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Original Research Article

A NEW VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF CITALOPRAM IN TABLET DOSAGE FORMS

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ABSTRACT

An accurate high performance liquid chromatographic method was developed for quantification of citalopram in its tablet dosage forms. Ideal separation of the drug was achieved on an Agilent Eclipse XDB C_{18} column (150 x 4.6 mm; 5µ) by eluting with a mobile phase consisting of a mixture of acetate buffer (pH 4.5) and acetonitrile (65:35 v/v) at a flow rate of 1.0 mL/min. The drug in the eluates was monitored by U V detection at 240 nm. Under optimized conditions, the retention time obtained for the drug was 3.72 min. The relevant calibration plot was linear in the concentration range of 25-150 µg/mL of the drug. The validation of the method was done by following the ICH guidelines. The proposed method could be applied for determination of citalopram in its tablet dosage forms without any interference from normal excipients. The method thus, is suitable for routine quality control analysis of citalopram.

Key words: Citalopram, Estimation, Tablets, HPLC.

INTRODUCTION

Citalopram hydrobromide [(±)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1, 3-dihydroisobenzofuran -5-carbonitrile, hydrobromide] is an orally active selective serotonin reuptake inhibitor (SSRI) and is used in the management of depression[1], obsessive-compulsive disorder and anxiety disorder[2]. It has a chemical structure unrelated to those of the other SSRIs and other available antidepressant agents. The mechanism of action of citalopram hydrobromide as an antidepressant is presumed to be linked to the potentiation of serotonergic activity in the central nervous system[3]. A literature survey revealed the report of some analytical methods for the quantification of citalopram in pharmaceutical dosage forms by HPLC[4-11] technique. We have developed a new, accurate and precise RP-HPLC method with short retention and run times for the determination of citalopram in bulk drug samples and in tablet dosage forms. The developed method was duly validated as per ICH guideline.[12]

MATERIALS AND METHODS

Drugs, chemicals, and solvents

The pure reference sample of citalopram was obtained from Aurobindo Pharmaceuticals, Hyderabad. The commercial tablet formulation of citalopram 'Celepra' (10 mg) manufactured by Micro Labs Ltd., Bangalore was purchased from the local market. Ammonium acetate, triethylamine, glacial acetic acid and HPLC grade acetonitrile, methanol and water were purchased from Rankem Fine chemicals Ltd., Mumbai.

Equipment and chromatographic conditions

A Waters Alliance liquid chromatograph (model 2695) fitted with a diode array detector (model 2996) and running on Empower2 data handling system was employed in the study. An Agilent Eclipse XDB C_{18} column (150 x 4.6 mm; 5µ) was used for analyzing the drug. All the chromatographic runs were carried out by using a mobile phase consisting of a mixture of acetate buffer (pH 4.5) and acetonitrile (65:35 v/v) in isocratic mode at a flow rate of 1.0 mL/min. The injection volume of the samples was 10 µL. The detector wavelength was set at 240 nm. The chromatographic run time was set as 8.0 min.Under these optimized conditions, the retention time obtained for citalopram was 3.727 min.

Preparation of the acetate buffer

To prepare the acetate buffer 0.385 gm of ammonium acetate and 0.5 mL of triethylamine were transferred into a1000 mL beaker and mixed with about 800 mL of milli-Q water. The contents were sonicated and the volume was made up to 1000 mL. The pH of the solution was then adjusted to 4.5 with glacial acetic acid. It was then filtered through a 0.45µ membrane filter.

Preparation of the mobile phase

The optimized mobile phase consisted of a mixture of the above-mentioned acetate

buffer (pH 4.5) and acetonitrile in a ratio of 65:35 v/v.

Preparation of the diluent

A mixture of the acetate buffer (pH 4.5) and acetonitrile in the ratio of 50:50 v/v was employed as the diluent for preparing some drug solutions.

Preparation of the working standard solution of citalopram

50 mg of citalopram reference standard was accurately weighed and transferred into a 50 mL volumetric flask. To this, 25 mL of acetonitrile was added, sonicated for 5 minutes and the volume was made up with a further quantity of acetonitrile. This was used as the standard stock solution. The working standard solution was prepared by diluting 1.0 mL of the standard stock solution to 10 mL with the diluent in a volumetric flask.

Calibration plot

Solutions of citalopram at different concentration levels including the working standard concentration were prepared in the diluent. Ten microlitres of each concentration was injected three times into the HPLC system (n=3). The response was read at 240 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas at the different concentration levels were calculated and the linearity plot of mean peak areas over their concentrations was constructed.

Estimation of the drug from the tablet dosage forms

15 tablets (Celepra; 10 mg) were crushed into a fine powder, and a quantity of the powder equivalent to 100 mg of citalopram was transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated for 25 min. Then, the volume was made up with the diluent, mixed well and the contents filtered through a 0.45µ nylon filter. From the filtered solution, a quantity of 1.0 ml was pipetted out into a 10 ml volumetric flask and diluted upto the mark with the diluent. This solution was then chromatographed six times. From the peak areas obtained in the chromatograms, the average drug content in the formulation was calculated.

RESULTS

Table.1. Optimized chromatographic conditions

Stationary phase	**Agilent Eclipse XDB C18 (150 x 4.6 mm; 5µm)**
Mobile phase	Acetate buffer (pH 4.5) - Acetonitrile (65:35 v/v)
Flow rate	1.0 mL/min
Column temperature	30°C
Injection volume	10 µL
Detection wavelength	240 nm
Run time	8 min

Table.2. Accuracy data of the proposed method

Analyte	Amount of the analyte taken (µg/mL) (n=3)	Mean recovery (µg/mL) ± SD	% Mean recovery ± SD
Citalopram	50	49.00 ± 0.341	98.01 ± 0.683
	100	101.40 ± 0.313	101.40 ± 0.313
	150	149.57 ± 0.098	99.71 ± 0.065

Table.3. Precision data of the proposed method

S.No.	Intra-day precision	Inter-day precision
1	1617890	1609754
2	1621056	1612498
3	1612908	1604753
4	1603209	1610784
5	1603742	1617354
6	1617460	1603498
Average	1612711	1609774
SD	7612.93	5108.73
%RSD	0.47	0.32

Table.4. System suitability parameters of the proposed method

Parameter	Value
Retention time (min)	3.691
Tailing factor	1.01
Theoretical plates	8461
HETP	0.0177

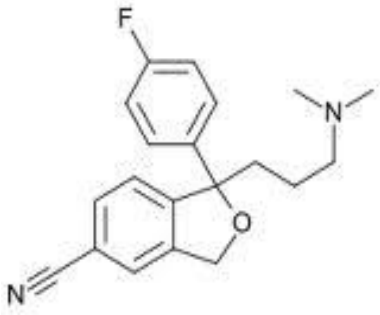

Fig.1.Structure of citalopram

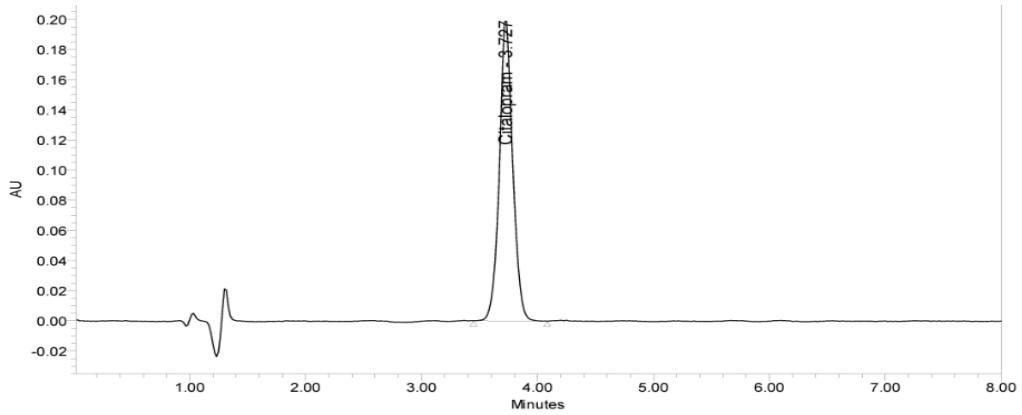

Fig.2. Chromatogram of citalopram from working standard solution

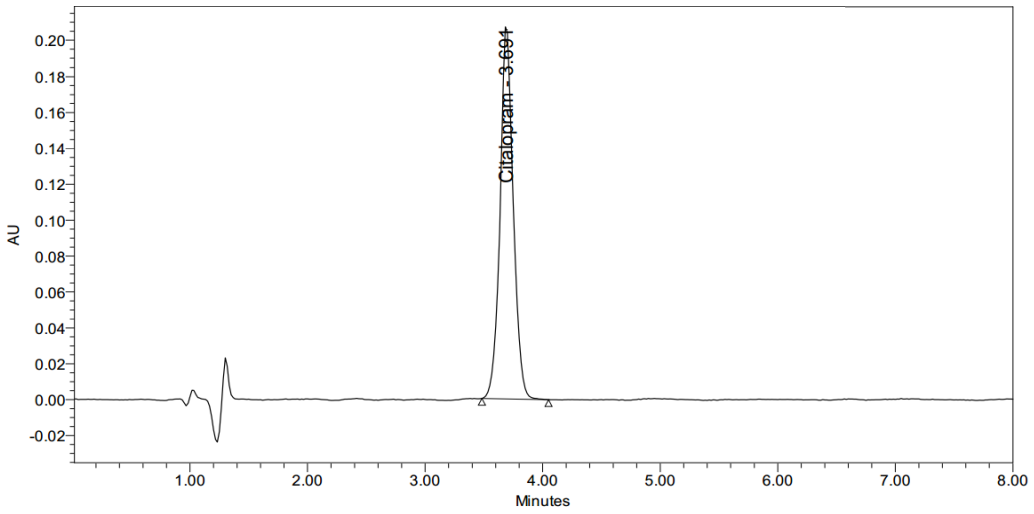

Fig.3. Chromatogram of citalopram from its tablet dosage form

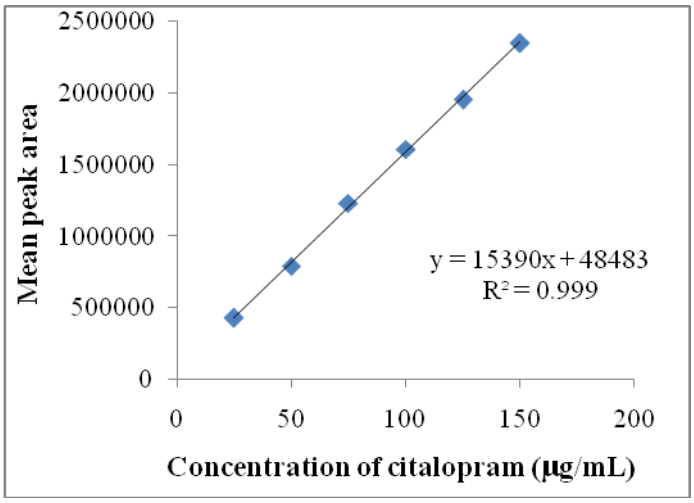

Fig.4.Linearity plot for citalopram

DISCUSSION

During the method optimization studies, various combinations and proportions of the solvents and buffers were examined on an Agilent Eclipse XDB C_{18} column for efficient separation of citalopram. Using a mobile phase consisting of a mixture of acetate buffer (pH 4.5) and acetonitrile in the ratio of 65:35 v/v, a good resolution and baseline separation of the drug peak was obtained. All the chromatographic conditions were optimized by evaluating the column efficiency parameters like theoretical plates and tailing factor (Table 1). Under these optimized conditions, the retention time obtained for citalopram was 3.72 min (Figure 2) in a run time of 8.0 min. The method was then validated as per the ICH guideline. The proposed method was also found to be applicable for the analysis of citalopram in tablet formulations.

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of probable interferences from its solvent as well as from the excipients of its formulation. Figure 2 shows good chromatographic baseline separation of citalopram from its working standard solution. Figure 3 demonstrates that no interfering peaks were observed at the retention time of citalopram arising due to the excipients of its tablet.

Linearity

The calibration curve (n=3) constructed for the drug was linear over the concentration range of 25 – 150 µg/mL. The regression of the plot was computed by least square regression method and is shown in Figure 4. The correlation coefficient is greater than 0.99 and the %RSD at each concentration studied was less than 2.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percent recovery with its standard deviation were calculated (Table 2). The high percentage of recovery indicates that the proposed method is quite accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were made and the percent RSD was calculated (Table 3).

System suitability parameters

System suitability parameters were studied with six replicate injections of the standard solution and the results are presented in Table 4.

Method suitability

The commercial tablet formulation, Celepra (10 mg) was analyzed by the proposed method. The recovery obtained (100.1%) by the proposed method was found to be in good agreement with the labelled amount of the drug, which confirms the suitability of the method for the analysis of citalopram in tablet dosage forms.

CONCLUSION

The proposed RP -HPLC method is sensitive, precise and accurate and can be used for the routine determination of citalopram in its tablet dosage forms.

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