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Simultaneous Estimation Of Escitalopram Oxalate And Fesoterodine In Oral Dosage Forms By Validated Reverse Phase High Performance Liquid Chromatography

Linganaboina Sirisha[1] , MD.Parveen[2] , Sayed Sana[3] , Sayyada Saleha Momina[4] , Mohammed Tajuddin[5]

[1,2,3,4]Max Institute of Pharmaceutical Sciences, Velugumatla, Khammam, Telangana – 507318, India[1234]

[5]Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan – 333001, India

ABSTRACT

A new, precise, rapid, accurate HPLC method was developed for the simultaneous estimation of Escitalopram oxalate and Fesoterodin in oral dosage forms. HPLC method for estimation of drug escitalopram in tablets of Cita S-10 (Cresent) was developed by using Kromosil C18 (250 mm x 4.6 mm) 5 µm column with mobile phase composition of acetonitrile : methanol : ammonium acetate buffer Ph 3.0 in the ratio 30:20:50 by isocratic elution technique, the flow rate was 1.0 mL /min and UV detection at 238 nm. The developed HPLC technique is a simple and accurate method for the estimation of API Escitalopram in tablet dosage forms. HPLC method for estimation of drug Fesoterodine in solid dosage form capsules of Roliten OD (Ranbaxy) was developed by using Kromosil C18 (250 mm x 4.6 mm) 5 µm column with mobile phase composition of acetonitrile: methanol: ammonium acetate buffer pH 3.0 in the ratio 30:30:40 by isocratic elution technique, the flow rate was 1.0 mL / min and UV detection at 281 nm. The correlation coefficient r^2 values were found to be both the drugs 0.9997 for Escitalopram oxalate. RP-HPLC method for estimation of Fesoterodine in solid dosage form capsules of Roliten OD (Ranbaxy) was developed by using Kromosil C18 (250 mm x 4.6 mm) 5 µm column with mobile phase composition of acetonitrile: methanol: ammonium acetate buffer pH 3.0 in the ratio 30:30:40 by isocratic elution technique, the flow rate was 1.0 mL/min and UV detection at 281 nm. The correlation coefficient R2 value was found to be 0.9991 for Fesoterodine.

Key words: Escitalopram oxalate and Fesoterodin; RP-HPLC method; UV-VIS detector; oral dosage forms.

Introduction

Escitalopram oxalate (EST) (Figure 1a) is chemically known as S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluro-phenyl)- 5-phthalancarbonitrile oxalate, which fits to the class of compounds known as antidepressant and is the Senantiomer of racemic citalopram1. Escitalopram is spontaneously soluble in dimethlysulfoxide (DMSO), methanol and, sparingly soluble in ethanol and water. Escitalopram is insoluble in heptane but slightly soluble in ethyl acetate. Escitalopram oxalate originates in category of oral selective serotonin reuptake inhibitor (SSRI) and proved highly potent with in vitro and in vivo studies. Escitalopram mainly used for the management of generalized Anxiety Disorder and major depressive disorder. Escitalopram worksby specific competitive inhibition of the membrane transporter

of serotonin2. As per studies Escitalopram discovered to be more than twice as potent as citalopram and is the highly selective drug in its class2- 3.Several analytical methods have been developed for the estimation of escitalopram oxalate in pharmaceutical formulations and/or biological fluids include liquid chromatography coupled with mass spectrometry[4].

Fesoterodine Fumarate (FST) is a new antimuscarinic agent developed for the treatment of overactive bladder1-4. 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 respectively6-9. 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 C30H41NO7 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 column10. 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) method11. A UV spectrophotometry method was published for determination of Fesoterodine in Extended Release Tablets12 . 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 validated13 . 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

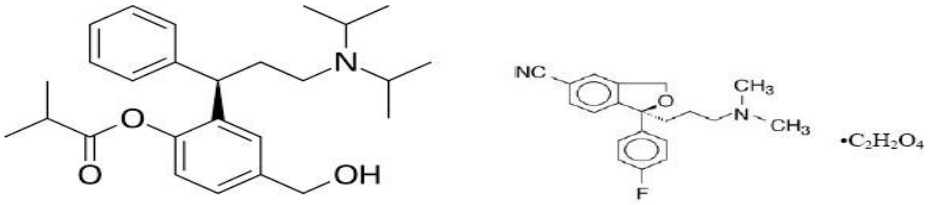


Fig.1.Chemical structure of Fesoterodine and Escitalopram

MATERIALS AND METHODS

PART A-HPLC of escitalpram oxalateSelection of wavelength

10 mg Esc is dissolved in 10 ml methanol, pipetted 1 mL, made 10 ml, pipetted 1ml, made 10 ml to get final conc of 10µg/ml and scanned between 200 to 400 nm and 238 nm was selected as the optimum wavelength.

Preparation of standard solution

10.8 mg of escitalopram is dissolved in 10 ml with methanol, pipetted 2.5ml, made 100mL with mobile phase.

Preparation of sample solution

10.3 mg of escitalopram is dissolved in 100 ml with methanol, pipetted 1ml, made 10ml with mobile phase.

Assay procedure

20 µL each of standard and sample were injected in to HPLC port and performed four trials using different solvents in different ratios and 4th trial was optimized for the assay.

Trials

- In trial 1, 1% acetic acid : ACN 20:80 was used as the mobile phase.
- In trial 2, 0.1% SLS buffer pH 2.4: ACN pH 2.8, 60:40 was used.
- In trial 3, ammonium acetate buffer pH3.0: methanol: ACN 40:30:30 was used as the mobile phase.
- In trial 4, 5mM ammonium acetate buffer pH 3.0: methanol: ACN 50:20:30 was used as the mobile phase.
- The above fourth trial was selected as the mobile phase for assay of both standards and samples.

VALIDATION

Accuracy

Preparation of 50% solution

51.8 mg of tablet powder was made to 100 ml with methanol and pipetted 1 ml, make up to 10 ml with mobilephase.

Preparation of 100% solution

103.6 mg of tablet powder was made to 100 ml with methanol and pipetted 1 ml, make up to 10 ml with mobilephase.

Preparation of 150% solution

155.4 mg of tablet powder was made to 100 ml with methanol and pipetted 1ml, make up to 10 ml with mobilephase.

PRECISION

The standard solution of escitalopram containing 10.18 µg/mL was given in 6 replicate injections.

LINEARITY

Preparation of standard Stock Solutions

10.8 mg of standard Esc was made to 10 ml with methanol, pipetted 2.5 ml, made to 100 ml with mobile phase.

- **Preparation of 50% Linearity mixture**

- To 2 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to10 ml.

- **Preparation of 75% Linearity mixture**
- To 3 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to10 ml.

- **Preparation of 100% Linearity mixture**
- To 4mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to10 ml.

- **Preparation of 125% Linearity mixture**
- To 5 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to10 ml.

- **Preparation of 150% Linearity mixture**
- To 6 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to10 ml.

- **RUGGEDNESS**
- 103.6 mg of tablet powder transferred to 100 ml volumetric flask, dissolved in methanol by sonication for 5 mins. From the above solution, 1ml was pipetted in to 10ml volumetric flask and final the volume was madewith mobile phase.
- From this 20 µL was injected in to HPLC port separately by two different analysts in the same HPLC system and same column.

- **ROBUSTNESS**
- Solution of standard mixture was injected into HPLC sample port with varying wave length (236, 238 and 240 nm) and varying flow rate (0.8, 1.0 and 1.2 ml/min).

Part B-HPLC of Fesoterodine

- **Selection of wavelength**
- 10 mg of Fesoterodine was made upto 10 mL with methanol.
- From this 1 mL was pipetted and made upto 10 mL with methanol and again 1ml was pipetted and madeupto 10 ml with mobile phase to get the final concentration of 10 µg/mL of Fesoterodine.
- The wavelength was selected by scanning between 200 to 400 nm and optimized as 281 nm.

- **Preparation of standard solution**
- 10.6 mg of tablet powder was made upto 10 ml with methanol, pipetted 2.5ml, made 50 ml with mobilephase, pipetted 4 ml, make up to 10 ml with mobile phase.

- **Preparation of sample solution**
- 159.6mg of tablet powder was made upto 100 ml with methanol, pipetted 5ml, made 10 ml

with mobilephase.

VALIDATION

- **ACCURACY**

- **Preparation of 50% solution**
- 82.3 mg of tablet powder made to 100 mL with methanol and pipetted 5 ml, made to 10 ml by mobilephase.

- **Preparation of 100% solution**
- 159.6mg of tablet powder made to 100 mL with methanol and pipetted 5 ml, made to 10 ml by mobilephase.

- **Preparation of 150% solution**
- 239.4 mg of tablet powder made to 100 mL with methanol and pipetted 5 ml made to 10 ml by mobilephase.

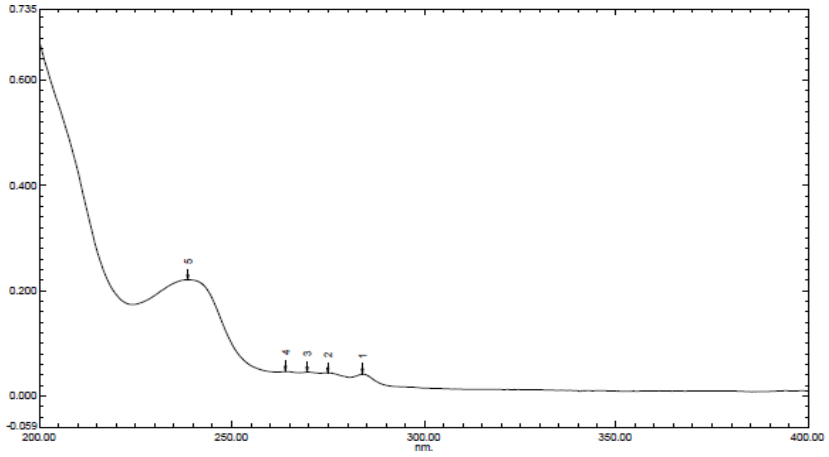
VALIDATION

- **PRECISION**
- The standard solution of Fesoterodine containing 20.12 µg/mL was injected in 6 replicates, andthe result of the injections were observed.

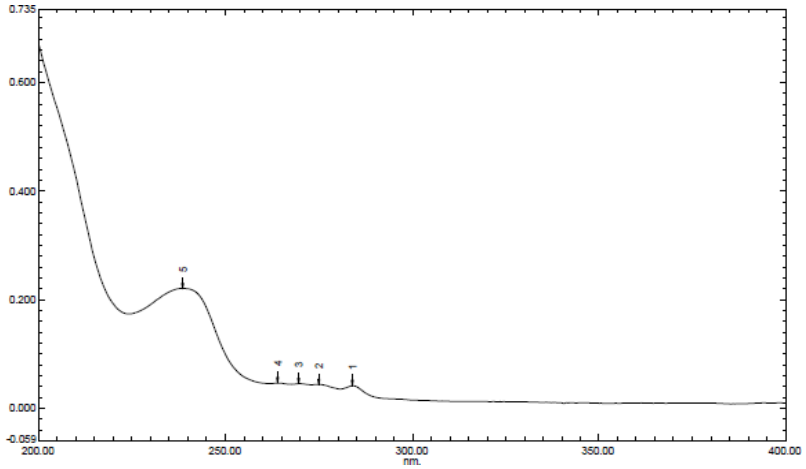
- **LINEARITY**
- Preparation of Stock Solutions of Fesoterodine.
- To 10.6mg of Fesoterodine in 10ml volumetric flask, add methanol was added and sonicated for 5min. from the above solution 2.5ml was pipette out in 50ml volumetric flask , the final volume was made up with mobile phase.
- Preparation of 50% Linearity mixture
- To 2 mL of standard stock solution of Fesoterodine in 10ml volumetric flask, mobile phase wasadded and volume was made.
- Preparation of 75% Linearity mixture
- To 3 mL of standard stock solution of Fesoterodine in 10ml volumetric flask, mobile phase was addedand volume was made.

RESULTS-PART A

Selection of wavelength

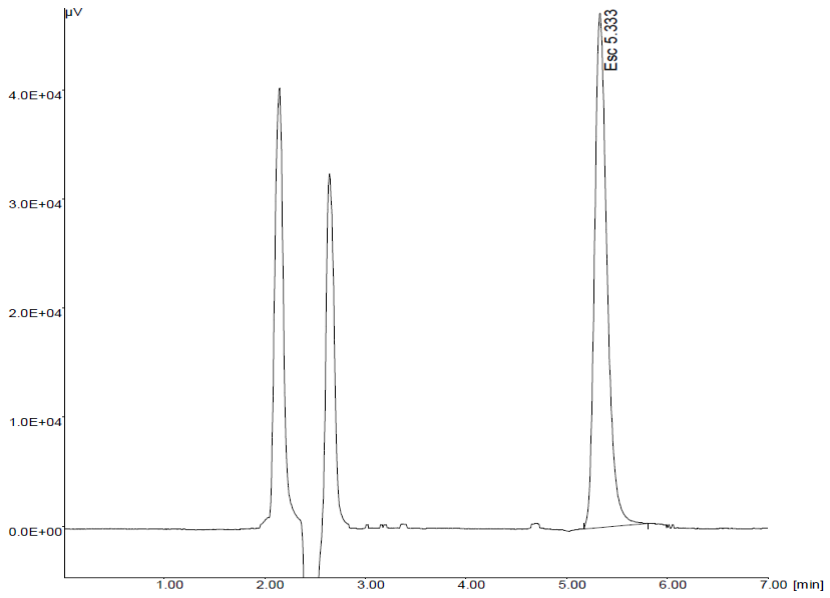


- **Chromatogram of tablet sample injection – I**

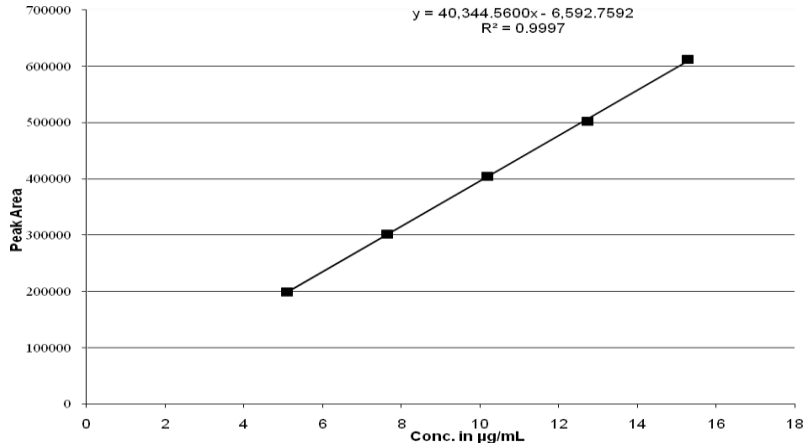


Chromatogram of tablet sample injection – II

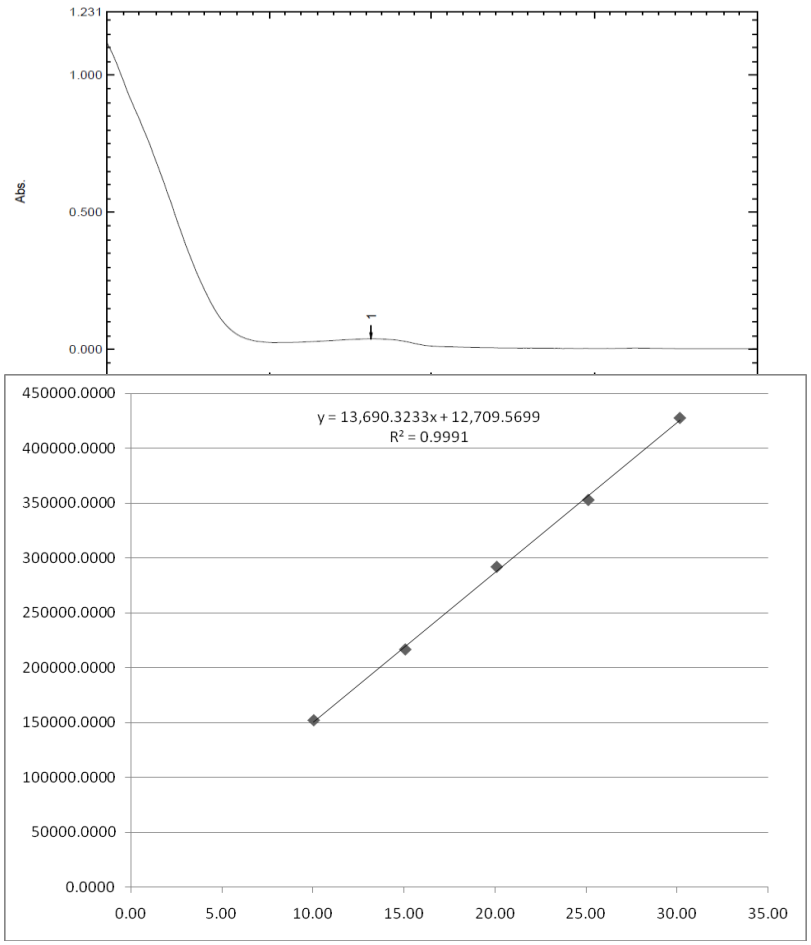
Chromatogram of tablet sample injection - III



Linearity of standard Escitalopram



PART B-HPLC of Fesoterodine Selection ofwavelength



Linearity of standard Fesoterodine

DISCUSSION

- Initially, various mobile phase compositions were tried to elute the drug and the mobile phase and flow rate selection was based on peak parameters such as peak height, peak capacity, number of theoretical plates, tailing or symmetry factor, run time and resolution.

- **Escitalopram oxalate**
- In trial 1, peak shape was broad and very close RT was observed; so ion pairing agent 1% SLS was added for trial 2, but peak shape was split in nature, so methanol and ammonium acetate buffer were used in trial3, again very close RT was observed, therefore mobile phase ratio of trial 3 was changed for trial 4.
- Finally the mobile phase containing the mixture of acetonitrile: Methanol: ammonium acetate buffer pH 3.0 in the ratio 30:20:50 was selected / optimized for escitalopram oxalate.

The optimum wavelength selected for detection was 238 nm where better detector response was obtained withretention time of 5.3 minutes for escitalopram.

Discussion-Fesoterodine by HPLC

- In trial 1, using 50 mM phosphate buffer: acetonitrile 60:40, peak shape was broad, so to improve the peak quality, ammonium acetate buffer was added in trial 2 showed broad peak with far RT.
- In trial 3, 70:30 ammonium acetate buffer : acetonitrile, very close RT of 2.3 was observed, so mobile phaseratio was changed and methanol was added in trial 4.
- Finally the mobile phase containing the mixture of acetonitrile: Methanol: 5mM Acetate buffer pH 3.0 inthe ratio 30:30:40 was selected/ optimized for Fesoterodine .
- The optimum wavelength selected for detection was 281 nm where better detector response was obtainedwith retention time of 5.03 minutes for Fesoterodine.

TABULATION

Parameter	Escitalopram	Fesoterodine	Ich limit
Assay	101.86 %	102.65	98.0-103.0%
Accuracy	100.73 %.	102.53 %.	% recovery 98.0-102.0%
Precision	0.59	1.38	% RSD NMT 2.0
Linearity	0.9997	0.9991	Correlation coefficient

			0.999
Ruggedness	102.63	102.67	% RSD NMT 2.0

CONCLUSION

PART - A - Escitalopram-HPLC

HPLC method for estimation of drugs escitalopram in tablets of Cita S-10 (Cresent) was developed by using Kromosil C_{18} (250 mm x 4.6 mm) 5 µm column with mobile phase composition of acetonitrile: Methanol: ammonium acetate buffer pH 3.0 in the ratio 30:20:50 by isocratic elution technique, the flow rate was 1.0 mL/min and UV detection at 238 nm. The developed HPLC technique is a simple and accurate method for the estimation of API Escitalopram in tablet dosage forms.

PART - B - Fesoterodine HPLC

HPLC method for estimation of drug Fesoterodine in solid dosage form capsules of Roliten OD (Ranbaxy) was developed by using Kromosil C_{18} (250 mm x 4.6 mm) 5 µm column with mobile phase composition of acetonitrile: Methanol: ammonium acetate buffer pH 3.0 in the ratio 30:30:40 by isocratic elution technique, the flow rate was 1.0 mL/min and UV detection at 281 nm. The developed HPLC technique is a simpleand accurate method for the estimation of API Fesoterodine in capsule dosage forms.

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