A New and Novel Assay Method Validation, Development and Degradation Study of Fenspiride HCI

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Abstract

A selective and sensitive reversed phase UPLC method was developed and validated for the simultaneous determination of assay of fenspiride HCI drug substance. The chromatographic separation was performed on Acquity BEH C18 column (2.1 mm x 100 mm), 1.7 µm using gradient elutionof 0.1% methane sulfonic acid in water and mixture of water and acetonitrile(50:50 v/v) at flow rate of 0.6 mL/min. UV and PDA detector are used for assay determination. The total run time was 3 min, within which fenspiride and its three impurities were well separated. The developed method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision solution stability ,robustness and degradation study. The calibration curve shows good linearity over the concentration range of 80-120ppm for fenspiride. The correlation coefficient obtained was >0.9998 in each case. The wide linearity range, short retention time, stability indicating and simple mobile phase showed that the method is suitable for routine analysis of fenspiride and the developed method was adopted in commercial batches.

KEYWORDS: Fenspiride HCI, RP-UPLC, Assay, Degradation study, Precision, Validation

INTRODUCTION

Fenspirideis an oxazolidinonespiro compound used as a drug in the treatment of certain respiratory diseases.Fenspiride alsoknown its brand names such as, Eurespal and Pneumorel. The pharmacotherapeutic classification is antitussives. In Russia it is approved for the treatment of acute and chronic inflammatory diseases of organs (ear, nose, throat) and the respiratory tract (like rhinopharyngitis, laryngitis, tracheobronchitis, otitis and sinusitis), as well as for maintenance treatment of asthma.The chemical name of fenspiride hydrochloride is 1-Oxa-3,8-diazaspiro[4,5]decan-2-one,8-(2-phenylethyl)-monohydrochloride. The molecular formula $C_{15}H_{20}N_2O_2$.HCl.The molecular weight is 296.79.

The first method for the quantification of Fenspiride in plasma and urine which was described in 1989 (1) included liquid–liquid extraction of analyte from the biomatrix with a mixture of organic solvents, re-extraction with aqueous acid and back-extraction with an organic solvent. The extract was evaporated to dryness and reconstituted in the mobile phase. The quantification of fenspiride was run by HPLC method on the reversed-phase column, while electrochemical detection was used for concentration range from 2 to 100 mg/mL and ultraviolet detection was used for concentration range from 100 to 1000 mg/mL. With the help of this quite complex method the phase I pharmacokinetic study of fenspiride in 12 healthy volunteers was conducted in 1993 (2). In the paper (3) fenspiride and its metabolites in equine plasma and urine were detected with the help of capillary

gas chromatography-mass spectrometry method. The method is also characterized by complex sample preparation which covers solid-phase extraction, analyte elution from the sorbent by strong base in ethyl acetate, evaporation to dryness under N_2 and obtaining of trimethylsilyl derivates of fenspiride.

The selective LC-MS/MS method was developed for more than 250 basic drugs screening including fenspiride in the supernatant of enzyme hydrolyzed equine urine after extraction on the short Oasis HLB® column (4). One more method based on gas chromatography-mass spectrometry was developed for narcotics and stimulants in equine urine screening (5). Fenspiride and other analytewere extracted from biomatrix by organic solvents, extracts were evaporated to dryness under N_2, after that the derivates of N-methyl-N-trimethylsilyl trifluoroacetamide were obtained. Fenspiride was determined in human plasma using the liquid-liquid extraction of fenspiride and the internal standard in 1-octanol, followed by direct injection of large volume aliquot (75µL) of 1-octanol containing the analyte in the reversed-phase chromatography column and MS/MS detection(6).New rapid UPLC-MS/MS method for the quantification of fenspiride in human plasma. The lower limit of quantification (LOQ), was 2 mg/mL by using 200µL aliquot of human plasma and simple precipitation procedure(7).There was no HPLC and UPLC Related substances method specified for Fenspiride API in United States pharmacopeia (8). Hence there was a need to develop it which is became the purpose of the further study.

Present work describes the development of simple, selective, rapid, accurate, precise and cost effective RP-UPLC method for the determination of Assay of fenspiride in pharmaceutical dosage forms.

This method is validated as per ICH guidelines in terms of linearity, precision, accuracy, specificity,robustness and degradation study.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade acetonitrile was purchased from Merck (Mumbai, India). Analytical grade Methane sulfonic acidwas purchased fromSigma-Aldrich (St.Louis, MA, USA). Working standard and test sample were obtained from Emcure Pharmaceuticals Limited, Analytical research Center Hinjewadi, Pune. Water was purified with a Milli-Q .

Instrumentation

The instrumentation used was UPLC coupled with PDA detector (H-Class, Waters, USA). Empower 2 software used for data acquisition and calculations.

Preparation of Standard Solution

The working standard solution was prepared by accurately weighing about 50 mg of fenspiride into 50 mL volumetric flask and made up to the mark with diluent .further pipette 5 ml of this solution into 50 ml volumetric flask diluted up to mark with diluent. The concentration of the standard solution and samples were optimized to achieve a desired satisfactory peak shape. All the standard were Sonicated well degassed before the analysis.

Chromatographic Conditions

All chromatographic experiments were carried out on an Acquity UPLC system coupled with PDA detector. The analytical column used was Acquity BEH C18 (100 x 2.1 mm, 1.7µm). Thegradient elution employed with mobile phase A and mobile phase B components. The solution of mobile phase A is a 0.1 % methane sulfonic acid and mobile phase B having a mixture of acetonitrile- water (50:50, v/v).The flow rate of the mobile phase was set at 0.6 mL/min and column temperature was maintained at 60°C.

The gradient program was set as follows: time/% mobile phase A: 0/90, 3.0/90, filter through0.22µm nylon filter before the analysis.

PDA Detector

The Waters UPLC system with was used with PDA detector. The control of the system and datacollection was done by empower 2 software. 210 nm wavelength and sampling rate is 5 points/seconds selected for the fenspiride analysis.

Validation Study

The developed method was validated in terms of specificity, linearity, accuracy, precision, robustness, solution stability and forced degradation study by following ICH guidelines.

The linearity of the method was evaluated by preparing and analyzing five point calibrators of 80-120.0ppm for fenspiride. The slope, intercept and regression coefficient values were determined by the least squares linear regression analysis. System precision of the mass spectrometric response was established by making six injections of the standard solution. The method precision was evaluated bysix analyte and determining the %RSD. The intermediate precision was evaluated by six analyte and determining the %RSD. Calculate the % RSD of twelve injections (six from method precision and six from intermediate precision). Stability of the fenspiride insample solution was done by analyzing sample solution at different time intervals at roomtemperature.In force degradation study fenspiride was subjected to Stress condition observed as Photolysis, Humidity, Thermal, Hydrolysis, Acid, Alkali, Peroxide degradation.

RESULTS AND DISCUSSION

Method Development

The main objective of the present study was to determine % assay of drug substance using UPLC. UPLC is very rapid technology as compared to HPLC. Now a day UPLC is prepared for the method development. During development of Assay method for fenspiride UPLC was preferred. During different trials, selected mobile phase, column and diluent and finally this method was selected for the assay method for fenspiride.

Column Selection and Separation

The main objective of the present study was to achieve better separation among the closely eluting impurities with symmetrical peak shapes, and the method should be able to determine all the impurities in a single run. Moreover, the developed method should be linear, rapid, accurate, reproducible, robust, stability indicating and enough for routine use in quality control laboratory.

Several attempts were made with different C18 UPLC columns (Acquity CSH Phenyl-hexyl (2.1 mm x100 mm)1.7µmand YMC Triart C18, 100 mm x 2.0 mm, 1.7µm), using gradient elution. Using the above columns the separation of the impurities was not satisfactory and peak shape of fenspiride not symmetrical. On Acquity BEH C18 column (100 mm x 2.1mm, 1.7 µm) separation and response for all the four impurities were found to be satisfactory and peak shape of fenspiride also symmetrical. On this column the impurities were well retained and separated from the fenspiride drug substance peak. The sample and standard preparation 100 ppm was prepared in the diluent
(Water: acetonitrile, 50:50 v/v).

Method Validation

The developed method was fully validated by standard procedure to ensure adequate selectivity,linearity, precision, accuracy, solution stability force degradation study and robustness(9,10,11).The system suitability was checked by injecting 100ppm fenspiride standard solution checking the tailing factor and theoretical plates throughout the validation.

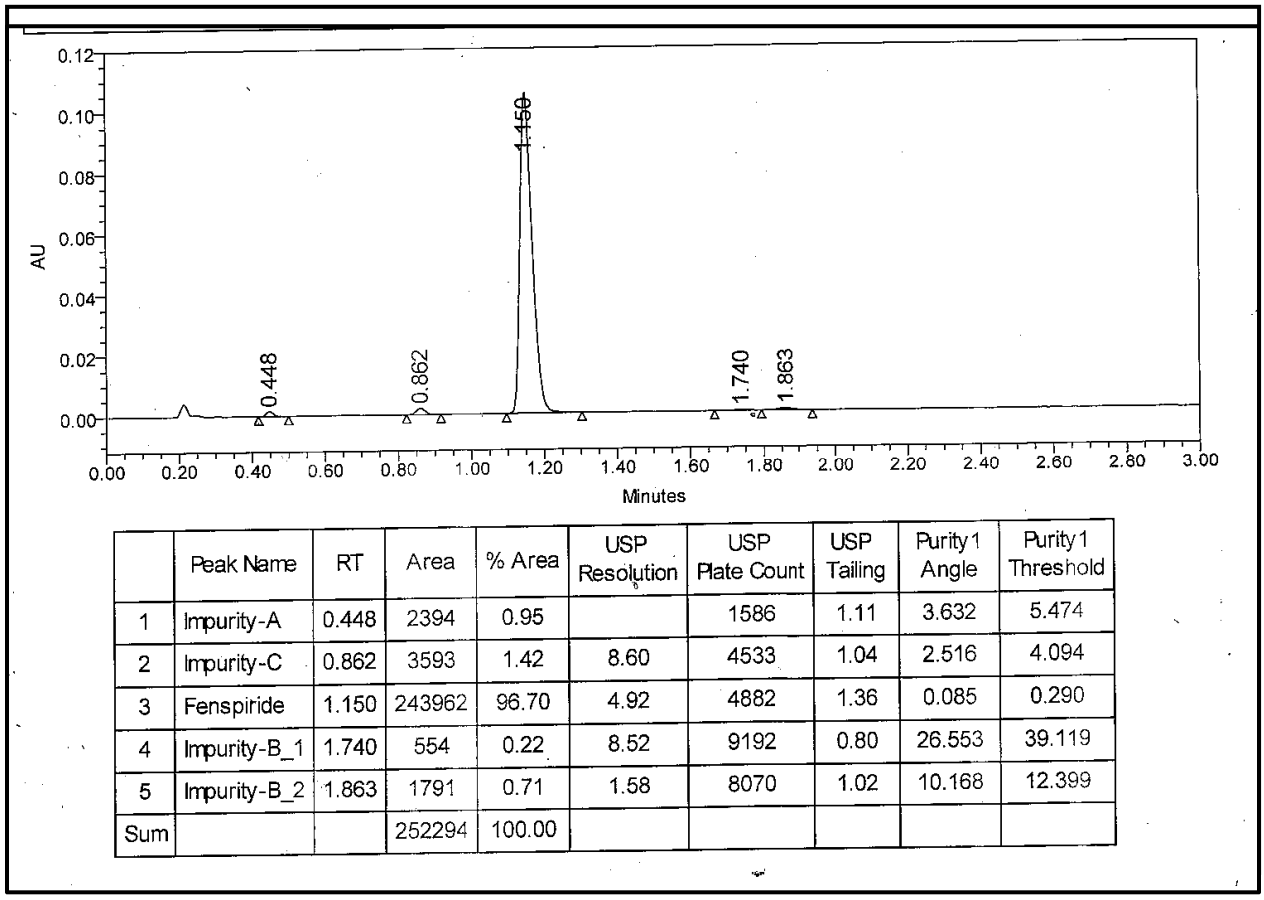
Specificity

An investigation of specificity should be conducted during the validation of identification test, the determination of impurities, the procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte. In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

Method should involve demonstration of the discrimination of the analyte in the presence of impurities. This can be done by spiking pure substances with appropriate levels of impurities (0.5ppm) and demonstrate the results is unaffected by presence of this impurities.

All known and unknown impurities are well resolved from Fenspiride and peak purity of known impurities and Fenspiride are passed, hence it shows that impurities and Fenspiride peaks are homogeneous. Interfering peak was observed for any of the impurity with fenspiride.

The system suitability parameters such as tailing factor, theoretical plates and resolution are within acceptance criteria. Hence it is concluded that method is selective for assay analysis of Fenspiride. The specificity chromatogram was shown in Fig.-1.



	Peak Name	RT	Area	% Area	USP Resolution	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Impurity-A	0.448	2394	0.95		1586	1.11	3.632	5.474
2	Impurity-C	0.862	3593	1.42	8.60	4533	1.04	2.516	4.094
3	Fenspiride	1.150	243962	96.70	4.92	4882	1.36	0.085	0.290
4	Impurity-B_1	1.740	554	0.22	8.52	9192	0.80	26.553	39.119
5	Impurity-B_2	1.863	1791	0.71	1.58	8070	1.02	10.168	12.399
Sum			252294	100.00					

Fig.-1: Typical Chromatogram showing separation of all the impurities from fenspiride.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in thesample. It may be directly demonstrated on the analyte, or on spiked samples using at leastfive concentrations over the whole working range. Moreover a visual evaluation of theanalyte signal as a function of the concentration, appropriate statistical calculations arerecommended, such as a linear regression. The parameters slope and intercept, residual sum of squares and the coefficient of correlation should be reported. A graphical presentation of the data and residuals is recommended.

In assay method was determined by using standard solution of with 80% to 120% of specification limit of Fenspiride (80 %, 90%, 100%, 110%,120%). The peak area verses concentration data was treated by least squares linear regression analysis. The Correlation coefficient obtained was greater than 0.999.The % Y intercept of calibration curve not more than 2. The Results and Linearity graph shown in Table- 1 and Figure-2

Table- 1: Linearity of fenspiride by using standard solution of with 80% to 120% ofspecification limit.

Levels	Concentration	Average Area
1	Level 1 (80%)	190110
2	Level 2 (90%)	213532
3	Level 3 (100%)	234015
4	Level 4 (110%)	260048
5	Level 5 (120%)	284964
	Correlation coefficient	0.99909
	Intercept	2373.3744
	Slope	-440.5627

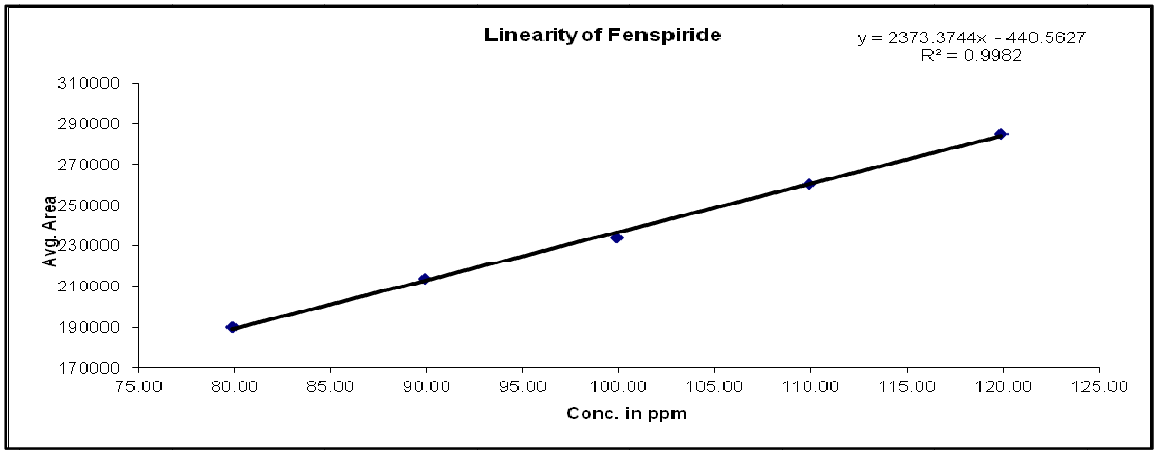


Fig.-2: Typical graph showing linearity of fenspiride.

Precision

Precision provides an indication of random errors and can be broken down into repeatability and intermediate precision. This procedure should only be performed when the entire analytical method procedure is finalized.

Repeatability represents the simplest situation and involves analysis of replicates by the same analyst, generally one injection after the other.

Intermediate precision includes the influence of additional random effects according to the intended use of the procedure in the same laboratory and can be regarded as an (initial) estimate for the long-term variability. Relevant factors, such as operator, instrument, and days should be varied. Intermediate precision is obtained from several independent series of applications of the (whole) analytical procedure to (preferably) authentic, identical samples.

In repeatability, System precision carried out by performed by five replicates injection of fenspiride standard.

And calculated the % RSD in method precision % Assay was performed by injecting six independent samples.
In intermediate precision analysis done with different day, different UPLC system, different analyst. % Assay was performed by injecting six independent test samples. Calculated the overall %RSD of twelve samples (six from repeatability and six from intermediate precision.
All results are within acceptance criteria. Hence it is conclude that method is precise for assay analysis of Fenspiride. The %RSD was found to be less than 2.0% in both the cases, these results confirmed the overall precision of the method (Table- 2).

Table- 2: Repeatability and intermediate precision data of fenspiride

ID	% Assay	
	Repeatability	Intermediate precision
Sample-1	100.05	100.35
Sample-2	100.08	100.25
Sample-3	100.40	100.80
Sample-4	100.36	100.27
Sample-5	100.30	100.36
Sample-6	100.28	100.24
Mean (n=6)	100.25	100.38
Standard Deviation(n=6)	0.1461	0.2127
% RSD(n=6)	0.15	0.21
Mean (n=12)	100.33	
Standard Deviation(n=12)	0.1926	
% RSD(n=12)	0.19	

Accuracy
The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness ofagreement between the value that is adopted, either as a conventional, true, or acceptedreference value, and the value found.
In assay method validation of drug substances accuracy proved by Linearity and repeatability data. This data shows that the method is accurate.

Robustness
The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.
To determine the robustness of the developed method the experimental conditions were

Deliberatelychanged and the impact on chromatographic performance was observed. To study the effect of flow rate itwas changed to 0.54 and 0.66 mL/min (altered by 10% of flow). The effect of column temperature wasstudied at 55°C and 65°C. However, in all these experiments the mobile phase components were notchanged. In all the deliberately varied chromatographic conditions the selectivity as well as theperformance of the method was unchanged, which proves the robustness of the method.

Stability in Solution and in the Mobile Phase

The solution stability of sample preparation was performed by day basis (1 day, 2 days, 3 days) at the room temperature. The % assay of fenspiride was calculated for the study period of sample preparation.

The percentage of Assay test solution at different time intervals were within therange of 99.20-101.26% . The results from the solution stability experimentconfirmed that sample solutions were stable up to 3 days during the analysis. The corresponding solutionstability data was presented in Table- 3.

Table- 3: Solution stability data of fenspiride.

ID	% Assay	Cumulative %RSD
Sample preparation-Fresh	99.20	-
Sample preparation-1 day	99.48	0.20
Sample preparation-2 days	100.96	0.95
Sample preparation-3 days	101.26	1.03

Forced degradation Study:

Forced degradation study was also performed on Fenspiride to provide an indication of the stability indicating property.% degradation was determined by comparing with untreated test sample preparation. Untreated test sample was also analyzed along with the degraded sample. The degradation was compared with untreated test and percentage degradation was determined.

In thermal degradation test sample was heated at 105°C for 24 Hrs. Humidity degradation carried out by 40°C, 75 % RH for 24 hrs and Photolytic degradation test sample treated with Light energy of 1.2 million lux hours and near UV 200 watt hrs./m^2.

All above test were analyzed as per the analytical method according to the concentration.

No any physical appearance changed in heat, humidity and photolytic condition.

No degradation observed in heat, humidity and photolytic condition. Fenspiride molecule is stable for heat, humidity and photolytic stress conditions.

In acid degradation study, test sample exposed to 5.0 mL 5.0 M HCI kept at 80°C on oil bath for 24 Hours, after 24 Hrs cooled it and neutralized with alkali. In fenspiride acid degradation, hydrochloric acid interactedwith fenspiride and degradation product is observed. Impurity-A is the degradation product of the Acid degradation. In this condition 15.08 % degradation observed.

In alkali degradation study, test sample exposed to 5.0 ml 1.0 M NaoH kept at 80°C on oil bath for 5 min., after 5 min cooled it and neutralized with hydrochloric acid. In Fenspiride alkali degradation, sodium hydroxide interact with Fenspiride and degradation

product is observed. Impurity-A, is the degradation product of the alkali degradation. In this condition 15.16 % degradation observed.
In peroxide degradation study, test sample exposed to 5ml 50% H_2O_2kept at 80°C on oil bath for 11 min. In Fenspiride peroxide degradation, peroxide interacted with Fenspiride and degradation product is observed. Impurity-B, is the degradation product of the peroxide degradation. Impurity- B is major degradants. In this condition 18.75 % degradation observed. The corresponding degradation study data was presented inTable-4.

Table:-4 Summary of Forced Degradation study of fenspiride.

Stress Condition	Exposure period	% Assay	% degradation
Untreated Test Preparation	-	99.37	---
Humidity degradation	40°C, 75% RH for 24 Hrs.	100.43	---
Thermal degradation	105°C for 24 Hrs.	99.22	---
Photolytic degradation	Light energy of 1.2 million lux hours and near UV 200 watt hrs./m^2	98.25	---
Aqueous degradation	5 mL 24 hrs at room temperature.	99.34	---
Acid Degradation	5.0 mL 5.0 M HCI kept at 80°C on oil bath for 24 Hours	84.29	15.08
Alkali Degradation	5.0 mL1.0 M NaoH kept at 80°C on oil bath for 5 min.,	84.21	15.16
Peroxide degradation	5 mL 50% H_2O_2kept at 80°C on oil bath for 11 min.	80.62	18.75

CONCLUSION:
A new, accurate, linear, stability indicating and selective UPLC method was proposed for the determination of assay method for Fenspiride in active pharmaceutical ingredients. This method was validated as per ICH guideline Q_2R_2. Newly developed method was found to be ease to handle,selective, precise, accurate, costeffective, stability indicating and robust .The Method was completely validated showing satisfactory data for all the method parameters tested. Themethod was also applied for the determination of mentioned impurities from the formulation batches of fenspiride. The developed UPLC method is stability indicating and used for routine analysis of Fenspiride.

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