Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(11):4804-4809

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development of normal phase chiral liquid chromatographic method for estimation of escitalopram oxalate and determination of R-citalopram enantiomer from escitalopram oxalate in bulk drug and tablet

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ABSTRACT

A simple, sensitive, precise and high-performance liquid chromatographic (HPLC) method has been developed and validated for escitalopram oxalate and R-enantiomer of escitalopram oxalate in bulk drug and tablet using uv detector at 240 nm. The developed method was able to separate R-enantiomer of escitalopram oxalate from its bulk drug within 25 min. The chromatographic separation was carried out by normal phase chromatography using column of cellulose based chiral stationary phase (Chiralcel-OD 250mm x 4.6mm x 10µm) with mobile phase comprising of n-heptane, isoropanol, diethylamine (94.5:5:0.5, v/v/v) at a flow rate of 1.0 ml/min at 25°C temperature. The limit of detection (LOD) and limit of quantitation (LOQ) of R-enantiomer of escitalopram oxalate were found to be 0.16 µg/ml and 0.50 µg/ml respectively. The linearity of response of R-citalopram oxalate was in the range of 0.05 µg/ml to 7.5 µg/ml with r > 0.9999. The percentage recovery of the escitalopram oxalate for bulk drug sample ranged between 98.20 to 98.97 and for tablet ranged between 97.07 to 99.61. The percentage recovery of the R-citalopram oxalate from escitalopram oxalate bulk drug sample ranged between 97.41 to 100.79 and for escitalopram oxalate tablet ranged between 98.82 to 99.61. The method was validated and found to suitable for estimation of escitalopram oxalate and determination of R-citalopram oxalate from escitalopram oxalate bulk drug and tablet.

Keywords: Escitalopram oxalate, R-Enantiomer (R-citalopram oxalate), High performance liquid chromatography, Method validation and quantitation

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INTRODUCTION

Depression, anxiety and obesity are some of the most common and serious health problems of the people today. Development of therapeutic agents to treat these disorders is of significant interest in recent times. Escitalopram oxalate or S-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofuran-carbonitrile oxalate is one of the novel drugs belonging to the group of selective serotonin reuptake inhibitors (SSRI) for the treatment of various affective disorders[1]. It is active not only against major depression, but also against panic, anxiety, obsessive compulsive disorder pathological laughing and crying [2-3]. Many of pharmaceutical formulation products contain racemic mixture escitalopram oxalate, while recent ones contain only the active escitalopram. Its pharmacological effect is mainly due to the escitalopram oxalate while R-citalopram oxalate is considered to be inactive. Owing to the pharmacological and toxicological differences between these isomers, it is quite important to develop stereo specific assay for separation these of drugs.

Literature survey revealed that various methods have been reported for the determination of escitalopram in human plasma and are used in bioequivalence study [4], fluorimetry, quantitation of plasma [5], validation of capillary electrophoresis for simultaneous determination of impurities [6], chiral HPLC method for separation of enantiomers [7-12], simultaneous determinations of escitalopram and clonazepam in combined dosage form by UV, HPLC [13-

15] and HPTLC methods [16-17]. However normal phase LC method for quantitation of R-enantiomer from escitalopram oxalate using chiralcel OD column within 25 min. has not been reported in the literature.

In the present research work, a simple, sensitive and accurate normal phase HPLC method to separate R-enantiomer of escitalopram oxalate in bulk drugs and tablets using polysaccharide-based (chiralcel OD) column has been reported for first time. The method was also validated to ensure the compliance in accordance with the ICH guidelines [18].

EXPERIMENTAL SECTION

2.1 Materials and reagents

HPLC grade n-heptane, isopropanol, methanol and diethylamine were purchased from Merck (Mumbai, India) and escitalopram oxalate reference standard and R-citalopram oxalate reference standard were obtained from LGC Promochem India Private Limited. Sample of escitalopram oxalate was obtained from the process development laboratory of API plant unit of RPG Life Sciences Ltd, Navi Mumbai, India. NEXITO 5 tablet (B. No.: BSJ0439, each tablet contains escitalopram oxalate equivalent to 5.0 mg of escitalopram, Manufacturing Batch-03/2010 and Expiry-02/2012) was obtained from market sample of Sun Pharma Private Limited, Sikkim, India. All solutions were filtered through 0.45µm membrane filters.

2.2 Instrumentation

The HPLC system composed of LC-2010 CHT, with variable wavelength UV-Visible detector, auto injector and LC solution data processor. Chiral column chiralcel OD (250 mm x 4.6 mm) 10 µm, with Chiralcel OD (50 mm x 4.6 mm) guard column,(Daicel chemical Industries, Japan) were used for separation. The chromatographic and integrated data were recorded using HP computer system.

2.3 Chromatographic conditions

Chromatographic separation was achieved on Chiralcel OD (250 mm x 4.6 mm) 10 µm column attached with chiralcel OD (50 mm x 4.6 mm) guard column with mobile phase consisting of n-heptane, isopropanol and diethylamine in the volume ratio (94.5:5:0.5) at 25°C. The flow rate was 1.0 ml/min and detector wavelength was kept at 240 nm for monitoring separation. 10 µl volume was injected into the system with total run time of 25 min.

2.4 Preparation of standard stock solution of R-citalopram oxalate (25 µg/ml)

2.5 mg of R-citalopram oxalate was accurately weighed and dissolved in 2.0ml methanol first and diluted to the mark in 100ml standard volumetric flask with n-heptane and isopropanol (50:50 v/v) to get the concentration of 25 µg/ml. This stock solution was stored in a refrigerator at 5°C.

2.5 Preparation of working standard solution of R-citalopram oxalate (5 µg/ml)

Working standard solution of R-citalopram oxalate was prepared by diluting aliquot of 5.0 ml of stock solution of R-citalopram oxalate in 25 ml standard volumetric flask with mobile phase to get the concentration of 5 µg/ml.

2.6 Preparation of standard solution of escitalopram oxalate (500 µg/ml)

25 mg of escitalopram oxalate standard was accurately weighed and dissolved in 2.0 ml methanol first and diluted to the mark in 50ml standard volumetric flask with n-heptane and isopropanol (50:50 v/v) to get the concentration of 500 µg/ml.

2.7 Preparation of sample solution of escitalopram oxalate (500 µg/ml)

25 mg of escitalopram oxalate bulk drug sample was accurately weighed and dissolved in 2.0 ml methanol first and diluted to the mark in 50ml standard volumetric flask with n-heptane and isopropanol (50:50 v/v) to get the concentration of 500 µg/ml.

2.8 Preparation of sample of escitalopram oxalate tablet (500 µg/ml)

Twenty tablets of escitalopram oxalate were weighed accurately and finely powdered. The powder equivalent to 50 mg of escitalopram oxalate was accurately weighed and dissolved in 10.0 ml methanol first, sonicated for 10 minutes and diluted to the mark in 50 ml standard volumetric flask with n-heptane and isopropanol (50:50 v/v) to get the concentration of 1000 µg/ml and filtered through 0.45µ membrane. Further 5.0 ml of this solution was diluted to the mark in 10 ml standard volumetric flask with n-heptane and isopropanol (50:50 v/v) as diluents to give a solution of concentration of 500 µg/ml of escitalopram oxalate.

3.0 METHOD VALIDATION

3.1 **Method validation parameters**

3.1.1. **Specificity**

Specificity is the ability of the method to measure the analyte response in the presence of its impurity. Enantiomer resolution of R-enantiomer and baseline separation was achieved using chiralcel OD column. There was no interfering peak co-eluted with the compound of interest. This has indicated appropriate specificity of elaborated procedure. The order of elution was determined using UV detector, the retention times of R-citalopram and escitalopram oxalate were approximately 14.6 min and 17.3 min, respectively. A typical chromatogram of R-citalopram oxalate along with escitaloptam oxalate has been represented in Fig. 1.

3.1.2. **Precision**

The method was validated in terms of system precision, method precision and intermediate precision. The system precision was studied by separate, repetitive injections (n=6) of standard solution of escitalopram oxalate (500 µg/ml) and R-citalopram oxalate (5.0 µg/ml), in the chromatographic system under the specified conditions. The percent relative standard was found to be less than 1. The method precision was evaluated by carrying out six replicates of escitalopram oxalate bulk drug sample. The values of percent relative standard deviation of retention time and peak areas for escitalopram oxalate and R-citalopram oxalate were found to be less than 1. The intermediate precision of the method was evaluated using on different days in the same laboratory. The values of percent relative standard deviation of retention time and peak areas for escitalopram oxalate and R-citalopram oxalate for intermediate precision were found to be less than 1.The results indicate that method is precise and reproducible.

3.1.3. **Limit of Detection (LOD) And Limit of Quantitation (LOQ) of the escitalopram oxalate and R-citalopram oxalate**

The limit of detection and limit of Quantitation of the escitalopram oxalate and R-citalopram oxalate were estimated at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of diluted solution of escitalopram oxalate and R-citalopram oxalate with known concentrations. The values of LOD and LOQ were found to be 0.16 µg/ml and 0.50 µg/ml respectively.

3.1.4. **Linearity of the escitalopram oxalate and R-citalopram oxalate**

Linearity was evaluated by analyzing working standard solutions of escitalopram oxalate in the concentration range 0.50 µg/ml to 600 µg/ml and R-citalopram oxalate in the concentration range 0.50 µg/ml to 7.50 µg/ml. The solutions were injected in duplicate in the chromatographic system under optimized conditions describe earlier. The calibration plot for escitalopram oxalate was found to be linear in the concentration range 0.50 µg/ml to 600 µg/ml, with correlation coefficient r as 0.9999. Similarly R-citalopram oxalate was found to be linear in the concentration range 0.50µg/ml to 7.50 µg/ml with correlation coefficient r as 0.9992.

3.1.5. **Accuracy**

The accuracy of the method was established by performing recovery experiment using weight variation method for escitalopram oxalate and standard addition method for R-citalopram oxalate. For zero level, only sample solution was analyzed by HPLC in triplicates. To 500 mg of escitalopram oxalate sample, pure standard solutions of R-citalopram oxalate with varying concentrations (0.50 µg/ml, 4.0 µg/ml, 5.0 µg/ml, 6.0 µg/ml) respectively were added. The solutions were prepared and analyzed by HPLC for each level and mean amounts of escitalopram oxalate and R-citalopram oxalate present in each level of sample solution were determined. The value of percent recovery for escitalopram oxalate in bulk drug was found to be 98.67 and percent recovery in escitalopram oxalate tablet was found to be 98.25. The average value of percent recovery for R-citalopram oxalate in escitalopram oxalate bulk drug was found to be 99.37 and percent recovery for R-citalopram oxalate in escitalopram oxalate tablet was found to be 99.19. As the values are close to 100 %, it indicates a good accuracy of the method.
The results are given in Table 1 and Table 2.

3.1.6. **Robustness**

Robustness of the method was determined by making small deliberate changes in the chromatographic conditions utilized in present method parameter, with changes made to the chromatographic conditions described as, change in the mobile phase composition (+/- 10 % of organic phase used in mobile phase), flow rate (+/- 10 %) and wavelength (+/- 2 nm).

The amounts of escitalopram oxalate and R-citalopram oxalate from escitalopram oxalate sample in bulk drug and tablet obtained by altered method to that obtained by normal method were found to be similar and modifications in the chromatographic method did not affect the system suitability criteria. The resolution between escitalopram oxalate and R-citalopram oxalate was greater than 2.5; under all separation conditions tested, demonstrating

sufficient robustness. As deliberate changes made to the chromatographic method did not affect the results, it can be concluded that the method is robust.

RESULTS AND DISCUSSION

The aim of present work was to separate the R-enantiomer of escitalopram oxalate. The escitalopram oxalate and R-citalopram oxalate are stereoisomers.

The mobile phase comprising of n-heptane, isopropanol and diethylamine in the volume ratio (94.5:5:0.5) in the present research work shows a better resolution of different components present in escitalopram oxalate bulk drug and tablet. Addition of diethylamine in the mobile phase had a significant effect on the resolution and tailing of compounds, it gave symmetrical, sharp and well-resolved peaks were observed for escitalopram oxalate and R-citalopram oxalate from its sample. Isopropanol used in the mobile phase is easily miscible with n-heptane , has low viscosity, low surface tension, and is economical. The wavelength selected for analysis is 240 nm, which gave better sensitivity for R-enantiomer of escitalopram oxalate. The flow of mobile phase was 1.0 ml/min.

The effect of column temperature on resolution and retention of escitalopram oxalate was studied using chiralcel OD column at 25°C, 30°C and 35°C. On increasing temperature, retention as well as resolution was decreased. Hence column temperature used was 25°C.

The values of limit of detection (LOD) and limit of quantification (LOQ) concentration were found to 0.16 µg/ml and 0.50 µg/ml for R-citalopram and escitalopram oxalate. Good linearity was observed for R-citalopram oxalate over the concentration range of 0.50 µg/ml -7.50 µg/ml, with linear regression equation y = 20967 x – 2915 and curves were linear with correlation coefficient of 0.9992. Similarly, good linearity was observed for escitalopram oxalate over the concentration range of 0.50 µg/ml - 600 µg/ml, with linear regression equation y = 21571 x – 5258 and correlation coefficient value was 0.9999. In the repeatability study, the relative standard deviation (RSD) was less than 1.0 % for the retention times and peak areas of the R-citalopram and for escitalopram peak area. The accuracy of method was evaluated by using freshly prepared solution of R-citalopram oxalate at four concentration levels of 0.50 µg/ml, 4.0 µg/ml, 5.0 µg/ml, 6.0 µg/ml of analyte concentration. The percentage recovery values of R-citalopram oxalate and escitalopram oxalate were in the range of 97.5 to 100.79. The resolution between escitalopram and R-citalopram was greater than 2.5; under all separation conditions tested, demonstrating sufficient robustness. As deliberate changes made to the chromatographic method did not affect the results, it can be concluded that the method is robust.

Several chromatographic methods have been reported in literature for the determination of escitalopram oxalate and R-citalopram oxalate in bulk drug or its formulations likewise.

In reverse phase chromatography, use of chiral-recognition protein column (Ultron ES-OVM) (150 mm x 4.6 mm x 5µm) is being used in the United States Pharmacopeia method [8]. The mobile phase comprised of 0.05 M monobasic potassium phosphate pH-7, and acetonitrile (17:3 v/v). Retention time of escitalopram oxalate is 13.7 min. and retention time R-citalopram oxalate is 12.5 min. After few numbers of injections into HPLC system, the resolution between R-citalopram oxalate from escitalopram oxalate bulk is minimize using Ultron ES-OVM column in the USP method. Life of the column is less and column is costly. In present research work the components, escitalopram oxalate and R- citalopram oxalate were quantitated at single wavelength of 240 nm with a good sensitivity and using chiralcel OD column of 250 mm x 4.6 mm, 10 µm particle size attached with chiralcel OD (50 mm x 4.6 mm) guard column. It increased the separation efficiency of both the standards from sample and gave symmetrical, sharp and well-resolved peaks of escitalopram oxalate and R-citalopram oxalate. By using the guard column, the life of the Chiralcel OD column was increased. After analyzing more than 300 samples of escitalopram oxalate, it was found that resolution between escitalopram oxalate and R-citalopram oxalate is more than 2.5.

The normal phase HPLC method has been reported in literature for the determination of enantiometric purity of citalopram hydrobromide in bulk drugs and pharmaceuticals [10]. The HPLC analysis was carried out using chiralcel OD-H (250 mm x 4.6 mm) 5 µm column, using a mobile phase containing n-hexane,2-propanol, triethylamine (95:5:0.1 v/v/v). Clopidogrel hydrogen sulphate was used as an internal standard (IS) for quantitative determinations using UV detector at λ = 240 nm. Retention time of S-citalopram is about 15.5 min., retention time R-citalopram is about 12.5 and retention time of internal standard is about 6.0 min. In present research work the components, R-citalopram oxalate and escitalopram oxalate were quantitated at single wavelength of 240 nm using UV detector with a good sensitivity and using chiralcel OD column of 250 mm x 4.6 mm, 10 µm particle size attached with chiralcel OD (50 mm x 4.6 mm) guard column. Diethylamine is used as additive to minimise the peak tailing & improve the peak shape in the sample.

Table 1. Recovery results of studies of R-citalopram from escitalopram oxalate bulk drug using the proposed HPLC method

Recovery level	Amount of R-citalopram oxalate added (µg/ml)	*Mean amount of R-citalopram oxalate found (µg/ml)		
		Mean amount found	% Average Recovery	% RSD
1	0.50	3.05	99.71	0.14
2	4.0	6.62	98.70	0.42
3	5.0	7.80	101.64	0.15
4	6.0	8.85	101.60	0.30

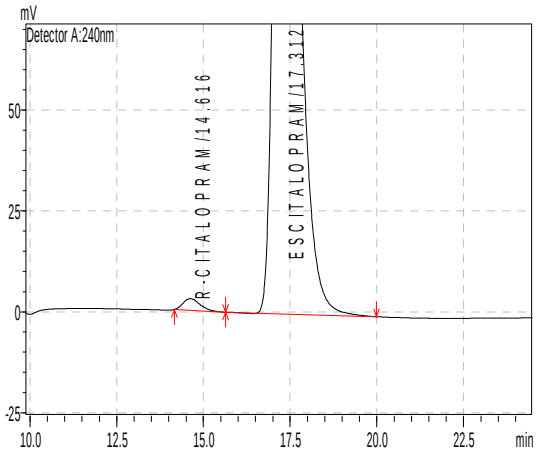
**n=3*

Table 2. Recovery results of studies of R-citalopram from escitalopram oxalate tablet using the proposed HPLC method

Recovery level	Amount of R-citalopram oxalate added (µg/ml)	*Mean amount of R-citalopram oxalate found (µg/ml)		
		Mean amount found	% Average Recovery	% RSD
1	4.0	3.97	99.06	0.32
2	5.0	4.96	99.22	0.30
3	6.0	5.94	99.30	0.30

**n=3*

Fig. 1. A typical chromatogram of R-citalopram oxalate spiked with escitalopram oxalate bulk drug



CONCLUSION

An isocratic, stereo selective and rapid chiral liquid chromatographic method was developed for enantiomeric separation and quantitative determination of escitalopram oxalate from its bulk drug. The method was found to be precise, sensitive, accurate and specific in bulk active substances. The method was completely validated showing satisfactory data for all validation parameters tested. The developed method could be used for quantification of R-citalopram in bulk samples of escitalopram oxalate.

Acknowledgments
The authors wish to thank the management of RPG LIFE SCIENCES Group, Navi Mumbai for all kind of supports for this research work. Authors wish to acknowledge the API group for providing the samples for our research.

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