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**[Research article]**

# Development and validation of HPLC method for the estimation of Escitalopram oxalate in tablets.

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## ABSTRACT

A simple, specific, robust, accurate and precise isocratic HPLC method has been developed and subsequently validated for simultaneous determination of escitalopram (ESP) in pharmaceutical dosage forms. Kromosil (250x4.6)mm 5µ with flow rate of 1ml/ min by using JASCO PU-1580 and UV/VIS JASCO UV-1570 at 238 nm. The separation was carried out using a mobile phase consisting of acetonitrile, methanol and 5mM ammonium acetate buffer (pH 3.0) in the ratio 30:20:50 respectively. The retention time for escitaloparm was found to be 5.36 minutes respectively. The correlation coefficient was found to be 0.9997 (ESP). The mean percentage recovery was found to be 101.86 respectively. The % estimation of the drugs was found near to 100 % representing the accuracy in the method. The proposed method was also validated and applied for the analysis of drugs in tablet formulation.

**KEYWORDS:** HPLC, Escitoparm (ESP), Method development and Validation.

## INTRODUCTION

Escitalopram oxalate is chemically S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate, is selective serotonin reuptake inhibitor (SSRI). Escitalopram is the pure S-enantiomer (single isomer) of the racemic bicyclic phthalane derivative citalopram[1-2]. Literature survey revealed HPLC, Spectrophotometric and HPTLC methods were reported for multicomponent mode analysis but no validated HPLC method studies of escitalopram in individually in pharmaceutical preparation have been found in literature[3-19]. The proposed methods presented here is the simple, fast, accurate, precise, sensitive, robust, rapid and economic methods for quantitative determination of escitalopram tablets. The method was validated as per ICH guidelines.

## INSTRUMENT

Instrument used in the present study was JASCO. HPLC. The pump used was JASCO PU-1580 pump. The samples were applied Kromosil (250x4.6)mm 5µ column with Rhedyne injector. The sample was performed using UV/VIS JASCO UV-1570 detector with flow rate 1ml/min and operated by JASCO LC –NeT II/ADC interface. The Shimadzu electronic balance (0.001 gm sensitivity) was used for weighing purpose.

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## MATERIALS USED

Escitalopram was kindly supplied by Unichem Lab. Ltd. Mumbai, India. CITA S-10 was taken for study, which contains Escitalopram-10mg. Methanol (Merck Ltd., Mumbai, India) and HPLC grade acetonitrile (Molychem, mumbai), methanol, ammonium acetate, glacial acetic acid (Finar chemicals, ahmedabad and HPLC grade water.

### Preparation of mobile phase

Acetonitrile: Methanol: 5mM Ammonium acetate buffer pH (3.0) in the ratio of 30: 20:50 were mixed, sonicated for 10 minutes and filtered through the membrane filter of micron 0.45µ.

### Preparation of standard solution

10 mg of escitalopram were dissolved in methanol by sonication and makeup to 10 mL. 1 mL and 10 mL respectively. The worked final concentrations were prepared 10µg/mL respectively.

### Preparation of sample solution

A quantity of tablet powder equivalent to 10 mg (103.6 mg) was accurately weighed and transferred to 100 mL volumetric flask, dissolved in few mL of methanol, sonicated for 15 mints and made up to the volume by mobile phase acetonitrile: methanol: 5mM ammonium acetate buffer pH (3.0)in the ratio of 30: 20:50. This will give the concentration of 10 µg of escitalopram per millilitre solution of tablet sample.

## METHOD DEVELOPMENT

### Selection of wavelength

Stock solutions of 10mg/ml were prepared for escitalopram drug in methanol and further diluted to get the concentration of 10µg/mL for escitalopram was prepared with methanol. The wavelength was selected by scanning the above standard drugs between 200 to 400 nm. The scanned results showed that reasonably maximum absorbance for escitolapram and was recorded at 238 nm. Therefore, 238 nm was selected as the detection wavelength for the HPLC investigation. [Figure1].

### Method

The samples were applied Kromosil (250x4.6)mm 5µ column with Rhedyne injector in reverse saturation mode using acetonitrile: methanol: 5mM ammonium acetate buffer pH (3.0)in the ratio of 30: 20:50 as mobile phase with flow rate of 1.0 mL/min. The sample was performed using UV/VIS JASCO UV-1570 detector with flow rate 1ml/min. The instrument computes accurate results within minimal time. The retention time was obtained 5.36 for escitolapram respectively. The result of assay was reported in the table-1and figure-2.

## VALIDATION OF THE METHOD

### Accuracy

Accuracy was determined by tablet samples with different known concentrations of the drug (50%, 100% and 150%). Each concentration was injected in triplicate and the assay was performed as per the test method. From this % recovery and the amount present or recovered were calculated. Results of recovery study are reported in table-2.

### Precision

Standard stock solutions of escitalopram were prepared in the same manner for the standard preparation and prepared the mixed standard solution. This solution containing 10µg/mL of escitalopram. The repeatability was performed for three times. Results of precision are reported in table-3.

### Linearity

Linearity was determined in the range of 50-150 % (50, 75, 100, 125 and 150%) targeted concentration of assay procedure. five series of standard solutions containing 5.09,7.64,10.18,12.73 and 15.27 µg/mL of escitalopram were injected. Linearity of each drug was observed and response ratio of each drug was found out linearity of each drug is reported in table-4 and graph1.

### Ruggedness

The above sample prepared solution and diluted to get the concentration of 10 µg of escitalopram per millilitre of tablet sample. From this 20 µL was injected through column separately by two different analysts in the same HPLC system and same column. The result was reported in a table -5.

### Robustness

The above prepared standard mixture was determined by the variation of flow rate and variation of wavelength. The result was reported in a table -6.

## RESULT AND DISCUSSION

The present study was aimed to develop an accurate precise and linear HPLC method for analysis of escitalopram (ESP) and in

pharmaceutical dosage forms as per ICH guidelines. Escitalopram dosage form showed the linearity response over range 5.09-15.27 µg/mL. The correlation coefficient for three drugs was found to be 0.9997. The recovery studies of these three drugs were found to be 101.86 (ESP). The precision % RSD was found to be 0.68 for (ESP) respectively. The raggedness and robustness were studied with replicates standard solution of these drugs, and the result was found to be acceptance criteria.

**Fig-1- Overlain UV spectra of standard**

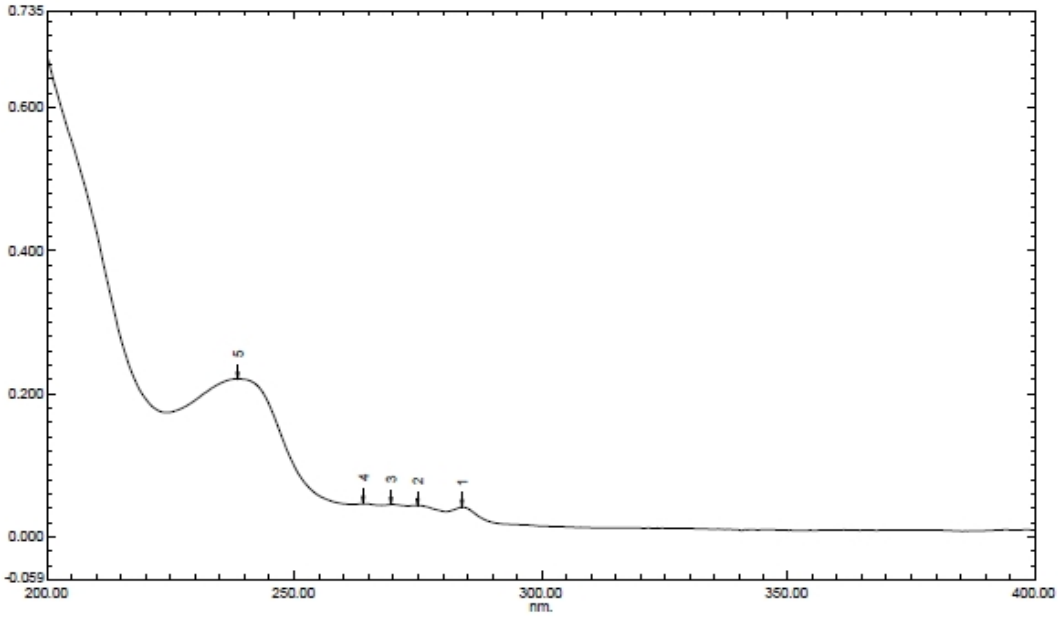
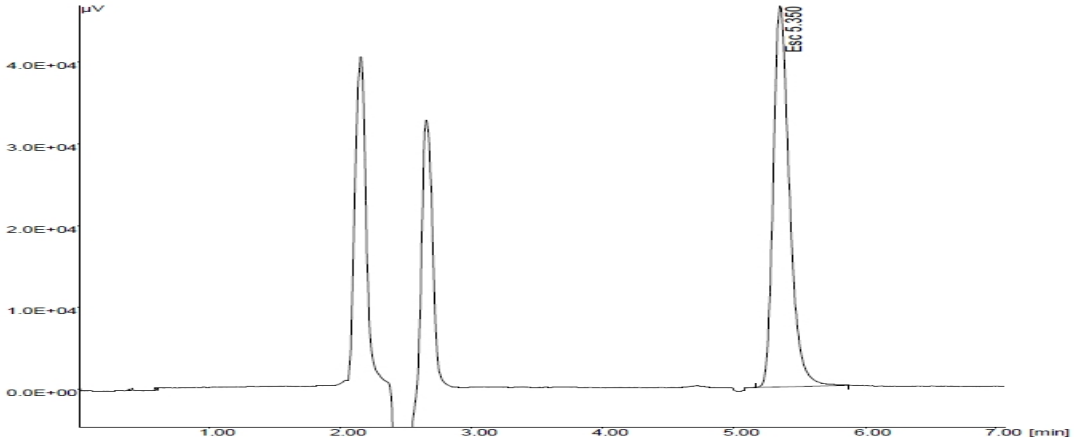



**Fig-2- Assay chromatogram of ESP**



**Graph -1- Linearity chart of standard escitalopram**

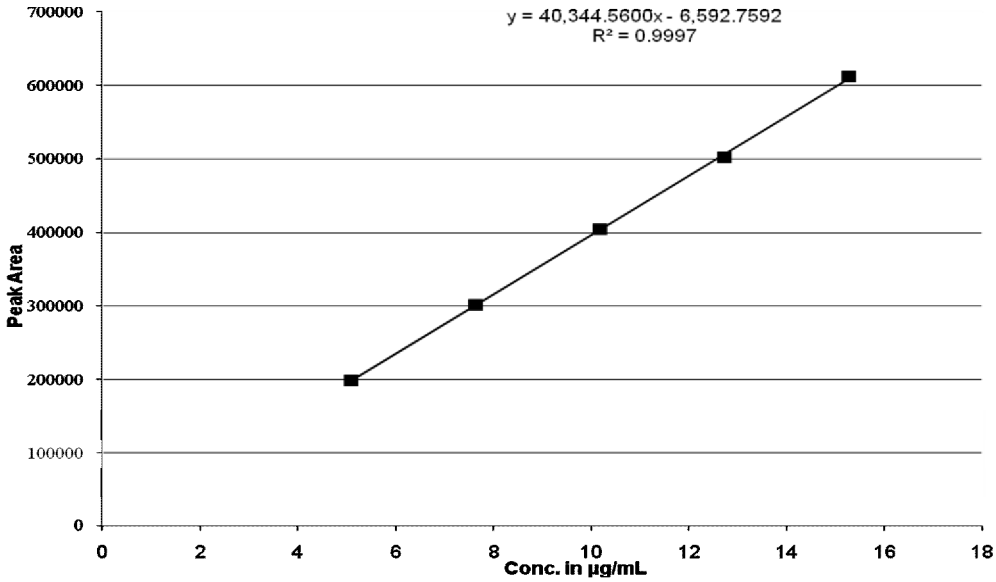



**Table -1- Results of assay**

| Name of the drug | Amount present per tablet(mg) | % of assay |
|---|---|---|
| Escitalopram (ESP) | 10 | 101.8 |

**Table -2- Results of recovery studies**

| Name of the drug | Amount taken (mg) | Amount found (mg) | % recovery | % of mean recovery |
|---|---|---|---|---|
| Escitalopram (ESP) | 5 | 5.07 | 100.73 | 100.50 |
| | 10 | 10.18 | 101.86 | 100.65 |
| | 15 | 15.09 | 100.95 | 101.08 |

**Table -3- Results of precision study**

| Injection No. | Retention time | Peak area |
|---|---|---|
| 1. | 5.350 | 400836.000 |
| 2. | 5.342 | 402876.563 |
| 3. | 5.342 | 406464.000 |
| 4. | 5.333 | 403422.750 |
| 5. | 5.342 | 407893.500 |
| 6. | 5.350 | 406821.250 |
| **Avg** | **5.3432** | **404719.0105** |
| **SD** | **0.0063** | **2745.7042** |
| **%RSD** | **0.12** | **0.68** |

**Table 4- Results of linearity studies of standard escitalopram**

| Concentration in µg/mL | Standard peak area |
|---|---|
| 5.0 | 199295 |
| 7.5 | 301794 |
| 10.0 | 404719 |
| 12.5 | 502375 |
| 15.0 | 612389 |

**Table-5-Ruggedness data of analyst – I and II**

| Analyst | Mean of peak area | %RSD | Recovery Quantity | % Recovery |
|---|---|---|---|---|
| Analyst-I | 403392 | 0.71 | 10.18 | 101.86 |
| Analyst-II | 404399 | 0.81 | 10.34 | 103.41 |

**Table -6- Robustness study.**

| Parameters | Variations Flow rate at 0.8ml\min | Flow rate at 1.2ml\min | Wavelength at 236 nm | Wavelength at 240 nm |
|---|---|---|---|---|
| Retention Time | 6.7 | 4.4 | 5.3 | 5.3 |
| Peak area | 507187 | 328262 | 377228 | 399867 |

## CONCLUSION

The proposed method gives good resolution of escitalopram within short analysis time (7minutes). The validation parameters are validated, and the results are complied with in the ICH guidelines. The method is very simple, specific, accurate, rapid and precise for the simultaneous determination of escitalopram dosage form. Therefore, the method can be used for routine quality control analysis of these drugs.

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