

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF ESCITALOPRAM OXALATE AND TOLTERODINE TARTRATE IN ORAL DOSAGE FORMS

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ABSTRACT

A new, precise, rapid, accurate HPLC method was developed for the estimation of Escitalopram oxalate and Tolterodine tartrate in oral dosage forms. HPLC method for estimation of drug escitalopram in tablets of Cita S-10 (Crescent) 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 tolterodine

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 0.9997 for Escitalopram oxalate. HPLC method for estimation of drug tolterodine 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 Tolterodine tartrate.

INTRODUCTION

- **HPLC**

- It is the major and integral analytical tool applied in all stages of drug discovery, development and production.
- The principle of separation in normal phase and reverse phase mode is adsorption.
- When mixture of components are introduced into HPLC column, they travel according to their relative affinities towards the stationary phase.
- The components which has more affinity towards the stationary phase travels faster.
- Since no components have the same affinity towards the stationary phase, the components are separated.

- **VALIDATION**

- It is a process of providing documented evidence that the method does what it is intended to do.
- It is a process of method validation and ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use.
- Validation is an act of providing that any procedure, process, equipment, material, activity or system performs as expected under the given set of conditions.

PLAN OF WORK

- **PART A and B: HPLC**

- Selection of Drugs
- Selection of analytical method
- Selection of wavelength
- Optimization of mobile phase
- Assay of standards and API in Tablets
- Validation of developed method using the parameters like accuracy, linearity, precision, LOD, LOQ, ruggedness and robustness as per the ICH guidelines, 2005.
- Statistical evaluation of assay and validation parameters using SD and % RSD.

DRUG PROFILE

Escitalopram oxalate

MOLECULAR FORMULA: C₂₀H₂₁N₂O₄

MOLECULAR WEIGHT: 414.40 g/mol

CATEGORY: Antidepressant, SSRI.

MECHANISM OF ACTION: Selective serotonin reuptake inhibitor-SSRI.

USE: Depression, generalized anxiety disorders.

SOLUBILITY: Freely soluble in methanol and DMSO, soluble in isotonic saline solution, sparingly soluble in water and ethanol, slightly soluble in ethyl acetate, and insoluble in heptane.

Tolterodine tartrate

MOLECULAR FORMULA: C₂₆H₃₇NO₇

MOLECULAR WEIGHT: 475.6 g/mol

CATEGORY: Antispasmodic.

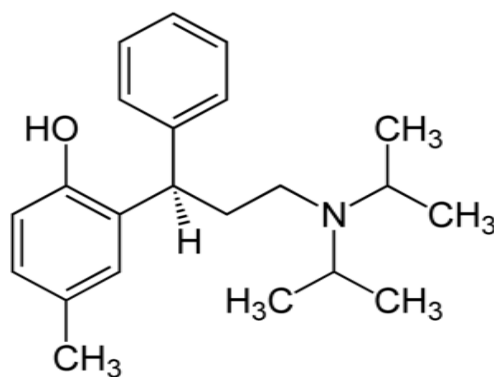
CATEGORY: Muscarinic receptor antagonist-MRA.

USE: Urinary bladder incontinence.

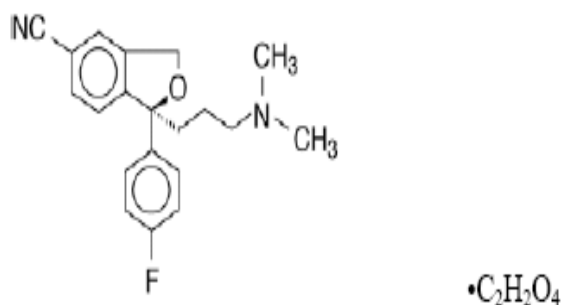
SOLUBILITY: Soluble in methanol, slightly soluble in ethanol and practically insoluble in toluene.

DRUG PROFILE

Tolterodine Tartrate



Escitalopram Oxalate



MATERIALS AND METHODS

PART A-HPLC of escitalpram oxalate

Selection 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 10 μ g/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 μ L 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 mobile phase.

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 mobile phase.

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 mobile phase.

PRECISION

The standard solution of escitalopram containing 10.18 $\mu\text{g/mL}$ 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 to 10 ml.
- **Preparation of 75% Linearity mixture**
- To 3 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to 10 ml.
- **Preparation of 100% Linearity mixture**
- To 4mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to 10 ml.
- **Preparation of 125% Linearity mixture**
- To 5 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to 10 ml.
- **Preparation of 150% Linearity mixture**
- To 6 mL of standard stock solution of Escitalopram, mobile phase was added and volume was made up to 10 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 made with mobile phase.
- From this 20 μ L 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 tolterodine

- **Selection of wavelength**
- 10 mg of tolterodine 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 made upto 10 ml with mobile phase to get the final concentration of 10 μ g/mL of tolterodine.
- 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 mobile phase, 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 mobile phase.

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 mobile phase.

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

VALIDATION

• PRECISION

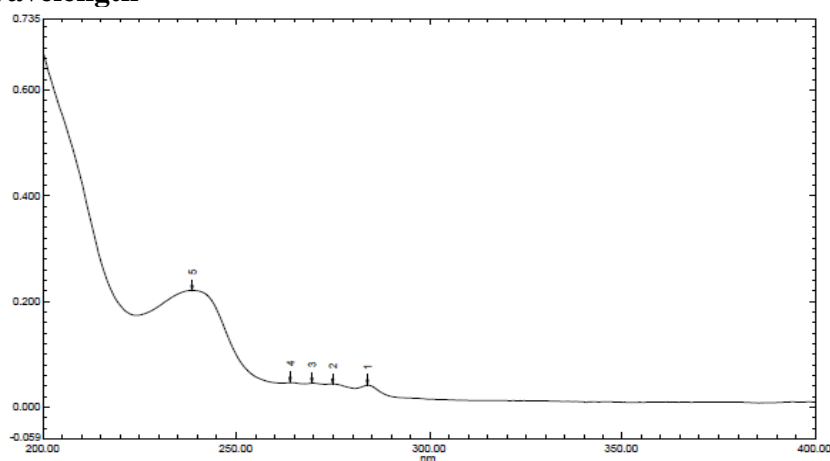
- The standard solution of Tolterodine containing 20.12 $\mu\text{g/mL}$ was injected in 6 replicates, and the result of the injections were observed.

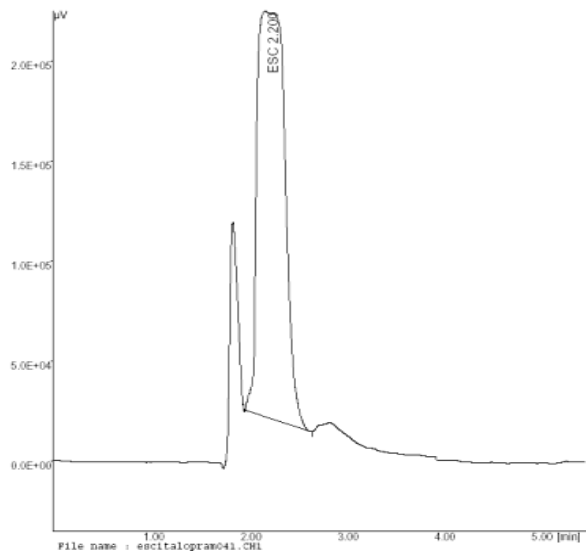
• LINEARITY

- Preparation of Stock Solutions of tolterodine.
- To 10.6mg of tolterodine 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 Tolterodine in 10ml volumetric flask, mobile phase was added and volume was made.
- Preparation of 75% Linearity mixture
- To 3 mL of standard stock solution of tolterodine in 10ml volumetric flask, mobile phase was added and volume was made.

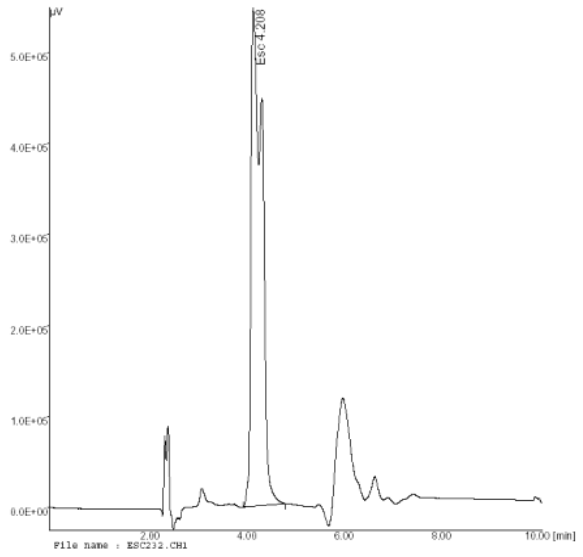
RESULTS-PART A

Selection of wavelength

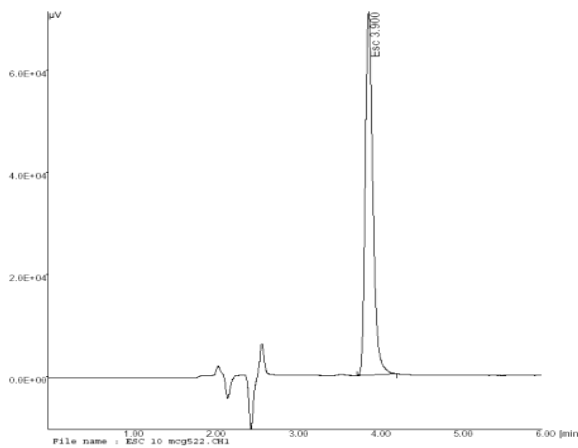




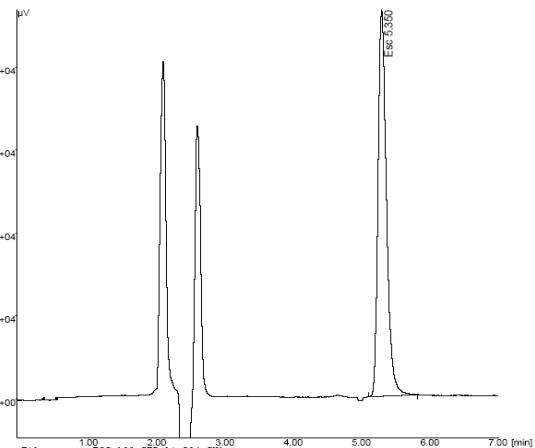
TRIAL 1



TRIAL 2



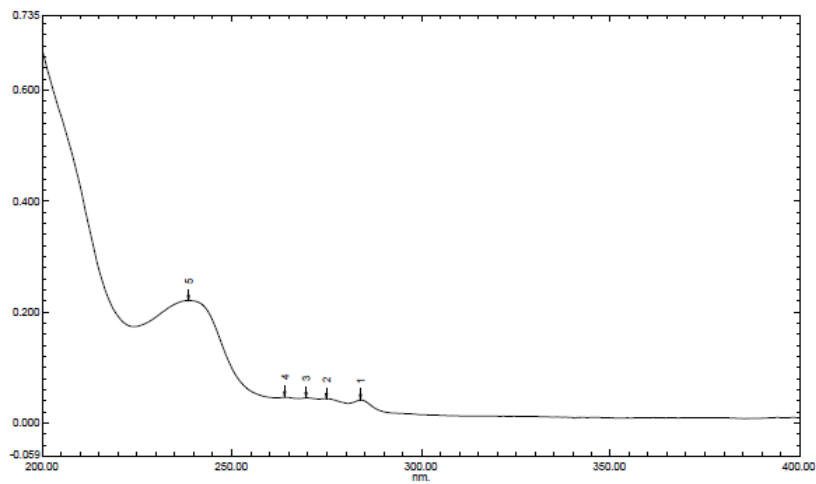
TRIAL 3



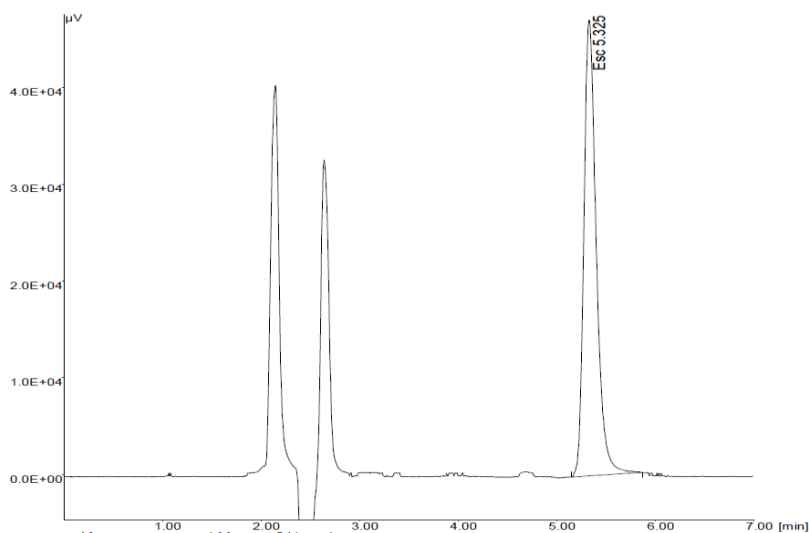
TRIAL 4

• **ASSAY CHROMATOGRAMS of escitalopram oxalate**

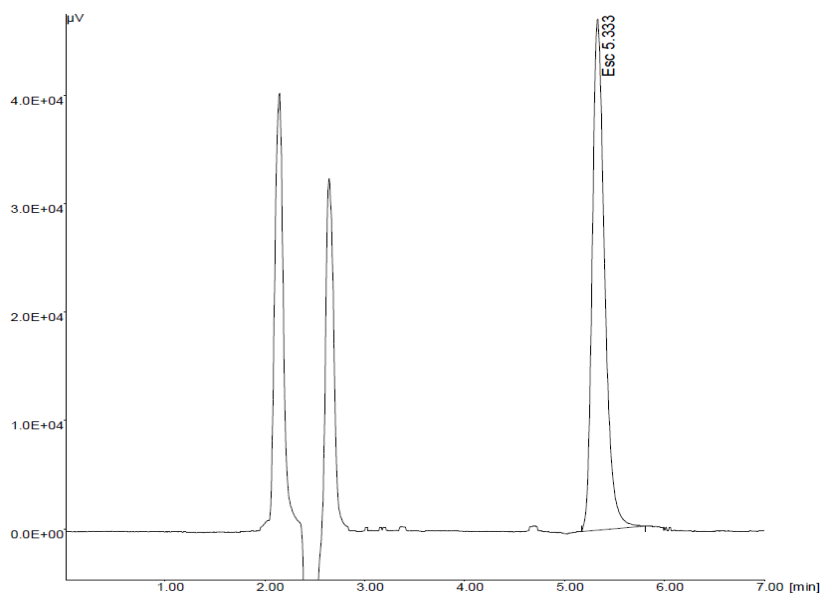
Chromatogram of tablet sample injection - I



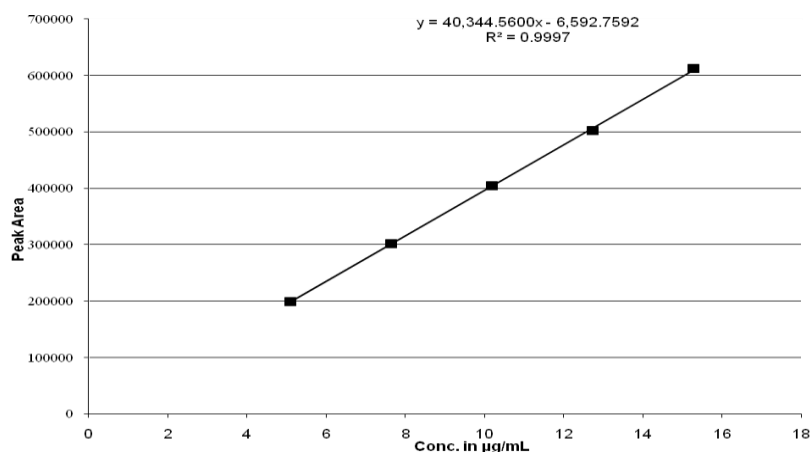
Chromatogram of tablet sample injection - II



Chromatogram of tablet sample injection - III

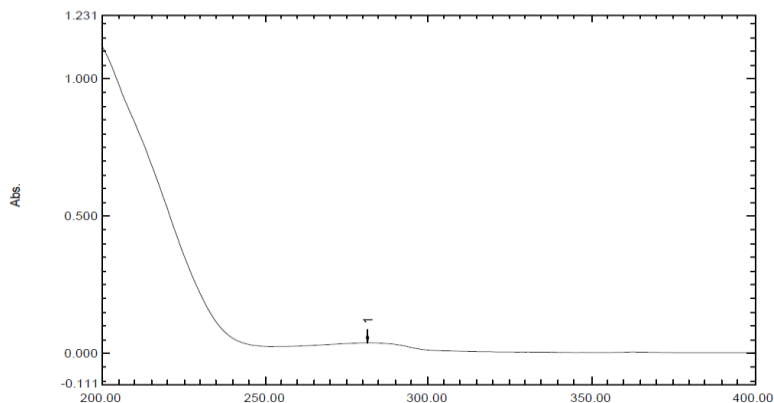


Linearity of standard Escitalopram

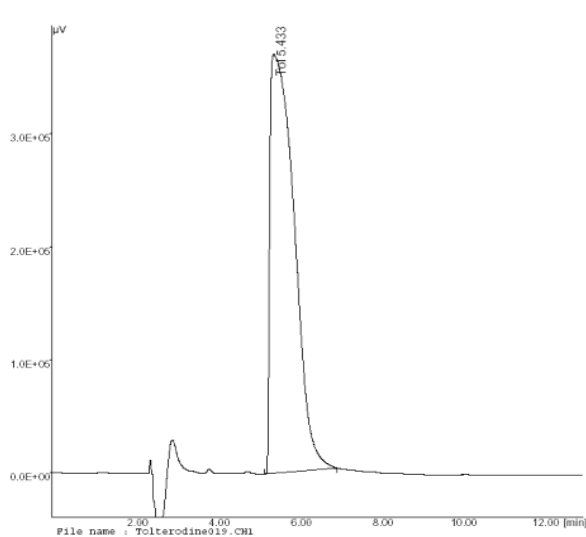


PART B-HPLC of tolterodine

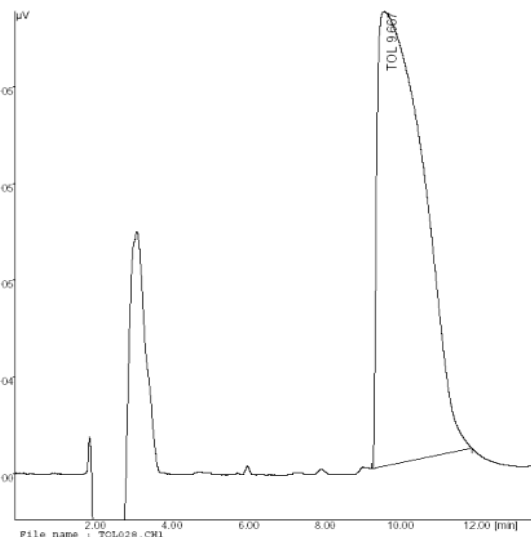
Selection of wavelength



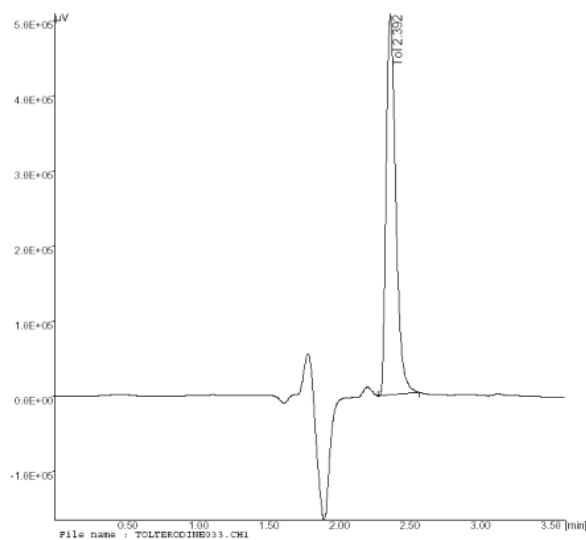
Optimization of mobile phase and flow rate using standards



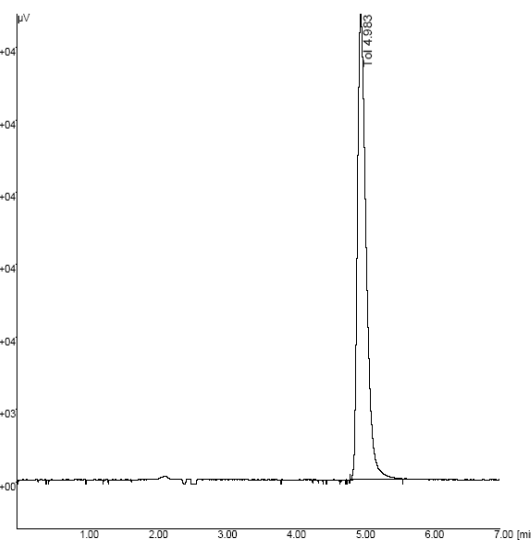
TRIAL 1



TRIAL 2



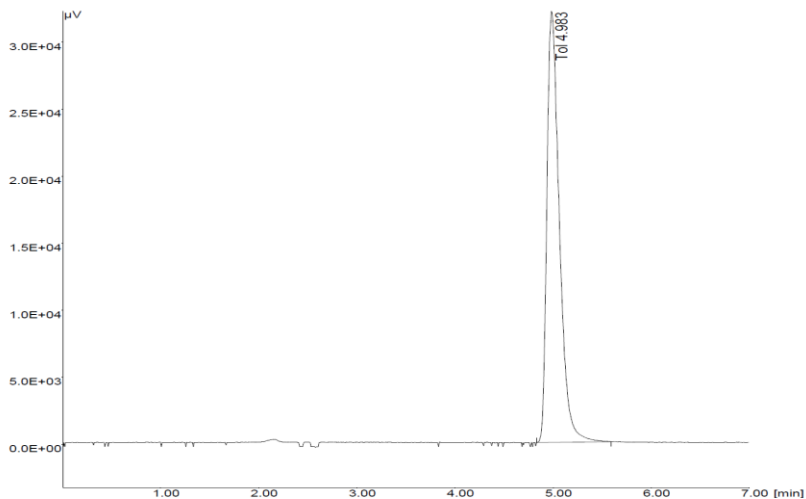
TRIAL 3



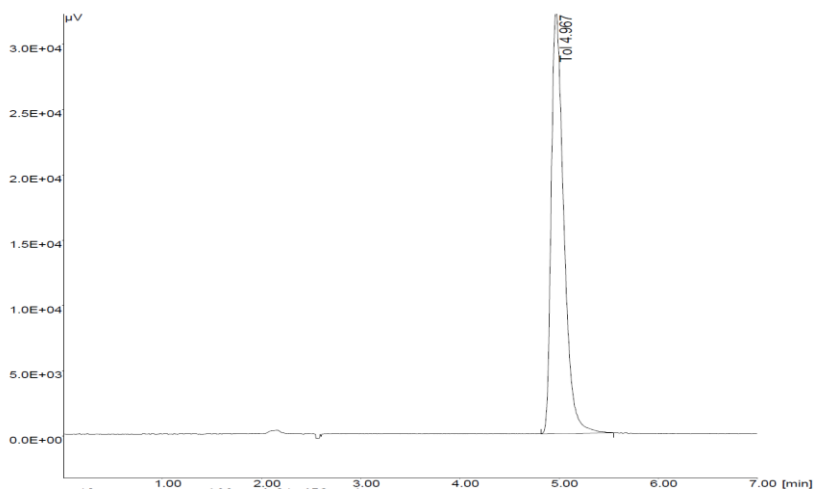
TRIAL 4

ASSAY CHROMATOGRAMS OF TOLTERODINE

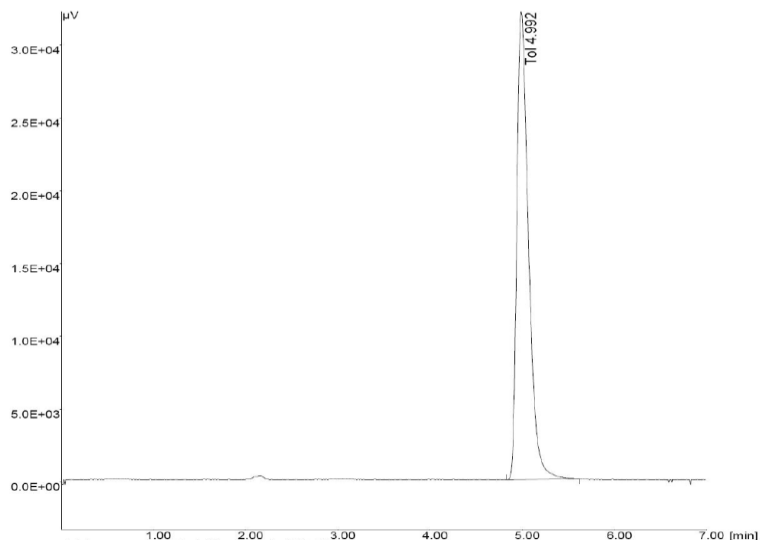
Chromatogram of tablet sample injection-I



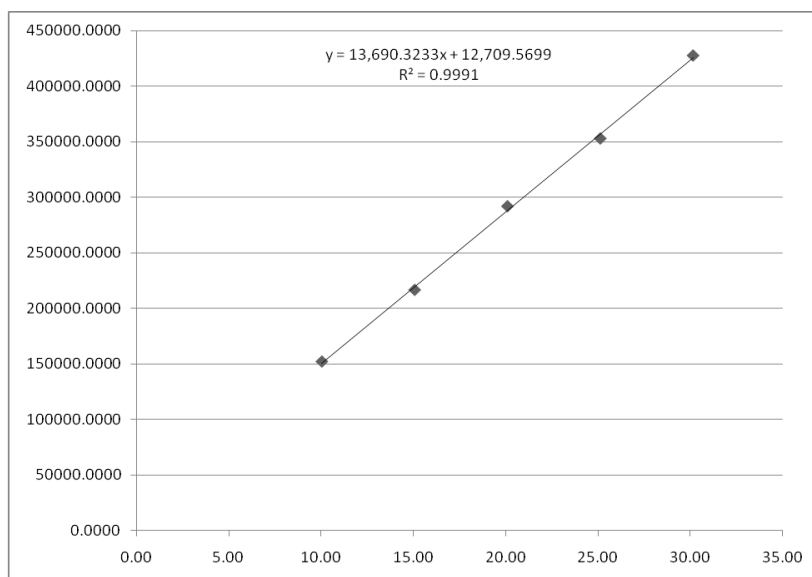
Chromatogram of tablet sample injection-II



Chromatogram of tablet sample injection-III



Linearity of standard tolterodine



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 trial 3, 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 with retention time of 5.3 minutes for escitalopram.

Discussion-tolterodine tartrate 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 phase ratio 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 in the ratio 30:30:40 was selected/ optimized for tolterodine tartrate.
- The optimum wavelength selected for detection was 281 nm where better detector response was obtained with retention time of 5.03 minutes for tolterodine.

TABULATION

Parameter	Escitalopram	Tolterodine	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 (Crescent) 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.

PART - B - Tolterodine HPLC

HPLC method for estimation of drug tolterodine 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 developed HPLC technique is a simple and accurate method for the estimation of API tolterodine in capsule dosage forms.

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