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A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF FLUPENTIXOL AND MELITRACEN IN ITS PURE AND PHARMACEUTICSL DOSAGE FORM AS PER ICH GUIDELINES

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Abstract:

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Flupentixol and Melitracen in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 275nm. The retention time of the Flupentixol and Melitracen was found to be was 2.133, 3.692 ± 0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Flupentixol and 10-30mg/ml of Melitracen. The inter-day and intra-day precisions were found to be within limits. 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: *Flupentixol and Melitracen, RP-HPLC, Validation, Accuracy, Precision.*

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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*".

"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.

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).

High Performance Liquid Chromatography (HPLC) [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

Working Principle of HPLC [8]

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography.

The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been

developed. For example, if a compound can absorb Ultra Violet light, a UV-absorbance detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an Evaporative-Light-Scattering Detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a Mass Spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

MATERIALS AND METHODS:

Flupentixol -Sura labs, Melitracen-Sura labs, Water and Methanol for HPLC LICHROSOLV (MERCK),Acetonitrile for HPLC Merck

Hplc method development:

Trails:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Flupentixol and Melitracen 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 0.3 ml of Flupentixol and 0.6ml of Melitracen 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.

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 : Methanol: TEA Buffer (65:35 v/v)
Flow rate : 1ml/min
Wavelength : 230nm
Injection volume : 10µl
Run time : 6minutes

Validation

Preparation of mobile phase:

Preparation of mobile phase:

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) 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 : Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 275nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

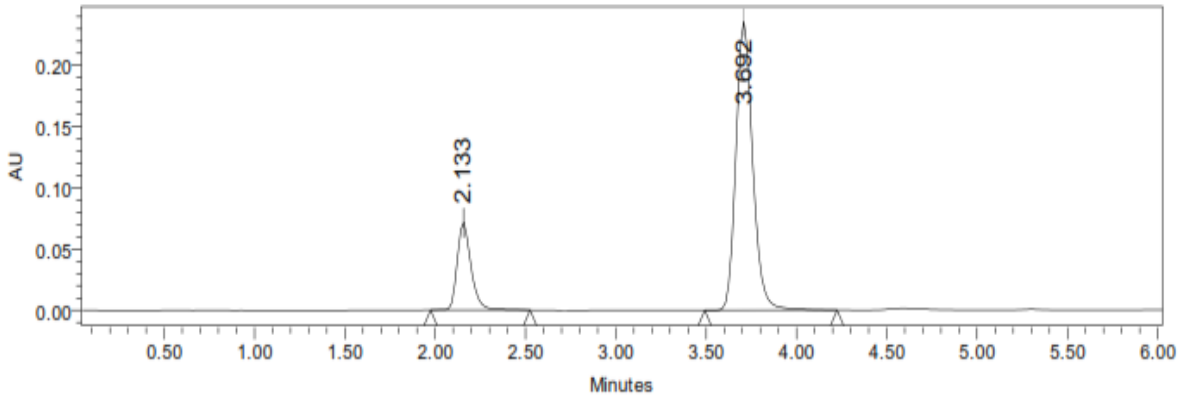


Figure-: Optimized Chromatogram (Standard)

Table No.18: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Flupentixol	2.133	526388	86757	1.57	5678	
2	Melitracen	3.692	1687286	367533	1.78	8686	9.8

Optimized Chromatogram (Sample)

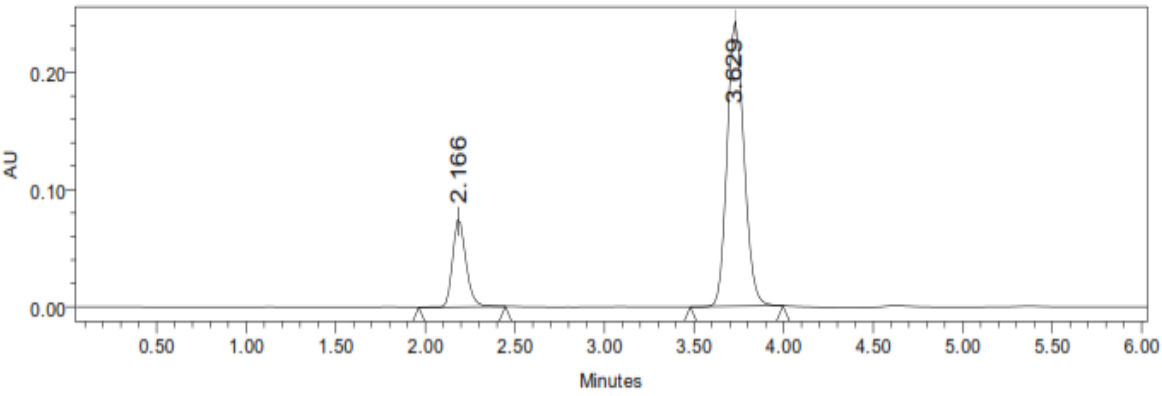


Figure-: Optimized Chromatogram (Sample)

Table No. 19: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Flupentixol	2.166	536588	77465	1.56	5788	
2	Melitracen	3.629	1695847	378563	1.81	8796	10.02

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-: Peak results for assay standard

Flupentixol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Flupentixol	2.152	526359	86599	1.56	5699	1
2	Flupentixol	2.198	526585	86785	1.57	5688	2
3	Flupentixol	2.179	529659	86254	1.56	5638	3

Melitracen

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Melitracen	3.646	1687588	365878	1.80	8658	1
2	Melitracen	3.604	1685986	365855	1.79	8698	2
3	Melitracen	3.610	1685975	369853	1.80	8676	3

Assay (Sample):

Table-: Peak results for Assay sample

Flupentixol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Flupentixol	2.152	536858	87585	1.58	5788	1
2	Flupentixol	2.150	532655	87966	1.59	5785	2
3	Flupentixol	2.187	532686	87466	1.58	5768	3

Melitracen

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Melitracen	3.646	1698569	378563	1.81	8758	1
2	Melitracen	3.651	1698575	375846	1.80	8796	2
3	Melitracen	3.601	1698546	376585	1.81	8744	3

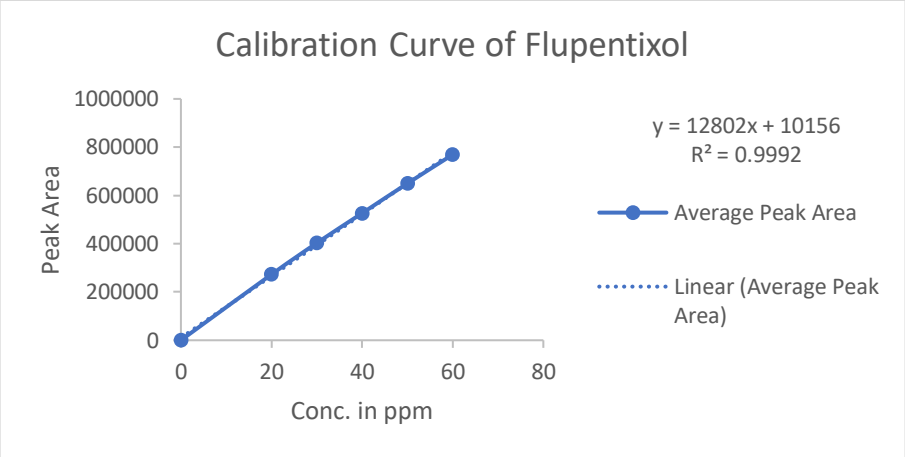
$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Flupentixol and Melitracen in pharmaceutical dosage form was found to be 99.89%

Linearity:
Chromatographic data for linearity study:
Flupentixol:

Concentration µg/ml	Average Peak Area
20	272898
30	402987
40	526388
50	649786
60	769288



Melitracen:

Concentration µg/ml	Average Peak Area
10	1000238
15	1448769
20	1887286
25	2365898
30	2826846

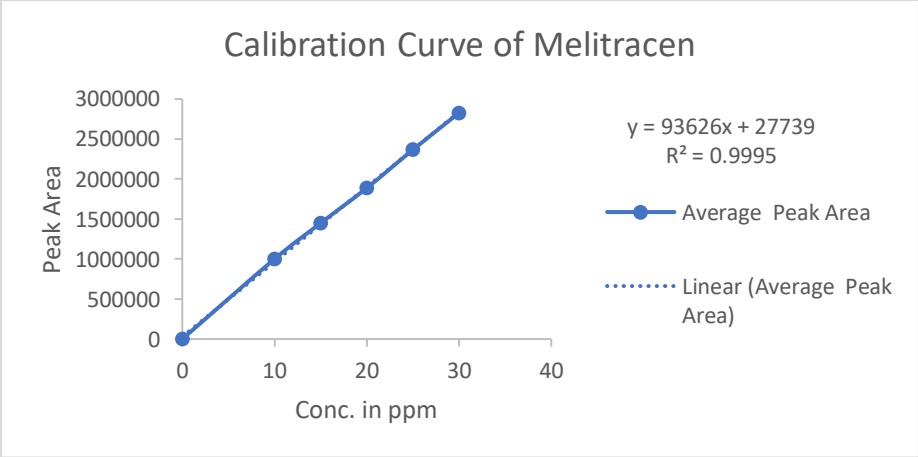


Fig: Chromatogram showing linearity level

Repeatability:

Table-: Results of Repeatability for Flupentixol:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Flupentixol	2.157	526359	86599	5688	1.56
2	Flupentixol	2.159	524857	86543	5688	1.57
3	Flupentixol	2.186	526986	86579	5685	1.56
4	Flupentixol	2.160	528655	86355	5688	1.56
5	Flupentixol	2.170	528458	86959	5638	1.56
Mean			527063			
Std.dev			1569.114			
%RSD			0.297709			

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 Melitracen:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Melitracen	3.603	1687588	367858	8658	1.79
2	Melitracen	3.608	1685988	368548	8678	1.80
3	Melitracen	3.600	1685986	367986	8646	1.80
4	Melitracen	3.696	1685755	365875	8694	1.79
5	Melitracen	3.629	1685986	364588	8626	1.79
Mean			1686261			
Std.Dev			748.7896			
%RSD			0.044405			

Intermediate precision:

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Flupentixol	2.198	546586	87588	5899	1.58	100%
2	Flupentixol	2.196	548759	87986	5878	1.59	100%
3	Flupentixol	2.160	549855	87453	5867	1.58	100%
4	Flupentixol	2.160	548799	87422	5846	1.59	100%
5	Flupentixol	2.160	542658	87964	5897	1.58	100%
6	Flupentixol	2.186	548755	87255	5875	1.59	100%
Mean			547568				
Std. Dev.			2631.576				
% RSD			0.480593				

Acceptance Criteria:

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

Table : Results of Intermediate precision day1 for Melitracen

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Melitracen	3.623	1698588	385483	8788	1.81	9.8	98%
2	Melitracen	3.611	1698575	385699	8758	1.80	9.8	98.2%
3	Melitracen	3.696	1698533	385749	8755	1.81	9.9	98.7%
4	Melitracen	3.696	1698575	386957	8756	1.81	10.01	99.7%
5	Melitracen	3.696	1698533	385756	5797	1.80	9.98	98.5%
6	Melitracen	3.642	1698548	386559	8763	1.80	10.02	98.2%
Mean			1698559					
Std. Dev.			23.77114					
% RSD			0.001398					

Acceptance Criteria:

- %RSD of six 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	536855	8759	5788	1.58
2	Flupentixol	2.196	536986	8796	5727	1.59
3	Flupentixol	2.178	536588	8747	5743	1.58
4	Flupentixol	2.142	532547	8755	5745	1.59
5	Flupentixol	2.177	534588	8726	5799	1.58
6	Flupentixol	2.177	538599	8727	5786	1.59
Mean			536026.3			
Std. Dev.			2131.493			
% RSD			0.397648			

Acceptance Criteria:

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

Table: Results of Intermediate precision Day 2 for Melitracen

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing	Resolution
1	Melitracen	3.611	1678599	356876	8876	1.82	9.9
2	Melitracen	3.623	1678986	358984	8857	1.83	10.01
3	Melitracen	3.684	1678985	358753	8863	1.82	9.9
4	Melitracen	3.697	1678986	352411	8848	1.83	10.01
5	Melitracen	3.684	1678548	358988	8874	1.82	9.9
6	Melitracen	3.684	1678985	358987	8843	1.83	10.01
Mean			1678849				
Std. Dev.			212.8049				
% RSD			0.012675				

Acceptance Criteria:

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

Accuracy:

Table-: The accuracy results for Flupentixol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267012.3	20	20.064	100.316%	100.28%
100%	523753.3	40	40.119	100.294%	
150%	778457.4	60	60.134	100.222%	

Table : The accuracy results for Melitracen

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972877.3	10	10.095	100.95%	100.49%
100%	1900123	20	19.999	99.98%	
150%	2851151	30	30.155	100.53%	

Robustness

Table-: Results for Robustness
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	526388	2.133	5678	1.56
Less Flow rate of 0.9 mL/min	542686	2.210	5265	1.54
More Flow rate of 1.1 mL/min	526484	2.184	5427	1.52
Less organic phase	516855	2.200	5164	1.57
More Organic phase	506899	2.172	5097	1.51

Table : Results for Robustness- Melitracen

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687286	3.692	8686	1.78
Less Flow rate of 0.9 mL/min	1725469	4.498	8264	1.68
More Flow rate of 1.1 mL/min	1652848	3.505	8416	1.59
Less organic phase	1687486	4.504	8327	1.62
More organic phase	1674523	3.512	8416	1.63

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 Flupentixol and Melitracen in bulk drug and pharmaceutical dosage forms.

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

Flupentixol was found to be freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. Melitracen (hydrochloride) was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, soluble in water.
Methanol: Phosphate Buffer (pH-4.2) (37:63 v/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 Flupentixol and Melitracen in bulk drug and in Pharmaceutical dosage forms.

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