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Research Article

TO DEVELOP ARP-HPLC ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM&CLONAZEPAM FORIT'S QUANTIFICATION IN FINISHED DOSAGE FORM

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ABSTRACT

A Simple, efficient and reproducible reverse phase high performance liquid chromatographic method was developed and validated for the Simultaneous determination of Escitalopram oxalate and Clonazepam in combined dosage form.The process was carried out on aInertsil C18 ODS 3V, 15 cm X 4.6 mm, 5µm or equivalent column. The flow rate was 1ml/min and eluent was monitored by absorbance at 254 nm using a mixture of Methanol and Buffer (pH 7.0) in the ratio of 55:45 (v/v). The retention times of Escitalopram and Clonazepam was found to be 17.3 and 10.02 min respectively. Calibration plots were linear in the concentration range of 80-120ppm and 8-12ppm for Escitalopram and clonazepam respectively. All the analytical validation parameters were determined and foundto be within the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of Escitalopram oxalate and Clonazepam in tablet dosage forms.

Key Words:*Escitalopram,Clonazepam,RP-HPLC,Validation, ICH Guidelines.*

INTRODUCTION

Escitalopram oxalate (ESC; S-(+)-1-(3-(dimethylamino) propyl)-1-(p-fluorophenyl)-5-phthalan carbonitrile oxalate; (as shown in Figure 1a) is the S enantiomer (single isomer) of the racemic bicyclic phthalane derivative of citalopram.Escitalopram is freely soluble in methanol and dimethlysulfoxide (DMSO), sparingly soluble in water and in ethanol, slightly soluble in ethyl acetate, insoluble in heptane & it has selective serotonin reuptake inhibitor activity.

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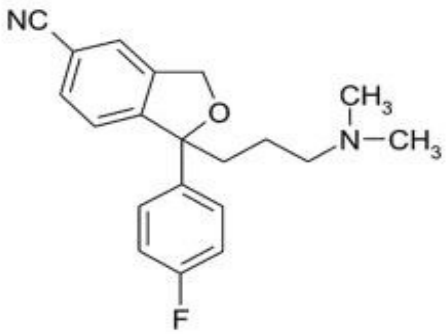
Clonazepam (CLO; 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepine-2-one; (as shown in Figure 1b) is a benzodiazepine derivative, Clonazepam is slightly soluble in acetone, chloroform, acetic anhydride, hardly soluble in methanol, isopropanol, ether, almost insoluble in water having antiepileptic activity [1]. ESC is used in the management of major depressive disorder and generalized anxiety disorder whereas CLO is used in the management of epileptic disorder.

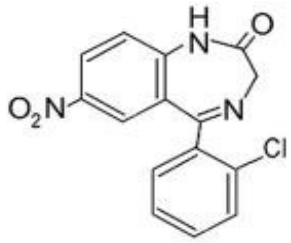
Literature survey revealed several spectroscopic [2-4], HPLC [5,6] and HPTLC [7-16] methods for estimation of Escitalopram and clonazepam individually as well as combination with other drugs. All the reported HPLC methods used buffer in the mobile phase and having longer retention time. The

present investigation describes a rapid, accurate and precise RP-HPLC method for the determination ofEscitalopram oxalate and Clonazepam from bulk sample and pharmaceutical combined dosage forms and the method was validated as per ICH guidelines.



Escitalopram oxalate ($C_{20}H_{21}N_2O.\ C_2H_2O_4$)

(a)



Clonazepam ($C_{15}H_{10}ClN_3O_3$)

(b)

Figure 1

Structural formulas of escitalopram oxalate and clonazepam.

MATERIALS & METHODS

a.Chemicals:

Bulk samples of ESC and CLZ were obtained from Medley Pharmaceuticals Ltd. Mumbai, India. Methanol (HPLC Grade), Water (HPLC Grade), Triethyl amine (HPLC Grade) Potassium dihydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) was purchased from RANCHEM (India). Mili-Q water was used throughout the experiment.

b. Equipment's:

Quantitative HPLC was performed on a isocratic HPLC of SHIMADZU prominence consisting of LC-2010CHT liquid pump, manual with 100µL sample injection loop and SPD 20A UVVisible absorbance detector. The output signal was monitored and integrated by Shimadzu classVP software.

c. Liquid chromatographic conditions:

Chromatographic conditions were obtained using aInertsil column (C18, ODS 3V, 15 cm X 4.6 mm, 5µm or equivalent), which was maintained at 40^0C. The analytical wavelength was set at 254 nm and samples of 50µl were injected to HPLC system. The mobile phase was methanol and Phosphate Buffer in ratio of (45:55v/v) (pH=7) at a flow rate of 1ml/min. The mobile phase was filtered through 0.22µm filter and degassed for 10 minutes by ultra-sonication.

d. Diluent

Water: Methanol (50:50v/v) was used as a diluent.

e. Preparation of standard stock solutions:

Clonazepam Reference Stock Solution

Weighed and transferred accurately about 20.0 mg of Clonazepam working standard into a 100 ml of volumetric flask. Added 70 ml of diluent and sonicated to dissolve. Cooled & made up the volume to 100 ml with diluent. (Conc.: 200 ppm of Clonazepam)

Reference Solution

Weighed and transferred accurately about 20.0 mg of Escitalopram Oxalate working standard into a 200 ml of volumetric flask. Added 100 ml of diluent, 10.0 ml of Clonazepam Reference Stock Solution (1) and sonicated to dissolve. Cooled & made up the volume to 200 ml with diluent. Mix and inject the solution.

Filtered the solution through 0.45 µ nylon syringe filter and injected. (Conc.: 100 ppm of Escitalopram Oxalate & 10 ppm of Clonazepam)

f. Preparation of Sample solutions:

Weighed accurately 20 tablets and calculated average weight. Crushed 20 tablets by suitable means to fine powder.Weighed powder equivalent to 10.0 mg of Escitalopram and 1.0 mg of Clonazepam in to a 100 ml of volumetric flask. Added 50 ml of diluent and sonicated for 15 minutes. Cooled and diluted to volume with diluent.

Filtered the solution through 0.45 µ nylon syringe filter and injected. (Conc.: 100 ppm of Escitalopram Oxalate & 10 ppm of Clonazepam)

g. Determination of Assay:

Five replicates of sample in equal volume (20µL) were injected separately into the stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug present per tablet was calculated by comparing a sample peak with that of standard solution.

METHOD VALIDATION[17, 18, 19]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Specificity:

Amount of Escitalopram& Clonazepamtest, blank& Placebo is spiked and the sample is analysed for Escitalopram& Clonazepam recovery by HPLC.

Acceptance criteria:% recovery of Escitalopram should be within 100±2%

Linearity of Response:

To demonstrate the linearity of response, series of solutions ranging from 80-120 ppm and 8-12 ppm for ESC and CLZ respectivelywere made and injected into the HPLC system following the described conditions. The graph was constructed between concentration vs. peak area and it was found that correlation co-efficient and regression analysis were within the limits

Acceptance Criteria:The correlation co-efficient (R2) should be not less than 0.98.

Accuracy

To establish the accuracy of the test method, sample solutions in triplicate by spiking the test solutions withEscitalopram& Clonazepam at 50%, 100% and 150% of the specification were prepared and injected into the HPLC system as per the test procedure. The 'amount added', 'amount found' and average % recovery for Escitalopram& Clonazepam at 50%, 100% and 150% spike levels were calculated and the results are summarized.

Acceptance criteria:The mean recovery should be within 100±2%.

Robustness

The robustness of an analytical procedure has been defined by the ICH as a "measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. The typical variations studied under this parameter are Flow rate,Temperature& mobile phase.

Acceptance criteria

RSD for the peak areas of five replicate injections of the Standard is not more than 2.0%.

RESULTS &DISCUSSION

To develop a suitable and robust LC method for the simultaneous estimation of ESC and CLZ different mobile phases and columns were employed to achieve the efficient separation and resolution. The criteria employed for selecting the mobile phase for the analysis of the drugs were cost and timeinvolved required for the analysis. Attempts with different chromatographic conditions & reverse phase columns presented poor peak symmetry and tailing problem. The proposed method was able to selectively separate ESC in a short chromatographic run with the use of buffer mobile phase having pH 7. The retention time for ESC and CLZ are

17.308 and 10.050 min respectively. The chromatogram is shown in Fig No.2

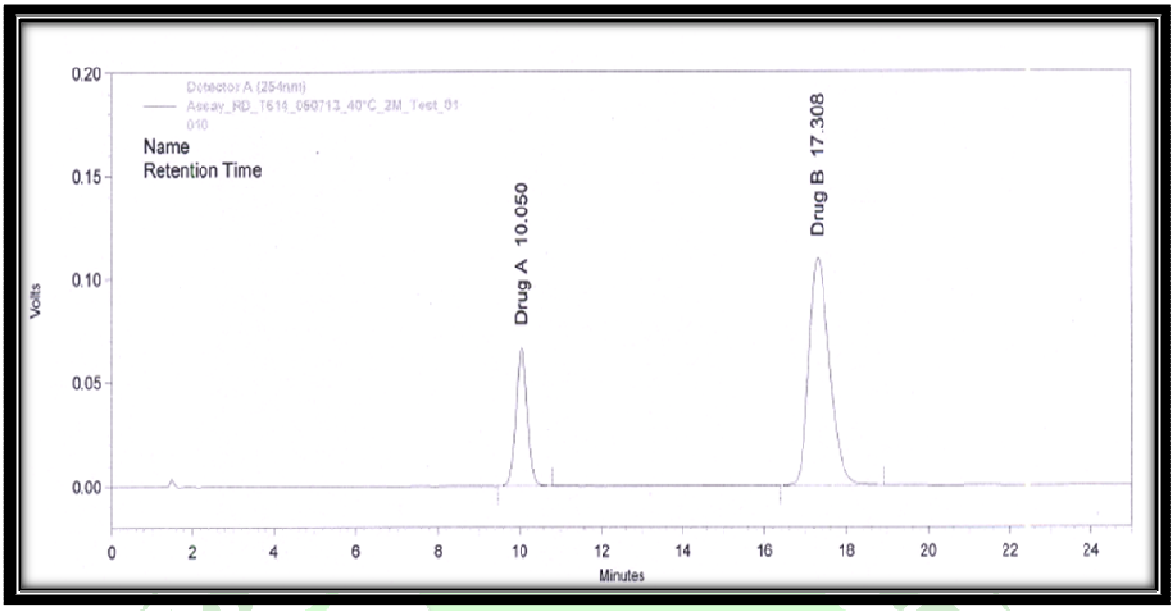


Fig No.2:-A typical Representative chromatogram of Test (Drug A: Clonazepam, Drug B: Escitalopram)

Specificity

The overlay spectra of Placebo , Blank and sample is shown in Fig.no.3

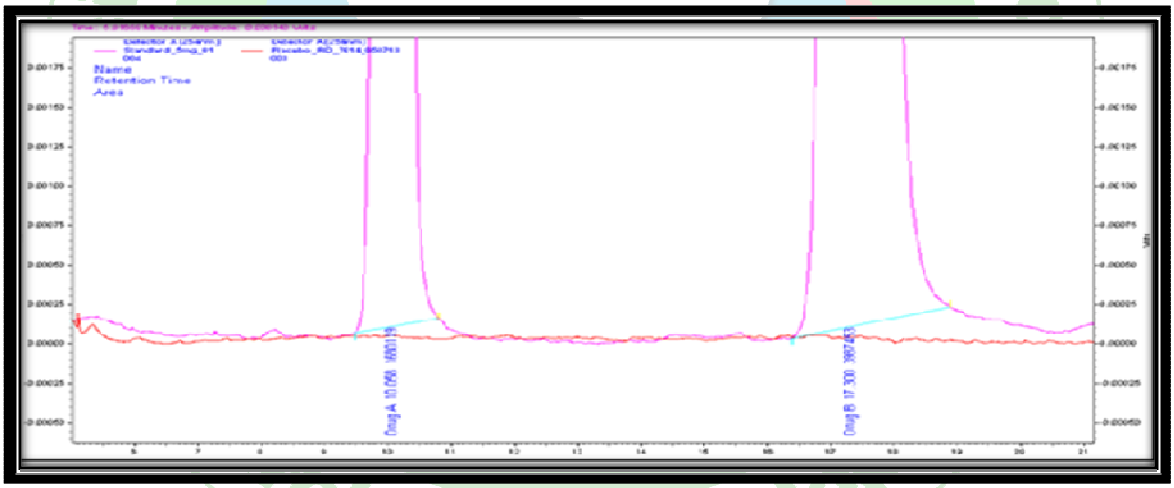


Fig No.3:-A typical representative Chromatogram of Overlay view of placebo, blank & sample.

Linearity

Linearity was evaluated by analysis of working standard solutions of ESC and CLZ ofsix different concentrations. The response for the drug was linear in the concentration range between 80-120 ppm and 8-12 ppm for ESC and CLZ respectively. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The regression data obtained are represented in Table 1. The results shows that linearity was with-in the concentration range mentioned above and there was an excellent correlation between peak area and concentration of drug. (Graph of Concentration vs. Area is obtained as Shown in fig.4 & 5)

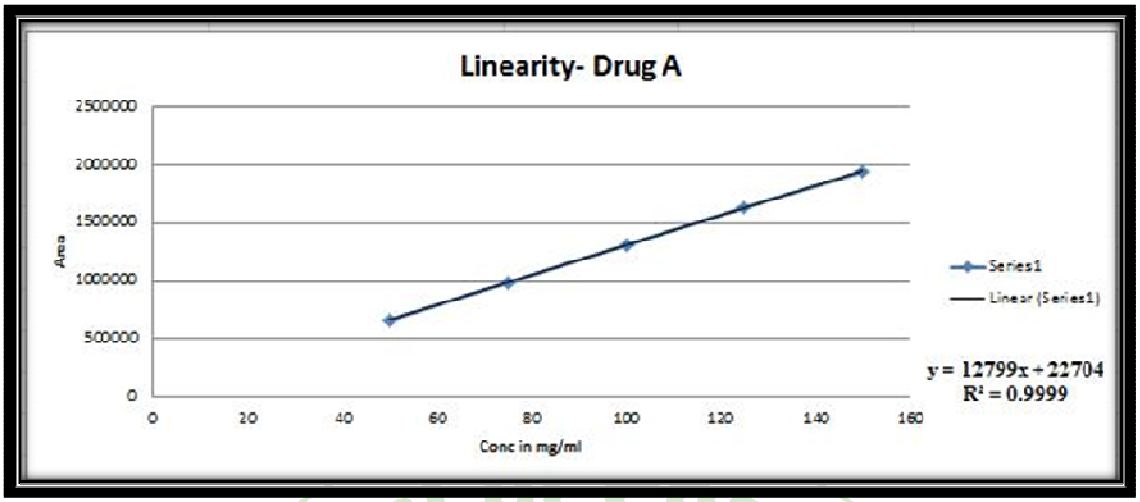


Fig No.4:-Linearity for the Escitalopram

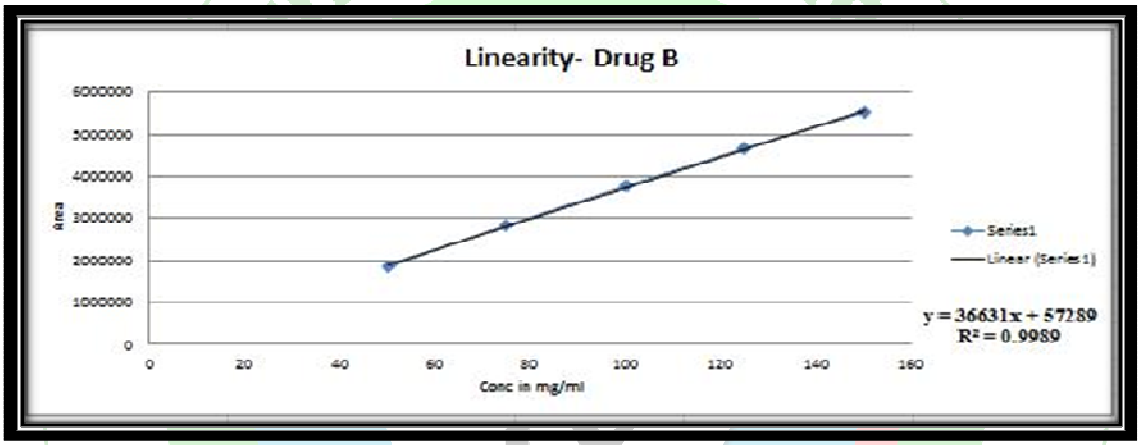


Fig No.5:-Linearity for the Clonazepam

Table No.1:-Linearity of the Escitalopram& Clonazepam

Parameters	**Values obtained For Escitalopram**	**Values obtained For Clonazepam**
Correlation coefficient	0.999	0.999
%Y-intercept	1.53	1.74
Slope of regression line	3663	12799
Residual sum of Square	3774905495	3774905495

Accuracy:

Good recoveries were obtained when a mixtures of sample was spiked with the drug. The accuracy data are shown in Table 2.

Table No.2:-Accuracy of Escitalopram& Clonazepam

Concentration	**% Recovery**	
	Escitalopram	**Clonazepam**
50%	101	102
100%	102.5	101
150%	102	101.5

Robustness:

The recovery studies for both flow rate & Change in mobile phase & temperature showed good recovery which indicate that the method is robust enough to withstand the variations in flow rate, mobile phase & Temperature.

CONCLUSION

All these factors lead to the conclusion that the proposed validated method for estimation ofEscitalopram& Clonazepam by RP-HPLC is Specific, Linear,Accurate, Precise, Fast and cost savingand also selective for simultaneous determination & quantification ofEscitalopram& Clonazepam in finished dosage formulation & can be used by Pharmaceutical industry.

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