

Analytical method development and validation for clomipramine and fluvoxamine by using RP-HPLC technique

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ABSTRACT

A Precise, Specific, Linear, Accurate and Robust method development and validation for clomipramine and fluvoxamine by using RP-HPLC technique and Validated as per ICH Validation guidelines. Method was optimized by Inersil ODS (150mm x 4.6mm, 5 μ m) column at a flow rate of 0.8ml/min, Mobile phase wasp H 3.0 Phosphate buffer, Methanol (30:70 respectively). The linearity range of Clomipramine and Fluvoxamine were found to be from 100-500 μ g/ml of Clomipramine and 1-5 μ g/ml of Fluvoxamine. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Clomipramine and Fluvoxamine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: RP-HPLC, Clomipramine Hydrochloride,

INTRODUCTION

Clomipramine is a strong, but not completely selective serotonin reuptake inhibitor (SRI), as the active main metabolite desmethyclomipramine acts preferably as an

inhibitor of noradrenaline reuptake. α 1-receptor blockage and β -down-regulation have been noted and most likely play a role in the short term effects of clomipramine. A blockade of sodium-channels and NDMA-receptors might, as with other tricyclics, account for its effect in chronic pain, in particular the neuropathic type¹.

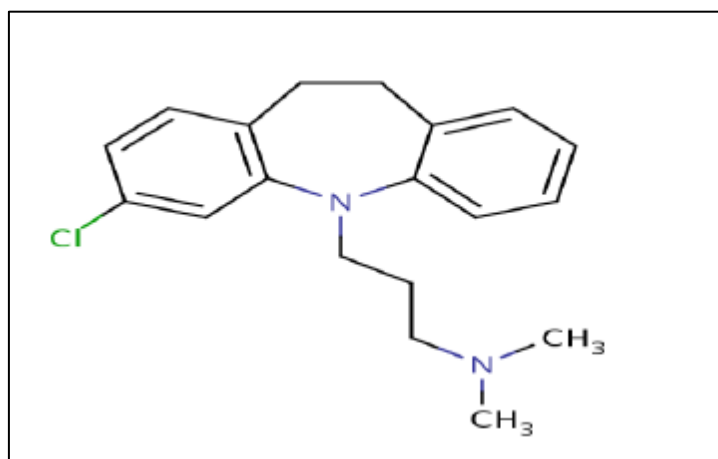


Fig 1: Structure of Clomipramine

Fluvoxamine is a potent and selective serotonin reuptake inhibitor with approximately 100-fold affinity for the serotonin transporter over the nor epinephrine transporter. It has negligible affinity for the dopamine transporter or any other receptor, with the sole exception of the σ_1 receptor. It

behaves as a potent agonist at this receptor and has the highest affinity of any SSRI for doing so. This may contribute to its antidepressant and anxiolytic effects and may also afford it some efficacy in treating the cognitive symptoms of depression².

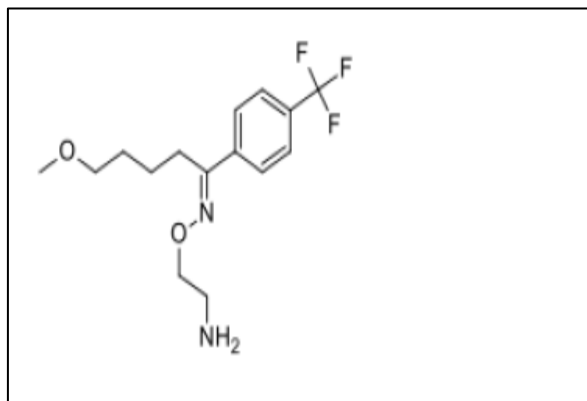


Fig 2: Structure of Fluvoxamine

MATERIALS AND METHODS

Instruments was Used

Shimadzu, model No. SPD-20MA LC+20AD HPLC, Software- LC-20 Solution, LABINDIA UV 3000+ UV/VIS spectrophotometer, Adwa – AD 1020 pH meter

Drug Samples

Clomipramine and fluvoxamine Active pharma ingredients and Marketed samples of clomipramine and fluvoxamine Tablet.

Chemicals and Reagents

Potassium di hydrogen ortho phosphate (Make: Merck and Grade: Empata ACS), Orthophosphoric acid (Make: Merck

and Grade: Emparta ACS), Acetonitrile and Methanol (Make :Merckand Grade: HPLC)

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wave length selection

UV spectrum of 10 μg / ml Clomipramine and Fluvoxamine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

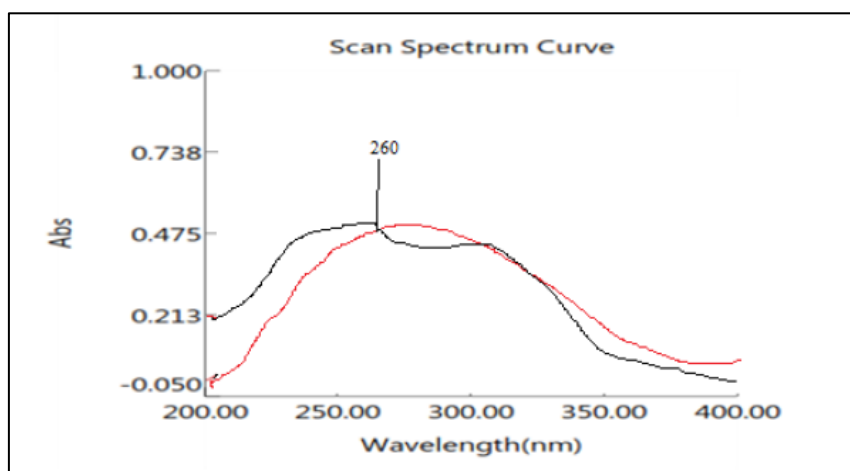


Fig 3: Spectrum of Clomipramine and Fluvoxamine

Optimization of Chromatographic conditions

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5 μ m) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow at 260nm with 10 μ L injection volume.

Preparation of Phosphate buffer

Accurately weighed 6.8 grams of KH₂PO₄ was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Optimized Chromatographic conditions

Column	Inertsil ODS (4.6 x 150mm, 5 μ m)
Mobile Phase and Composition	pH 3.0 Phosphate Buffer: Methanol (30:70)
Flowrate	0.8mL/min
Column oven Temperature	25°C
Injection volume	10 μ L
Detection wavelength	260nm
Auto sampler Temperature	25°C
Retention Times	2.153min of Clomipramine and 3.792min of Fluvoxamine (Total Run time 8.0min)

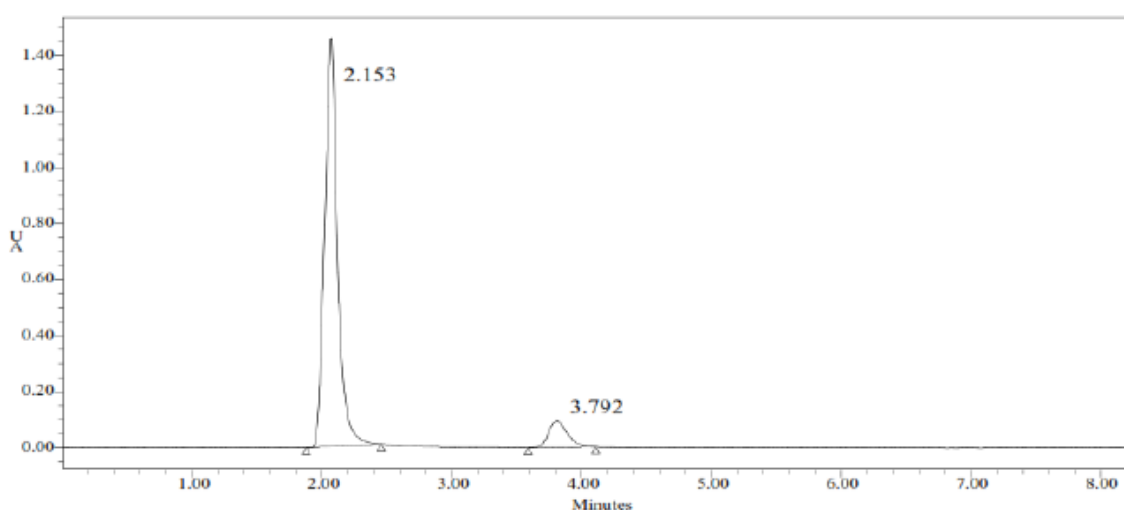


Fig 4: Optimised Chromatogram of Clomipramine and Fluvoxamine

Method Validation

By using Optimised condition Analytical Method of Assay carried out by ICH Guideline Q2B.³⁻⁴ The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

Diluent Preparation

The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Clomipramine and Fluvoxamine 10mg of working standard into a 10mL & 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 3ml & 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Clomipramine and Fluvoxamine (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of Clomipramine e and Fluvoxamine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

System Suitability and System Precision⁵⁻¹¹

According to ICH guidelines³⁻⁴ System suitability checking out is an integral part of many analytical procedures. The tests are based on the idea that the equipment, analytical operations and samples to be analyzed represent an integral system that can be evaluated as such. System suitability check parameters to be established for

a particular procedure depend on the type of procedure being validated. According to ICH Specifications: Theoretical Plates should not be less than 2000, Tailing factor should not be 0.9 to 2.0 and Resolution should not be less than 2.0 between Clomipramine and Fluvoxamine. System Precision

Specification:%RSD for Area and Retention time for the six replicate injections should not be more than 2.0 of each analyte and system suitability results were shown in table 2 and system precision were summarized in Table 1.

Table 1: Results of System suitability and system precision

S.No	Clomipramine			Fluvoxamine			Resolution
	Rt in min	Plate count	Tailing Factor	Rt in min	Plate count	Tailing Factor	
1	2.569	4668	1.3	3.842	6090	1.3	4.0
%RSD for Area and Retention time for the six replicate injections was not more than 2.0							

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be

present. Typically these might include impurities, degradants, matrix, etc. Procedure for Specificity: Blank (Diluent used as a Blank) and Placebo solutions were injected into HPLC system. The blank and placebo chromatograms were shown in Fig.5 and Fig.6 respectively.

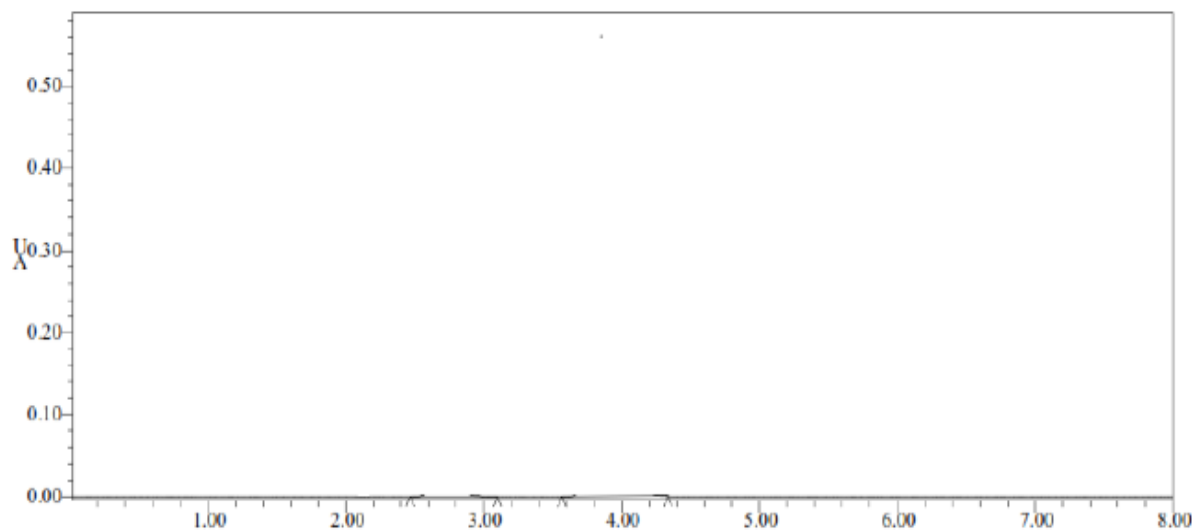


Fig 5: Blank of Clomipramine and Fluvoxamine

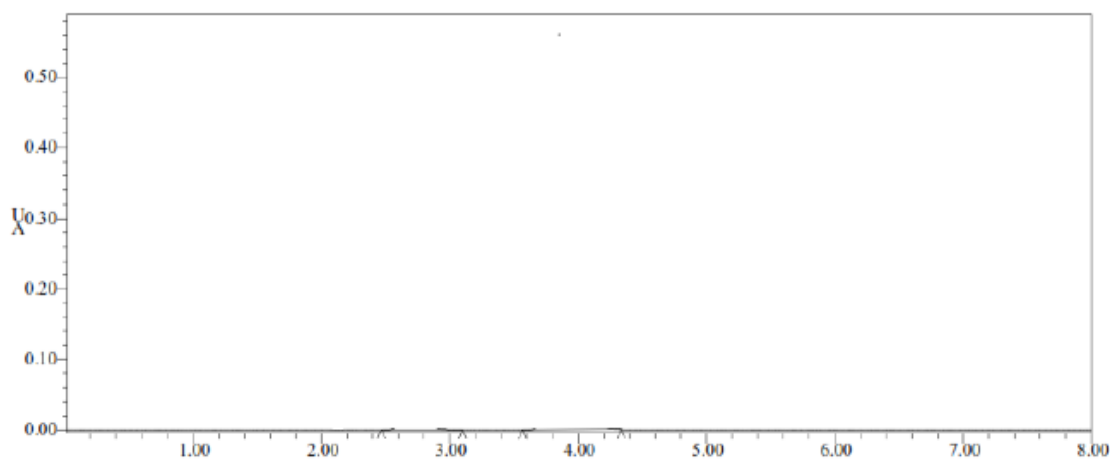


Fig 6: Placebo of Clomipramine and Fluvoxamine

Blank solution and Placebo solution should not be interfered at the retention time of the three main Analyte peaks. observation: No Blank and Placebo interference was

observed at the retention times of the two main Analyte peaks.

Method Precision

Closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample³⁻⁴.

Sample solutions were six prepared individually and each injected in to HPLC System, Calculated %Assay by using average area of Six Standards. The %RSD for % Assay of Clomipramine and Fluvoxamine were calculated and summarized in the table 2.

Name of the Sample	%Assay of Clomipramine	%Assay of Fluvoxamine
Method Precision-01	99.2	98.9
Method Precision-02	99.1	98.7
Method Precision-03	99.7	99.5
Method Precision-04	99.4	101.0
Method Precision-05	99.6	98.9
Method Precision-06	99.7	98.7
AVG	99.5	99.3
Std dev	0.8	0.9
%RSD	0.8	0.9

Average and individual %Assay was obtained between 95.0 to 105.0% individual preparation and %RSD for %Assay of six replicate preparations was obtained below 2.0.

Accuracy And Recovery Studies

Expresses the closeness of agreement between the value which is accepted either as a conventional true value and the value found³⁻⁴. Three levels (50%, 100% and 150%) of

accuracy sample were prepared in triplicate by Standard API addition method to the Placebo, At each level API taken 50%, 100% and 150% respectively in the presence of Placebo. The accuracy, recovery and %RSD of Clomipramine and Fluvoxamine were calculated and summarized in the table 3.

Name of the Level	Clomipramine	Fluvoxamine
50% Accuracy	100.7	100.8
100% Accuracy	100.0	100.0
150% Accuracy	98.8	99.7
Mean	99.8	100.5
Std dev	0.7	0.8
%RSD	0.8	0.8

Linearity and Range³⁻⁴

Five linearity solutions (50%, 80% 100%, 120% and 150%) were prepared from standard stock solution i.e., 100µg/mL

to 500µg/mL for Clomipramine, 1µg/mL to 5µg/mL for Fluvoxamine. The linearity of different concentrations of the Clomipramine and Fluvoxamine were calculated and summarized in table 4 and graphs were shown in Fig 7.

Clomipramine		Fluvoxamine	
Conc. in µg/mL	Area	Conc. in µg/mL	Area
40	668934	4	66510
60	956781	6	94710
80	1313873	8	124802
100	1563458	10	152731
120	1867084	12	179732
Correlation coefficient	0.999	Correlation coefficient	0.999

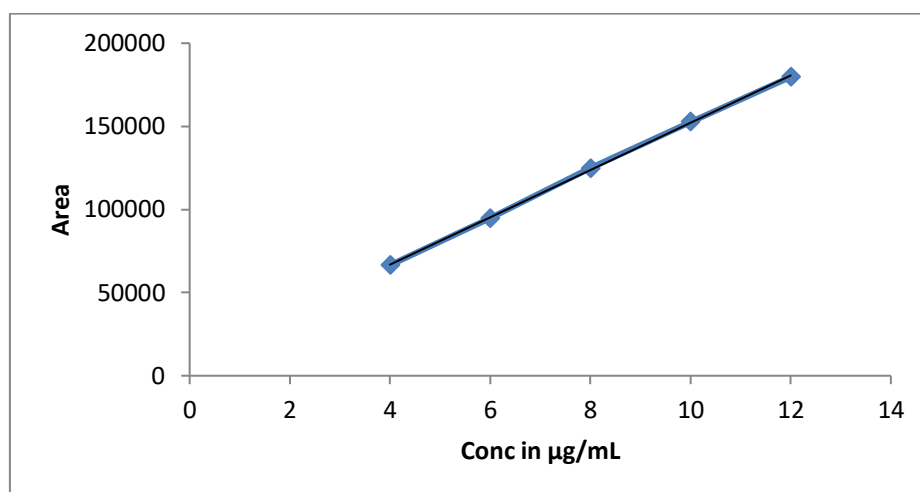
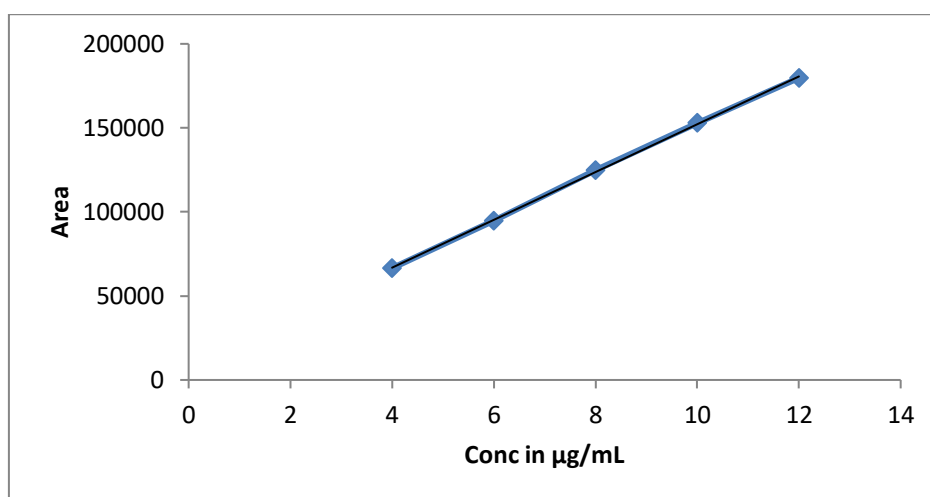


Fig 7: Calibration Curve of Clomipramine



The Correlation coefficient was found 0.999 for both Clomipramine and Fluvoxamine

Fig 8: Calibration Curve of Fluvoxamine

ROBUSTNESS⁵⁻¹¹

Robustness conditions like flow (0.8mL/min±0.1mL) and Wavelength (260m±5nm) was maintained in the HPLC System and injected six standards replicate injections in

each condition. System suitability parameters were within the acceptance criteria, %RSD for Area of six standard injections for within the limit. Robustness of different conditions were conducted, calculated and summarized in the table 5.

Table 5: Robustness

S. No.	Condition	%RSD for Clomipramine	%RSD for Fluvoxamine
01	Flow Rate_0.7mL/min	0.62	0.35
02	Flow Rate_0.9mL/min	0.37	0.47
03	Wavelength (255nm)	0.35	0.50
04	Wavelength (265nm)	0.60	0.54

CONCLUSION

A Specific, Accurate, Precise and Robust indicating Assay method was developed for the simultaneous estimation of the, Clomipramine and Fluvoxamine in Tablet dosage form, For the optimised conditions of Assay method was Validated by Using ICH Q2B guidelines. Method was shown precise, Specific, accurate, linear and robust results. % RSD of the Clomipramine and Fluvoxamine were and

found to be 0.2 and 0.9% respectively in system precision. In method precision sample has shown precise %Assay results. That was 95.0 to 105.0%. % Recovery was obtained as 99.8% and 100.5% for Fluvoxamine respectively. Linearity was obtained as 0.999, 0.999 and 0.999 for Fluvoxamine respectively. So this method very use full to Routine analysis like in Quality control to reduce the time and cost.

DECLARATION

I hereby declare that the seminar report entitled "ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR CLOMIPRAMINE AND FLUVOXAMINE BY USING RP-HPLC TECHNIQUE." is being submitted in the partial fulfillment of curriculum prescribed by the

Jawaharlal Nehru Technological University, Hyderabad, is a record of bonafide work carried out by me under the guidance of Mrs. SRI LAKSHMI M.Pharm.,(Ph.D.)in the Dept. of Pharmaceutical Chemistry, during the academic year 2020-21, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad,Telangana.

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