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A VALIDATED STABILITY-INDICATING HPLC ASSAY METHOD FOR DETERMINATION OF FESOTERODINE FUMARATE

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ABSTRACT

A novel stability indicating reverse-phase high performance liquid chromatographic method has been developed for quantitative determination of Fesoterodine Fumarate, new antimuscarinic agent for the treatment of overactive bladder. The chromatographic separation was achieved using an Inertsil ODS-3V (150mm × 4.6mm × 5µm) in isocratic mode employing Buffer (1.15g of Ammonium dihydrogen orthophosphate, 2.0mL Triethylamine in 1000mL of water. Adjust pH of the solution to 3.0±0.05 with Orthophosphoric acid solution) and Methanol in the ratio of 42:58(v/v) with a 1.0 mL/min flow rate was chosen. Detector wavelength monitored at 210nm. The column temperature was maintained at 30°C.Fesoterodine Fumarate was exposed to thermal, photolytic, acid, base and oxidative stress conditions. Considerable degradation of the drug substance was found to occur under acid, base and oxidative stress conditions. Peak homogeneity data of Fesoterodine Fumarate obtained by photodiode array (PDA) detection demonstrated the specificity of the method in the presence of degradants. The degradation products were well resolved from main peak of Fesoterodine Fumarate thus proved the stability, indicating power of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, precision, linearity, accuracy and robustness.Regression analysis showed correlation coefficient value greater than 0.999. Accuracy of the method was established based on the recovery obtained between 96.9% and 101.5% for Fesoterodine Fumarate.

Keywords: Development, Validation, Stability Indicating Method, Fesoterodine Fumarate, Forced degradation.

INTRODUCTION

Overactive bladder, also called urge incontinence, is caused by urinary muscle spasms that cause an urgency to urinate. An overactive bladder is a condition that results from sudden, involuntary contraction of the muscle in the wall of the urinary bladder. Overactive bladder causes a sudden and unstoppable need to urinate, even though the bladder may only contain a small amount of urine. There are several medications (anticholinergics) recommended for the treatment of overactive bladder (Darifenacin HBr, Fesoterodine Fumarate, Oxybutynin, Solifenacin Succinate, Tolterodine Tartrate and Trospium). Using these medications in conjunction with behavioral therapies has shown to increase the success rate for the treatment of overactive bladder.

Fesoterodine Fumarate (FST) is a new antimuscarinic agent developed for the treatment of overactive bladder[1-4]. Fesoterodine itself is inactive and is rapidly and extensively converted by ubiquitous esterases to its principal active moiety,5-hydroxymethyl tolterodine (5-HMT)[5]. 5-HMT (Here in referred as Impurity-A) is formed via biotransformation of both Fesoterodine and tolterodine, albeit by different metabolising enzymes, viz. esterases and CYP2D6 respectively[6-9]. Fesoterodine Fumarate is commercially available under the brand name of Toviaz. Chemically, Fesoterodine Fumarate is designated as isobutyric acid 2-((R)-3-diisopropylammonium-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 and Impurity-A is shown in (Fig.1).

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[10]. 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[11]. A UV spectrophotometry method was published for determination of Fesoterodine in Extended Release Tablets[12].

However extensive survey revealed that no stability indicating HPLC method for quantitative determination of Fesoterodine Fumarate in active pharmaceutical ingredient. Therefore it was felt necessary to develop an accurate, rapid, specific and stability indicating method for the determination of assay of Fesoterodine Fumarate.

The present ICH drug stability test guideline suggests that stress studies should be carried out on a drug substance to establish its inherent stability characteristics, leading to separation of degradation impurities and hence supporting the suitability of the proposed analytical procedure, which must be fully validated[13].

To our present knowledge we have developed a new accurate and stability indicating HPLC assay method for determination of Fesoterodine Fumarate in Bulk drugs. The main advantage of this method is simple and accurate with shorter run time.

EXPERIMENTAL

Materials

Samples of FTS reference standard, Impurity-A and test samples were received from Analytical Research and Development department of Hetero Drugs Limited, Hyderabad, India. HPLC grade Methanol was purchased from Merck, Darmstadt, Germany. Analytical reagent grade Ammonium dihydrogen orthophosphate, Orthophosphoric acid and Triethylamine were purchased from Qualizens Fine Chemicals, Mumbai, India. High pure water was prepared by using Millipore Milli 'Q' plus purification system.

Equipment

The HPLC system used for initial chromatographic development was Waters alliance HPLC (Milford, MA, USA) 2695 separation module equipped with quaternary gradient pumps, inbuilt auto injector, 270852 thermostatic compartment and 2487 UV detector. Empower chromatography manager software was used for data acquisition and system suitability calculations. Photo diode array detector was used for determining peak purity.

Chromatographic conditions

The chromatographic separation was achieved on Inertsil ODS-3V 150 x 4.6 mm, 5µm column. The mobile phase composition was the buffer (1.15g of Ammonium dihydrogen orthophosphate, 2.0mL Triethyl amine in 1000mL of water and adjusted to pH 3.0 ± 0.05 with Orthophosphoric Acid solution) and Methanol in the ratio of 42:58 (v/v). The mobile was filter and degassed through 0.22µm filter paper. The flow rate of the mobile phase was kept 1.0 mL/minute. The column temperature was maintained at 30°C and the detector wavelength was monitored at 210 nm. The injection volume was 10 µL. Mobile phase used as diluent. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

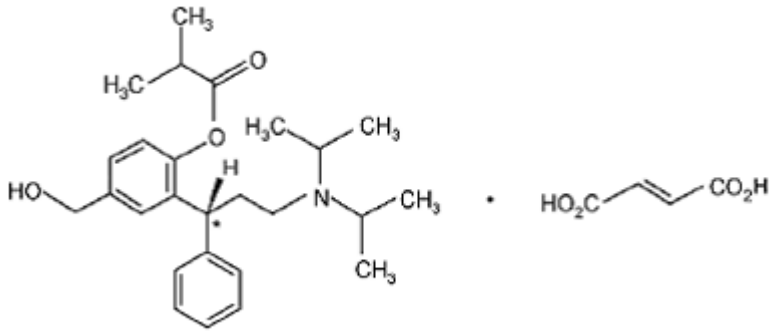
Preparation of standard solutions

Two milligrams of the standard sample were placed in 10-mL volumetric flask, dissolved and diluted to the mark with diluent. Working solution of (0.2 mg/mL) test solution was prepared by dissolving appropriate amount of test in the diluent.

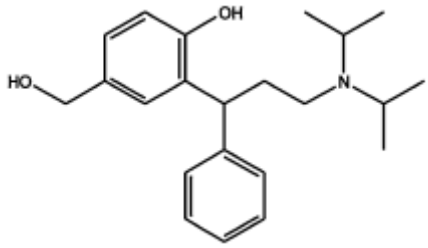
RESULTS AND DISCUSSION

Method development and optimization

The primary criteria for the development of a successful HPLC method for the determination of assay of Fesoterodine Fumarate was that the method should be able to separate impurities and degradants within shorter run time and should be accurate, reproducible, robust, indicative of stability, free of interferences from degradation products and impurities and straight forward enough for routine use in quality control laboratory. The main objective of the chromatographic method was to achieve the separation of degradation products and other known and unknown impurities of Fesoterodine Fumarate by using different stationary phases like C18, C8 and cyano, different mobile phases containing buffers like phosphate, acetate and Formate with different pH(2-7) and using organic modifiers like acetonitrile and methanol in the mobile phase. The chromatographic separation was achieved on Inertsil ODS-3V 150 x 4.6 mm, 5µm column. The system suitability parameters are USP tailing factor of not more than 2.0 and %RSD for five replicate injections of standard solution is not more than 1.0. The developed method is specific for Fesoterodine Fumarate and its degradation products.



Fesoterodine Fumarate



Fesoterodine Fumarate Impurity-A

Fig.-1: Chemical structures of Fesoterodine Fumarate and its Impurity-A

Method validation Results

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[14]. 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 performed on Fesoterodine Fumarate to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes Light 1.2 million LUX hrs (carried out as per ICH Q1B), Thermal (80°C, 7days), Acid hydrolysis (0.1N HCl, 30 minutes heating at 80°C), Base hydrolysis (0.1N NaOH, 5minutes at Ambient temperature), Water hydrolysis (8hrs heating at 80°C) and Oxidation (10% H_2O_2, 30 minutes heating at 80°C). Stressed samples of Fesoterodine Fumarate generated were checked for peak purity of by using Waters Photo-

diode array detector (PDA). The purity Angle is within the threshold limit obtained in all stressed samples, demonstrates the analyte peak homogeneity. Assay studies were carried out for stress samples against quailed reference standard and the mass balance (%assay+%impurities+%degradation products) was calculated. Typical HPLC assay chromatogram of Fesoterodine Fumarate in the developed method is shown in (Fig.2).

Precision

The system precision of the assay method was evaluated by carrying out six replicate injections of Fesoterodine Fumarate standard solution. The method precision of the assay method was evaluated by carrying out six independent assays of test sample of Fesoterodine Fumarate against qualified reference standard. The %RSD for six assay values obtained was calculated. The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory.

The %RSD for assay of Fesoterodine Fumarate during assay system precision and method precision study was within 1.0. The %RSD of assay results obtained in intermediate precision study was within 0.32% thus confirming good precision of the method.

Linearity

The Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of the analyte in the sample. Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 80 to 120% of assay analyte concentration (80, 90, 100, 110, and 120%). The peak area versus concentration data was performed by least-squares linear regression analysis.

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e.0.16-0.24mg/mL and the correlation coefficient obtained was greater than 0.999. The %RSD values for each level is within 1.0 and %Y-intercept of the calibration curve is -1.20 (within$\pm$2). These results show that an excellent correlation existed between the peak area and concentration of the analyte. The Linearity results are tabulated in Table-1.

Accuracy/Recovery

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Fesoterodine Fumarate. The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 80, 100 and 120% of the analyte concentration (0.2 mg/mL) in bulk drug sample. The percentage of recoveries was calculated.

The percentage recovery of Fesoterodine Fumarate in assay method is within the limit (Specification limit considered as, 98-102). The percentage recovery of the Fesoterodine Fumarate results listed in Table-1.

Robustness

To determine the robustness of the developed assay method, experimental conditions were purposely altered and assay content of the Fesoterodine Fumarate was evaluated. The flow rate of the mobile phase was 1.0 mL/minute. To study the effect of flow rate on the assay, it was changed by 0.2 units, from 0.80 to 1.2 mL/minute, while other mobile phase components were held constant as stated in section 2.3. The effect of change in the composition of organic modifier was checked by changing in the mobile phase composition within ± 2.0%. The effect of pH on the assay was studied by varying ± 0.2 pH units (at 2.8 and 3.2 instead of 3.0). The effect of column temperature on assay was studied at 28°C and 32°C instead of 30°C. In the all above varied conditions, the components of the mobile phase were held constant as stated in Section 2.3. In all the deliberate varied chromatographic conditions (Flow rate, pH, mobile phase composition and column temperature), no significant change in the assay value was observed. The system suitability parameters like tailing factor and the %RSD values are well within the limits, which confirm the robustness of the developed method. Robustness results data shown for assay method in Table-1.

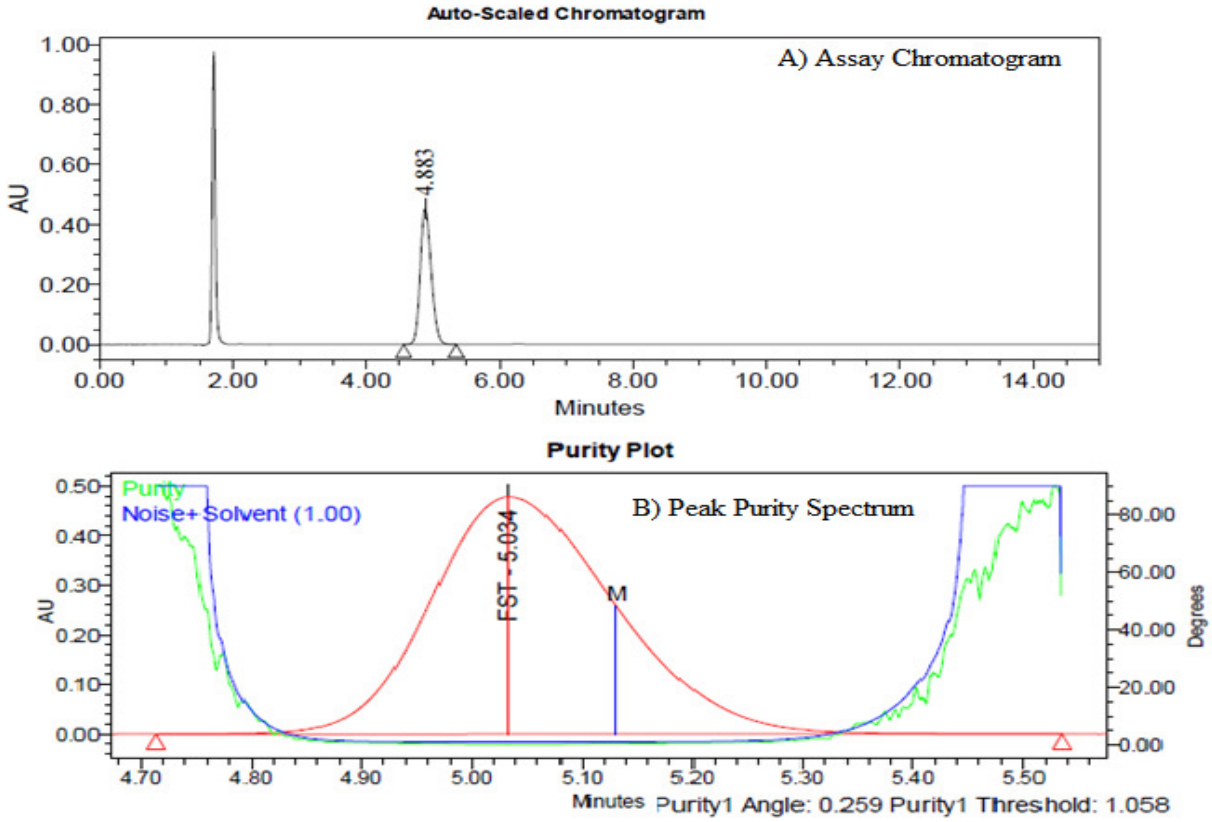


Fig.-2: Typical A) Fesoterodine Fumarate Assay Chromatogram B) FST Peak Purity Spectrum

Test Solution stability and mobile phase stability

The test solution stability of Fesoterodine Fumarate in the assay method was carried out by leaving both the test solution and reference standard in tightly capped volumetric flasks at room temperature for 12hrs and 24hrs. The same sample solutions were assayed for 6 hours interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for 6 hours interval up to 24hours. Mobile phase prepared was kept constant during the study period. The percentage recovery of assay of Fesoterodine Fumarate was calculated for the study period during mobile phase and solution stability experiments.

The %RSD of the assay of Fesoterodine Fumarate during solution stability and mobile phase stability experiments was within 1.0%. No significant changes were observed in the content of assay of Fesoterodine Fumarate during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiment data confirms that sample solutions and mobile used during the assay are stable up to 24h.

Table-1: Validation Results

Linearity Data							
Level	Concentration (X axis)(mg/mL)	Replicate-01	Replicate-02	Replicate-03	Avg. Area	STDEV	%RSD
1	0.160	4154329	4152369	4165241	4157313	6935.44	0.17
2	0.179	4626872	4652413	4663587	4647624	18820.17	0.40
3	0.199	5184899	5173568	5184578	5181015	6451.29	0.12
4	0.219	5645965	5658421	5622141	5642176	18434.45	0.33

5	0.239	6253247	6239898	6300014	6264386	31568.13	0.50
Correlation co-efficient					0.9991		
Intercept					-62273		
% Y Intercept					-1.20		
Slope(m)					26309113		
Recovery results							
Level-I(80%)			Level-II (100%)		Level-III (120%)		
100.9-101.5			99.7-100.0		96.9-97.4		
Robustness Results							
Robust Condition			%Recovery Range		Tailing Factor	Theoretical plates	
Flow rate 0.80mL/min			99.7-99.8		1.22	4806	
Flow rate 1.2mL/min			100.1-100.7		1.17	3732	
Mobile phase pH 2.8±0.05 instead of 3.0±0.05			99.9-100.6		1.18	4772	
Mobile phase pH 3.2±0.05 instead of 3.0±0.05			99.7-100.3		1.20	3868	
Mobile phase Composition 42:60 Instead of 42:58(v/v)			99.9-100.1		1.18	4199	
Mobile phase Composition 42:56 Instead of 42:58(v/v)			99.7-100.3		1.20	4250	
Column Temperature 28°C Instead of 30°C			99.7-100.1		1.19	4063	
Column Temperature 32°C Instead 30°C			99.8-100.3		1.20	4381	
Precision(%RSD)[a]			0.21				
Intermediate Precision(%RSD)[a]			0.32				

[a] Six determinations.

Table-2: Summary of forced degradation results

S.No	Stress condition	Time	%Assay of active substance	Mass Balance (%Assay+%Impurities +%degradation imp)	Remarks
1	Acid hydrolysis (0.1N HCl Heating at 80°C)	30minutes	93.7	99.8	Degradation was observed
2	Base hydrolysis (0.1N NaOH) at Ambient Temperature	5minutes	96.2	100.8	Degradation was observed
3	Oxidative (10 %H2O2)	30minutes	89.3	99.6	Degradation was observed
4	Water hydrolysis (Heating at 80°C)	4hours	99.6	100.7	No Degradation was observed
5	Thermal (105°C)	Week days	99.3	100.1	No Degradation was observed
6	Ambient sample (25 ± 2°C)	---	99.8	100.5	No Degradation was observed
7	Light (Photolytic)	1.2 million Lux Hrs &	99.2	99.9	No Degradation was observed

		200 Watt-Hours/Sq.mts			

Results of forced degradation studies

Significant degradation was not observed in Fesoterodine Fumarate stressed sample that were subjected to Light, Heat and Hydrolysis. The degradation of drug substance was observed under acid, base and oxidative stress conditions. Peak purity test results derived from photo diode array detector (Fig.2), confirmed by that Fesoterodine Fumarate peak is homogeneous and pure in all the analyzed stress samples. The mass balance of samples (Table-2) was closed to 100.5%. The %assay of Fesoterodine Fumarate is unaffected in the presence of impurities and its degradation products, confirm the stability indicating power of the developed method. The Acid, base, Peroxide Degradation chromatograms are shown in (Fig.3).

CONCLUSIONS

The newly developed RP-HPLC isocratic method for determination of Fesoterodine Fumarate assay in bulk active pharmaceutical ingredients was found to be specific, Precise, accurate and robust. The stability indicating nature of the proposed method was established by performing forced degradation, which provided degradation behavior of Fesoterodine Fumarate under various conditions. The proposed method was completely validated as per ICH guidelines. The method validation data showing satisfactory results for all the method parameters tested. Hence the developed HPLC method is stability indicating and can be used for routine analysis of production samples and also to check the stability of bulk samples of Fesoterodine Fumarate.

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