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**Research Article**

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# Stability Indicating HPLC Method for the Enantiomeric Separation of Fesoterodine Fumarate in Drug Product and Drug Substance Using Chiral Stationary Phase

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**ABSTRACT**

*A chiral liquid chromatographic method was developed for the enantiomeric purity of Fesoterodine Fumarate in drug substance as well as in drug product. The chromatographic separation was achieved on Chiralpak IC-3, column using a mobile phase system consisting of n-hexane, isopropyl alcohol and diethyl amine in the ratio of 950:50:1 (v/v/v). The mobile phase was pumped through column at the flow rate of 1 mL min$^{-1}$. Addition of diethyl amine in the mobile phase enhanced chromatographic efficiency and resolution between the enantiomers. The resolution between the enantiomers was found to be more than three. The developed method was subsequently validated and proved to be accurate, specific and precise. The experimentally established limit of detection and quantification for (S)-enantiomer of Fesoterodine were found to be 0.509 µg mL$^{-1}$ and 1.316 µg mL$^{-1}$ respectively for 20 µl injection volumes. The percentage recoveries of (S)-enantiomer was ranged between 95 to 105 % in drug product as well as in drug substance. The proposed method was found to be suitable and accurate for the quantitative determination of chiral purity of Fesoterodine Fumarate in drugs substance as well as in drug product.*

**Keywords:** Fesoterodine Fumarate, Chiral Purity, Normal Phase, Development, Validation

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## 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].

Enantiomeric separations have acquired importance in all stages of drug development and commercialization process. Therefore, the development of new methods for efficient chiral separations mainly based on HPLC, capillary electrophoresis (CE) or gas chromatography (GC) is more than necessary. The chromatographic separation of enantiomers using high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) is one of the most useful and popular techniques for enantiopurity analysis in pharmaceutical preparations and biological

fluids [8-10]. Hence our focus is for the chiral separation of final drug substance as well as drug product and at intermediate stage. Our previous work has been reported for the determination of chiral purity at intermediate stage [11].

Antimuscarinic medications are the first-line pharmacotherapy for overactive bladder (OAB) and tolterodine has been the treatment of choice for many patients. However, optimal efficacy may be difficult to achieve because of tolterodine's limited dosing options. Fesoterodine Fumarate 2-[(1R) - 3- [bis (propan-2-yl) amino]-1 -phenylpropyl]-4- (hydroxymethyl) phenyl 2-methylpropanoate, a nonselective oral antimuscarinic with the same active metabolite as tolterodine, provides an alternative option [12].

The thorough literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these two compounds under investigation for the determination chiral purity. However few articles are available for the determination of Fesoterodine Fumarate using LC-MS [13] and its related substance using LC-MS [14]. Hence it is desirable to develop a liquid chromatographic method for the determination of chiral purity of Fesoterodine Fumarate in final drug substance as well as in drug product which serves a reliable, accurate, sensitive and stability indicating.

## EXPERIMENTAL SECTION

### Chemicals

Fesoterodine Fumarate and its respective (S)-enantiomers were kindly supplied by Research and Development department of Wockhardt Limited, Aurangabad, India, and the chemical structure is shown in figure-1. HPLC grade n-hexane, methanol, isopropyl alcohol (IPA), diethyl amine (DEA), trifluoroacetic acetic acid (TFA) and hydrogen peroxide were purchased from Merck Ltd, India.

### Equipment

A Waters 2695 separation module (Waters, Milford, MA, USA) coupled to Photo Diode Array (PDA) detector equipped with an auto injector and thermostatic column oven compartment was utilized for method development and validation. Empower software was used for data acquisition and system suitability calculations.

### Solution preparation

**Preparation of Mobile Phase:**

Prepared a mixture of n-hexane, isopropyl alcohol and diethyl amine in the ratio of 950:50:1 (v/v/v) mixed and degassed.

**Preparation of diluents:**

Mobile phase was used as blank.

**Preparation of system suitability solution** (For Fesoterodine Fumarate drug substance as well as for drug product)
Accurately weighed and transferred about 1 mg each of Fesoterodine Fumarate working standard and its (S) enantiomer in to a 50 mL volumetric flask. About 10 mL of isopropyl alcohol was added, sonicated to dissolve and diluted to volume with mobile phase. The solution was filtered through 0.45 µm nylon filter with discarding first few milliliter of filtrate.

**Preparation of standard solution**:

Accurately weighed and transferred about 100 mg of Fesoterodine Fumarate working standard in to a 100 mL volumetric flask. About 10 mL of isopropyl alcohol was added, sonicated for about 20 minutes with intermittent shaking and diluted to volume with mobile phase. The resulting solution was further diluted with mobile phase to get a solution containing 10 µg $mL^{-1}$ concentration. The solution was filtered through 0.45 µm nylon filter with discarding first few milliliter of filtrate.

**Sample preparation (For Fesoterodine Fumarate drug substance)**

Accurately weighed and transferred about 100 mg of Fesoterodine Fumarate in to a 100 mL volumetric flask. About 10 mL of isopropyl alcohol was added, sonicated for about 20 minutes with intermittent shaking and diluted to volume with mobile phase. The solution was filtered through 0.45 µm nylon filter with discarding first few milliliter of filtrate.

**Sample preparation (For Fesoterodine Fumarate drug product)**
Accurately weighed and transferred tablet powder equivalent to 100 mg of Fesoterodine Fumarate in to a 100 mL volumetric flask. About 10 mL of isopropyl alcohol was added, sonicated for about 20 minutes with intermittent shaking and diluted to volume with mobile phase. The solution was filtered through 0.45 µm nylon filter with discarding first few milliliter of filtrate.

**Chromatographic conditions**
The chromatographic conditions were optimized using chiral stationary phase (CSPs) Chiralpak IC-3, 250 mm X 4.6 mm, 3 µm, (Daicel, Tokyo, Japan). The mobile phase consisting of n-hexane, isopropyl alcohol and diethyl amine in the ratio of 950:50:1 (v/v/v) was pumped through the column at the flow rate of 1.0 ml $min^{-1}$. The column oven compartment was maintained at 40°C and the detection was carried out at a wavelength of 235 nm. The injection volume was 20µl.

## RESULTS AND DISCUSSION

**Optimization of chromatographic conditions**
The aim of this work is to separate the enantiomers and for the accurate quantification of (S)-enantiomer. A solution containing 1 mg $mL^{-1}$ of Fesoterodine Fumarate and 0.01 mg $mL^{-1}$ of S-isomer prepared in mobile phase was used in the method development. To develop a rugged and suitable LC method for the separation of enantiomers, different mobile phases and various chiral stationary phase (CSPs) columns were employed. To achieve the chromatographic separation various chiral columns like Chiral AGP, YMC Chiral NEA (S), Chiralcel OD-RH, Chiralcel OD-H, Chiralpak AD-H, and Chiralpak IC-3 (Diacel) were used. Various experiments were conducted, to select the best chiral stationary phase and mobile phases that would give optimum resolution and selectivity for the enantiomers. But satisfactory separation was not found in all above experiments. There is an indication of separation on Chiralpak IC-3 column using a mobile phase consisting of n-hexane, isopropyl alcohol in the ratio of 950:50 (v/v) but the peak shapes were found broad with poor efficiency and poor resolution. Introduction of diethyl amine in the mobile phase improved the chromatographic efficiency and resolution between the enantiomers. A satisfactory separation was achieved on Chiralpak IC-3 column (resolution between enantiomers was found greater than 3) using the mobile phase system n-hexane, isopropyl alcohol, diethyl amine in the ratio of 950:50:1 (v/v/v) (Figure – 2). It is presumed that it could be due to high probability of interaction, better resolution was found on Chiralpak IC-3 column. Due to the better chromatographic results obtained on the Chiralpak IC-3 column, further method validation was carried out on the same column. In the optimized method, the typical retention times of Fesoterodine Fumarate and its (S)-enantiomer were found about 10.3 min and 11.8 min, respectively. 5-hydroxy methyl tolterodine is an active metabolite of Fesoterodine Fumarate which is also a degradation product of Fesoterodine Fumarate . The proposed method is also capable for the chiral separation of 5-hydroxy methyl tolterodine and the typical retention times of 5-hydroxy methyl tolterodine and its (S)-enantiomer were found about 21 min and 16.5 min, respectively. Hence it is very easy to check the chiral purity of active metabolite as well as at final drug substance and drug product. The typical chromatogram of resolution between Fesoterodine Fumarate and its S-Isomers is shown in figure – 2.

**Method validation**
The optimized chiral purity method for Fesoterodine Fumarate was validated according to ICH guidelines [15], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability parameters were also assessed.

**System suitability test**
The system suitability test was performed according to USP 30 [16] and BP 2007 [17] indications. The observed RSD values at 1% level of analyte concentration were well within the usually accepted values ($\leq$2%). Theoretical plates (N), USP tailing factor ($T_f$) and USP resolution ($R_s$) between Fesoterodine Fumarate and S-isomer were also determined. The results obtained were all within acceptable limits (Table-1).

**Specificity**
The specificity of the method was checked by injecting blank solution, excipient (for drug product) solution without drug substance, sample solution and sample solution spiked with all related known impurities at 1% level (for drug substance as well as drug product). There was no interference from blank, excipient and related known impurities at the retention time of analyte peak. Specificity was also checked by exposing the sample under stressed conditions

like acid hydrolysis (1mL Trifluoro acetic acid), base hydrolysis (2mL, Diethyl amine), Oxidation (30% hydrogen peroxide 2mL), heat (80°C), light (1.2 million lux hours), humidity (40°C/75%RH). The results are tabulated in table 2. The peak purity indices for the analytes in stressed solutions and spiked sample were determined with PDA detector under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference.

**Method precision and ruggedness**

In order to determine the **Method precision and ruggedness** of the method, six independent preparation of sample solution containing 1.0 mg $mL^{-1}$of Fesoterodine Fumarate spiked with (S)-enantiomer (0.5%) was prepared and calculated the percentage (% w/w) of (S)-isomer and was determined of percentage (weight / weight) of each enantiomer. The results are tabulated in table - 4.

**Linearity and range**

The nominal concentration of Fesoterodine Fumarate in test solution was 1 mg $mL^{-1}$. Taking into account that typical impurity tolerance levels is 0.5 % for S-isomer and response function was determined by preparing standard solution of S-isomer at different concentration levels ranging from lower limit of quantification to 150 % of impurity tolerance level. The regression statistics are shown in table 4.

**Determination of limit of quantification and detection (LOQ and LOD)**

The linearity performed above, used for the determination of limit of quantification and detection. Residual standard deviation (σ) method was applied and were predicted the LOQ and LOD values using following formula (a), (b) and established the precision at these predicted levels. The results are tabulated in table-3

$$LOQ = \frac{10X\sigma}{S} \qquad \text{(a)}$$

$$LOD = \frac{3.3X\sigma}{S} \qquad \text{(b)}$$

Where

σ = Residual standard deviation of response, S = Slope of the calibration curve

**Accuracy**

Accuracy was evaluated by the determination of S-isomer in solution prepared by standard addition method. The experiment was carried out by adding known amount of S-isomer corresponding to three concentration levels of 50 %, 100 % and 150 % of the impurity tolerance level in sample solution (Drug substance as well as in drug product). The samples were prepared in triplicate at each level. The quantification of added analyte (% weight/weight) was carried out by using an external standard of S-isomer prepared at the analytical concentration. The experimental results revealed that approximate 95 - 105 % recoveries were obtained for S-isomer in drug substance as well as in drug product. Therefore, based on the recovery data (Table - 4) the estimation of S-isomer that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

**Table 1: System suitability parameters**

| Analyte (n=6) | Tailling factor ($T_f$)[a] | Efficiency (plates m-1)[a] | Resolution ($R_s$)[a] | RSD (%) |
|---|---|---|---|---|
| S-Isomer | 1.07 | 8983 | 2.78[b] | 0.86 |

*a calculated according to the USP. b Resolution between Fesoterodine and its S-isomer. c Resolution between 5-Hydroxy methyl Tolterodine and its S-isomer.*

**Table 2: Results of stress study**

| Condition | Conditions | Time | Temperature (°C) | %Degradation |
|---|---|---|---|---|
| Acid hydrolysis | 1mL, TFA | 1Hr | 25°C± 2°C | 1.31 |
| Base hydrolysis | 2mL, DEA | 2Hr | 60°C± 2°C | 4.13 |
| Oxidation | 2mL, 30% H2O2 | 2Hr | 60°C± 2°C | 6.67 |
| Thermal | - | 24Hrs | 80°C | 1.12 |
| Photolytic | 250 watt h/$m^2$ | 22Hrs | - | 0.87 |
| Humidity | 40°C/75% RH | 8 days | - | 0.45 |

**Table –3: Validation results of precision, Regression statistics, LOD and LOQ**

| **Validation parameters** | **Results** |
|---|---|
| **Repeatibility (n=6, %RSD)** | |
| Retention time (S)-enantiomer | 0.61 |
| Retention time (R)-enantiomer | 0.76 |
| Peak area (S)-enantiomer | 1.30 |
| % w/w of (S)-enantiomer | 3.53 |
| **Intermediate Precision (n=12, %RSD)** | |
| Retention time (S)-enantiomer | 0.54 |
| Retention time (R)-enantiomer | 0.62 |
| Peak area (S)-enantiomer | 0.91 |
| % w/w of (S)-enantiomer | 3.28 |
| **Regression statistics (S-enantiomer)** | |
| Calibration range (µg/mL) | 1.271 to 7.628 µg $mL^{-1}$ |
| t-stat | 150.56 |
| P-value | 7.15E-24 |
| 95% confidence interval (Lower) | 9.206 |
| 95% confidence interval (Upper) | 9.472 |
| Correlation coefficient | 0.999078 |
| **LOD-LOQ (S-enantiomer)** | |
| Limit of detection (µg/mL) | 1.316 |
| Limit of quantification (µg/mL) | 0.509 |
| Precision at LOQ (%RSD) | 8.23 |

**Table – 4: Recovery Data**

| Spike level (%) | Amount added (%w/w) | Amount recovered (%w/w) | Recovery (%) | % RSD |
|---|---|---|---|---|
| Drug Substance | | | | |
| 40% | 0.203 | 0.208 | 102.46 | 3.47 |
| | 0.197 | 0.204 | 103.55 | |
| | 0.201 | 0.195 | 97.01 | |
| 100% | 0.503 | 0.499 | 99.20 | 2.65 |
| | 0.502 | 0.489 | 97.41 | |
| | 0.497 | 0.510 | 102.62 | |
| 150% | 0.754 | 0.749 | 99.34 | 1.59 |
| | 0.745 | 0.754 | 101.21 | |
| | 0.747 | 0.766 | 102.54 | |
| Drug product | | | | |
| 40% | 0.203 | 0.199 | 98.03 | 2.90 |
| | 0.198 | 0.205 | 103.54 | |
| | 0.196 | 0.201 | 102.55 | |
| 100% | 0.504 | 0.492 | 97.62 | 3.30 |
| | 0.498 | 0.507 | 101.81 | |
| | 0.496 | 0.517 | 104.23 | |
| 150% | 0.755 | 0.740 | 98.01 | 2.92 |
| | 0.749 | 0.777 | 103.74 | |
| | 0.752 | 0.768 | 102.13 | |

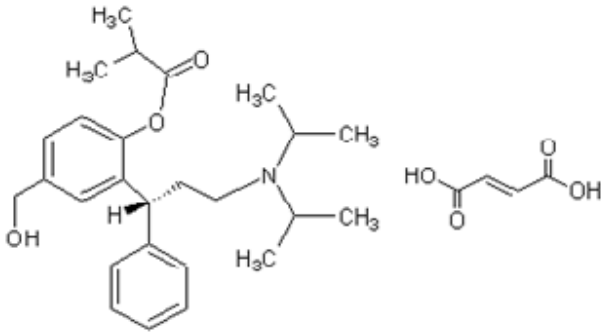

**Figure 1: Chemical structure of Fesoterodine Fumarate**

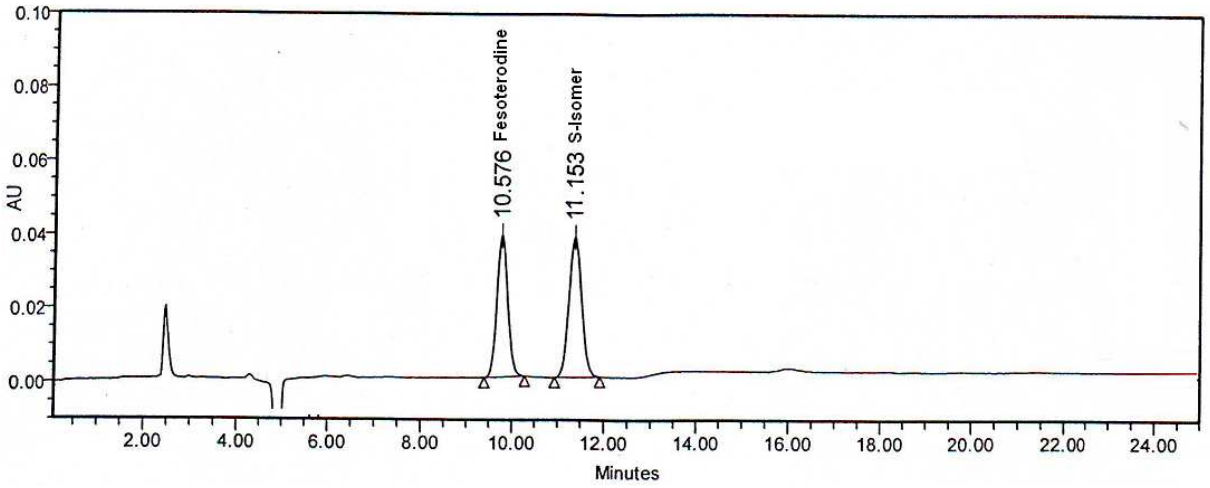


**Figure 2: Typical HPLC chromatograms of resolution between Fesoterodine and S-Isomer**

## CONCLUSION

A simple, rapid and accurate normal phase chiral LC method was described for the enantiomeric separation of Fesoterodine Fumarate in drug substance as well as in drug product. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the quantitative determination of chiral impurity ((S)-enantiomer) in drug substances and drug product.

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