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A NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FLOUXAMINE, CHLOMIPRAMINE HCL IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:

The objective of the current study was the development of a simple, precise and accurate isocratic reversed-phase High Performance Liquid Chromatography [RP-HPLC] assay method and validated for determination of Flouxamine and Chlomipramine Hcl in capsule dosage forms. Isocratic separation was achieved on a C18 Inertsil ODS (5µ, 250mm× 4.6mm) with flow rate of 1.0ml/min using UV detection at 230nm. Different mobile phase compositions of pH 3.5 phosphate buffer: ACN (30:70) in order to determine the best conditions for the effective separation and elution of the analytes. The mobile phase consisting of pH 3.5 buffer: ACN (30:70) was selected. *The injection volume was 10.0 µl and the detection was carried out at 254 nm by using photo-diode array detector. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The proposed method was successfully used to determine the drug content of marketed formulation.*

Keywords: *Flouxamine, Chlomipramine Hcl , RP-HPLC,Methanol and Acetonitrile*

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INTRODUCTION:

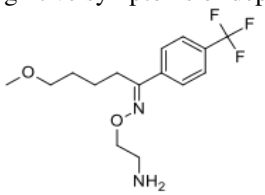
High performance liquid chromatography is a very sensitive analytical technique most widely used for

quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

In the modern pharmaceutical industry, high-performance liquid chromatog- raphy (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chem- ical entities (NCEs) is comprised of two major activities: drug discovery and drug development. The goal of the drug discovery program is to investigate a plethora of compounds employing fast screening approaches, leading to gen- eration of lead compounds and then narrowing the selection through targeted synthesis and selective screening (lead optimization). This lead to the final selection of the most potentially viable therapeutic candidates that are taken forward to drug development. The main functions of drug development are to completely characterize candidate compounds by performing drug metabolism, preclinical and clinical screening, and clinical trials.

Concomi- tantly with the drug development process, the optimization of drug synthesis and formulation are performed which eventually lead to a sound and robust manufacturing process for the active pharmaceutical ingredient and drug product. Throughout this drug discovery and drug development paradigm, rugged analytical HPLC separation methods are developed and are tailored by each development group (i.e., early drug discovery, drug metabolism, pharmokinetics, process research, preformulation, and formulation). At each phase of development the analyses of a myriad of samples are performed to adequately control and monitor the quality of the prospective drug candidates, excipients, and final products Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization[1,2].

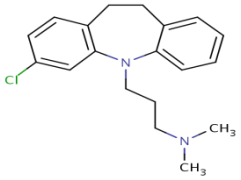
Fluvoxamine is a potent and selective serotonin reuptake inhibitor with approximately 100-fold affinity for transporter over the nor epinephrine transporter. It has negligible affinity for the dopamine transporter or any other receptor, with the sole exception of the σ_1 receptor. It behaves as a potent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



FLOUXAMINE[3,4]

For the treatment of major depressive disorder (MDD), and anxiety disorders such as panic disorder, social anxiety disorder, post-traumatic stress disorder (PTSD), andobsessive-compulsive spectrum disorders.

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. α1-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 type and for the treatment of malaria.



CHLOMIPRAMINE HCL[5,6]

4.3. MATERIALS AND METHODS:

Instruments used:

High performance liquid chromatography equipped with Auto Sampler and PDA detector Empower 2 (WATERS)**.**Column :Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters)

Orthophosphoric Acid**,** Potassium dihydrogen Ortho phosphate**,** Chlomipramine Hcl& Flouxamine Working Standards**,** Chlomipramine Hcl& Flouxamine Tablets

A good method development strategy should require only as many experimental conditions as necessary to achieve the desired final result. Finally, method development should be as simple as possible, yet it should allow the use of sophisticated tools such as computer modeling, if these are available[7,8,9].

Selection of mobile phase:

- Pure drug of Flouxamine and Chlomipramine Hcl mixed standard stock solution (10 µg/mL of Flouxamine and 10 µg/mL of Chlomipramine Hcl) were taken and 10µL sample was injected in to RP-HPLC system and run in different solvent systems.
- Different mobile phase compositions of

pH 3.5 phosphate buffer: ACN (30:70) in order to determine the best conditions for the effective separation and elution of the analytes.

- The mobile phase consisting of pH 3.5 buffer: ACN (30:70) was selected.

Preparation of Phosphate buffer: (P^H:3.5)

Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mix a mixture of above Buffer 250 mL (25%):750 mL of Acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluent.

Selection of Flow rate:

A chromatogram was run with the optimized mobile phase, and some different flow rates of 0.8mL/min, 1mL/min, 1.2 mL/min and were tried. The best retention time and separation was obtained at 1.0mL/min, so the flow rate of 1.0 mL/min has been selected.

Selection of analytical wavelength;

By appropriate dilutions of the standard stock solutions with methanol, various concentrations of Flouxamine and Chlomipramine Hcl were prepared separately and their overlain spectra was obtained using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. From the overlain spectra, it was observed that Flouxamine and Chlomipramine Hcl exhibited strong absorbance at about 254 nm (it is the coinciding maximum absorbance where the two drugs can be detected sufficiently enough for quantitative evaluation) which was selected as the analytical wavelength for further analysis.

Standard Solution Preparation:

Accurately weigh and transfer 12 mg of Chlomipramine Hcl and 10mg of Flouxamine working standard 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 0.3&0.6ml of the above stock solution into a 10ml

Volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSIONS:

Method Validation of Flouxamine and Chlomipramine Hcl In Tablet Dosage Form By RP-HPLC Respectively

System suitability studies:

System suitability studies were carried out as specified in the United States Pharmacopoeia (USP). These parameters include column efficiency, resolution, capacity factor, tailing factor and HETP were calculated in present study.

PRECISION STUDIES:

Expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions the conditions.

Repeatability/ Intra Repeatability :(Intra--assay precision)

Precision under same operating conditions over a short interval of time interval time.

Intermediate precision Intermediate precision: Precision within laboratories variation Precision variation--days, equipment, analyst, equipment, analyst.

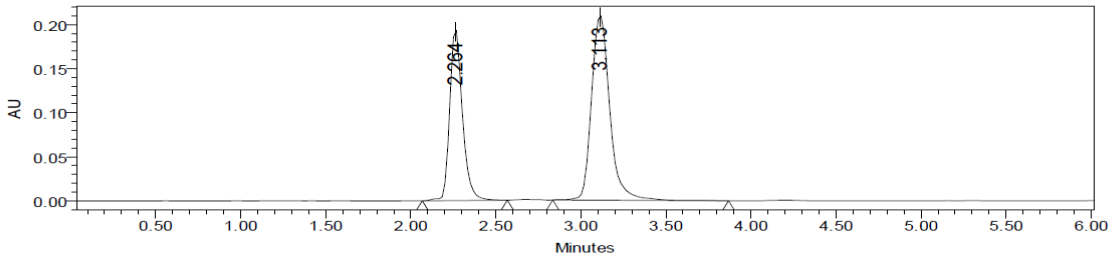
Reproducibility:

Inter laboratories precision laboratories precision--technology transfer technology transfer.

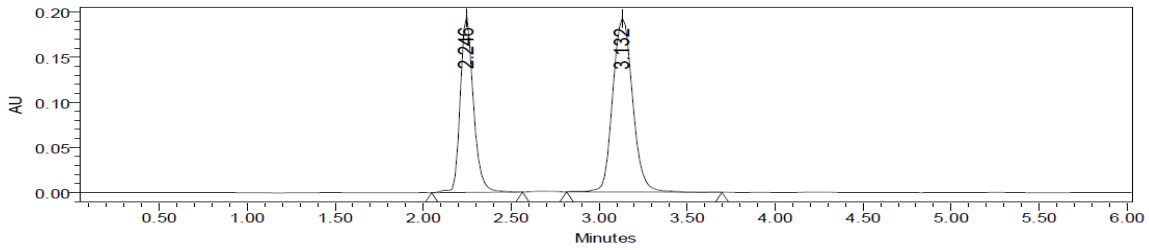
Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

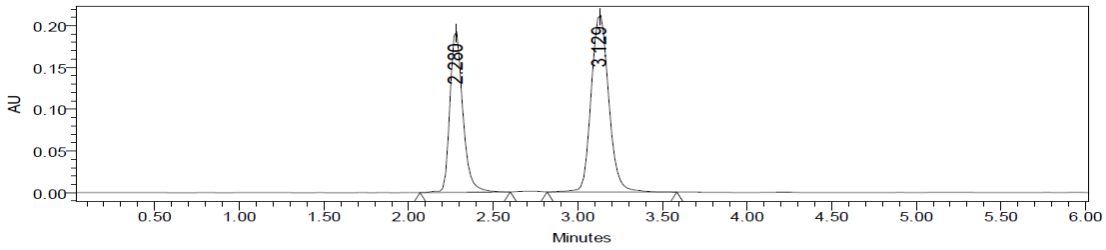
Precision Studies chromatograms:



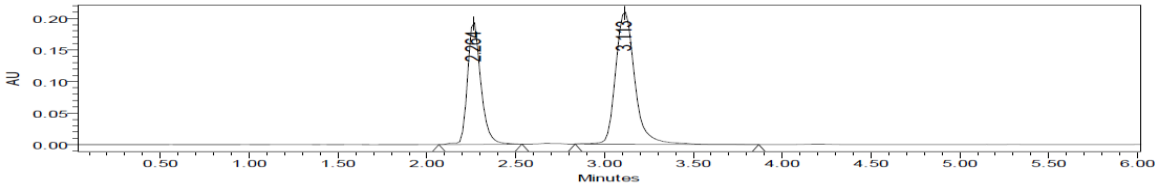
Chromatogram 1: Precision Studies njection-1



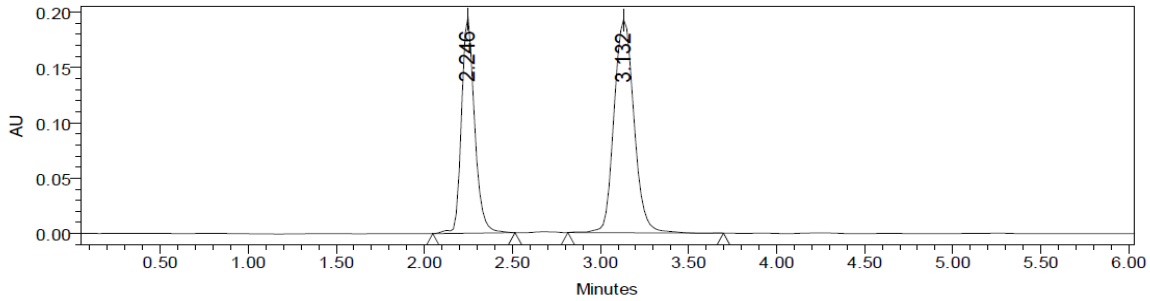
Chromatogram 2: Precision Studies njection-2



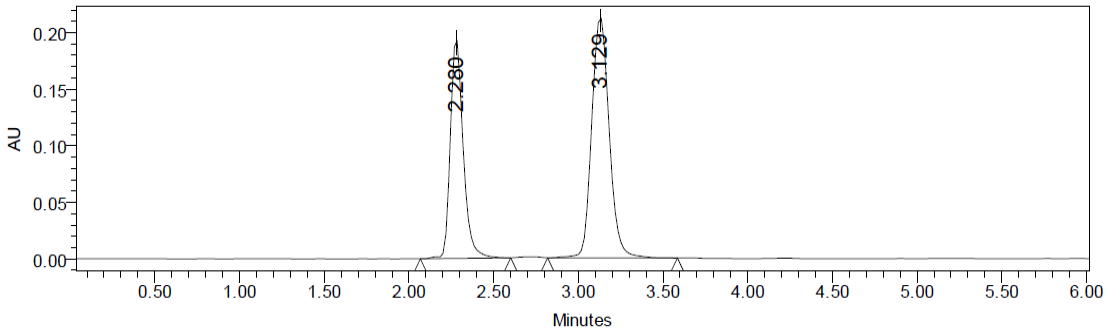
Chromatogram 3: Precision Studies njection-3



Chromatogram 4: Precision Studies injection-4



Chromatogram 5: Precision Studies injection-5



Chromatogram 6: Precision Studies injection-6

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%.

ACCURACY STUDIES:

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Chlomipramine HCl& Flouxamine and calculate the individual recovery and mean recovery values.

Table 1 The accuracy results for Chlomipramine Hcl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	605652.5	6	5.8	98.1%	100.0%
100%	1246314	12	12.1	101.0%	
150%	1869868	18	18.1	101.0%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

Table 2 The accuracy results for Flouxamine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	774787	5	5.0	101.3%	100.3%
100%	1537580	10	10.0	100.3%	
150%	2285575	15	14.9	99.4%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

Accuracy Studies chromatograms:

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

b). The Organic composition in the Mobile phase was varied from 70% to 80%.

Standard solution 36 µg/ml of Chlomipramine Hcl & 60µg/ml of Flouxaminewas prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized:

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

Table 3 System suitability results for Chlomipramine Hcl:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2028.0	0.9
2	*Actual	4750.0	1.2
3	10% more	2013.0	1.0

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Table 4 System suitability results for Flouxamine:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3035.0	1.0
2	*Actual	3744.0	1.2
3	10% more	3002.0	1.0

* Results for actual Mobile phase composition (25:75Buffer: Acetonitrile) have been considered from Accuracy standard.

LIMIT OF DETECTION: (for Chlomipramine Hcl)

Preparation of 36 µg/mL solution:

Accurately weigh and transfer 12 mg of Chlomipramine Hcl working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.03 µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents Pipette 0.9 mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 163/55 = 2.96

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

(b) LIMIT OF DETECTION: (for Flouxamine)

Preparation of 60 µg/ml solution:

Accurately weigh and transfer 10 mg of Flouxamine working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.6 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mark with diluent.

Preparation of 0.05 µg/ml solution):

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Pipette 0.9 mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N =166/55 =3.01

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION: (for Chlomipramine HCl)

Preparation of 30 µg/ml solution:

Accurately weigh and transfer 12 mg of Chlomipramine Hcl working standard 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 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.09 µg/mL solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 2.7 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 55 µV

Signal Obtained from LOQ solution : 543µV

S/N = 543/55 = 9.87

Acceptance Criteria:

- S/N Ratio value shall be 10 for LOQ solution.

LIMIT OF QUANTIFICATION: (for Flouxamine)

Preparation of 60 µg/mL solution:

Accurately weigh and transfer 10mg of Flouxamine working standard 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 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.18 µg/mL solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.3mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

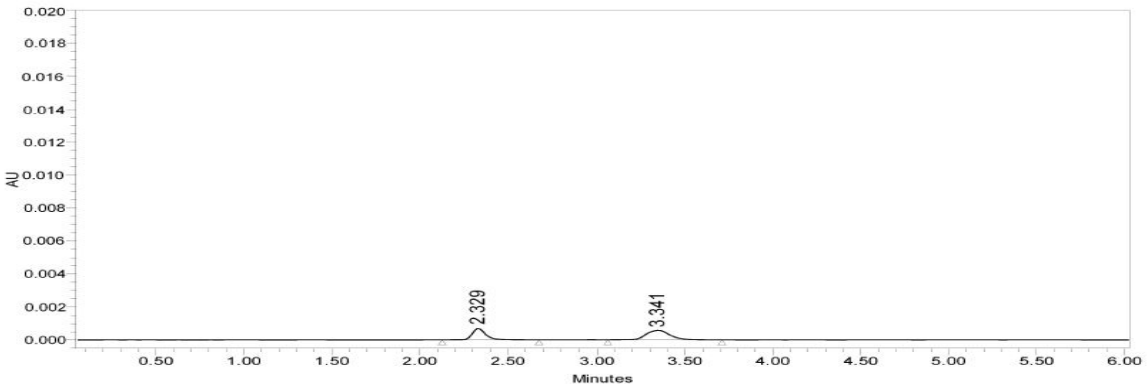
Signal Obtained from LOQ solution

S/N = 548/55 = 9.96

Acceptance criteria:

• S/N Ratio value shall be 10 for LOQ solution.

Limit of quantification of Flouxamine and Chlomipramine Hcl chromatogram:



Chromatogram LOQ

VALIDATION OF THE METHOD:

The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs and presented. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found.

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by preparing 3 individual samples with same procedure from the formulation and injecting. The percentage recovery and percentage relative standard deviation of the percentage recovery was calculated and presented in Tables 1 & 2 . From the data obtained, added of standard drugs were found to be accurate.

The precision of the method was demonstrated by system and method precision. All solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated . From the data.

The standard drug solution of varying concentration ranging from 12-60 µg / mL for Chlomipramine Hcl and 20-100µg / mL for Flouxamine. The response factor, slope, intercept and correlation co-efficient were calculated. The slope, intercept, correlation co-efficient were found to be 0.999 for The Chlomipramine 0.999, for Flouxamine calibration curves were plotted using response factor Vs concentration of standard solutions . The calibration graph shows that linear response was obtained over the range of concentration used in the assay procedure. These data demonstrates that the method have adequate sensitivity to the analytes. The range demonstrate that the method is linear outside the limits of expected use.

The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters (table 1&2)and hence developed method is said to be robust and ruggedness.

Parameters	Flouxamine	Chlomipramine Hcl
Accuracy	% Recovery =100.0%	% Recovery =100.3
precision	% RSD =1.6%	% RSD =0.5%
Id precision	% RSD = 0.9%	% RSD = 0.7%
Linearity	R^2 =0.999	R^2 =0.999
Range	12-60	20-100
Limit of detection	0.2 µg/ml	1.0 µg/ml
Limit of quantitation	0.5 µg/ml	1.2 µg/ml

CONCLUSION

The results indicating that the proposed methods are precise, accurate, specific and simple. These methods were developed and validated according to the ICH guidelines. So the developed methods can be easily applied for routine analysis.

It is clear from the present study that the RP-HPLC method for the determination of Acitretin is simple, accurate, specific and precise. This method was validated statistically. The results of recovery studies were in good agreement with the respective label claim of the formulation. Thus the method is less time consuming and can be employed for routine batch analysis of Flouxamine and Chlomipramine Hcl.

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