopyrrolate.

Formoterol		Glycopyrrolate	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
1.5	146747	25	708980
3	264901	50	1306214
4.5	385670	75	1891231
6	506387	100	2463403
7.5	641313	125	3104995
9	756622	150	3679424

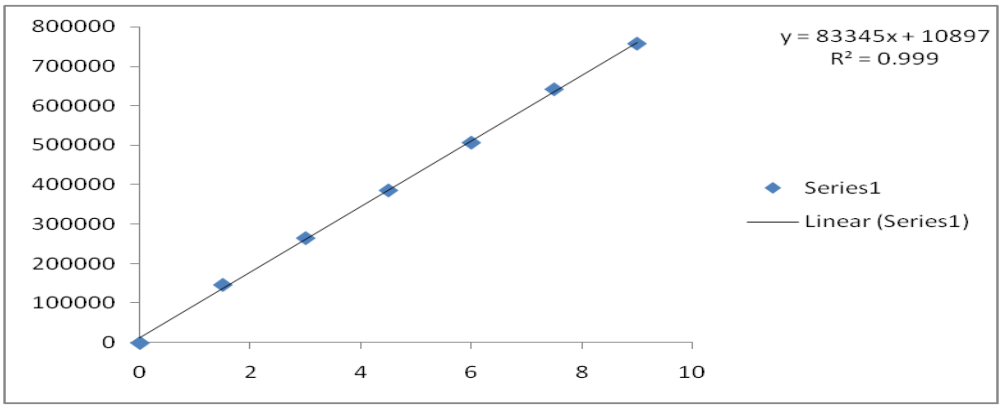

Fig No. 6.13 Calibration curve of Formoterol.

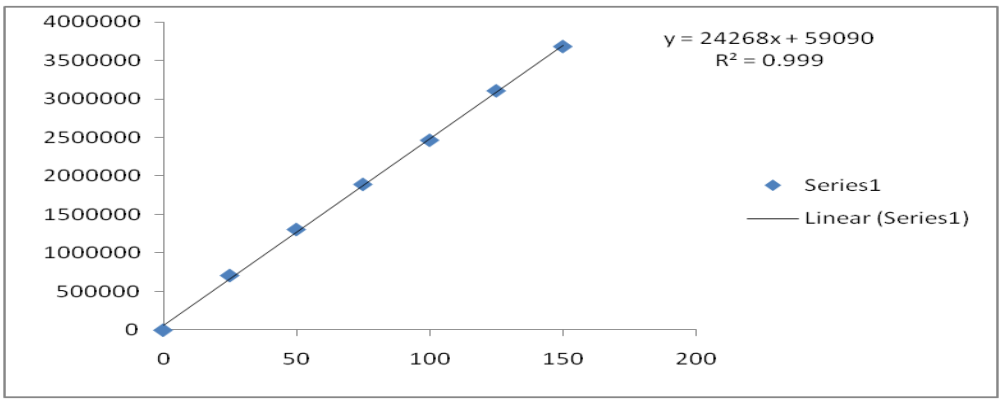

Fig No. 6.14 Calibration curve of Glycopyrrolate.

Precision

System Precision

Table 6.3 System precision table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol	Area of Glycopyrrolate
1.	504172	2420728
2.	505762	2434175
3.	500807	2418171
4.	500690	2400879
5.	507112	2422375
6.	509239	2430032
Mean	504630	2421060
S.D	3436.1	11575
%RSD	0.7	0.5

Repeatability

Table 6.4 Repeatability table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol	Area of Glycopyrrolate
1.	505200	2419181
2.	502334	2402490
3.	502117	2420882
4.	504411	2428161
5.	503153	2401371
6.	507843	2414533
Mean	504176	2414436
S.D	2155.4	10638.2
%RSD	0.4	0.4

Intermediate precision (Day_Day Precision)

Table 6.5 Intermediate precision table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol	Area ofGlycopyrrolate
1.	504458	2424512
2.	505540	2422230
3.	502577	2451595
4.	503808	2422284
5.	502598	2414662
6.	506029	2427654
Mean	504168	2427156
S.D	1453.0	12717.0
%RSD	0.3	0.5

Accuracy

Table 6.6 Accuracy table of Formoterol.

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	3	2.96	98.62	99.10%
	3	2.95	98.32	
	3	2.97	99.11	
100%	6	5.97	99.54	
	6	6.07	101.09	
	6	5.91	98.44	
150%	9	8.86	98.47	
	9	9.00	99.97	
	9	8.85	98.33	

Table 6.7 Accuracy table of Glycopyrrolate.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.56	99.12	99.40%
	50	49.81	99.63	
	50	49.48	98.96	
100%	100	100.38	100.38	
	100	100.34	100.34	
	100	100.54	100.54	
150%	150	147.76	98.51	
	150	147.28	98.19	
	150	148.48	98.99	

Sensitivity

Table Sensitivity table of Formoterol and Glycopyrrolate.

Molecule	LOD	LOQ
Formoterol	0.03	0.10
Glycopyrrolate	0.35	1.06

Robustness

Table Robustness data forFormoterol and Glycopyrrolate.

S.no	Condition	%RSD of Formoterol	%RSD of Glycopyrrolate
1	Flow rate (-) 0.7ml/min	1.2	1.6
2	Flow rate (+) 0.9ml/min	1.5	1.1
3	Mobile phase (-) 55B:45A	0.6	0.4
4	Mobile phase (+) 45B:55A	1.1	0.5
5	Temperature (-) 25°C	1.4	1.7
6	Temperature (+) 35°C	0.7	0.1

Table 6.10Assay Data of Formoterol.

S.no	Standard Area	Sample area	% Assay
1	504172	505200	100.01
2	505762	502334	99.45
3	500807	502117	99.40
4	500690	504411	99.86
5	507112	503153	99.61
6	509239	507843	100.54
Avg	504630	504176	99.81
Stdev	3436.1	2155.4	0.43
%RSD	0.7	0.4	0.43

Table 6.11 Assay Data of Glycopyrrolate

S.no	Standard Area	Sample area	% Assay
1	2420728	2419181	99.82
2	2434175	2402490	99.13
3	2418171	2420882	99.89
4	2400879	2428161	100.19
5	2422375	2401371	99.09
6	2430032	2414533	99.63
Avg	2421060	2414436	99.6
Stdev	11575	10638.2	0.4390
%RSD	0.5	0.4	0.4

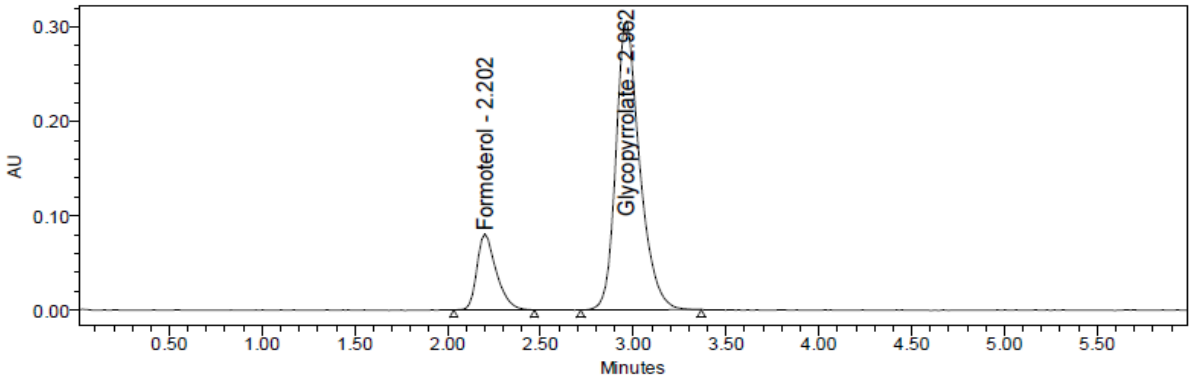

Fig. Chromatogram of working standard solution.

Table 6.12 Degradation Data of Formoterol.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.51	0.306	0.412
2	Alkali	2.72	0.305	0.423
3	Oxidation	1.91	0.354	0.468
4	Thermal	0.71	0.351	0.409
5	UV	0.50	0.284	0.392
6	Water	0.98	0.383	0.431

Table 6.13 Degradation Data of Glycopyrrolate.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.60	0.151	0.369
2	Alkali	2.96	0.140	0.368
3	Oxidation	1.77	0.240	0.395
4	Thermal	0.68	0.152	0.374
5	UV	0.80	0.129	0.364
6	Water	0.84	0.146	0.372

SUMMARY AND CONCLUSION

Parameters		Formoterol	Glycopyrrolate	LIMIT
Linearity Range(µg/ml)		1.5-9 µg/ml	25-150 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		83345	24261	
Intercept(c)		10897	59983	
Regression equation (Y=mx+c)		y = 83345x + 10897	y = 24261x + 59983	
Assay (% mean assay)		99.81%	99.94%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.7	0.5	NMT 2.0%
Method precision %RSD		04	0.4	NMT 2.0%
Accuracy%recovery		99.10%	99.40%	98-102%
LOD		0.03	0.10	NMT 3
LOQ		0.35	1.06	NMT 10
Robustness	**FM**	1.2	1.6	%RSD NMT 2.0
	FP	1.5	1.1	
	MM	0.6	0.4	
	MP	1.1	0.5	
	TM	1.4	1.7	
	TP	0.7	0.1	

CONCLUSION

For the concurrent estimation of Formoterol and Glycopyrrolate in dosage form, an easy, accurate and linearpractice was created. It contains a single sample preparation and direct injection step operation. The preparation of samples and the analytical procedure run times are brief so that this practice can be used effectively for routine quality control valuation. The technique created was easy and low-priced, which can be accepted in industry in periodic quality control tests.

REFERANCES

1. Swartz M., Ultra Performance Liquid Chromatography. LCGC. 2005; 23(1): 46–53.
2. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA,Geneva, (1996).
3. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2010; 2: 1657-1658.
4. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 1408-1409 2 (2011).
5. https://www.drugbank.ca/drugs/DB00986
6. https://www.drugbank.ca/drugs/DB00983
7. **N. Md. Akram* and M. Umamahesh**a. New validated RP-HPLC method for the determination of glycopyrrolate and formeterol fumarate in its bulk and pharmaceutical dosage forms. WJPPS. 2017; 6(6): 903-917.
8. K. Srinivasu, j. Venkateswara Rao*, N. Appala Raju and K. Mukkanti.Simultaneous RP-HPLC method for the estimation of formoterol fumarate and tiotropium bromide in pharmaceutical dosage forms. AJC. 2010; 22(5): 3943-3948**.**
9. Kumaraswamy.Gandla,Parameshwar.Pabba,Ganesh. Akula. RP-HPLC method development and validation for estimation of glycopyrrolate in bulk and tablet dosage forms. AJPCR. 2011; 4(4): 0974-244.
10. Mohd. Gousuddin, S. Appala Raju,Sultanuddin &Shobha manjunath. Development and validation of spectrophotometric methods for estimation of formoterol bulk drug and its pharmaceutical dosage forms. IJPPS. 2011; 3(3): 0975-1491.
11. Hanan A.Mereysally, S.el-Mosallamynagiba, Y.Hassanbadr &A.el-Zeany. Validated chromatographic methods for the simultaneous determination of mometasone furoate and formoterol fumarate dihydrate in a combined dosage form. 2016.
12. Kusum malik, Davinder kumar, Vivek tomar, Satish. Kaskhedikar, Love soni. Simultaneous Quantitative Determination of Formoterol Fumarate and Fluticasone Propionate by Validated Reversed-Phase HPLC Method in Metered dose inhaler.PRL. Der Pharmacia Sinica, 2011; 2(6): 77-84.
13. Priyank P. Raval*, Dr. J. B. Dave, Mittal P. Joshi, and Bhargav K. Solanki Estimation of Fluticasone propionate in nasal preparation and in combination with formoterol fumarate in inhalation preparation. WJPPS. 3(6): 1148-1160.
14. N.M. Gowekar · S.J. Wadher Simultaneous estimation of formoterol fumarate dihydrate and Fluticasone in dry powder inhalation formulation by RP-HPLC. Research gate. 2016; 9: 164-170.