Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(5):115-122
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Stability indicating HPLC method for the quantification of fesoterodine fumarate and its related substances

Narasimha Reddy Kotla*[ab], Nagaraju Ch. V. S.[a], Rajan S. T.[a], Eshwaraiah S.[a], Sampath Kumar Reddy G.[b], Rakesh M.[a] and Chakravarthy I. E.[b]

[a]*R&D Center, MSN Laboratories Pvt. Limited, Plot No. 12, Phase-IV, Pashmylaram, Hyderabad, AP, India*
[b]*Rayalaseema University, Kurnool, Andhra Pradesh, India*

ABSTRACT

A reverse phase liquid chromatographic (RP-LC) method was developed for the quantification of the related impurities of Fesoterodine Fumarate drug substance. The method was optimized using buffer (prepared by dissolving 3mL H_3PO_4 (85%) and 1.0gm 1-Octane sulphonoic acid sodium salt anhydrous taken in 1000mL milli-Q-water and then pH was adjusted to ~ 7.2 with dilute potassium hydroxide solution) taken along with Acetonitrile 60:40v/v as mobilephase-A, and Acetonitrile: water in the ratio of 90:10 v/v as mobile phase-B. The flow rate was set at 1.2 mL min^{-1}, wavelength at 220nm respectively and the column temperature was maintained at 45°C. The capability of stability indicating method developed was demonstrated by studying the degradation products generated during the forced degradation studies under the following conditions i) water hydrolysis, ii) at 75% relative humidity, iii) oxidative, iv) thermal v) photolytic, vi) acid, vii) base, and viii) photolytic degradation. The developed method can be used for the determination of synthetic and degradation impurities in the regular quality control analysis for the drug substance.

Keywords: Fesoterodine Fumarate, Reverse phase liquid chromatography, Stability-indicating methods, method development, Method validation, Stress conditions, ICH.
MSNRD Communication No.011

INTRODUCTION

The biological activity of chiral substances often depends upon their stereochemistry. A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective differences in their pharmacokinetics and pharmacodynamics [1-3]. Analysis of the enantiomeric purity of chiral drug candidates has become very important particularly in the pharmaceutical and biological fields, because few enantiomers of racemic drugs have relatively different pharmacokinetic properties and diverse pharmacological or toxicological effects [4-7]. apart from this the International Conference on Harmonization (ICH) guide-lines [8-10] emphasizes that the purity and assay of drug susceptible to change during storage, must be determined by using validated stability- indicating methods, which can selectively determine the drug in presence of its process (including the other isomers) and degradation impurities. Fesoterodine Fumarate is a new antimuscarinic agent developed for the treatment of overactive bladder [11-14]. Fesoterodine itself is inactive and is rapidly and extensively converted by ubiquitous esterases to its principal active moiety,5-hydroxymethyl tolterodine (5-HMT)[15]. 5-HMT is formed via biotransformation of both Fesoterodine and tolterodine, albeit by different metabolising enzymes, viz. esterases and CYP2D6 respectively [16-19]. Fesoterodine Fumarate is commercially

available under the brand name of Toviaz. Chemically, Fesoterodine Fumarate is designated as iso butyric acid 2-((R)-3-isopropylammonium-1-phenylpropyl)-4-(hydroxymethyl) phenyl ester hydrogen Fumarate. The empirical formula is $C_{30}H_{41}NO_7$ and its molecular weight is 527.66 and the chemical structure of Fesoterodine Fumarate is shown in (Fig.1).

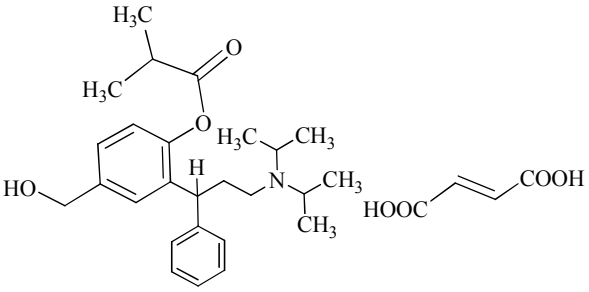

Fig-1
Fesoterodine Fumarate
Isobutyric acid 2-((R)-3-diisopropylammonium-1- phenylpropyl)-
4- (hydroxymethyl)phenyl ester hydrogen fumarate

Recently, a stability-indicating liquid chromatography (LC) method was developed and validated for determination of Fesoterodine in commercial tablet dosage forms using a monolithic column [20]. Moreover, for the fast determination of the drug in tablets with very low levels of residues produced, validated a specific and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was disclosed by Sangoi, M.S [21]. A UV spectrophotometry method was published for determination of Fesoterodine in Extended Release Tablets [22]. A liquid chromatographic method for the determination of chiral purity [23] and stability indicating HPLC assay method for determination of Fesoterodine Fumarate bulk drugs methods was published [24]. However extensive survey revealed that stability indicating HPLC method for quantitative determination of Fesoterodine Fumarate and its related impurities in active pharmaceutical ingredient was not reported till date. Therefore it was felt necessary to develop an accurate, rapid, and specific stability indicating method for the determination of 'Related substances' of Fesoterodine Fumarate. We have developed a new accurate and stability indicating HPLC method for the determining the 'Related substances' of Fesoterodine Fumarate.

MATERIALS AND METHODS

Chemicals and Reagents

Fesoterodine fumarate and its impurities viz. Diol impurity, Propionate impurity, Toluoyl hydroxy impurity, Double bond impurity and Benzyloxy impurities were obtained from MSN Laboratories Private Limited, Hyderabad, India. HPLC-grade of acetonitrile and AR grade of potassium di hydrogen phosphate, ortho phosphoric acid, potassium hydroxide, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Rankem, New Delhi, India. Milli Q Millipore (USA) purification system was used to prepare high pure water.

HPLC Instrumentation and Conditions

The method development attempts, forced degradation studies and the method validation was performed in Agilent 1200 series LC systems with a diode array and variable wave length detectors (Agilent Technologies, Waldbronn, Germany). The data were collected and processed using Ez chrom Elite software. The peak homogeneity was studied by using Agilent 1200 series DAD detector.

Chromatographic conditions

The chromatographic separation was optimized in the Inertsustain C-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved with the buffer (3.0mL H3PO4 (85%) and 1.0gm 1-Octane sulphonoic acid sodium salt anhydrous in 1000mL of milli Q water (100%) and pH adjusted to7.2 with diluted potassium hydroxide solution) and Acetonitrile 60:40v/v as mobilephase-A, and acetonitrile: water in the ratio of 90:10 (*v/v)* as mobile phase B. The HPLC gradient program was set as (time/% mobile phase- B) 0.01/5, 14/19, 35/70, 45/70, 45.5/5, 55/5. The flow rate of the mobile phase and the column temperature and auto sampler temperature was set as 1.2 mL min^{-1} and 45°C and 5°C. The detection wave length was optimized at 220 nm, 20µL

injection volume. A mixture of Mobile phase-A was used as diluent, and Acetonitrile: Methanol 1:1v/v used for needle wash purpose.

Preparation of standard solutions:
Buffer and Acetonitrile in the ratio of 60:40v/v was used as diluent. A standard solution (Reference solution) 0.001mg/mL of Fesoterodine Fumarate solution was prepared in the diluent. A stock solution with the blend of Diol impurity, Propionate impurity, Toluoyl hydroxy impurity, double bond impurity and benzyloxy impurity was also prepared in diluent for the preparation of system suitability solution (0.15% solution with respect to 1.0mg/mL Fesoterodine Fumarate test concentration).

RESULTS AND DISCUSSION

Method development and optimization
The HPLC method was optimized so as to obtain stability– indicating method that it could resolve degradation impurities from Fesoterodine Fumarate. Different stationary phases with different selectivity were used for the determination of Fesoterodine Fumarate and it's impurities as the initial attempts. However good peak shape with less peak width and the resolution of all the related impurities were achieved satisfactorily in Inert-sustain C-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved a buffer (3.0mL H3PO4 and 1.0 gm 1-Octane sulphonoic acid sodium salt anhydrous in 1000mL of milli Q water (100%) and pH adjusted to 7.2 with diluted potassium hydroxide solution) and Acetonitrile 60:40v/v as mobile phase A, and acetonitrile: water in the ratio of 90:10 (*v/v)* as mobile phase B. The HPLC gradient program was set as time/% mobile phase- B: 0.01/5, 14/19, 35/70, 45/70, 45.5/5, 55/5. The flow rate of the mobile phase and the column temperature and auto sampler Temperature was set as 1.2 mL min^{-1} and 45°C and 5°C. The detection wave length was optimized at 220nm, 20µL injection volume. A mixture of Mobile phase-A was used as diluent, and Acetonitrile: Methanol 1:1v/v used for needle wash purpose.

The system suitability parameters are resolution between Double bond impurity and Fesoterodine should not be less than 1.5, detector sensitivity for reference solution the S/N ratio should not be less than 30 and tailing factor not more than 2.0. The developed method is specific for Fesoterodine Fumarate and its degradation products. The structures and chemical names of the impurities are given below:

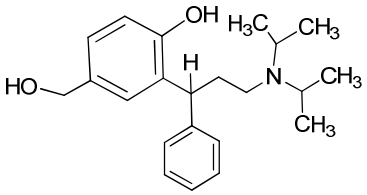

Fig-2 Diol impurity
(R)-2-(3-diisopropylamino-1-phenyl propyl) 4-hydroxy methyl phenol

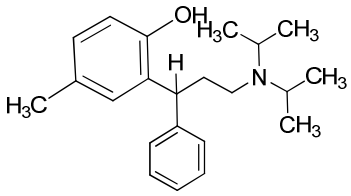

Fig-4 Toluoyl hydroxy impurity
(R)-2-[3-[bis(1-methylethyl)-amino] 1-phenylpropyl]-4-methylphenol

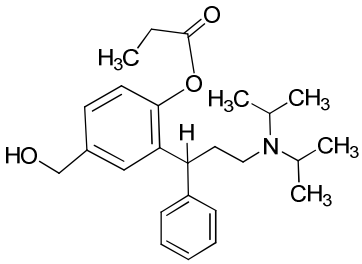

Fig-3 Propionate impurity
(R)-2-(3-(diisopropylamino)-1-phenyl propyl)-4-(hydroxymethyl)phenyl propionate

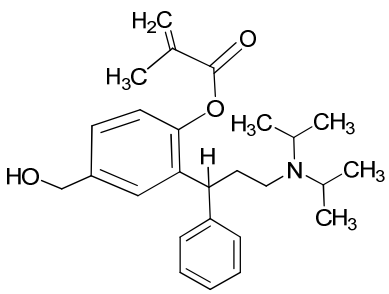

Fig-5 Double bond impurity
(R)-2-(3-diisopropylamino)-1-phenylpropyl)-4-(hydroxymethyl)phenylmethacrylate

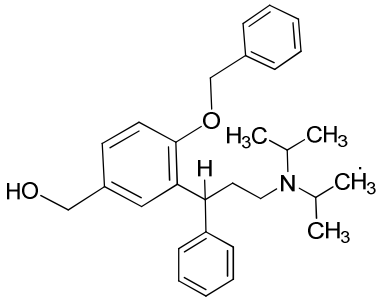

Fig-6 Benzyloxy impurity
(R)-[4-benzyloxy-3-(3-diisopropylamino-1-phenyl propyl)-phenyl] methanol

Chromatograms: A typical chromatogram of Blank, System suitability solution, Reference solution, mother sample are given below

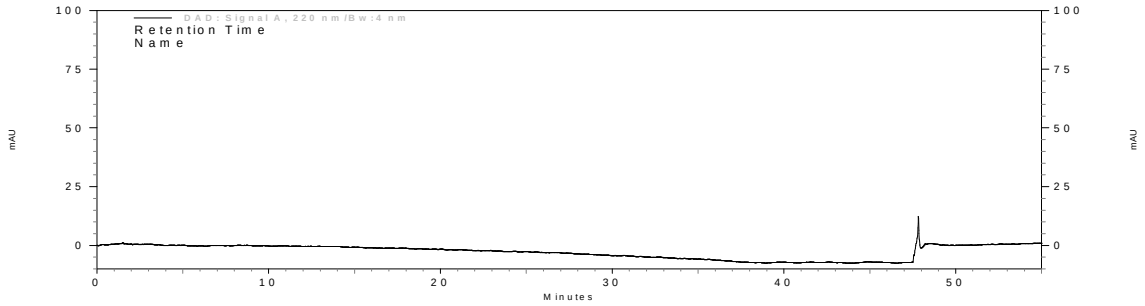

Fig 7: Diluent

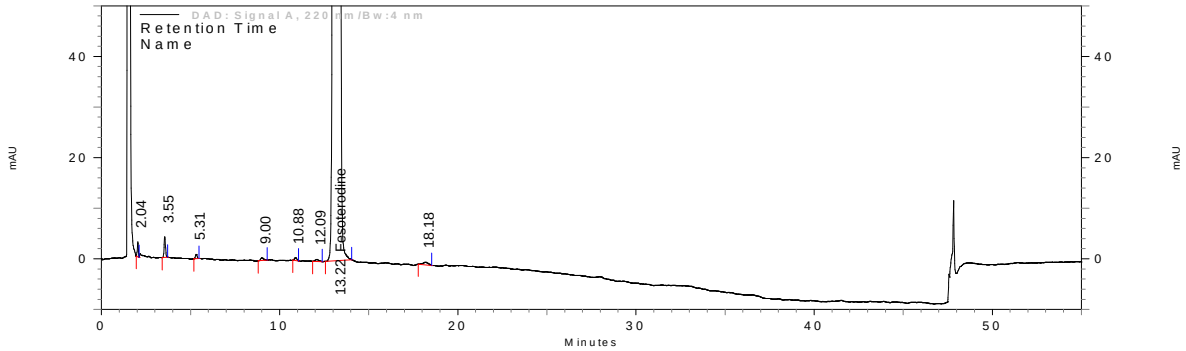

Fig 8: Mother solution

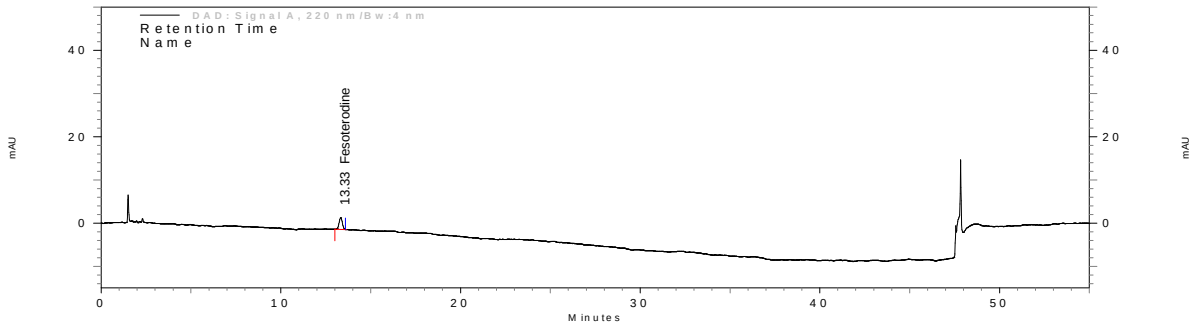

Fig 9: Reference solution

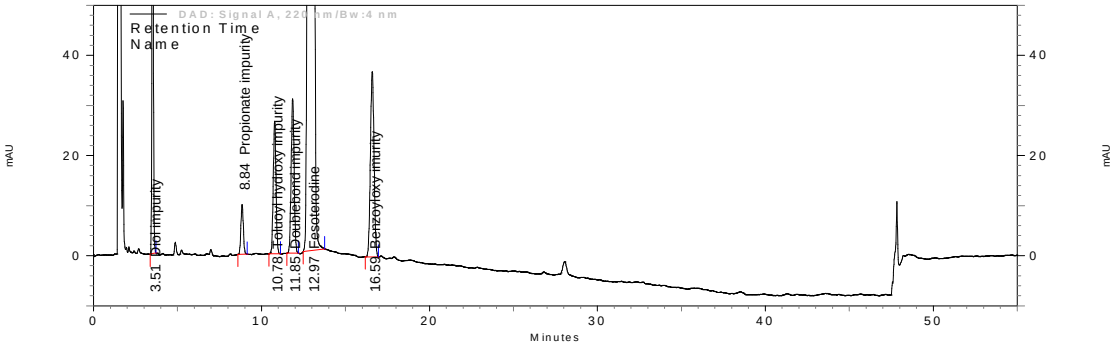

Fig10: 0.15% all impurities spiked to Fesoterodine Fumarate sample

Method validation Results

The developed method was validated as per ICH guidelines and the results are given (Table I). Stress testing of the drug substance can help to identify the degradants, which in turn help to evaluate the stability-indicating nature of the developed method. The specificity of the developed HPLC method for Fesoterodine Fumarate was determined in the presence of its process and degradation impurities. All the stressed samples of Fesoterodine Fumarate and all degradation impurities were well resolved from one another and from Fesoterodine Fumarate. The analysis was carried out by HPLC with Diod array detector. The chromatographic peak purity tool, applied to Fesoterodine Fumarate and its impurities peaks, demonstrated that all the peaks were pure in all cases conform the absence of other impurities co-eluting in the same retention time and there by signifying the specificity and stability indicating nature of the method. The detection limit (DL) and quantification limit (QL) for Diol impurity, Propionate impurity, Toluoyl hydroxyl impurity, Double impurity and Benzyloxy impurity were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of Diol impurity, Propionate impurity, Toluoyl hydroxyl impurity, Double impurity and Benzyloxy impurity. Linearity test solutions for purity determination were at six concentration levels from QL to 150 % of the specification level (0.15%). Peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 50%, 75%, 100%, 125% and 150% level with respect to specification 0.15%. The percentages of recoveries for impurities were calculated.

Table-1: Validation data of the developed method

Parameter	Diol imp.	Propionate imp.	Toluoylhydroxy imp.	Double bond imp.	Benzyl oxy imp.	Fesoterodine
DL (%)	0.0008	0.00195	0.0024	0.0018	0.00155	0.0020
QL (%)	0.0027	0.0075	0.0085	0.0060	0.0066	0.0080
Method Precision (%RSD)#	0.83	0.65	0.67	1.73	1.27	---
Intermediate precision (%RSD)#	0.23	0.44	0.27	0.24	0.27	---
Accuracy[a] (% recovery) at:-						
QL	102.1	101.4	101.1	100.9	99.5	
50%	100.7	100.5	100.2	100.6	100.5	
75%	101.1	101.2	101.4	100.7	100.0	
100%	100.9	100.1	100.3	100.4	99.5	
125%	101.6	101.1	100.8	101.4	99.9	
150%	100.6	100.0	101.3	101.2	100.1	

[a] *Carried at QL,50%,75%, 100% and 150% level with respect to specification (0.15%)*

The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Fesoterodine Fumarate, Diol impurity, Propionate impurity, Toluoyl hydroxyl impurity, Double bond impurity and Benzyloxy impurity. Flow rate was changed by $\pm$ 0.1 units, pH was varied by $\pm$ 0.2 units and column temperature was studied at 40°C and 50°C instead of 45°C and Auto sampler temperature 10°C instead of 5°C in all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed.

Significant changes were not observed in the contents of Diol impurity, Propionate impurity, Toluoyl hydroxyl impurity, Double impurity and Benzyloxy impurity. The stability data confirmed that sample solutions were stable up to 48hrs. The system suitability was established in terms of resolution between Double bond impurity and Fesoterodine Fumarate which was more than 1.5, when a 1.0mg/ml Fesoterodine Fumarate solution spiked with 0.15% of Diol impurity, Propionate impurity, Toluoyl hydroxyl impurity, Double impurity and Benzyloxy impurity were injected.

Forced degradation studies

The stability indicating power of the developed method was studied by conducting forced degradation studies on Fesoterodine Fumarate. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Fesoterodine Fumarate was determined in the presence of its impurities, and degradation products. Forced degradation studies were also performedon relative Humidity study stress at 75% Relative humidity for 10 days. The thermal stress was done at 60 °C for 10 days. The under photolytic stress studies conducted for 50 hours at under sunlight. The photolytic stressed studies were performed for UV Direct (200 watt hours/square meter), UV Indirect (200 watt hours/square meter), Lux direct (1.2 million LUX hours) and Lux in direct (1.2 million LUX hours). Water hydrolysis was performed for 48 hours at 60 °C. The acid stress was performed at 0.05 N HCl at the concentrated sample solution at ambient temperature for 48 hours and base stress was performed at 0.05N NaOH for 1.0 hours at ambient temperature and the oxidation stress was done using 1% hydrogen peroxide for 29 hours an ambient temperature. Stressed samples of Fesoterodine Fumarate generated were checked for peak purity of by using Agilent diod array detector (DAD). The peak purity is within the limit obtained in all stressed samples, demonstrates the analyte peak homogeneity. The Forced degradation studies results are given below (Table-2).

Table 2. Summary of forced degradation results

S.No	Stressed conditions		Duration	% of Total imp	% of Diol imp	% of Propionate imp	% of Toluoyl hydroxyl imp	% of Double bond imp	% of Benzyloxy imp
1	**Normal**		---	0.16	0.04	0.02	0.02	0.01	0.00
2	**Thermal at 60°C**		10 days	0.97	0.24	0.02	0.02	0.01	0.00
4	**At 75% Relative Humidity**		10 days	1.38	0.86	0.02	0.02	0.01	0.00
5	**Under Sunlight**		50 hours	6.87	0.32	0.02	0.02	0.04	0.05
6	***Photo Degradation***	**UV direct**	200watt hours/square meter	0.39	0.05	0.02	0.02	0.01	0.00
		UV indirect		0.21	0.05	0.02	0.02	0.01	0.00
		LUX direct	1.2 million LUX hours	0.24	0.05	0.02	0.02	0.01	0.00
		LUX indirect		0.19	0.04	0.03	0.02	0.01	0.00
7	**Acid hydrolysis (0.05N HCl at RT)**		After 48hrs	5.08	4.91	0.02	0.02	0.01	0.00
8	**Base Hydrolysis (0.05N NaOH at RT)**		After 1 hr	67.87	67.71	0.00	0.01	0.00	0.00
9	**Oxidation (1% H_2O_2 at RT)**		After 29 hrs	6.09	4.76	0.01	0.02	0.02	0.00
10	**Water Hydrolysis (at 60°C±5°C)**		After 48 hrs	0.64	0.50	0.02	0.02	0.01	0.00

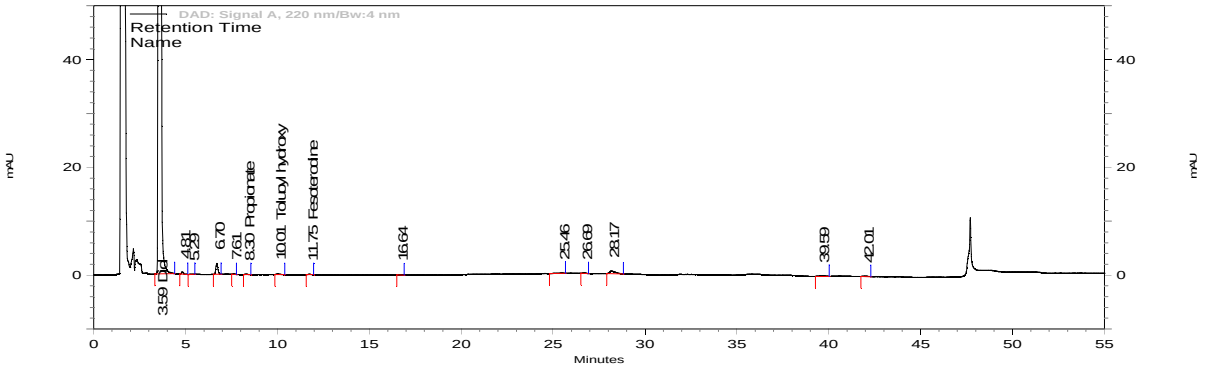

Fig 11: Typical chromatogram of 0.05N NaOH degradation solution

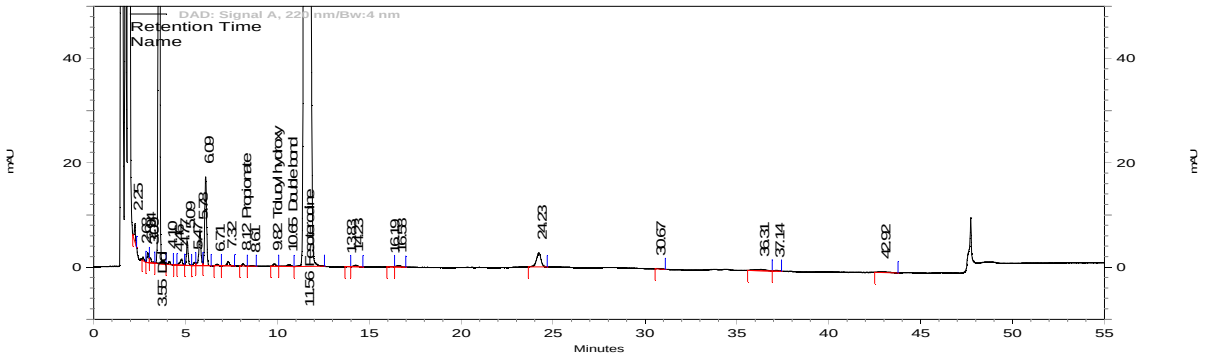

Fig12: Typical chromatogram of 1% H2O2 degradation solution

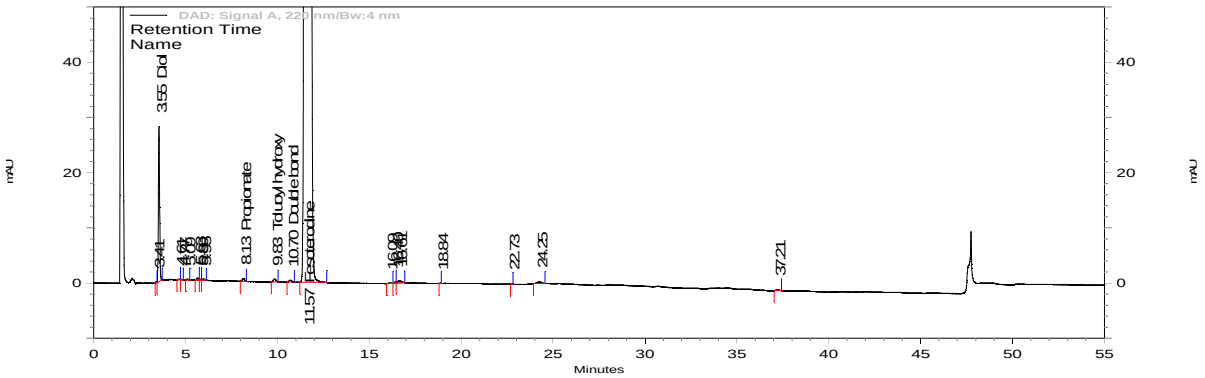

Fig 13: Typical chromatogram of degradation solution of Water at 60°.

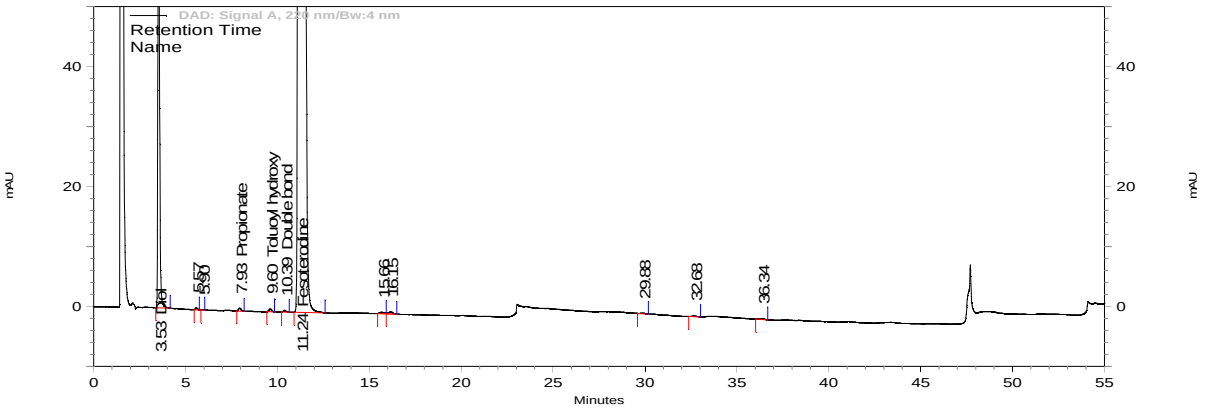

Fig 14: Typical chromatogram of 0.5N HCl degradation solution

Results of forced degradation studies

Significant degradation was observed in Fesoterodine Fumarate stressed sample that were subjected to very sensitive in Acid hydrolysis, Base hydrolysis, Peroxide hydrolysis and Sunlight degradation. Sensitive in Thermal, 75% relative humidity and Water at 60°C±5°C. And stable in Photo degradation(U.V direct and indirect, Lux direct and indirect). Peak purity test results derived from Diode array detector, confirmed by that Fesoterodine Fumarate peak is homogeneous and pure in all the analyzed stress samples. The Acid, base, Peroxide Degradation chromatograms are given below:

Solution stability and mobile phase stability

Solution stability and mobile phase stability provides an indication of its reliability during normal usage during the storage of the solutions used in the method. The solution stability of Fesoterodine Fumarate was established for 48

hours at 5°C. The solution stability studied by using Fesoterodine Fumarate sample and injected for every 12 hours. The content of impurities and Fesoterodine Fumarate were quantified at each interval up to the study period. The mobile phase stability was also established by quantifying the freshly prepared sample solutions against freshly prepared reference standard solutions for every 12 hours up to 48hrs. During the study period the prepared mobile phase was stable up to 48hrs at room temperature.

CONCLUSION

The developed stability-indicating analytical method for related substance determination of Fesoterodine Fumarate and its impurities is precise, accurate, linear and specific. The validation carried out for the method in accordance with the ICH requirements are satisfactory. The developed method can be used conveniently for the routine analysis of production samples and also to check the stability of bulk samples of Fesoterodine Fumarate during its storage. The same method can also be attempted for the drug products for the getting the information of impurities and degradation products at lower level.

Acknowledgments
One of the authors Narasimha Reddy thanks Dr. Chakravarthy. I.E, for guiding him in his PhD work. The authors wish to thanks Dr. MSN Reddy (MSN laboratories Pvt.Ltd) for providing facilities to do research work.

REFERENCES

[1] Stinson S.C., *Chiral drugs, Che. Eng. News*, **2002**, 43, 55–78.
[2] Stinson S.C., *Chiral chemistry, Chem. Eng. News*, **2001**, 45, 57.
[3] Thayer A.M., Interaction yields, *Chem. Eng. News*, **2008**, 12, 20.
[4] Ariens E.J., *Eur. J. Clin. Pharmacol*, **1984**, 26, 663.
[5] Ariens E.J., *Med. Res. Rev*, **1986**, 6, 451.
[6] Ariens E.J., Wuins E.W., *Clin. Pharmacol. Ther*. **1987**, 42, 361.
[7] FDA (Food and drug Administration) Policy Statement for the Development of New Stereoisomeric Drugs, 1992.
[8] International conference on Harmonization Guideline on stability testing of New Drug Substances and products, Q1A (R2), **2003**.
[9] International conference on Harmonization Guideline on Photo stability Testing of New Drug substances and products, Q1B, **1996.**
[10] International conference on Harmonization Guideline on Impurities in New Drug Substances and products, Q3B (R2), **2006.**
[11] Tzefos M., Dolder C., Olin J.L., Ann *Pharmacother*, **1992 (2009),** 43.
[12] Vella M., Cardozo L., *Expert Opin Drug Saf*, **2011,** 10, 805
[13] Gomelsky A., Dmochowski R.R., Drugs *Today (Barc)*, **2010**, 46, 81
[14] Ellsworth P., Berriman S. J., Brodsky M, *Am J Manag Care*, **2009,** 15, 115.
[15] McKeage K, Keating G M, *Drugs*, **2009,** 69, 731.
[16] Ney P., Pandita R.K., Newgreen D.T., Breidenbach A., Stohr T., Andersson K.E., *Bju Int.*, **2008,** 101, 1036.
[17] Nitti V.W., Dmochowski Sand P.K, Forst H.T, Hagg-Molkenteller C., Massow U., Wang J., Brodsky M., Bavendam T., *J.Urol*, **2007,** 178, 2488.
[18] Ellsworth P, Berriman S.J, Brodsky M, *Am. J. Manag. Care*, **2009,** 15, 115.
[19] Dmochowski R.R, Peters K.M, Morrow J.D, Guan Z.H, Gong F, Sun J, Siami P, Staskin, D.R, *Urology*, **2010,** 75, 62.
[20] Sangoi M.S., Todeschini V., Steppe M., *Talanta*, **2011,** 84, 1068.
[21] Sangoi M.S., Steppe M., *Eur J. Mass Spectrom (Chichester, Eng)*, **2010,** 16, 653.
[22] Sangoi, M.S., Vitor Todeschini and Martin Steppe, *Acta Chim Slov*, **2012,** 59, 136.
[23] Rajput A.P., Manohar C., Sonanis J., *Journal of Chemical and Pharmaceutical Research*, **2012**, 4(9), 4127-4133.
[24] Rami Reddy B.V., Reddy B., Sravan Kumar M., Rambabu C., *Rasayan J. Chem*, **2012,** 5(2), 239-24.