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RP-HPLC METHOD AND ITS VALIDATION FOR ANALYSIS OF FLUOXETINE HCL AND ALPRAZOLAM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Fluoxetine HCL and Alprazolam, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Fluoxetine HCL and Alprazolam was 2.061, 2.462 ±0.02min respectively. The method produces linear responses in the concentration range of 1-5µg/ml of Fluoxetine HCL and 100-500µg/ml of Alprazolam. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Fluoxetine HCL, Alprazolam, RP-HPLC, validation.*

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INTRODUCTION:

Analytical chemistry [1]:

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Electrophoresis's another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species.

Chromatography [2]:

Introduction:

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

" *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper,

thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Chromatographic Process [4]:
Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of Chromatography:
The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

MATERIALS AND METHODS:

Fluoxetine HCL -Sura labs, Alprazolam -Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck, Phosphate buffer-Sura labs.

HPLC METHOD DEVELOPMENT: TRAILS

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Fluoxetine HCL and Alprazolam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.03ml of Fluoxetine HCL and 3.0ml of Alprazolam from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and X-Bridge. Zorbax C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Zorbax C18 (4.6×150mm, 5µ)
Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Flow rate : 1ml/min
Wavelength : 255nm
Injection volume : 10 µl
Run time : 8 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer pH 3.9:
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 3.9.

Preparation of mobile phase:
Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)
Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Column : Zorbax C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 255 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 8minutes

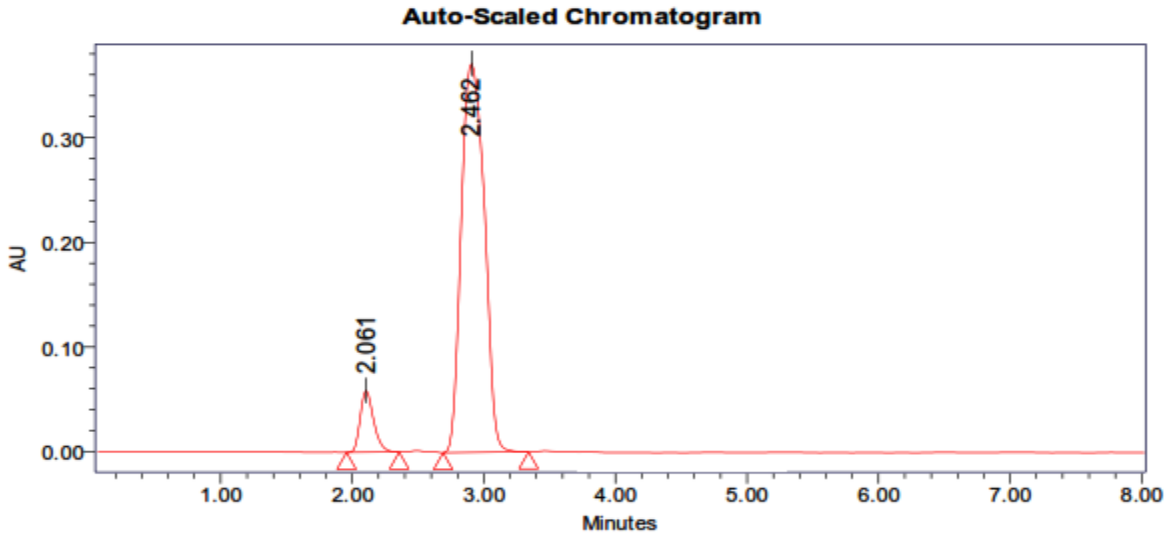

Figure 6: Optimized Chromatogram(Standard)

Table 6: - peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Fluoxetine HCL	2.061	247393	58953	1.2	7244
2	Alprazolam	2.462	3530867	371749	1.1	3388

Observation: From the above chromatogram it was observed that the Fluoxetine HCL and Alprazolam peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

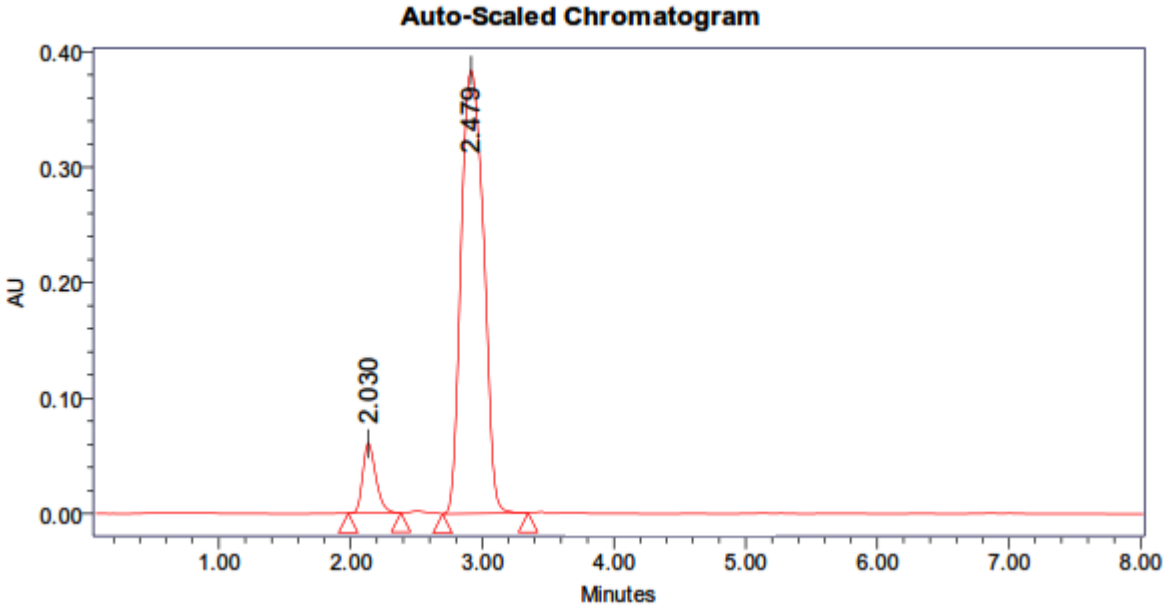

Figure 7: Optimized Chromatogram (Sample)

Table 7: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Fluoxetine HCL	2.030	240018	60879	1.2	7247
2	Alprazolam	2.479	3544381	384305	1.1	3376

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table 8: Results of system suitability for Fluoxetine HCL

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fluoxetine HCL	2.048	246714	73456	11319	1.1
2	Fluoxetine HCL	2.074	245618	78153	7106	1.2
3	Fluoxetine HCL	2.071	245831	78147	8975	1.2
4	Fluoxetine HCL	2.069	240553	78243	7088	1.2
5	Fluoxetine HCL	2.070	245726	77706	5123	1.2
Mean			244888.4			
Std. Dev			2463.26			
% RSD			1.005467			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 9: Results of system suitability for Alprazolam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Alprazolam	2.446	3363755	636863	8485	1.1
2	Alprazolam	2.490	3326433	641487	7888	1.0
3	Alprazolam	2.489	3345948	638082	7847	0.9
4	Alprazolam	2.488	3336622	617726	6773	0.9
5	Alprazolam	2.490	3355245	631711	6885	0.9
Mean			3345601			
Std. Dev			14753.44			
% RSD			0.44099			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Assay (Sample):

Table 10: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Fluoxetine HCL	2.068	244103	89283	1.2	5948
2	Alprazolam	2.489	3357567	576561	1.0	6867
3	Fluoxetine HCL	2.070	240053	88022	1.2	5862
4	Alprazolam	2.491	3371664	576998	1.0	6809
5	Fluoxetine HCL	2.067	243231	88883	1.2	5878
6	Alprazolam	2.489	3364002	570316	1.0	6824

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Fluoxetine HCL and Alprazolam in pharmaceutical dosage form was found to be 100.2 %.

LINEARITY:

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Fluoxetine HCL :

Concentration Level (%)	**Concentration µg/ml**	**Average Peak Area**
33.3	1	88442
66.6	2	165724
100	3	242754
133.3	4	315906
166.6	5	396371

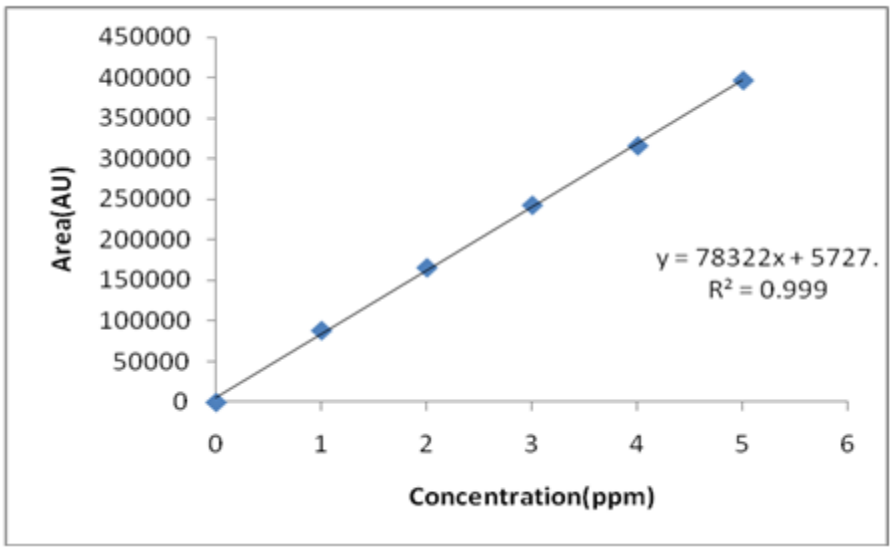

Figure 22: calibration graph for Fluoxetine HCL

Alprazolam

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	100	1131032
66	200	2345302
100	300	3355282
133	400	4429382
166	500	5623754

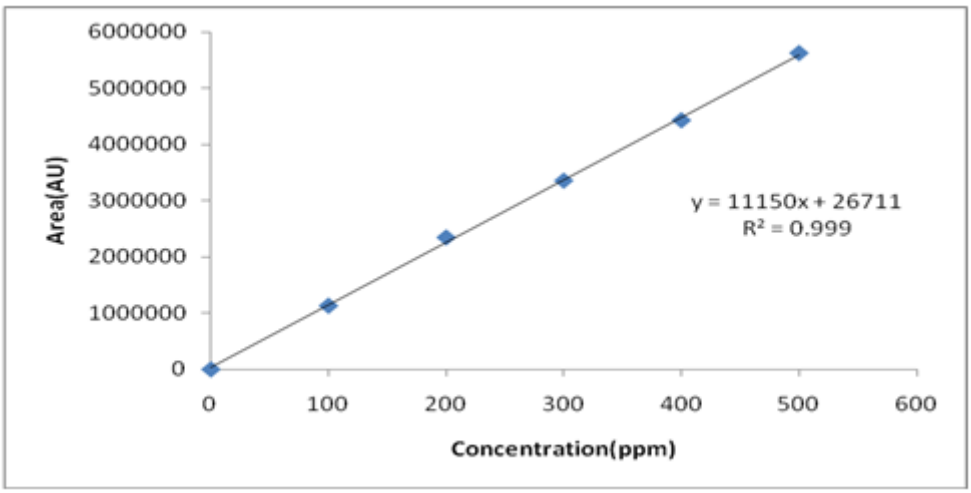

Figure 23:calibration graph for Alprazolam

REPEATABILITY:

Table 11: Results of repeatability for Fluoxetine HCL :

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fluoxetine HCL	2.065	249685	12078	5344	1.0
2	Fluoxetine HCL	2.064	249697	12069	5475	1.2
3	Fluoxetine HCL	2.064	246326	11948	5472	1.1
4	Fluoxetine HCL	2.065	249817	11812	5388	1.1
5	Fluoxetine HCL	2.067	249893	11736	5181	1.0
Mean			249083.6			
Std. Dev			1544.965			
% RSD			0.61987			

Acceptance criteria:

- %RSD for sample should be NMT 2

Table 12: Results of method precession for Alprazolam :

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Alprazolam	2.486	3233701	59096	6655	1.2
2	Alprazolam	2.484	3241324	57553	6523	1.3
3	Alprazolam	2.482	3245928	57214	6441	1.3
4	Alprazolam	2.483	3245926	57097	6412	1.4
5	Alprazolam	2.483	3222195	54364	6261	1.4
Mean			3237815			
Std. Dev			10060.63			
% RSD			0.310723			

Acceptance criteria:

- %RSD for sample should be NMT 2

Intermediate precision:

Table 13: Results of Intermediate precision for Fluoxetine HCL

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fluoxetine HCL	2.066	242722	11324	5273	1.21
2	Fluoxetine HCL	2.066	240156	11565	5169	1.16
3	Fluoxetine HCL	2.066	240944	11888	5311	1.14
4	Fluoxetine HCL	2.065	240387	11939	5276	1.19
5	Fluoxetine HCL	2.069	249921	11653	5079	1.10
6	Fluoxetine HCL	2.067	240822	11751	5226	1.17
Mean			243992			
Std. Dev			4641.98			
% RSD			1.6			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table 14: Results of Intermediate precision for Alprazolam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Alprazolam	2.477	3325308	54144	6148	1.25
2	Alprazolam	2.478	3323781	53741	6126	1.21
3	Alprazolam	2.483	3328192	54792	6609	1.28
4	Alprazolam	2.486	3329036	55099	6760	1.28
5	Alprazolam	2.489	3325969	52378	6708	1.30
6	Alprazolam	2.483	3327726	54776	6757	1.36
Mean			3326669			
Std. Dev			1985.642			
% RSD			0.059688			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table 15: Results of Intermediate precision Day 2 for Fluoxetine HCL

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fluoxetine HCL	2.067	249498	11595	5241	1.2
2	Fluoxetine HCL	2.069	240992	11358	5132	1.2
3	Fluoxetine HCL	2.068	240431	11879	5137	1.2
4	Fluoxetine HCL	2.069	241332	11746	5268	1.2
5	Fluoxetine HCL	2.067	240518	11831	5223	1.2
6	Fluoxetine HCL	2.067	240471	11476	5984	1.2
Mean			242208.7			
Std. Dev			3590.035			
% RSD			1.48223			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table 16: Results of Intermediate precision for Alprazolam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Alprazolam	2.485	3426978	53354	6701	1.3
2	Alprazolam	2.484	3446642	54455	6564	1.3
3	Alprazolam	2.496	3430607	53533	6856	1.3
4	Alprazolam	2.484	3430953	55158	6863	1.3
5	Alprazolam	2.490	3431677	56224	6941	1.3
6	Alprazolam	2.490	3429188	58579	6645	1.3
Mean			3433813			
Std. Dev			7041.408			
% RSD			0.205062			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

The accuracy results for Fluoxetine HCL

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124676.7	15	15.2	101%	100.4%
100%	242008.3	30	30.2	100.5%	
150%	357448	45	44.8	99.7%	

The accuracy results for Alprazolam

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696258	18.76	18.72	99.8%	99.2%
100%	3351662	37.4	37.3	99.4%	
150%	4975095	56.24	55.48	98.6%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

FLUOXETINE HCL:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247393	2.061	7244	1.2
Less Flow rate of 0.9 mL/min	69215	2.267	4712	1.3
More Flow rate of 1.1 mL/min	388839	1.864	4741	1.2
Less organic phase	445627	2.165	4708	1.2
More organic phase	69405	1.967	5591	1.4

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

ALPRAZOLAM:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530867	2.462	3388	1.1
Less Flow rate of 0.9 mL/min	527374	2.690	5276	1.0
More Flow rate of 1.1 mL/min	4363128	2.284	5612	1.0
Less organic phase	3965573	2.590	5551	1.0
More organic phase	527709	2.390	6274	1.0

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Fluoxetine HCL and Alprazolam in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Fluoxetine HCL and Alprazolam was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Fluoxetine HCL and Alprazolam in bulk drug and in pharmaceutical dosage forms.

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