A New and Novel Related Substances Method Developed for Fenspiride which includes Validation and Degradation Study of Drug Profile

Vitthal M Dhalape[a], Santosh T Khadangale[a], Neelakandan K[a]
[a]Emcure Pharmaceuticals Limited, Analytical research centre, Hinjwadi, Pune-411057, Maharashtra, India
Corresponding Author: Santosh T Khadangale

Abstract

A selective and sensitive reversed phase UPLC method was developed and validated for the simultaneous determination of related substances in fenspiride HCI drug substance. The developed method was validated as per ICH guidelines in terms of specificity, limit of detection(LOD) and limit of quantification (LOQ), linearity, accuracy, precision solution stability,robustness and degradation study. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration. Statistical analysis proved the method was specific, selective, precise, reproducible and accurate for the analysis of fenspiride and its related impurities. 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, Repeatability, Intermediate Precision

INTRODUCTION

Fenspirideis an oxazolidinonespiro compound used as a drug in the treatment of certain respiratory diseases[3]. Fenspiride also known 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 absorbent 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 analyte were 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).Newrapid 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 related substances of fenspiride in pharmaceutical dosage forms.

This method is validated as per ICH guidelines in terms of limit of detection, limit of quantitation, 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 acid, was purchased from Sigma-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 Stock and Standard Solutions

The working standard solution wasprepared by accurately weighing about 17.5 mg of fenspiride into 50 mL volumetric flask, dissolved and diluted to diluent. pipette 10 ml of this solution into 50 ml volumetric flask diluted up to mark with diluent. Further pipette 5 ml of this solution into 50 ml volumetric flask diluted up to mark with diluent. Prepared 15ppmimpurity-A and Impurity-C stock solution and 20 ppm of impurity-B stock solution.recovery preparation of 50 % 100 % and 150 % added 2.5 mL ,5.0 mL and 7.5 mL of impurity stock into 25 ml volumetric flask containing 50 mg of fenspiride sample.

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). The gradient 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: 0/98, 6.0/85, 10.0/85, 10.1/98, 12.0/98. The injection volume was 1.0µL both mobile phase filter through 0.22 µm nylon filter before the analysis.

PDA Detector

The Waters UPLC system with was used with PDA detector. Control of the system and datacollection was done by empower 2 software with 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, limit of quantification (LOQ), limitof detection (LOD), accuracy, precision, robustness, solution stability and forced degradation study by following ICHguidelines.The linearity of the method was evaluated by preparing and analyzing nine point .The slope, intercept and regression coefficient values were determined bythe least squares linear regression analysis. System precision of wasestablished by making six injections of the standard solution. The method precision was evaluated byspiking each analyte and determining the %RSD. Limit of detection (LOD) and limit of quantification(LOQ) were evaluated by considering the impurities concentration that would yield a signal–to–noise(S/N) ratios of 3:1 and 10:1 respectively. The LOD and LOQ values were experimentally verified by sixinjections of standard solutions of the compounds at the determined concentrations. Recovery of themethod was performed by standard addition method to evaluate accuracy and specificity. Accordingly,the accuracy of the method was determined by spiking at LOQ, 50 %,100 % and 150 % limit conc. of impurities. Stability of the impurities insample solution was done by analyzing spiked sample solution at different time intervals at roomtemperature. 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 achieve efficient separation between fenspiride,its related three impurities and its degradation products.New developed method would beease to handle, sensitive, precise, cost effective, accurate, robust and stability indicating.

Column Selection and Separation

The main objective of the present study was to achieve better separation among the closely eluting three impurities in fenspiride with symmetrical peak shapes, and the method should be able to determine all theimpurities in a single run. Moreover, the developed method should be linear, 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 Pheneyl-hexyl (2.0mm x100 mm)1.7 µm and YMC Triart C18, 100 mm x 2.0 mm, 1.9 µm), using gradient elution. Using the above columns theseparation of the impurities was not satisfactory. On Acquity UPLC BEH C18 column (100 mm x 2.1mm, 1.7 µm) separation and response for all the three impurities were found to be good. On this columnthe impurities were well retained and separated from the fenspiride drug substance peak.

The three impurities of fenspiride were subjected to separation by reversed-phase LC onWaters Acquity BEH C18 (100 mm x 2.1 mm, 1.7 µm) column with 0.1 % methane sulfonic acid mobile phase- A and homogeneous mixture of purified water and acetonitrile (80:20, v/v) as mobile phase- B, in this case impurities By changing the mobile phase- B composition to 50:50 ratio all the impurities were eluted withdesired peak shape as well as from the drug substance. Both isocratic and gradient elution modes wereevaluated during the optimization, among which gradient elution was observed to be more efficient inachieving optimum separation of all the three impurities from the drug substance.

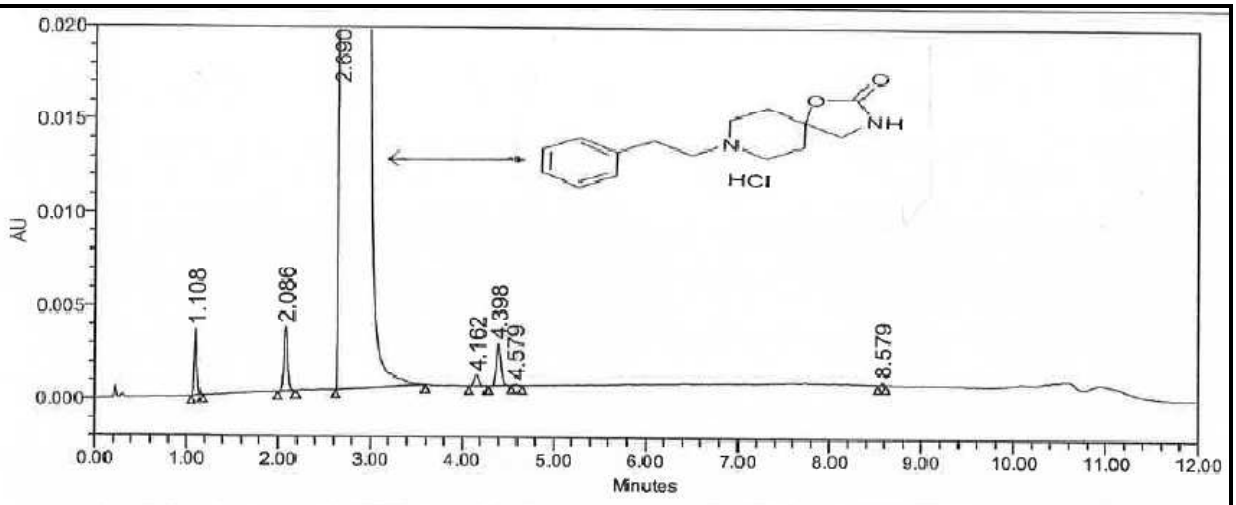
Method Validation

The developed new method was successfully validated by standard procedure to ensure adequate selectivity,sensitivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, solution stability robustness and forced degradation study(9,10,11).

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.

The ability of an analytical method to unequivocally assess the impurities in presence of the othercomponents can be demonstrated by evaluating the specificity of themethod. The specificity of method was verified by injecting a blank,individual impurity samples andspiking of impurity with its limit concentration in sample. No interfering peak was observed for any ofthe 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	1.108	7233	0.15		6021	1.08	0.968	1.461
2	Impurity-C	2.086	10093	0.21	14.38	11179	1.05	1.155	1.577
3	Fenspiride	2.690	4842628	99.42	3.07	1168	3.88	2.311	0.408
4	Impurity-B1	4.162	2216	0.05	6.94	23062	0.95	6.605	8.112
5	Impurity-B2	4.398	8293	0.17	2.19	27511	1.07	1.909	2.377
6		4.579	166	0.00	1.93	51176	1.41	43.553	90.000
7		8.579	319	0.01	57.55	349651	0.86	18.297	30.097
Sum			4870949	100.00					

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

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified. The limit of detection is frequently misguided with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass. In chromatography, the detection limit is the injected amount that results in a peak with a height at least two or three times as high as the baseline noise level.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and or degradation products. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. Furthermore this

signal/noise method describes three more methods (ICH) such as Visual inspection, Standard deviation of the response based on the standard deviation of the blank and Standard deviation of the response based on the slope of the calibration curve. From slope and residual standard deviation of regression line, limit of quantitation and limit of detection curve was plotted. The LOD, LOQ Concentration determined by S/N Ratio.LOD and LOQ of impurities were evaluated by injecting a series of dilute solutions of known concentration. Precision was also determined at LOD andLOQ levels by analyzing six individual preparations of all impurities and fenspiride, calculating their %RSDof the peak area for each impurity (Table- 1).

Linearity

The linearity of all the impurities was not upto the standard demonstrated with a nine point calibration graph between LOQ – 300% of specification limit with respect to the sample concentration. The average peak areas were plotted against the concentration of the analyte, resulting in a linear plot. The regression line was obtained by the least squares method, and expressed by the correlation coefficient (r) shown in Table-1.The result showed an excellent correlation between the peak and concentration of all impurities.

Relative response factor

Relative response factor (RRF) is alternative method for determination of quantity of impurities present in pharmaceutical products and amount of impurity can be calculated with the help of peak area of components. Relative response factor is the ratio of the response of impurity and active pharmaceutical ingredient (API) under the identical chromatographic conditions (Column oven temp, Flow rate Mobile phase)

$$\text{Relative response factor (RRF)} = \frac{\text{Response factor of Impurity}}{\text{Response factor of API}}$$

Response factor are different for different products and should be determined by individual products. RRF determined on a particular detector cannot be used in the calculation of product determine on the other detector because different detector shows different response of same Compound.

Relative Response Factor (RRF) is a common analytical parameter frequently used in many chromatographic procedures. The parameter is critical for quantitative or limit tests for impurities because in many cases the corresponding reference standards are not available. The RRF parameter is sensitive to the experimental parameters and hence the value changes on deviating the chromatographic conditions. While establishing the RRF, one should follow the exact method conditions without deviating from the original.

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.

The precision of the method was verified by repeatability and intermediate precision. Repeatability waschecked by injecting six individual preparations of fenspiride samplespiked with specification level of known impurities. The intermediate precision of the method was determined by performing theanalysis on different days, different analyst, different instrument and different column. %RSD area of for each impurity was

calculated for both repeatability as wellas intermediate precision. The %RSD was found to be less than 2.0% in both the cases, these resultsconfirmed the overall precision of the method (Table- 1).

Table- 1: Linearity, LOD, LOQ and Precision data of Impurity-A, Impurity-B, Impurity-C with respect to test concentration of fenspiride

Parameter	Impurity-A	Impurity-B	Impurity-C
Linearity range (% Conc.wrt .test prep.) slope r	 0.014-0.430 2532 0.9999	 0.019-0.575 2253 0.9999	 0.014-0.445 3305 0.9999
LOD Precision at LOQ	0.005 0.014	0.006 0.019	0.005 0.014
RRF	0.49	1.04	0.71
Precision Repeatability (n=6) % RSD	 0.140 1.07	 0.192 0.63	 0.148 0.54
Intermediate precision (n=6) % RSD	 0.139 1.08	 0.200 0.90	 0.146 0.89

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 Quantitative approaches, at least nine determinations across the specified range should be obtained, for example, three replicates at three concentration levels each. The percentage recovery or the difference between the mean and the accepted true value together with the confidence intervals are recommended. Accuracy study of impurities was carried out triplicate at its LOQ level, 50 % 100 %, and 150 % of the specification level in the test preparation. Therefore, the accuracy of the method in the range of LOQ-150% wasconfirmed by the recovery data which was shown in Table-2

Table- 2: Accuracy data of fenspiride spiked with its impurities at LOQ, to 150 % limit levelconcentrations.

Theoretical conc. (% Conc. wrt. Test preparation)	Impurity-A	Impurity-B	Impurity-C
Accuracy at LOQ level (n = 3)			
Amount added	0.014	0.019	0.015
Amount recovered	0.014	0.020	0.016
% recovery	97.62	108.77	104.45
% RSD	4.42	5.59	3.69

Accuracy at 50 % conc. (n=3)			
Amount added	0.072	0.096	0.074
Amount recovered	0.071	0.097	0.074
% recovery	98.15	101.39	100.45
Accuracy at 100 % conc. (n=3)			
Amount added	0.143	0.190	0.147
Amount recovered	0.139	0.192	0.148
% recovery	97.21	101.05	100.45
% RSD	0.70	0.52	0.39
Accuracy at 150 % conc. (n=3)			
Amount added	0.213	0.285	0.220
Amount recovered	0.211	0.286	0.222
% recovery	98.91	100.23	100.91
% RSD	0.28	0.53	0.28

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 assay and related substances analysis method, experimental condition were purposely altered and % related impurities was calculated. Flow rate of mobile phase was 0.6 mL /minute. The flow rate was changed by 10 % .The flow rate is 0.54 mL /minute and 0.66 mL /minute. The column oven temperature changes 5°C with experimental condition. The column oven temperature was 65°C and 55°C.

In all purposely varied chromatographic conditions (Flow rate, column oven temperature), no significant change in the related impurities values. The system suitability criteria like tailing factor, theoretical plates and % RSD value are well within acceptance criteria.

Hence it is conclude that method is robust with respect to flow and column oven temp for the assay and related substances analysis of Fenspiride.

Stability in Solution and in the Mobile Phase

No significant changes in the concentrations of all impurities were observed during solution stabilityexperiments. The percentage recoveries of standard solutions at different time intervals were within therange of 98.98-100.51 % of their nominal values. The results from the solution stability experimentconfirmed that standard 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 spiked with its impurities at limit level concentration.

Theoretical conc. (ppm)	Spiked test (Fresh)	Spiked test (1 day)	Spiked test (2 days)	Spiked test (3 days)
Impurity-A				
Theoretical conc. (In %)	0.132	0.132	0.132	0.132
Measured conc. (In %)	0.132	0.131	0.131	0.131
% recovery	100.00	99.24	99.24	99.24
Impurity-B				
Theoretical conc. (In %)	0.195	0.195	0.195	0.195
Measured conc. (In %)	0.195	0.196	0.194	0.196
%recovery	100.00	100.51	98.98	100.51
Impurity-C				
Theoretical conc. (In %)	0.147	0.147	0.147	0.147
Measured conc. (In %)	0.147	0.147	0.146	0.147
%recovery	100.00	100.00	99.32	100.00

Forced degradation Study:

Forced degradation study was also performed on Fenspiride to provide an indication of the stability indicating property.% of 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 in related substances analysis, test sample exposed to 5mL 5N HCI, Heat the solution at 80°C for 24 Hrs. after 24 Hrs cooled it and neutralized with alkali. In Fenspiride acid degradation, hydrochloric acid interact with Fenspiride and degradation product is observed. Impurity-A, is the degradation product of the Acid degradation. In this condition 20.96 % degradation observed and Impurity –A,major degradant and all degradants are known impurities.

In alkali degradation study in related substances analysis, test sample exposed to 5 mL 1N NaoH, heat the solution at 80°C for 5 min after 5 min cooled it and neutralized with acid. In Fenspiride acid degradation, sodium hydroxide interacts with Fenspiride and degradation product is observed. Impurity-A, is the degradation product of the Acid degradation. In this condition 17.36 % degradation observed and Impurity –A,is major degradants and all degradants is known impurity.

In peroxide degradation study in related substances method, test sample exposed to 5mL 50% H2O2(12) Kept for 5 hours at 80°C. In Fenspiride peroxide degradation, peroxide interact with Fenspiride and degradation product is observed. Impurity-B, is the degradation product of the peroxide degradation and Impurity-A is major degradants. In this condition 12.02% degradation observed and all degradants are known impurities.

Table:-4 Summary of forced degradation study of fenspiride.

Stress Condition	Exposure period	% Purity	% degradation
Untreated Test Preparation	-	99.97	---
Humidity degradation	40°C, 75% RH for 24 Hrs.	99.97	---
Thermal degradation	105°C for 24 Hrs.	99.97	---
Photolytic degradation	Light energy of 1.2 million lux hours and near UV 200 watt hrs./m^2	99.97	---
Aqueous degradation	5 mL 24 hrs at room temperature.	99.97	---
Acid Degradation	5.0 mL 5.0 M HCI kept at 80°C on oil bath for 24 Hours	79.04	20.96
Alkali Degradation	5.0 mL 1.0 M NaoH kept at 80°C on oil bath for 5 min.,	82.61	17.36
Peroxide degradation	5 mL 50% H_2O_2kept at 80°C on oil bath for 11 min.	87.98	12.02

CONCLUSION

A new,accurate, linear, stability indicating and selective UPLC method was proposed for the determination of related substances 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, cost effective,stability indicating and robust towards plant. The developed UPLC method is stability indicating and used for routine analysis of fenspiride at commercial scale.

ACKNOWLEDGEMENT:

The Author wish to thanks to Dr. Mukund Gurjar and Mr. Samit Mehta Emcure Pharmaceuticals Limited, Analytical research Centre, Hinjwadi, Pune for their support encouragement and permitting this work to communication for publication. Authors also wish to thanks the Analytical Method Validation and Development department in Emcure Pharmaceuticals Limited, Analytical research centre, Hinjewadi, Pune to provide the co-operation and valuable guideline.

REFERENCES

1. Sauveur, C;Baune A, Vergnes N, Jeanniot JP (1989) “The determination of fenspiride in human plasma and

urine by liquid chromatography with electrochemical or ultraviolet detection". *J Pharm Biomed Anal 7: 1799-*
1804.
2. Montes B, Catalan M, Roces A, Jeanniot JP, Honorato JM (1993) "Single dose pharmacokinetics of fenspiride
hydrochloride: phase I clinical trial". *Eur J ClinPharmacol 45: 169-172.*
3. Dumasia MC, Houghton E, Hyde W, Greulich D, Nelson T, et al. (2002) "Detection of fenspiride and
identification of in vivo metabolites in horse body fluids by capillary gas chromatography-mass spectrometry:
administration, biotransformation and urinary excretion after a single oral dose". *J Chromatogr B AnalytTechnol*
Biomed Life Sci 767: 131-144.
4. Stanley SM, Foo HC (2006) "Screening for basic drugs in equine urine using direct-injection differential-
gradient LC-LC coupled to hybrid tandem MS/MS". *J Chromatogr B AnalytTechnol Biomed Life Sci 836: 1-14.*5. Van Thuyne W, Van Eenoo P, Delbeke FT (2007) "Comprehensive screening method for the qualitative
detection of narcotics and stimulants using single step derivatisation". *J Chromatogr B AnalytTechnol Biomed*
Life Sci 857: 259- 265.
6. Medvedovici A, Udrescu S, Albu F, Tache F, David V (2011) "Large-volume injection of sample diluents not
miscible with the mobile phase as an alternative approach in sample preparation for bioanalysis: an application
for fenspiride bioequivalence". *Bioanalysis 3: 1935-1947.*
7. Pidpruzhnykov YV, Sabko VE, Iurchenko VV, Zupanets IA (2013) UPLC-MS/MS Quantification of Fenspiride
in Human Plasma. *J BioequivAvailab 5: 185- 190.*
8. United States Pharmacopeia 34 ,National Formulary 29United States pharmacopoeial convention 2011 :3602-
3605
9. A, Biksham Babu, A, Ramu, S,Venkatarao, S,Neeharika, *(2011.) Rasayan J. Chem., 4(2), 336*
10.V. KalyanChakravarthy, D,GowriSankar*(2012)*, *Rasayan J. Chem., 5(3), 293.*
11. V. KalyanChakravarthy, D,*GowriSankar (2012).Rasayan J. Chem., 5(1), 90*
12.Essam F. A, Al-Jumaily, Maha H.A, Al-Bahrani*(2012)OIIR journal, II(III), May-June 03-13*