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REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND FLUPENTIXOL IN PURE AND MARKETED FORMULATION

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Abstract:

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validation of Escitalopram and Flupentixol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Acetonitrile and water (75:25% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240nm. The retention time of the Flupentixol and Escitalopram was 2.121, 3.643 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Flupentixol and 66.6-330mg/ml of Escitalopram. 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: *Escitalopram, Flupentixol, RP-HPLC, validation.*

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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]

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).

Fig.No.1. Showing flow chart for classification of chromatography[4]

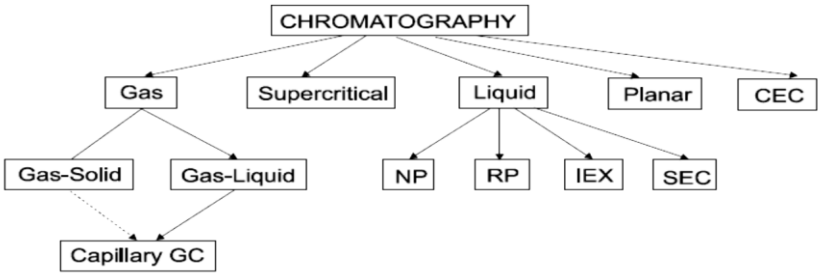
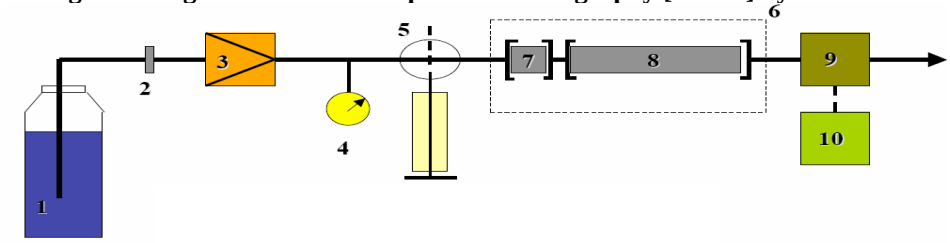


Fig.No.2. High-Performance Liquid Chromatography [HPLC] System



1 = eluent reservoir
2 = filter
3 = high pressure pump with pulse dampener
4 = pressure gauge
5 = sample injection valve with syringe
6 = column oven
7 = guard column
8 = column
9 = detector
10 = recorder (integrator, PC etc.)

Types of HPLC techniques [7]
Based on modes of separation

- Normal phase chromatography
- Reversed phase chromatography

ANALYTICAL METHOD VALIDATION:

Method validation can be defined as per ICH "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".

ICH Method validation parameters 18-19:
For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

- Specificity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness
- System suitability

MATERIALS AND METHODS:

Flupentixol(Pure) from Sura labs, Escitalopram(Pure) from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck

Hplc method development:
Trails:
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Flupentixol and Escitalopram 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 0.3ml of Flupentixol and 1.98ml of Escitalopram from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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 and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 75:25 v/v respectively.

Optimization of Column:
The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:
Instrument used :Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature :40ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase :Acetonitrile and water (75:25% v/v)
Flow rate : 1ml/min
Wavelength :240nm
Injection volume : 10µl
Run time : 6minutes

Validation:
Preparation of mobile phase:
Preparation of mobile phase:
Accurately measured 750ml of Acetonitrile (75%) of and 250ml of HPLC Water (25%) were mixed and degassed in a 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 ratio: Acetonitrile: Water(75:25 v/v)
Column: Phenomenex Gemini C18 (4.6×250mm) 5µ
Column temperature: 40ºC
Wavelength: 240nm
Flow rate: 1ml/min
Injection volume: 10µl
Run time: 6minutes

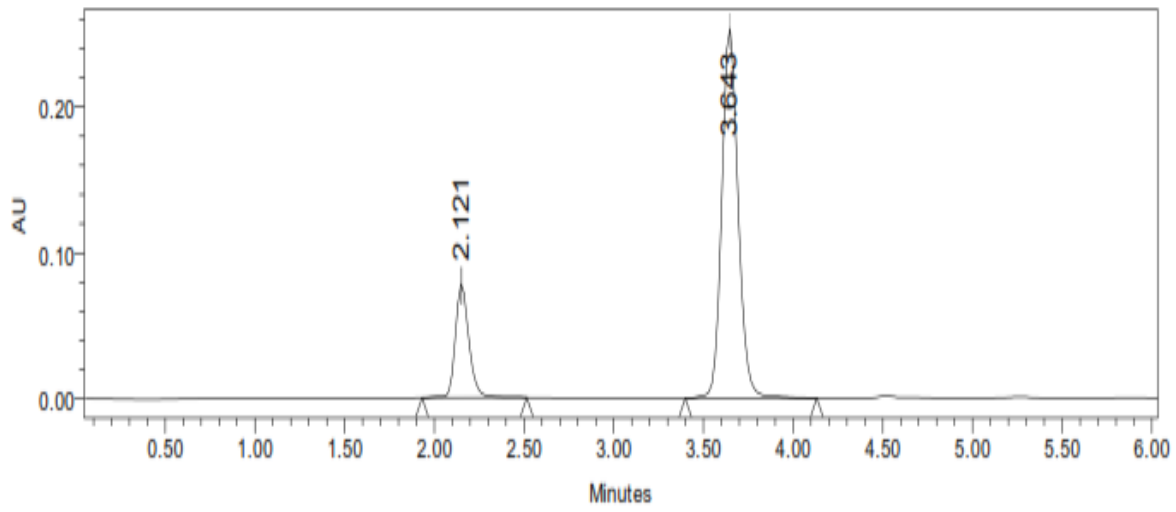


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Flupentixol	2.121	406433	77644	1.2	4009	
2	Escitalopram	3.643	1592811	251532	1.1	7849	9.8

Optimized Chromatogram

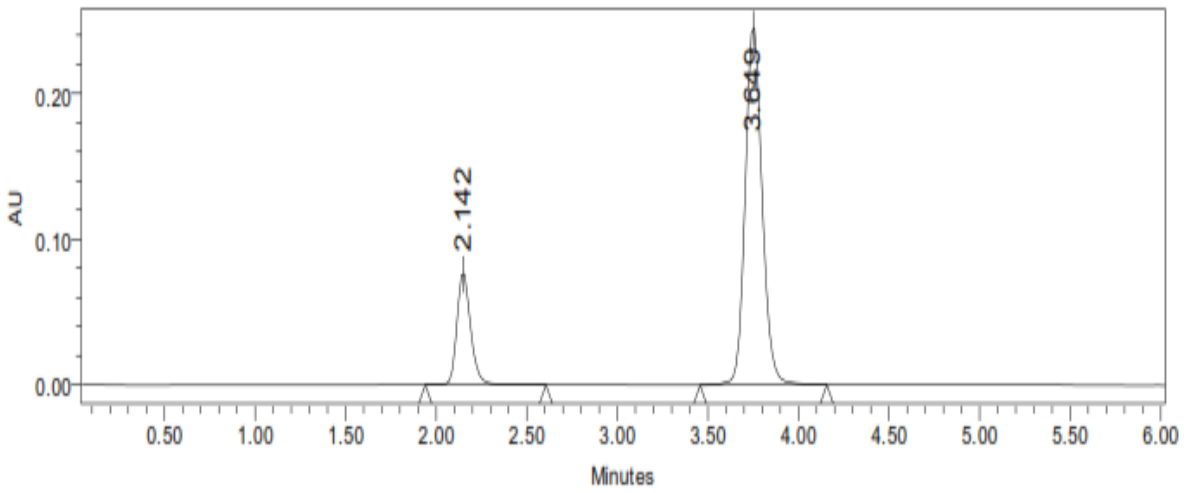


Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.no	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Flupentixol	2.142	403871	77464	1.2	4136	
2	Escitalopram	3.649	1573821	259361	1.1	7812	10.3

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and 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: Results of system suitability for Flupentixol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Flupentixol	2.152	382726	70725	5271	1.2
2	Flupentixol	2.157	382621	70625	5928	1.2
3	Flupentixol	2.141	389172	70617	5283	1.2
4	Flupentixol	2.133	384152	70718	5763	1.2
5	Flupentixol	2.166	389721	70172	6222	1.2
Mean			385678.4			
Std. Dev.			3497.932			
% RSD			0.906956			

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: Results of system suitability for Escitalopram

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Escitalopram	3.674	1562821	227365	5827	1.1	10.1
2	Escitalopram	3.631	1562726	226748	6183	1.1	10.1
3	Escitalopram	3.625	1567361	227163	5029	1.1	10.1
4	Escitalopram	3.692	1562811	226948	4920	1.1	10.1
5	Escitalopram	3.629	1563816	226452	5183	1.1	10.1
Mean			1563907				
Std. Dev.			1982.03				
% RSD			0.126736				

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.

Assay (Sample):

Table: Peak results for Assay sample of Flupentixol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Flupentixol	2.152	406538	77074	1.2	4009	1
2	Flupentixol	2.150	409975	76001	1.2	4136	2
3	Flupentixol	2.187	402911	77823	1.2	5173	3

Table: Peak results for Assay sample of Escitalopram

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Escitalopram	3.646	1609924	251956	1.1	7849	1
2	Escitalopram	3.651	1601840	246020	1.1	7819	2
3	Escitalopram	3.601	1603821	240291	1.1	6812	3

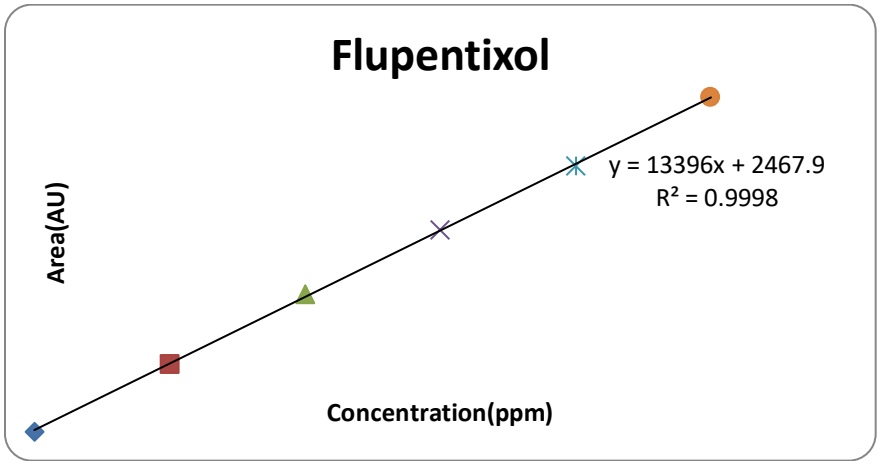
$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Flupentixol and Escitalopram in pharmaceutical dosage form was found to be 99.7%

Linearity

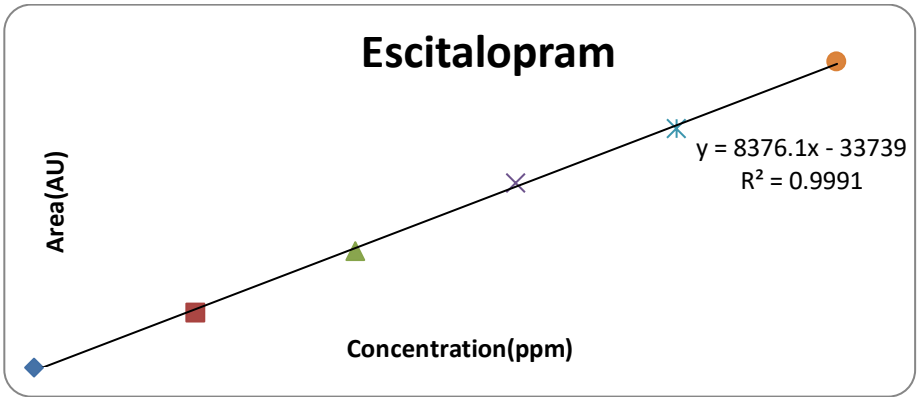
Chromatographic data for linearity study of flupentixol:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	135005
66	20	277120
100	30	405128
133	40	534643
166	50	672357



Chromatographic data for linearity study of escitalopram:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	66.6	489094
66	132	1049397
100	198	1657592
133	264	2150412
166	330	2748444



Repeatability:

Table: Results of repeatability for Flupentixol:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	%Assay
1	Flupentixol	2.157	400459	70717	1.2	4987	99%
2	Flupentixol	2.159	402118	71819	1.2	5019	99.4%
3	Flupentixol	2.186	405412	73930	1.2	5126	100%
4	Flupentixol	2.160	406506	73333	1.3	4999	100%
5	Flupentixol	2.170	407673	72623	1.2	5214	100%
Mean			404433.6				
Std.dev			2716.809				
%RSD			0.671757				

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.

Table: Results of repeatability for Escitalopram:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Escitalopram	3.603	1617864	226985	1.1	7045
2	Escitalopram	3.608	1618493	234764	1.1	7399
3	Escitalopram	3.600	1628262	227712	1.2	7159
4	Escitalopram	3.696	1615796	235459	1.1	7896
5	Escitalopram	3.629	1619626	242158	1.1	7965
Mean			1620008			
Std.dev			4310.623			
%RSD			0.266086			

Intermediate precision:

Table: Results of Intermediate precision Day 1 for Flupentixol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Flupentixol	2.198	405262	70572	5672	1.2
2	Flupentixol	2.196	405637	70516	5639	1.2
3	Flupentixol	2.160	405628	70572	6183	1.2
4	Flupentixol	2.160	405647	70372	5923	1.2
5	Flupentixol	2.160	405948	70592	6739	1.2
6	Flupentixol	2.186	408732	70526	5837	1.2
Mean			406142.3			
Std. Dev.			1287.197			
% RSD			0.316933			

Acceptance criteria:

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

Table: Results of Intermediate precision Day 2 for Escitalopram

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Escitalopram	3.623	1608292	235473	5372	1.1	10.1
2	Escitalopram	3.611	1609283	235938	5927	1.1	10.1
3	Escitalopram	3.696	1617836	235738	6129	1.1	10.1
4	Escitalopram	3.696	1619743	235963	5284	1.1	10.1
5	Escitalopram	3.696	1614262	231938	5284	1.1	10.1
6	Escitalopram	3.642	1608471	235948	6347	1.1	10.1
Mean			1611315				
Std. Dev.			6077.093				
% RSD			0.377151				

Acceptance criteria:

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

Table: Results of Intermediate precision Day 2 for Flupentixol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Flupentixol	2.198	405423	70572	5672	1.2
2	Flupentixol	2.196	405927	70516	5639	1.2
3	Flupentixol	2.178	405029	70572	6183	1.2
4	Flupentixol	2.142	405432	70372	5923	1.2
5	Flupentixol	2.177	405062	70592	6739	1.2
6	Flupentixol	2.177	408417	70526	5837	1.2
Mean			405881.7			
Std. Dev.			1283.857			
% RSD			0.316313			

Acceptance criteria:

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

Table: Results of Intermediate precision Day 2 for Escitalopram

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Escitalopram	3.611	1638732	244384	5363	1.1	10.1
2	Escitalopram	3.623	1637438	235827	6282	1.1	10.1
3	Escitalopram	3.684	1638474	236382	5938	1.1	10.1
4	Escitalopram	3.697	1634273	239183	6194	1.1	10.1
5	Escitalopram	3.684	1636372	231931	5402	1.1	10.1
6	Escitalopram	3.684	1639283	234356	5837	1.1	10.1
Mean			1637429				
Std. Dev.			1860.366				
% RSD			0.113615				

Acceptance criteria:

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

Accuracy:

The accuracy results for Flupentixol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	201472.3	15	14.9	99.3	99.4%
100%	406193	30	29.9	99.6	
150%	607144	45	44.8	99.5	

Acceptance Criteria:

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

The accuracy results for Escitalopram

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	826527.7	99	98.7	99.6	99.7%
100%	1622241	198	197.5	99.7	
150%	2422702	297	296.8	99.9	

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

Robustness

Table: Results for Robustness

Flupentixol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	406433	2.121	4009	1.2
Less Flow rate of 0.9 mL/min	398841	2.210	3800.8	0.9
More Flow rate of 1.1 mL/min	389947	2.184	4800.8	
Less organic phase	413898	2.200	4890.8	0.9
More Organic phase	389578	2.172	4190.8	0.7

Acceptance criteria:

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

Escitalopram

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1592811	3.643	7849	1.1
Less Flow rate of 0.9 mL/min	1613422	4.498	3312.2	0.9
More Flow rate of 1.1 mL/min	1619138	3.505	4312.2	0.8
Less organic phase	1616104	4.504	4392.2	0.9
More organic phase	1623185	3.512	4292.2	0.9

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 Escitalopram and Flupentixol bulk drug and pharmaceutical dosage forms.

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

Escitalopram and Flupentixol are freely soluble in ethanol, methanol and sparingly soluble in water.

Acetonitrile and water 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 Escitalopram and Flupentixol in bulk drug and in Pharmaceutical dosage forms.

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