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Article

Analytical Method Validation for Quantitative Estimation of Fenoterol Hydrobromide by Reversed-Phase High-Performance Liquid Chromatography Method

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ABSTRACT

A simple, accurate, and precise reverse-phase high-performance liquid chromatographic method was developed for the estimation of fenoterol hydrobromide in pharmaceutical formulations. A mixture of 70 volumes of buffer solution was prepared by adding 1 ml of triethylamine in 1000 ml of water adjust to pH = 5.0 with formic acid, and 30 volumes of acetonitrile at a flow rate of 1.0 ml/min with UV detection at 276 nm. The concentration range was from 50% to 150%, the retention time was 6.163 min and the correlation coefficient of the analytical curve was 0.999. The limit of detection and limit quantification were 0.002 mg/mL and 0.006 mg/mL, respectively. Intra- and inter-day relative standard deviations were ≤2.0%. The methodology accuracy showed the percentage between 98.61% and 101.11%. The described technique was found to be simple, rapid, precise, accurate, and sensitive; the advantages over the other current methodologies are the low-cost and low-polluting conditions. Owing to its simplicity and reliable results, this methodology is suitable to be used in the quality control of pharmaceutical drugs containing fenoterol hydrobromide as an active component.

Key words: Fenoterol hydrobromide, High-performance liquid chromatography, Stability indicating studies, Pharmaceutical drug validation.

1. INTRODUCTION

Fenoterol is chemically (RR, SS)-5-(1-hydroxy-2-{[2-(4-hydroxyphenyl)-1-methylethyl] amino} ethyl) benzene-1, 3-diol. It is a β2-adrenergic receptor (Figure 1) (β2-AR) agonist with 2 chiral centers resulting in 4 possible stereoisomers [1]. It is classed as a sympathomimetic β_2 agonist and asthma medication. Fenoterol hydrobromide is a short-acting β_2 agonist, which also stimulates β_1 receptors at doses above the recommended therapeutic doses. It has actions and uses similar to those of salbutamol and is used as a bronchodilator in the management of reversible airway obstruction, as occurs in asthma and some patients with chronic obstructive pulmonary disease. On inhalation, it acts within a few minutes and has a duration of action of about 3–5 h [2]. Recently, *in vitro* studies using a rat cardiomyocyte model have demonstrated that (R,R′)-Fen is a potent and selective β_2-AR agonist, which produces significant contractility in the model, whereas (S,S′)-Fen was essentially pharmacologically inactive [3,4].

This observation has led to the development of (R,R′)-Fen as a potential agent in the treatment of congestive heart failure. As part of the (R,R′)-Fen development program, authors have conducted an open-label, dose-escalation study in healthy volunteers to determine the safety, pharmacokinetics, and bioavailability of (R,R′)-Fen after oral administration and to compare the effects to equivalent doses of (R,R′; S,S′)-Fen. In humans, (R,R′; S,S′)-Fen has a low bioavailability (~2%) after oral administration due to extensive pre-systemic sulfation by sulfotransferase (SULT) enzymes [5,6].

Various analytical methods have been applied for the determination of fenoterol hydrobromide in raw materials, pharmaceuticals, and biological fluids. These methods include liquid chromatography (LC) [7-10], gas chromatography [11], voltammetry [12,13], fluoro-immunoassay [14], coulometry [15], colorimetric flow injection [16], electrophoresis [17-19], and spectrophotometry [20].

However, in the knowledge authors, no account has been reported for a stability-indicating assay method for the determination of fenoterol hydrobromide. It was felt necessary to develop a stability-indicating LC method for the determination of fenoterol hydrobromide as a bulk drug and pharmaceutical dosage form and separate the drugs from the degradation products [21].

2. EXPERIMENTAL METHODS

2.1. Instruments/equipments

High-performance liquid chromatography (HPLC) – Waters – Alliance 510 with UV – 484 Data Ace software (Instrument I.D:

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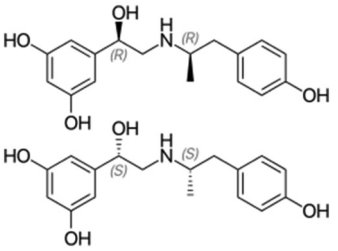


Figure 1: Chemical structure of fenoterol.

AL-011), HPLC – Agilent 1100 Series with software (Instrument I.D: AL-013), HPLC Analytical column Reverse-phase ODS – C18 15 cm × 3.9 mm × 5 µm, Analytical weighing balance – Mettler Toledo B204S and Millipore membrane 0.2 µm laboratory accessories.

2.2. Reagents, standards, and samples used

Fenoterol hydrobromide working standard, frandyl tablets 2.5 mg, triethylamine – AR Grade, formic acid – AR Grade, acetonitrile – HPLC Grade, Water – HPLC Grade.

2.3. Analytical method

The quantitative determination is carried out by HPLC system equipped with UV/VIS detector.

Chromatographic conditions	
Column	Reverse-Phase ODS – C18 15 cm × 3.9 mm × 5 µm
Mobile Phase	Prepare a mixture of 70 volumes of buffer solution prepared by adding 1 ml of triethylamine in 1000 ml water, adjust to pH 5.0 with formic acid, and 30 volumes of acetonitrile
Wavelength	276 nm
Flow rate	1.0 ml/min
Injection volume	10 µl
Run time	15 min
Diluent	A 0.1% v/v solution of triethylamine in Acetonitrile. 50:50

2.4. Validation method

2.4.1. Preparation of fenoterol hydrobromide standard solution

Weighed accurately 20 mg of fenoterol hydrobromide working standard and transferred it to a 50 ml volumetric flask. To this, 30 ml of diluent was added and sonicated to obtain a uniform solution. 1 ml of the solution was transferred to 10 ml of volumetric flask and diluted and mixed filtered through 0.2 µm nylon membrane filter.

2.5. Preparation of test solution

Weighed accurately about 200 mg of frandyl tablets powder (about 10 tablets) and transferred it to a 50 ml volumetric flask. To this 30 ml of diluent was added and sonicated to dissolve. The solution was filtered through a 0.2 µm nylon membrane filter. The solutions were prepared from the range starting from 50% to 150%.

2.6. System suitability solution

The fenoterol hydrobromide standard was used as a working solution as a system suitability solution.

2.7. Procedure

Separately, injected equal volumes of blank, five replicate injections of system suitability solution (fenoterol hydrobromide working standard solution) in to the system. Then, two injections of test solution were injected, and recorded the chromatograms. Ignored the peaks if any due to blank in the test solution. The % relative standard deviation (RSD) of five replicate injections of system suitability solution (fenoterol hydrobromide standard working solution) was calculated. The tailing factor and theoretical plates of the peak in the chromatogram obtained with the 5th injection of system suitability solution (fenoterol hydrobromide working standard solution) were checked.

The limits are designated as below:

1) Theoretical plates should be not <3000.
2) The tailing factor should be <2.0.
3) The % RSD should be not more than 2.0%.

The above summary and the validation data summarized in this document show that the analytical method of assay of fenoterol hydrobromide in frandyl tablets 2.5 mg by HPLC is found to be suitable, selective, specific, precise, linear, accurate, and robust. The analytical solution is found to be stable up to 48 h at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

2.8. Selectivity and specificity

The selectivity was assessed by comparing the chromatograms obtained from excipients (placebo). An amount of placebo equivalent to a sample containing 20 mg fenoterol hydrobromide was used. Ingredients to prepare the placebo were similar to those presented in the commercial formulations and in the same ratio. The system responses were examined in triplicate for the presence of interference or overlaps with fenoterol hydrobromide responses. The forced degradation studies should be performed using 5 N HCl and 5 N NaOH. The studies were performed on standard and sample separately.

2.9. Linearity and range

For the linearity study, five solutions of fenoterol hydrobromide were prepared in the range starting from 50% to 150% of the theoretical concentration of assay preparation. The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted, and the correlation coefficient was determined.

2.9.1. Acceptance criteria

The correlation coefficient should be ≥0.999. The average peak area of fenoterol hydrobromide peak at each concentration level was determined, and the linearity graph was plotted against the sample concentration in percentage. The results of the linearity study are given in Table 1. The linearity plot of peak area of fenoterol hydrobromide is presented in Figures 2 and 3.

3. PRECISION

3.1. System precision

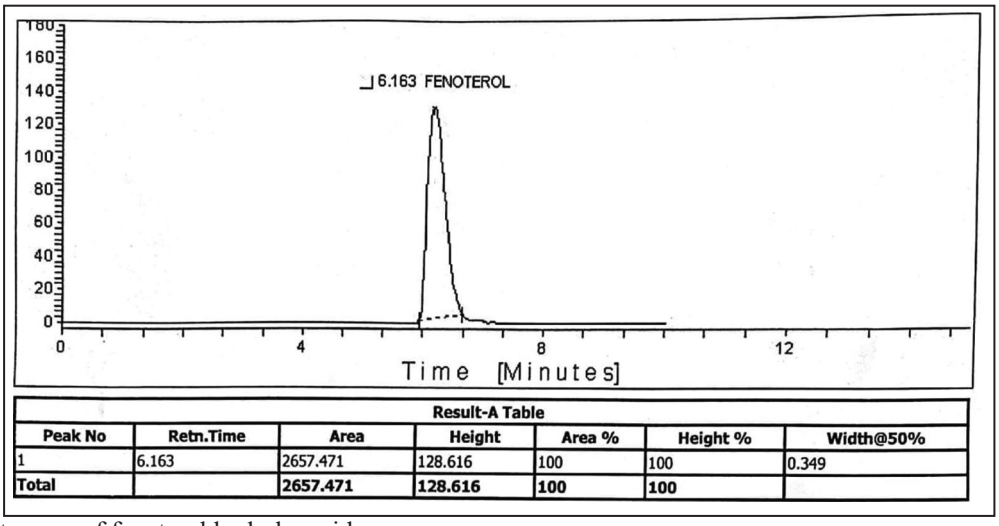
The system precision was performed by injecting ten replicate injections of system suitability solution and the chromatograms were reviewed for the system suitability criteria and the date in given in Table 2.

3.2. Acceptance criteria

The % RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per the analytical method.

Table 1: Results of linearity of fenoterol hydrobromide.

Linearity level	Concentration (in %)	Concentration (in ppm)	Peak area	Correlation coefficient
Level – 1	50	20	879.64	0.999
Level – 2	75	30	1292.23	
Level – 3	100	40	1746.27	
Level – 4	125	50	2243.70	
Level – 5	150	60	2672.37	



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	6.163	2657.471	128.616	100	100	0.349
Total		2657.471	128.616	100	100	

Figure 2: Chromatogram of fenoterol hydrobromide.

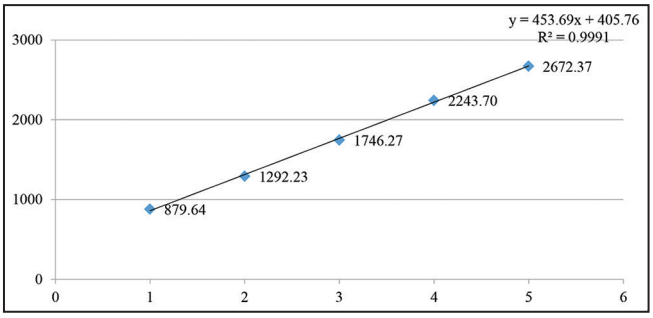


Figure 3: Linearity graph of fenoterol hydrobromide.

4. METHOD PRECISION

4.1. Procedure

Six test solutions of fenoterol hydrobromide in frandyl tablets 2.5 mg were prepared as per the analytical method. The % RSD assay of six test solutions was calculated.

4.2. Acceptance criteria

The % RSD of the results of six test solutions should not be more than 2.0%. The results of the assay obtained from six test solutions preparations are presented in Table 3.

5. INTERMEDIATE PRECISION

5.1. Procedure

Six test solutions of frandyl tablets 2.5 mg were prepared as per the analytical method on different days. These test solutions were analyzed by a different analyst using different HPLC columns of the same make but having different serial numbers and different HPLC system. The % RSD assay results of 12 test solutions (six samples from method precision and six samples from intermediate precision) were calculated.

5.2. Acceptance criteria

The % RSD of the results of 12 test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The results of the assay obtained from six test solutions are presented in Table 4. The % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table 5.

6. ACCURACY

6.1. Procedure

An accuracy study was performed by analyzing fenoterol hydrobromide test solutions which were prepared by mixing fenoterol hydrobromide API with placebo. These test solutions were prepared by adding a quantity of fenoterol hydrobromide API to placebo to produce different concentration solutions equivalent to 50%, 75%, 100%, 125%, and 150% of the test concentration.

6.2. Acceptance criteria

Mean recovery at each concentration level should be between 97.0% and 103.0%. The results of the accuracy study obtained are presented in Table 6.

7. FORCED DEGRADATION

7.1. Acid stress solutions

Transferred an accurately weighed quantity of about 20 mg of fenoterol hydrobromide working standard and of sample into three

Table 2: System precision.

S. No.	Area of fenoterol hydrobromide
1	1820.34
2	1843.36
3	1846.31
4	1874.01
5	1864.26
6	1860.34
7	1837.18
8	1870.59
9	1860.76
10	1867.59
Mean	1854.47
Standard deviation (±)	17.12
(%) Relative standard deviation	0.92

Table 3: Results of method precision.

Test solution	% Assay of fenoterol hydrobromide
1	98.91
2	99.55
3	98.64
4	98.70
5	99.50
6	99.35
Mean	99.11
Standard deviation (±)	0.41
(%) Relative standard deviation	0.41

Table 4: Results of intermediate precision.

Test Solution	% Assay of fenoterol hydrobromide
1	99.57
2	99.81
3	99.06
4	98.75
5	98.85
6	98.27
Mean	99.05
Standard deviation (±)	0.56
(%) relative standard deviation	0.57

separate 25 ml volumetric flasks. Moisten the contents of flasks with 1 ml of 5 N hydrochloric acid and the contents of the flasks were heated for 10 min at 60°C. After cooling, add 15 ml of diluents was added and sonicated for 5 min and finally filled up to the mark with diluent. 1.0 ml of solution was transferred into a 10 ml of volumetric flask and diluted to volume with the diluents and mixed thoroughly. The solution was filtered through 0.45 µ nylon filters. The same

Table 5: Results of 12 test solutions of fenoterol hydrobromide in six of method precision and six of intermediate precision.

Analysis performed during method precision study by analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of fenoterol hydrobromide**
1	98.91
2	99.55
3	98.64
4	98.70
5	99.50
6	99.35
Analysis performed during intermediate precision study by analyst 2 on system 2 and on column 2 on day 2	
Column S. No.	**015337030136 01**
Test solution	% Assay of fenoterol hydrobromide
7	99.57
8	99.81
9	99.06
10	98.75
11	98.85
12	98.27
Mean of twelve samples	99.08
Standard deviation (±)	0.47
(%) Relative standard deviation	0.47

procedure was used without the addition of HCL to get control solutions. Fenoterol hydrobromide chromatogram of acid degradation is presented in Figure 4.

7.2. Alkali stress solutions

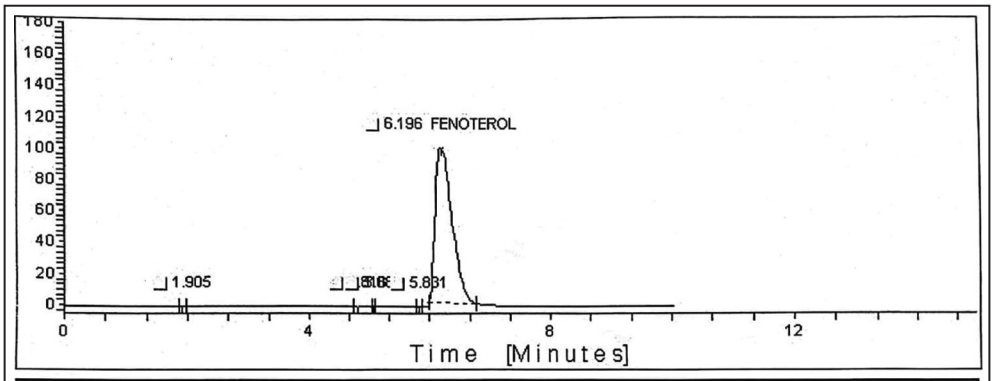
An accurately weighed quantity of about 20 mg of fenoterol hydrobromide working standard and also sample was transferred into three separate 25 ml volumetric flasks. Moisten the contents of flasks with 1 ml of 5 N sodium hydroxide and heated the contents of the flasks for 10 min at 60°C. After cooling, 15 ml of diluents was added and sonicated for 5 min and diluted up to the mark with diluent. Transferred 1.0 ml of the solution into a 10 ml of volumetric flask and make up to the mark with diluents and mixed well. The solutions were filtered through 0.45 µ nylon filters. The same procedure was used without the addition of NaOH to get control solutions. Fenoterol hydrobromide chromatogram of base degradation is presented in Figure 5.

7.3. Thermal/heat stress solutions

An accurately weighed quantity of about 20 mg of fenoterol hydrobromide working standard and 200 mg of sample into three separate 25 ml volumetric flasks were transferred. Keet the flasks in a hot air oven maintained at 105°C for 12 h. Cooled and added 15 ml of diluent and sonicated for 5 min and makeup to the mark with diluent. Transferred 1.0 ml of the solution into a 10 ml of volumetric flask and diluted up to the mark with the diluents and mixed well. The solution was filtered through 0.45 µ membrane filter. Fenoterol hydrobromide chromatogram of thermal degradation is given in Figure 6.

Table 6: Accuracy (% recovery) – results.

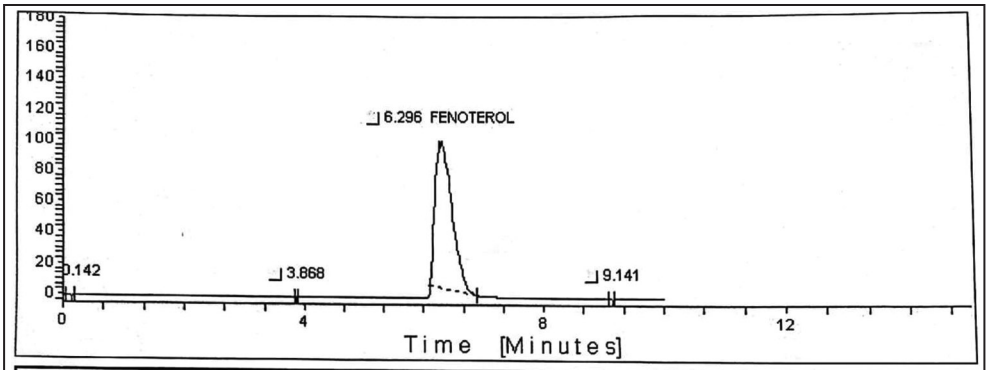
Level of addition	Amount of fenoterol hydrobromide added in mg	Amount of fenoterol hydrobromide found in mg	Recovery (%)
First level (Rec-50%)	10.70	10.59	98.97
Second level (Rec-75%)	15.40	15.56	101.04
Third level (Rec-100%)	20.80	21.03	101.11
Fourth level (Rec-125%)	27.40	27.02	98.61
Fifth level (Rec-150%)	31.90	32.18	100.88
Mean			100.12
Standard deviation (±)			1.23
(%) Relative standard deviation			1.22



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	1.905	0.227	0.074	0.012	0.074	0.066
2	4.816	0.025	0	0.001	0	0.033
3	5.082	0.037	0.021	0.002	0.021	0.033
4	5.831	0.225	0.064	0.012	0.064	0.067
5	6.197	1909.234	99.604	99.973	99.84	0.316
Total		**1909.748**	**99.763**	**100**	**100**	

Figure 4: Fenoterol hydrobromide chromatogram of acid degradation.



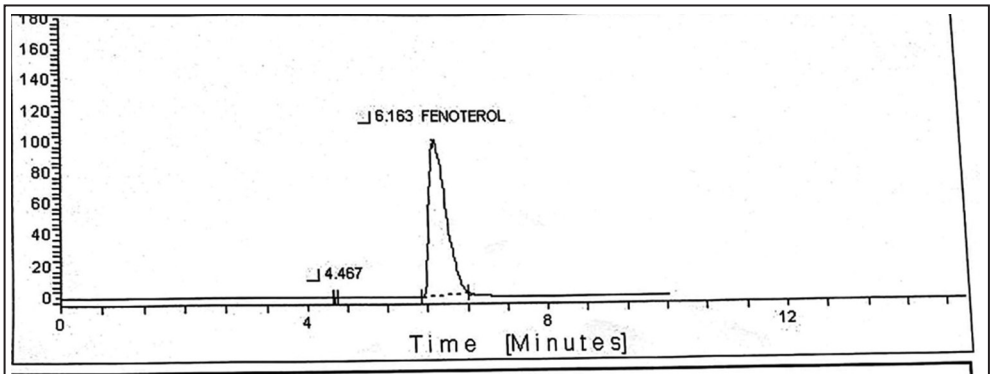
Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	0.142	0.594	0.095	0.032	0.099	0.116
2	3.868	0.193	0.077	0.01	0.08	0.05
3	6.297	1849.939	96.073	99.941	99.752	0.316
4	9.141	0.305	0.067	0.016	0.07	0.083
Total		**1851.031**	**96.312**	**100**	**100**	

Figure 5: Fenoterol hydrobromide chromatogram of base degradation.

7.4. UV stress solutions

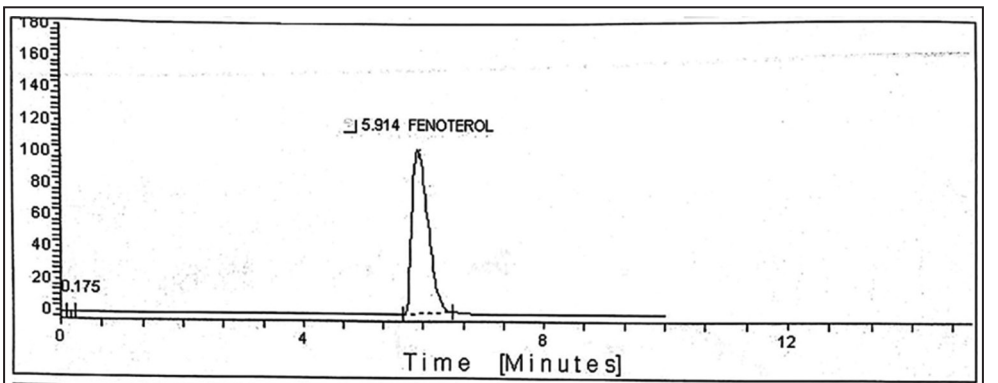
Transferred an accurately weighed quantity of about 20 mg of fenoterol hydrobromide working standard and of sample into three separate 25 ml volumetric flasks. Keet the flasks in the UV chamber and expose to UV-radiation for 7 days. Cooled and added 15 ml of diluents and sonicated for 5 min and diluted up to the mark with diluent. Transferred 1.0 ml of the solution into a 10 ml of volumetric flask and diluted to volume with the diluents and mixed thoroughly. The solution was filtered through 0.45 µ membrane filter. Repeated the same preparations without exposing the samples to UV rays, as a control



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	4.467	0.153	0.061	0.008	0.061	0.05
2	6.163	1994.286	100.911	99.992	99.939	0.333
Total		**1994.439**	**100.972**	**100**	**100**	

Figure 6: Fenoterol hydrobromide chromatogram of thermal degradation.



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	0.175	0.328	0.062	0.016	0.06	0.1
2	5.914	2004.198	103.662	99.984	99.94	0.333
Total		**2004.526**	**103.724**	**100**	**100**	

Figure 7: Fenoterol hydrobromide chromatogram of ultraviolet degradation.

Table 7: Results of filter validation.

Sample	Area of fenoterol hydrobromide	% Assay of fenoterol hydrobromide	Absolute difference
Unfiltered	1981.25	100.30	-
Filtered – 1	2083.04	99.93	0.37
Filtered – 2	2086.49	99.95	0.35
Filtered – 3	2093.28	100.03	0.27
Filtered – 4	2050.02	100.05	0.25
Filtered – 5	2095.93	99.90	0.40

Table 8: Results for change in flow rate.

Flow rate →	**0.8 ml/min**	**1.2 ml/min**
Sample	% Assay	
Test solution	99.41	100.14
Average assay result from method precision	99.11	99.11
Mean	99.26	99.63
Standard deviation (±)	0.21	0.73
(%) Relative standard deviation	0.21	0.73

Table 9: Results for change in wavelength.

Wavelength →	**274 nm**	**278 nm**
Sample	% Assay	
Test solution	99.55	99.57
Average assay result from method precision	99.11	99.11
Mean	99.33	99.34
Standard deviation (±)	0.31	0.33
(%) Relative standard deviation	0.31	0.33

Table 10: Results for change in composition of mobile phase.

Mobile phase composition	68B: 32 ACN	72B: 28 ACN
Sample	% Assay	
Test solution	99.99	98.88
Average assay result from method precision	99.11	99.11
Mean	99.55	99.00
Standard deviation (±)	0.62	0.16
(%) Relative standard deviation	0.63	0.16

Table 11: Results for solution stability.

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of fenoterol hydrobromide**
0^{th} h	98.28
12^{th} h	97.87
24 h	99.59
36 h	97.73
48 h	100.93
Mean	98.88
Standard deviation (±)	1.36
(%) Relative standard deviation	1.38

sample. Fenoterol hydrobromide chromatogram of UV degradation was presented in Figure 7.

7.5. Conclusion

There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well-separated from each other. The peak purity for fenoterol hydrobromide peak is passing. Hence, the method is said to be very precise, selective, and specific to the estimation of assay of fenoterol hydrobromide in frandyl tablets by HPLC and the same method is stable indicating, as the degraded products are well-separated from fenoterol hydrobromide and as well from each adjacent peaks.

8. FILTER VALIDATION

8.1. Procedure

Five tablets were weighed and transferred into 250 ml volumetric flask. About 180 ml of diluent was added and shaked manually for 20 min and further sonicated for 30 min, then the contents were diluted to volume with the diluent and mixed, centrifuged a portion of the resulting solution at about 8000 rpm for about 10 min. The supernatant solution was decanted into another test tube and transferred 10 ml of the supernatant solution into another 50 ml volumetric flask and made up the volume with diluent. This solution was used as an unfiltered test solution. 10 ml of the remaining portion of the supernatant solution was diluted to 50 ml with diluent and filtered through 0.45 µm nylon membrane filter paper, filled 5 vials of this solution and used this as filtered test solution.

8.2. Acceptance criteria

There should not be any significant difference between filtered and unfiltered test solutions. The results of the assay obtained for filtered and unfiltered test solutions are presented in Table 7 and reported along with the absolute difference between unfiltered and filtered test solution.

9. ROBUSTNESS

9.1. Experiment

To prepare two test solutions of the same lot (as used in 7.0.a and 7.0.b) of fenoterol hydrobromide in frandyl tablets of 2.5 mg as per the analytical method. Injected this solution along with diluent blank solution and system suitability solution at different chromatographic conditions as shown below:

9.2. Change in flow rate (± 0.2 ml/min)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The assay results obtained with different flow rate conditions are given in Table 8.

9.3. Change in wavelength (±2 nm)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The assay results obtained with different wavelength conditions are given in Table 9.

9.4. Change in the composition of mobile phase (Buffer: acetonitrile = 70:30)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical methods. The assay results obtained with the change in mobile phase composition are given in Table 10.

10. STABILITY OF ANALYTICAL SOLUTION

10.1. Procedure

System suitability solution and test solution of frandyl tablets 2.5 mg were prepared on the 0^{th}, 12^{th}, 24^{th}, 36^{th}, and 48^{th} h of the experiment and stored these solutions at room temperature for every time intervals up to 48 h and analyzed these solutions on 48 h with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of frandyl tablets 2.5 mg in the sample was calculated.

10.2. Acceptance criteria

The analyte is considered stable if there is no significant change in % assay. The assay results obtained during the solution stability experiment are given in Table 11

11. CONCLUSION

The proposed method for the determination of fenoterol hydrobromide in pharmaceutical formulations showed to be efficient and sensitive. Chromatographic parameters such as mobile phase, pH, and flow rate can be modified to control fenoterol hydrobromide retention time on column. The excipients of the commercial samples assayed did not interference in the analysis and the absence of interference demonstrated the specificity of the method. Stability-indicating methods were developed for fenoterol hydrobromide in pharmaceutical dosage form under hydrolytic stress conditions (5N HCL and 5N NaOH); oxidation condition (5% H_2O_2) and dry heat condition, thermal condition, and UV light. From this study, it was found that the drug is not susceptible for degradation to hydrolytic conditions, oxidation conditions, dry heat, UV light, and thermal conditions. The proposed method was found to be simple, rapid, precise, accurate, and sensitive. The method can be used for routine quality control of fenoterol hydrobromide in commercial samples.

12. ACKNOWLEDGMENT

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