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DEVELOPMENT OF VALIDATED ANALYTICAL METHOD FOR FORMOTEROL IN PURE AND DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A Simple, precise, accurate method was developed by simultaneous estimation of Formoterol 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 45:55 ratios was pumped through the column at a flow rate of 0.8 ml / min. The selected optimized wavelength was 223 nm. Formoterol retention time were obtained to be 2.2 min the %of RSD of Formoterol was 0.4, The % recovery for Formoterol was 99.10 % alone. LOD, LOQ values acquired from Formoterol were 0.03, 0.10. Formoterol regression equation is $y = 83345x + 10897$ and passes the regression coefficient 0.999. Retention time, runtime was reduced, so the technique created has been easy and economical that can be used in periodic quality control tests in industries.

KEYWORDS: Formoterol, Acetonitrile, O-Phosphoric acid, 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.Henceforth Analysis of unadulterated drug substances and their pharmaceutical measurement frames has a crucial role to play in assessing patient suitability for use.

Formoterol, also known as eformoterol, is a long-acting β2 agonist (LABA) used as a bronchodilator in the management of asthma and COPD. Formoterol has an extended duration of action (up to 12 h) compared to short-acting β2 agonists such as salbutamol (albuterol), which are effective for 4 h to 6 h. LABAs such as formoterol are used as "symptom controllers" to supplement prophylactic corticosteroid therapy. A "reliever" short-acting β2 agonist (e.g., salbutamol) is still required, since LABAs are not recommended for the treatment of acute asthma.
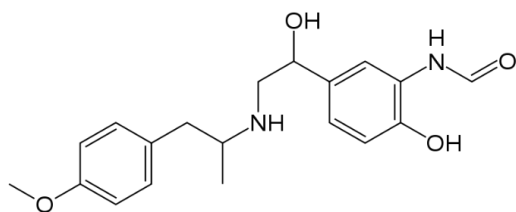

Fig: 1 Structure Of Formoterol.

MATERIALS AND METHODS
Materials

Formoterol API was procured from the sipra labs and, Formoterol dosage form capsule (Perforomist 6 mcg) was purchased from the local Market. The chemicals acetonitrile, water, phosphate buffer, Methanol, ortho phosphoric acid were AR Grade and purchased from the Merck and instruments like Electronics Balance from shimadzu, pH meter lico India, Ultrasonicator Lab man India, HPLC LC SYSTEM UV-VIS spectrophotometer PG Instruments T60 were used.

METHODS

Preparation of Standard stock solutions: Exactly weighed 1.5mg of Formoterol into volumetric flask 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: 1 RotacapInhaler (6µg 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.

Linearity
Table2: Preparation of drug concentrations for linearity.

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm(Formoterol)	%Linearity Level
1	0.25	10	1.5	25
2	0.5	10	3	50
3	0.75	10	4.5	75
4	1	10	6	100
5	0.25	10	7.5	125
6	0.50	10	9	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 flow rate, mobile phase ratio, and temperature, but the result has not been recognized and are within the range of ICH 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 sample stock solution. The alternatives were held at 60^0c for 30 minute. The resulting solution was diluted to achieve a solution of 6μg / 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 solutionwasdilutedtoobtain6μg/ml&100μg/ml solution.

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.

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

Dry Heat Degradation Studies:
The standard drug solution was placedinovenat105°C for1h after that the resultant solution was diluted to 6μg/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.

Neutral Degradation Studies
Sample stock solution refluxingthedruginwaterfor1hrs at a temperature of 60ºC. The resultant solution was diluted to 6μg/ml solution.

RESULTS AND DISCUSSION
Optimized method:
Chromatographic conditions:

Mobile phase	:	55% 0.1% OPA: 45%
Acetonitrile		
Flow rate	:	0.8ml/min

Column	:	BDS C8 (4.6 x 150mm, 5µm)
Column temperature	:	30°C
Injection volume	:	10µL
Wave length	:	223 nm
Runtime	:	6 min
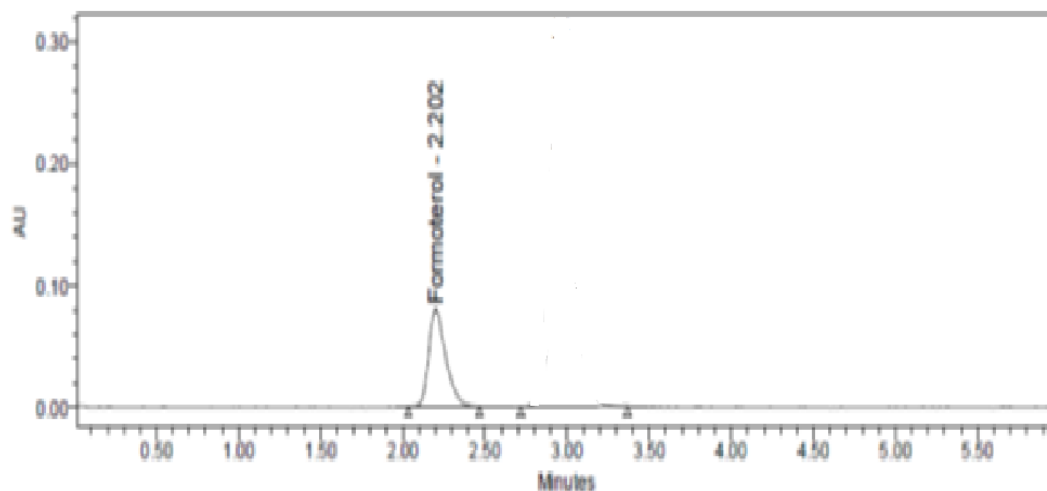
Fig 2: Optimized Chromatogram.

Table 3: system suitability of Formoterol.

S no	Formoterol		
Inj	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.161	3414	1.22
2	2.169	3188	1.23
3	2.182	2337	1.33
4	2.183	2387	1.35
5	2.187	2158	1.38
6	2.202	2119	1.36

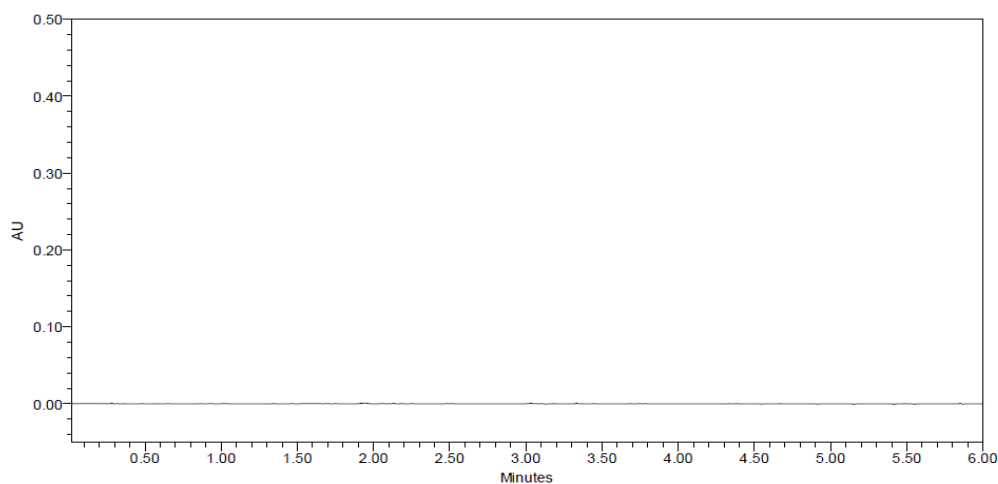**Validation**
Specificity

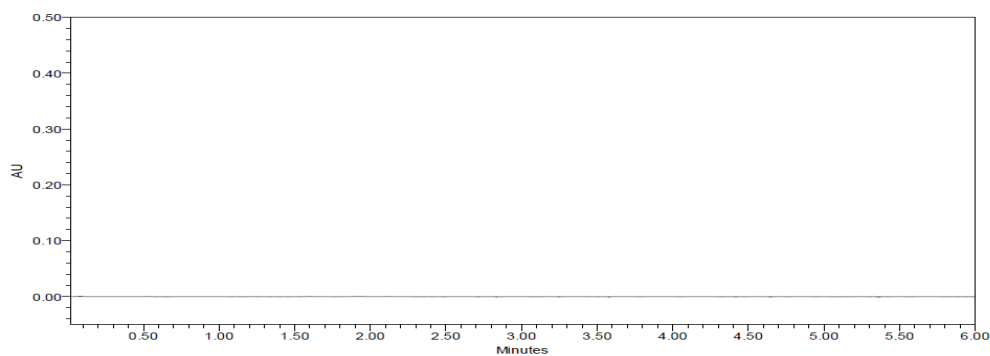**Figure No. 3 Chromatogramof blank.**

Figure No. 4: Chromatogramof placebo.
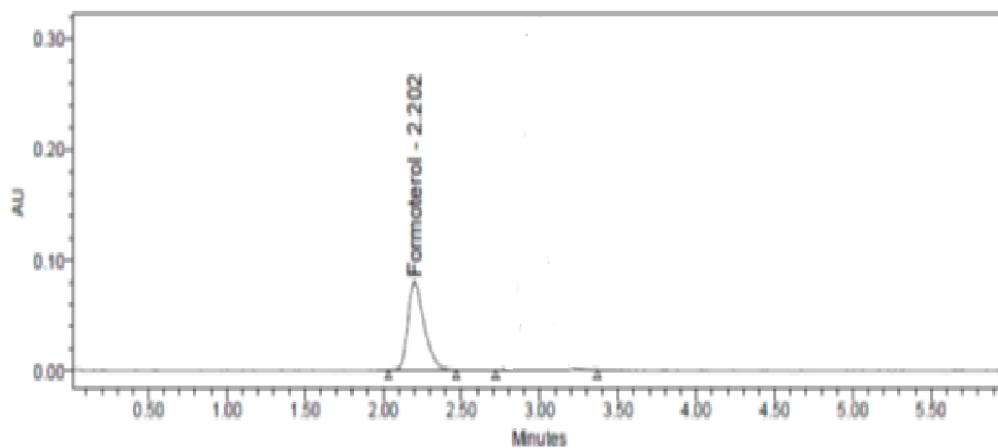
Fig 5: Typical Chromatogram of standard.

Linearity
Table 4: Linearity table for Formoterol.

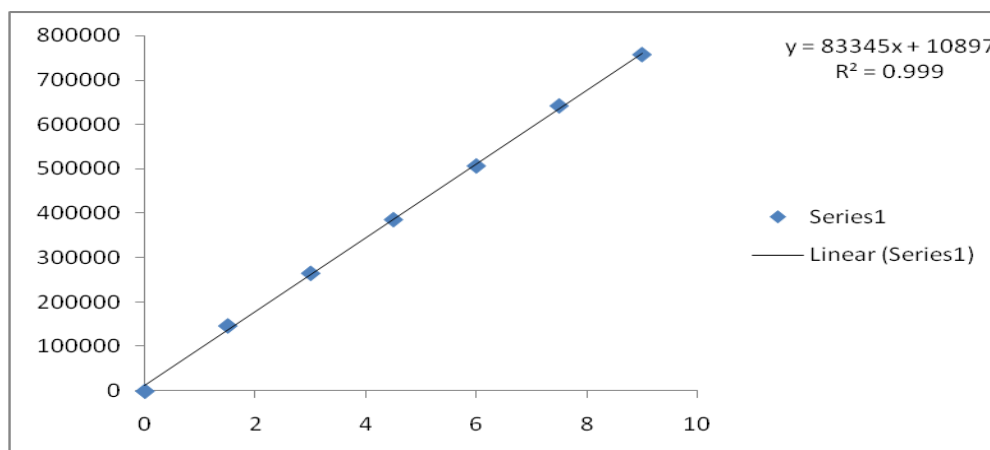| Formoterol	
Conc (µg/mL)	Peak area
0	0
1.5	146747
3	264901
4.5	385670
6	506387
7.5	641313
9	756622

Fig No. 6: Calibration curve of Formoterol.

Precision
System Precision
Table 5: System precision table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol
1.	504172
2.	505762
3.	500807
4.	500690
5.	507112
6.	509239
Mean	504630
S.D	3436.1
%RSD	0.7

Repeatability
Table 6: Repeatability table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol
1.	505200
2.	502334
3.	502117
4.	504411
5.	503153
6.	507843
Mean	504176
S.D	2155.4
%RSD	0.4

Intermediate precision (Day_Day Precision)
Table 7: Intermediate precision table of Formoterol and Glycopyrrolate.

S. No	Area of Formoterol
1.	504458
2.	505540
3.	502577
4.	503808
5.	502598
6.	506029
Mean	504168
S.D	1453.0
%RSD	0.3

Accuracy
Table 8: Accuracy table of Formoterol.

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

Sensitivity
Table 9: Sensitivity table of Formoterol and Glycopyrrolate.

Molecule	LOD	LOQ
Formoterol	0.029	0.087

Robustness
Table10: Robustness data for Formoterol.

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

Table 11: Assay 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

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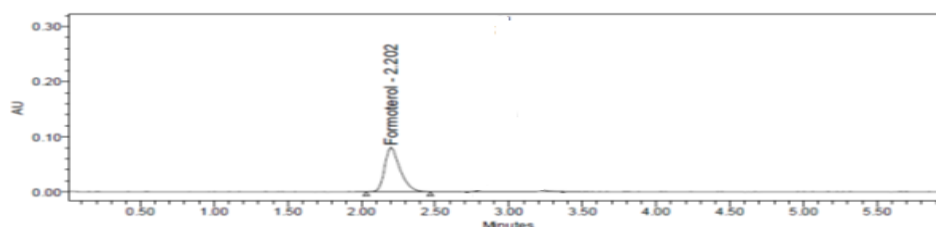
Fig 6: Assay Chromatogram of working standard solution.
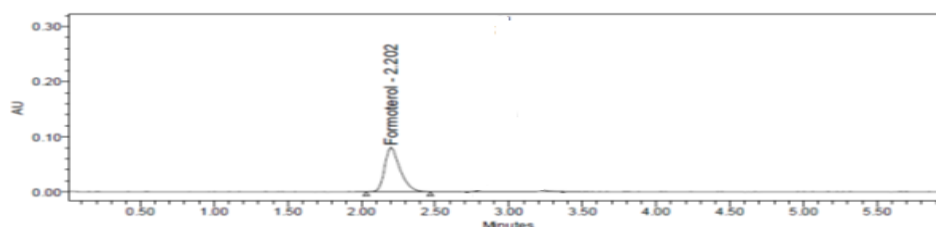
Fig 7: Assay Chromatogram of Marketed sample.

Table12: 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

SUMMARY AND CONCLUSION

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

CONCLUSION

For the concurrent estimation of Formoterol in dosage form, an easy, accurate and linear practice 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.

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