ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC.

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Olanzapine and Fluoxetine in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Olanzapine and Fluoxetine were (1.794, 3.440min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The

limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Olanzapine and 1.28µg/ml 3.84µg/ml for Fluoxetine respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Olanzapine and Fluoxetine in tablets.

Keywords: Olanzapine and Fluoxetine, RP-HPLC, Validation, Accuracy, Robustness.

INTRODUCTION

High performance liquid chromatography [4]

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows using a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Speed (many analysis can be accomplished in 20 min or less)
- Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low viscosity
- Easy sample recovery, handling and maintenance
- Instrumentation leads itself to automation and quantification (less time and less labour).

INSTRUMENTATION OF HPLC

The individual components HPLC and their working functions are described below.

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Column
- Detector
- Data system

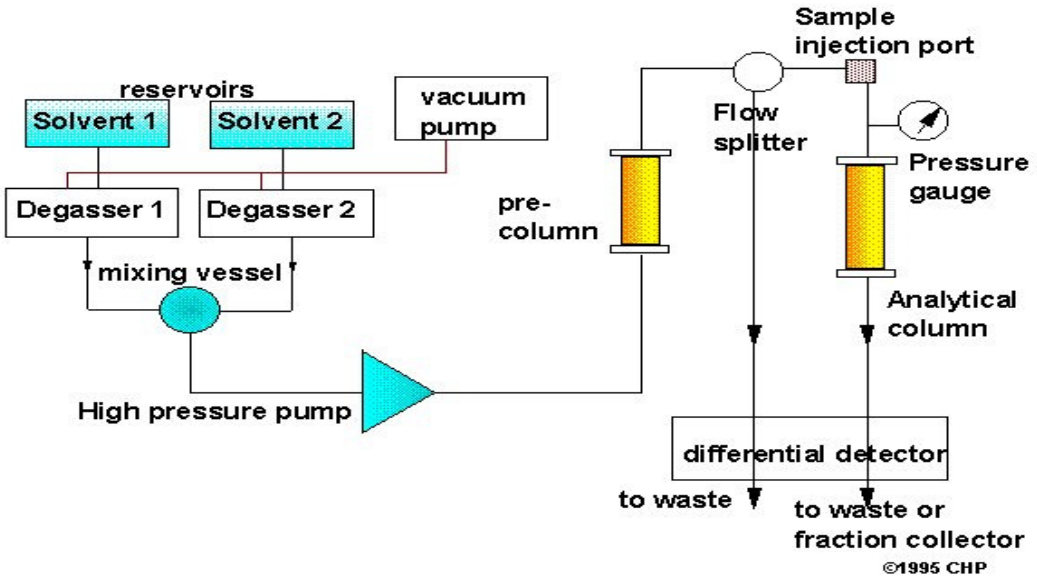


Figure: Instrumentation of HPLC

Applications of HPLC

- **Preparative HPLC** refers to the process of isolation and purification of compounds.
- **Chemical separations** can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates for a given set of column and mobile phase.

- **Identification of the compounds** by HPLC is a crucial part of any HPLC assay. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph.
- **Purification** refers to the process of separating or extracting the target compound from other (possibly structure related) compounds or contaminants.

MATERIALS

Olanzapine, Fluoxetine, Water and Methanol for HPLC, Acetonitrilefor HPLC from MERCK provided by **Sura labs, Dilsuknagar**, Telangana, India.

METHODOLOGY

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Olanzapine and Fluoxetine 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 required amount of the above Olanzapine and 0.3ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight ofOlanzapine andFluoxetine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette required amount of the sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Optimized chromatographic conditions:

Mobile phase ratio	: Methanol: Phosphate Buffer (pH-3.8) (28:72v/v)
Column	: Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Column temperature	: Ambient
Wavelength	: 252nm
Flow rate	: 1.0ml/min
Injection volume	: 20µl
Run time	: 8minutes

METHOD VALIDATION PARAMETERS

System Suitability:

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

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

Linearity:

Procedure:

Prepare concentrated solutions of drugs Artemether in the range of 60-140ppm, and Lumefantrine in the range of 100-500ppm using mobile phase as diluent.

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

***Intermediate Precision*:** To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Pipette out 0.5ml of Artemether and 1.5ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution:

Pipette out 1ml of Artemether and 3ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:

Pipette out 1.5ml of Artemether and 4.5ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Artemetherand Lumefantrine and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results

Effect of Variation of flow conditions:

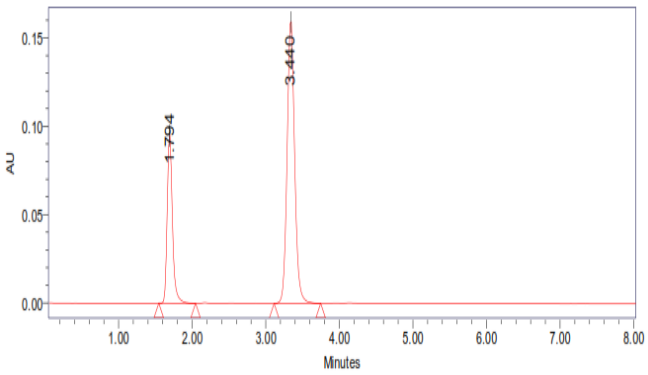
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

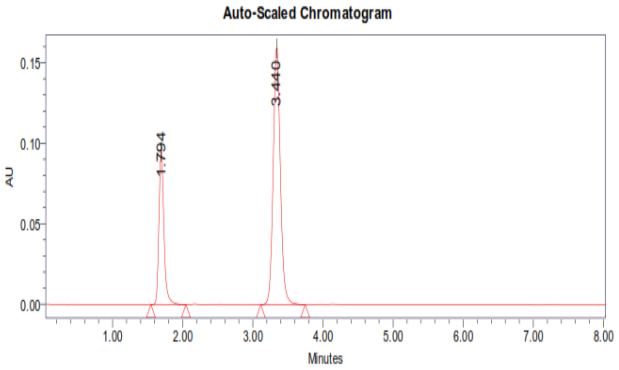
Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v)was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard) **Optimized Chromatogram (Sample)**





METHOD VALIDATION

SPECIFICITY

%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 Olanzapineand Fluoxetinein pharmaceutical dosage form was found to be 100.154%

LIMIT OF DETECTION FOR OLANZAPINEAND FLUOXETINE

LOD= 3.3 × σ / s

Result: Olanzapine =0.86µg/ml; **Fluoxetine** =1.28µg/ml

QUANTITATION LIMITFOR OLANZAPINEAND FLUOXETINE

LOQ=10×σ/S

Result: Olanzapine =2.58µg/ml; **Fluoxetine** = 3.84µg/ml

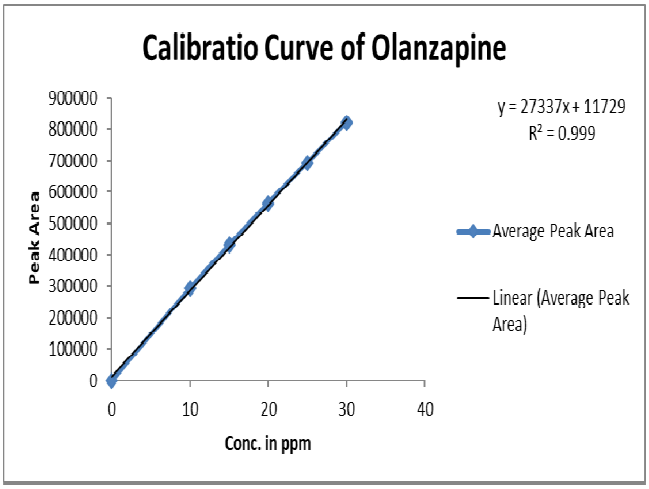
Where

σ = Standard deviation of the response ; S = Slope of the calibration curve

LINEARITY

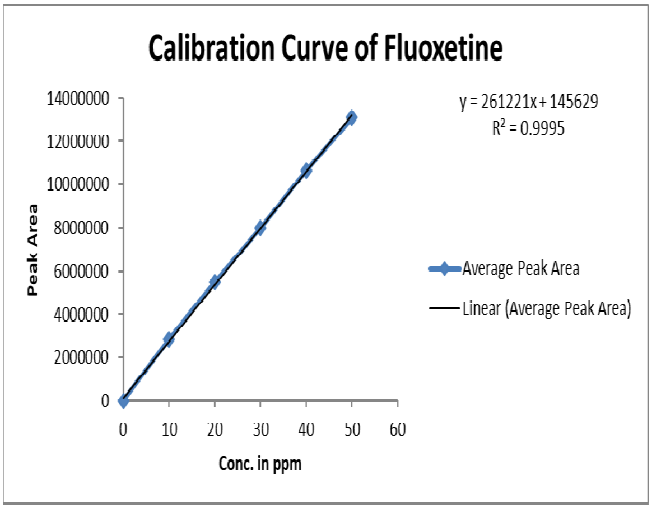
Chromatographic Data For Linearity Study For Olanzapine

Concentration µg/ml	Average Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584



Chromatographic Data For Linearity Study For Fluoxetine

Concentration µg/ml	Average Peak Area
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985



PRECISION:

REPEATABILITY

Table :Results of Repeatability for Olanzapine:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Olanzapine	1.792	548698	7458	7569	1.10
2	Olanzapine	1.791	548955	7485	7546	1.10
3	Olanzapine	1.790	548745	7469	7592	1.09
4	Olanzapine	1.790	549856	7463	7519	1.10
5	Olanzapine	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std.dev			1202.217			
%RSD			0.2191554			

Table: Results of Repeatability for Fluoxetine

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fluoxetine	3.435	7768958	43659	8659	1.12
2	Fluoxetine	3.428	7765984	43856	8647	1.13
3	Fluoxetine	3.419	7785469	43658	8675	1.12
4	Fluoxetine	3.414	7785498	43549	8652	1.12
5	Fluoxetine	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std.dev			9539.236			
%RSD			0.122689			

Intermediate precision:

Table: Results of Intermediate precision day1 for Olanzapine

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Olanzapine	1.787	556985	75986	7695	1.11
2	Olanzapine	1.789	558649	75986	7642	1.12
3	Olanzapine	1.789	557847	75689	7683	1.12
Mean			557827			
Std.Dev.			832.1803			
%RSD			0.149183			

Table: Results of Intermediate precision day1 for Fluoxetine

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Fluoxetine	3.482	7856982	44586	8758	1.13
2	Fluoxetine	3.477	7845285	44758	8769	1.14
3	Fluoxetine	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std.Dev.			6187.659			
%RSD			0.078801			

Table: Results of Intermediate precision Day 2 for Olanzapine

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing

1	Olanzapine	1.790	536598	7365	7459	1.08
2	Olanzapine	1.789	534875	7358	7436	1.07
3	Olanzapine	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std.Dev.			1049.608			
%RSD			0.196045			

Table: Results of Intermediate precision Day 2 for Fluoxetine

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Fluoxetine	3.474	7698521	42568	8582	1.11
2	Fluoxetine	3.473	7685985	42698	8546	1.10
3	Fluoxetine	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std.Dev.			27487.83			
%RSD			0.358064			

ACCURACY

Table:The accuracy results for Olanzapine

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

Table:The accuracy results for Fluoxetine

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

ROBUSTNESS

Variation in flow

Table: Results for Robustness -Olanzapine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min	526548	1.744	7425	1.12

Less organic phase(about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase(about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table: Results for Robustness-Fluoxetine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase(about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase(about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 252 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry (C18) (150mm x 4.6mm, 5µm) Column because it was giving good peak.

An ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (pH-3.8) (28:72v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 8 min because analyze gave peak around 1.794, 3.440 ±0.02min respectively and also to reduce the total run time.The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 10-30mg/ml of Olanzapineand 10-50mg/ml of Fluoxetineof the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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