ANALYTICAL METHOD VALIDATION FOR QUANTITATIVE ESTIMATION OF FENOTEROL HYDROBROMIDE BY RP-HPLC METHOD

B. Ramakrishna, M.Hanuman Nayak, N.S.Hanumantha Rao and N.V.S. Naidu*
Department of Chemistry, S.V.University, Tirupati-517 502, A.P, India.
Corresponding author E-mail: nvsn69@gmail.com

ABSTRACT

Simple, accurate, precise reverse phase high performance liquid chromatographic method for the estimation of Fenoterol Hydro bromide in pharmaceutical formulations and tabulate dosage form. The concentration range was from 50% to 150%, the retention time is 6.163 min and the correlation coefficient of analytical curve was 0.999. The limit of detection and limit of quantification were 0.002 mg/mL^{-1} and 0.006 mg/mL^{-1} respectively. Intra- and interday relative standard deviations were ≤ 2.0%. The methodology accuracy showed the percentage found between 98.61 to 101.11%. The described technique was found to be simple, rapid, precise, accurate and sensitive; the advantages over the others 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 quality control of pharmaceutical drugs containing Fenoterol Hydrobromide as active component.

Keywords: Fenoterol Hydrobromide, High Performance Liquid Chromatography, Stability indicating studies, Pharmaceutical drug validation.

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 (Fig. 1) (β2-AR) agonist with 2 chiral centers resulting in 4 possible stereoisomers [1]. It is classed as 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 airways obstruction, as occurs in asthma and in some patients with chronic obstructive pulmonary disease. On inhalation, acts within a few minutes and has duration of action of about three to five hours [2]. Recently, in vitro studies using a rat cardiomyocyte model has demonstrated that (R,R′)-Fen is a potent and selective β2-AR agonist, which produces

significant contractility in the model, while (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, we 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 presystemic sulfation by sulfotransferase (SULT) enzymes [5,6].

Various analytical methods have been applied for the determination of Fenoterol Hydrobromide in raw material, pharmaceuticals and biological fluids. These methods include liquid chromatography [7-10], gas chromatography [11], voltametry [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 stability indicating assay method for determination of Fenoterol Hydrobromide. It was felt necessary to develop a stability indicating liquid chromatography (LC) method for the determination of Fenoterol Hydrobromide as bulk drug and pharmaceutical dosage form and separate the drugs from the degradation products under the International Conference on Harmonization (ICH) suggested conditions (hydrolysis, alkali, UV and thermal stress) [21].

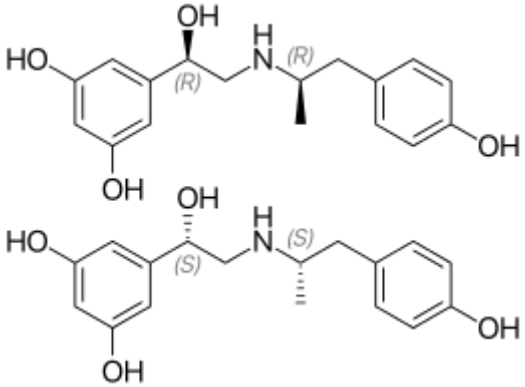


Fig: Chemical Structure of Fenoterol

Instruments / Equipments Used

H.P.L.C- Waters - Alliance 510 with UV- 484 Data Ace software (Instrument I.D: AL-011), HPLC - Agilent 1100 Series with Chromeleon software (Instrument I.D: AL-013), HPLC Analytical column Reverse Phase ODS – C18 15cm x 3.9mm x 5µm, Analytical

weighing balance - Mettler Toledo B204S and Millipore membrane 0.2µm Laboratory accessories.

Reagents, Standards & Samples Used

Fenoterol Hydrobromide working standard, Frandyl Tablets 2.5mg, Triethylamine–AR Grade, Formic Acid–AR Grade, Acetonitrile - HPLC Grade, Water - HPLC Grade

ANALYTICAL METHOD:

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

Chromatographic conditions:

Column	**:**	Reverse Phase ODS – C18 15cm x 3.9mm x 5µm
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 / minute
Injection volume	:	10 µl
Run time	:	15 minutes
Diluent	:	A 0.1 per cent v/v solution of triethylamine in Acetonitrile. 50:50

METHOD VALIDATION

Preparation of Fenoterol Hydrobromide Standard Solution

Weigh accurately about 20 mg of Fenoterol Hydrobromide working standard and transfer to a 50 ml volumetric flask. Add 30 ml of diluent and sonicate to dissolve. Dilute to volume with diluent. Transfer 1 ml of the solution to 10ml volumetric flask and dilute and mix Filter through 0.2µm nylon membrane filter.

Preparation of Test Solution

Determine the average weight of 10 tablets. Powder it and weigh accurately about 200 mg of Frandyl Tablets powder and transfer to a 50 ml volumetric flask. Add 30 ml of diluent and sonicate to dissolve. Dilute to volume with diluent. Filter the solution through 0.2µm nylon membrane filter. The solutions were prepared from the range starting from 50% to 150%.

System Suitability Solution

Use Fenoterol Hydrobromide standard working solution as system suitability solution.

Procedure

Separately inject equal volumes of blank, five replicate injections of system suitability solution (Fenoterol Hydrobromide working standard solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Fenoterol Hydrobromide standard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5^{th} injection of system suitability solution (Fenoterol Hydrobromide working standard solution).

The limits are as below,

1) Theoretical plates should be not less than 3000.
2) Tailing factor should be less than 2.0.
3) % RSD should be not more than 2.0%.

The above summary and the validation data summarized in this document shows that the analytical method of assay of Fenoterol Hydrobromide in Frandyl Tablets 2.5mg 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 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

Selectivity and Specificity

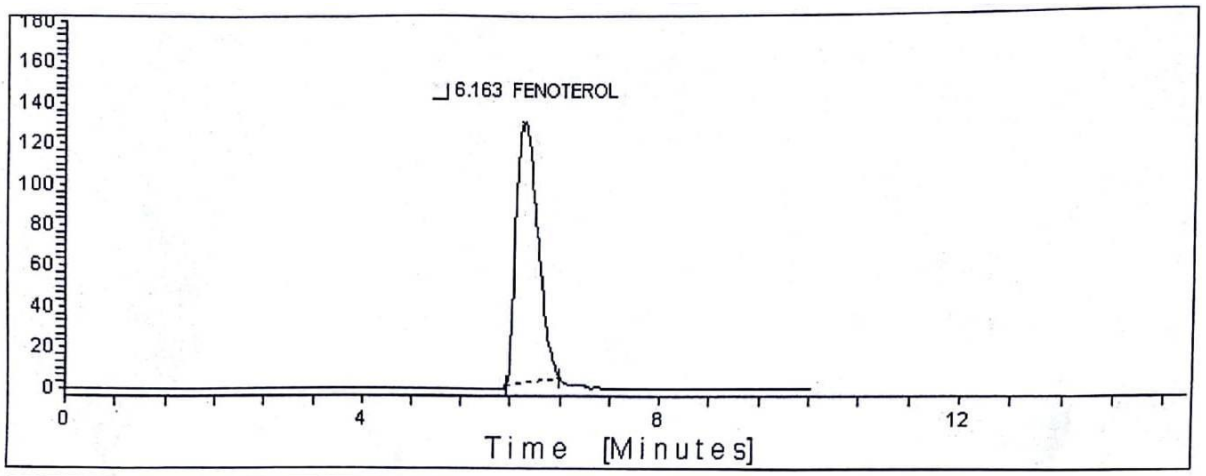
The selectivity was assessed by comparing the chromatograms obtained from excipients (placebo). An amount of placebo equivalent to sample containing 20mg Fenoterol Hydrobromide was used. Ingredients to prepare the placebo were similar to those presented in the commercial formulations and in the same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

Linearity and Range

For the linearity study five solutions of Fenoterol Hydrobromide were prepared from 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.

Acceptance criteria: Correlation coefficient should be greater than or equal to 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 linearity study are as given in Table - 1. The linearity plot of peak area of Fenoterol Hydrobromide is presented in figure-1&2.



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 1: Chromatogram of Fenoterol Hydrobromide

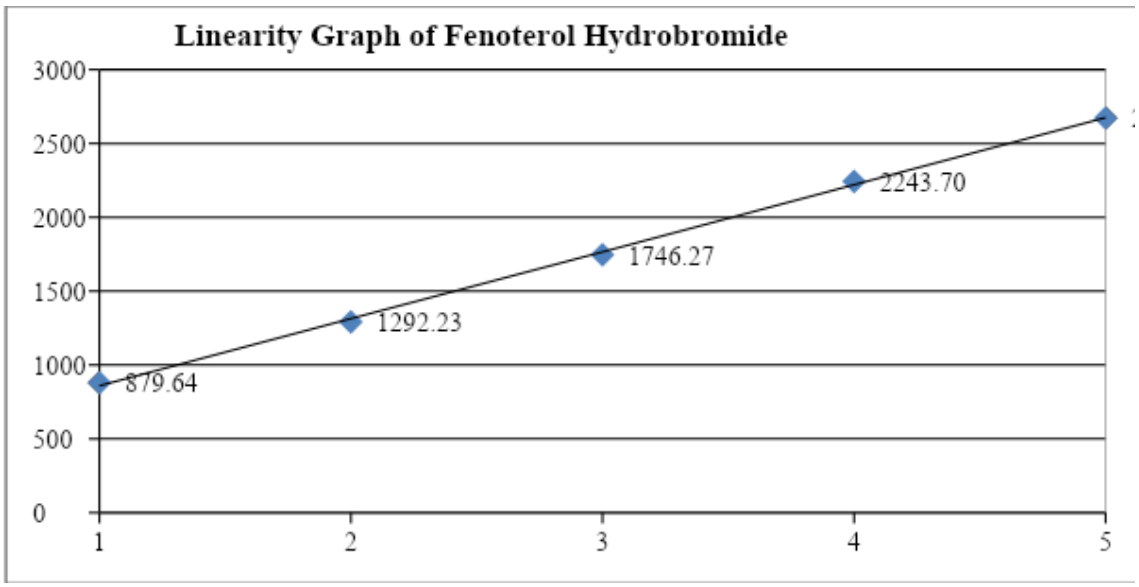


Figure 2: Linearity graph of Fenoterol Hydrobromide

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	

PRECISION:

System Precision

The system precision was performed by injecting 10 replicate injections of system suitability solution and the chromatograms are reviewed for the system suitability criteria.

Acceptance criteria: % 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 analytical method.

Table 2: System precision

Sr. 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**

METHOD PRECISION:

Procedure: Six test solutions of Fenoterol Hydrobromide in FRANDYL Tablets 2.5mg and were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated.

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

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**

INTERMEDIATE PRECISION:

Procedure: Six test solutions of Frandyl Tablets 2.5mg and were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

Acceptance criteria: % RSD of the results of twelve 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 assay obtained from six test solutions are presented in Table - 4. % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table - 1.

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**

Table - 5: Results of twelve test solutions of Fenoterol Hydrobromide in (six of method precision & 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 sr. 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**

ACCURACY:

Procedure: 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 three different concentration solutions equivalent to 50%, 75%, 100%, 125% and 150% of test concentration.

Acceptance criteria: Mean recovery at each concentration level should be between 97.0% and 103.0%. The results of accuracy study obtained are presented in Table-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**

Acceptance criteria:

System suitability criteria should pass as per analytical method and the % RSD between results obtained with changed condition and average result of method precision, should not be more than 2.0%.

FORCED DEGRADATION

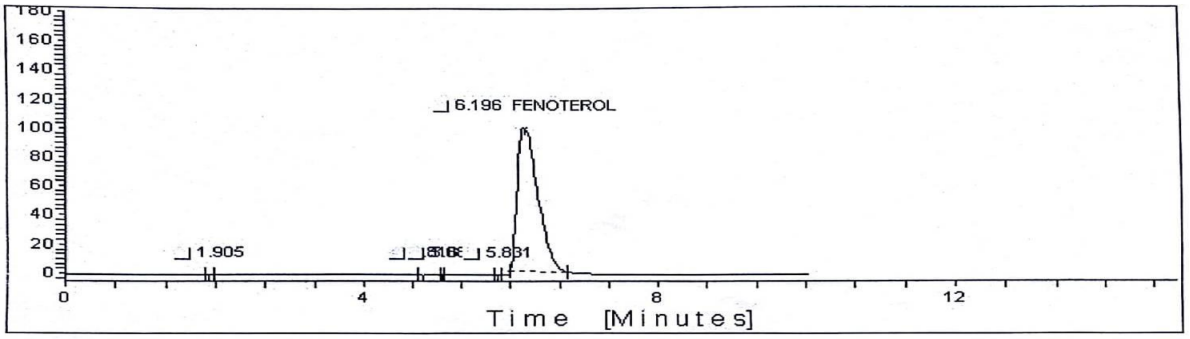
a) Acid stress solutions: Transfer an accurately weighed quantity of about 20mg of Fenoterol Hydrobromide working standard and of sample into three separate 25ml volumetric flasks. Moisten the contents of flasks with 1ml of 5N hydrochloric acid and heat the contents of the flasks for 10min at 60ºC. Cool and add 15ml of diluent, sonicate for 5minutes and dilute up to the mark with diluent. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluents and mix. Filter the solutions through 0.45µ nylon filters. Repeat the same preparations without addition of 5N hydrochloric acid, as control solutions.

b) Alkali stress solutions: : Transfer an accurately weighed quantity of about 20mg of Fenoterol Hydrobromide working standard and of sample into three separate 25ml volumetric flasks. Moisten the contents of flasks with 1ml of 5N Sodium Hydroxide and heat the contents of the flasks for 10min at 60ºC. Cool and add 15ml of diluent, sonicate for 5minutes and dilute up to the mark with diluent. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluents and mix. Filter the solutions through 0.45µ nylon filters. Repeat the same preparations without addition of 5N hydrochloric acid, as control solutions.

c) Thermal/Heat stress solutions. Transfer an accurately weighed quantity of about 20mg of Fenoterol Hydrobromide working standard and 200mg of sample into three separate 25ml volumetric flasks. Keep the flasks in a hot air oven maintained at 105ºC for 12 hours. Cool and add 15ml of diluent, sonicate for 5minutes and dilute up to the mark with diluent. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluents and mix. Filter the solution through 0.45µ membrane filter.

d) UV stress solutions: Transfer an accurately weighed quantity of about 20mg of Fenoterol Hydrobromide working standard and of sample into three separate 25ml volumetric flasks.

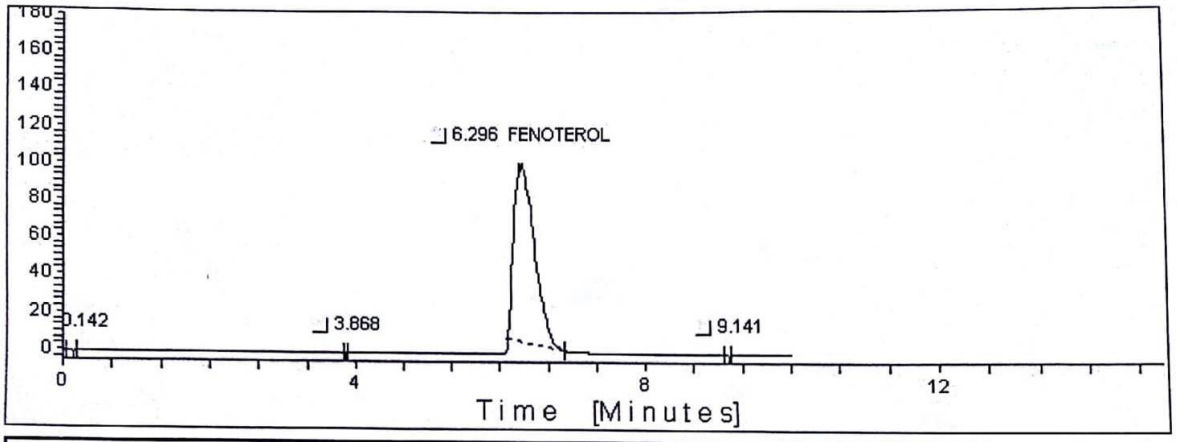
Keep the flasks in UV chamber and expose to UV-radiation for 7 days. Cool and add 15ml of diluent, sonicate for 5minutes and dilute up to the mark with diluent. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluents and mix. Filter the solution through 0.45µ membrane filter. Filter the solution through 0.45µ membrane filter. Repeat the same preparations without exposing the samples to UV rays, as control samples.



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**	

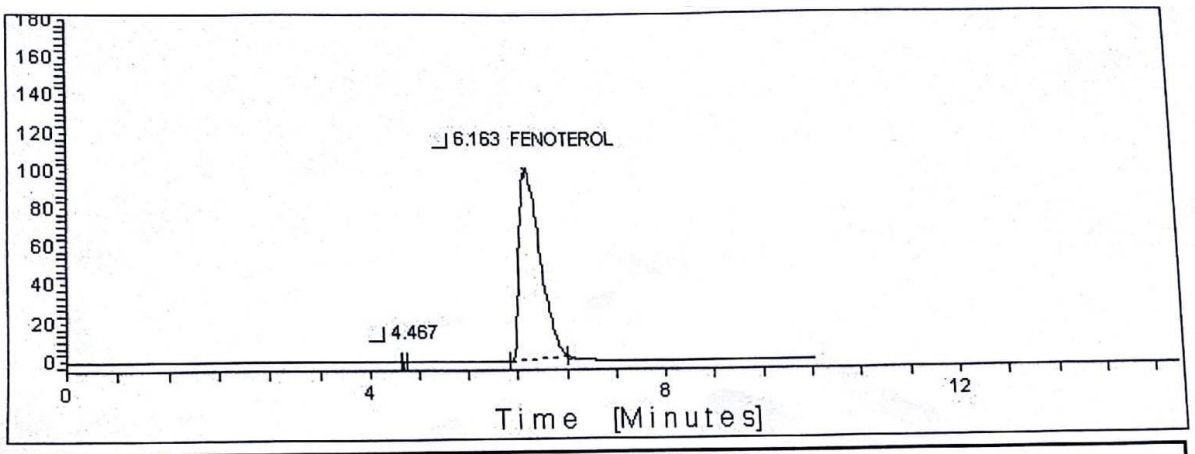
Figure: 3 Fenoterol Hydrobromide Chromatogram of Acid degradation



Result-A Table

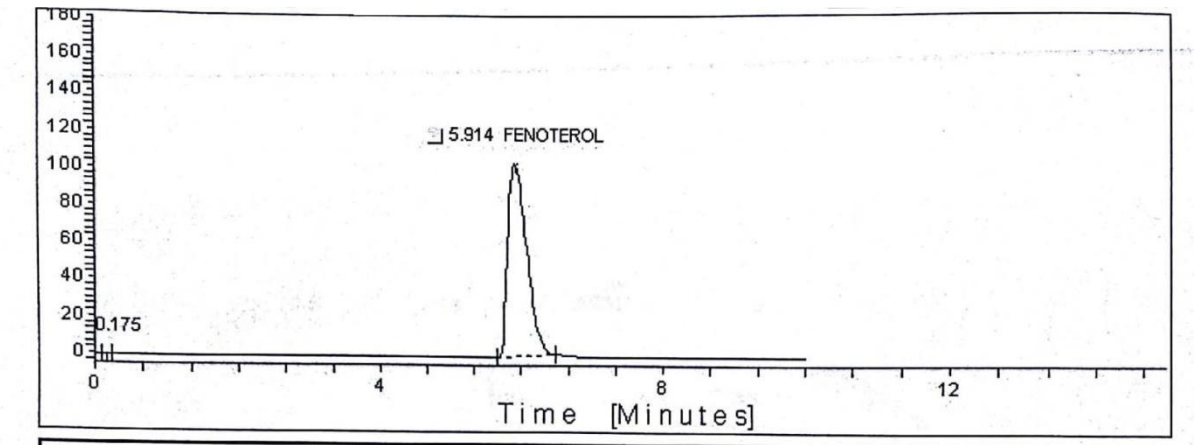
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	0.142	0.594	0.095	0.032	0.099	0.116
2	3.868	0.193	0.077	0.01	0.08	0.05
3	6.297	1849.939	96.073	99.941	99.752	0.316
4	9.141	0.305	0.067	0.016	0.07	0.083
Total		**1851.031**	**96.312**	**100**	**100**	

Figure: 4 Fenoterol Hydrobromide Chromatogram of Base degradation



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 UV degradation

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 very precise, selective and specific to the estimation of Assay of Fenoterol Hydrobromide in Frandyl Tablets by HPLC and the same Method is stability indicating, as the degraded products are well separated from Fenoterol Hydrobromide and as well from each adjacent peaks.

FILTER VALIDATION:

Procedure: Five tablets were weighed and transferred into 250 mL volumetric flask. About 180 mL of diluent was added and shaked manually for 20 minutes and further sonicated for 30 minutes, diluted to volume with the diluent and mixed, centrifuged a portion of the resulting solution at about 8000 rpm for about 10 minutes. The supernatant solution was decanted into another test tube and transferred 10 mL of supernatant solution into another 50 mL volumetric flask and made up the volume with diluent. This solution was used as unfiltered test solution. 10 mL of 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.

Acceptance criteria: There should not be any significant difference between filtered and unfiltered test solutions. The results of assay obtained for filtered and unfiltered test solutions are presented in Table 19 and reported along with the absolute difference between unfiltered and filtered test solution.

Table 3: 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

ROBUSTNESS

Experiment: Prepare two test solutions of the same lot (as used in 7.0.a and 7.0.b) of Fenoterol Hydrobromide in Frandyl Tablets 2.5mg as per analytical method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in Flow Rate (± 0.2 mL/minute):

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 as given in Table 4.

Table - 4: Results for change in flow rate

Flow rate →	**0.8 mL/minute**	**1.2mL/minute**
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**

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 as given in Table - 5.

Table - 5: Results for change in wavelength

Wavelength →	**274nm**	**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**

Change in change in 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 The assay results obtained with change in mobile phase composition are as given in Table - 6.

Table - 6: Results for Change in change in composition of mobile phase

Mobile phase composition	**68B:32ACN**	**72B:28ACN**

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**

Stability of Analytical Solution:

Procedure: System suitability solution and test solution of Frandyl Tablets 2.5mgwere prepared on 0^{th},12^{th}, 24^{th}, 36^{th} and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of Frandyl Tablets 2.5mgin the sample was calculated.

Acceptance criteria: The analyte is considered stable if there is no significant change in % assay. The assay results obtained during solution stability experiment are as given in Table-7

Table - 7: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Fenoterol Hydrobromide**
0^{th} hr	98.28
12^{th} hr	97.87
24 hr	99.59
36 hr	97.73
48 hr	100.93
Mean	**98.88**

Standard Deviation (±)	**1.36**
(%) Relative Standard Deviation	**1.38**

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 condition (5N HCL, 5N NaOH); Oxidation condition (5% H2O2) and dry heat condition, Thermal condition, UV light. From study, it was found that drug is not susceptible for degradation to hydrolytic condition, oxidation condition, dry heat, UV light, and thermal condition. 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.

ACKNOWLEDGEMENT

The author thanks to Analog labs, Prashanti Nagar, IDA, Balanagar, Hyderabad, Telangana and Department of Chemistry, Sri Venkateswara University, Tirupati, India for providing laboratory facilities.

REFERENCES

1. O'Donnell SR. Eur J Pharmacol. 1970; 12:35. [PubMed: 4394346].
2. Gleiter CH. Fenoterol: Pharmacology and Clinical Use. Cardiovasc Drug Rev. 1999; 17:87-106.
3. Beigi F, Bertucci C, Zhu W, Chakir K, Wainer IW, Xiao RP. Chirality. 2006; 18:822. [PubMed: 16917835].
4. Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA, Balke CW, Lakatta EG, Cheng H. Circulation. 2003; 108:1633. [PubMed: 12975249].
5. Rominger, KL.; Herman, M. Neuste Ergbnisse uber betamimitika Darmstadt. Jung, H.; Fendel, H.; Karl, C., editors. 1995. p. 62 .
6. Hildebrandt R, Wagner B, Preiss-Nowzohour K, Gundert-Remyk U. Xenobiotica. 1994; 24:71. [PubMed: 8165823].

7. Jacobson GA, Peterson GM. High-performance liquid chromatographic assay for the simultaneous determination of ipratropium bromide, fenoterol, salbutamol and terbutaline in nebulizer solution. J Pharm Biomed Anal. 1994; 12:825-32.
8. Polettini A, Montagna M, Hogendoorn EA, Dijkman E, van Zoonen P, van Ginkel LA. Applicability of coupled-column liquid chromatography to the analysis of β-agonists in urine by direct sample injection I. Development of a single-residue reversedphase liquid chromatography-UV method for clenbuterol and selection of chromatographic conditions suitable for multiresidue analysis. J Chromatogr A. 1995; 695:19-31.
9. Kramer S, Blaschke G. High-performance liquid chromatographic determination of the β2-selective adrenergic agonist fenoterol in human plasma after fluorescence derivatization. J Chromatogr B. 2001; 751:169-75.
10. Meineke I, Steinmetz H, Kramer S, Gleiter CH. Determination of fenoterol in human plasma by HPLC with fluorescence detection after derivatization. J Pharm Biomed Anal. 2002; 29:147-52.
11. Henze MK, Opfermann G, Spahn-Langguth H, Schänzer W. Screening of β-2 agonists and confirmation of fenoterol, orciprenaline, reproterol and terbutaline with gas chromatography–mass spectrometry as tetrahydroisoquinoline derivatives. J Chromatogr B. 2001; 751:93-105.
12. Boyd D, Barreira-Rodriguez JR, Miranda-Ordieres AJ, TunonBlanco P, Smyth MR. Voltammetric study of salbutamol, fenoterol and metaproterenol at unmodified and Nafion-modified carbon paste electrodes. Analyst. 1994; 119:1979-84.
13. Belal F, AL-Malaq HA, AL-Majed AA. Voltammetric determination of isoxsuprine and fenoterol in dosage forms and biological fluids through nitrosation. J Pharm Biomed Anal. 2000; 23:1005-15.
14. Haasnoot W, Stouten P, Lommen A, Cazemier G, Hooijerink D, Schilt R. Determination of fenoterol and ractopamine in urine by enzyme immunoassay. Analyst. 1994; 119:2675-80. 15. Nikolic K, Arsenijevec L, Bogavac M. Coulometric determination of some antiasthmatics. J Pharm Biomed Anal. 1993; 11:207-10.
15. Tanabe S, Togawa T, Kawanabe K. Colorimetric flow injection determination of resorcinol-type β2-adrenergic drugs with phenanthro[9,10-d]imidazole-2-N-chloroimide. Anal Sci. 1989; 5:513-6.

16. Wachs T, Sheppard RL, Henion J. Design and applications of a self-aligning liquid junction-electrospray interface for capillary electrophoresis-mass spectrometry. J Chromatogr B. 1996; 685:335-42.
17. Sirichaia S, Khanatharana P. Rapid analysis of clenbuterol, salbutamol, procaterol, and fenoterol in pharmaceuticals and human urine by capillary electrophoresis. Talanta. 2008; 76:11948.
18. Ullrich T, Wesenberg D, Bleuel C, Krauss G, Schmid MG, Weiss M et al. Chiral separation of the β2-sympathomimetic fenoterol by HPLC and capillary zone electrophoresis for pharmacokinetic studies. Biomed Chromatogr. 2010; 24:1125-9.
19. El-Shabrawy Y, Belal F, Sharaf El-Din M, Shalan S. Spectrophotometric determination of fenoterol hydrobromide in pure form and dosage forms. Il Fármaco. 2003; 58: 1033-8.
20. ICH Steering Committee, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology, Q2(R1), novembro, Genebra, Suíça, 2005.
21. Association of Official Analytical Chemists International. Official Methods of Analytical Chemists of AOAC International, 17. ed., XX, 200.