International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Prospective Study Method Development and Validation of Flupentixol and Melitracen in Pure API and Combined Dosage Form by RP-HPLC

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Flupentixol and Melitracen was done by RP-HPLC. The Phosphate buffer was p^H 4.5 and the mobile phase was optimized with consists of ACN: Phosphate buffer mixed in the ratio of 80:20 % v/v. Kromosil C_{18} Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 252 nm. The solutions were chromatographed at a constant flow rate of 0.8ml min^{-1}. The linearity range of Flupentixol and Melitracen were found to be from 100-500 µg/ml of Flupentixol and 1-5µg/ml of Melitracen. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Flupentixol and Melitracen. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Kromosil $C_{18,}$ Flupentixol and Melitracen, RP HPLC

ARTICLE HISTORY: Received 11 March 2020, **Accepted** 22 May 2020, **Available Online**27 June 2020

Citation: Gampa Vijaya Kumar, *et al.* Prospective Study Method Development and Validation of Flupentixol and Melitracen in Pure API and Combined Dosage Form by RP-HPLC, 8(6), 2020: 130-134.

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1. Introduction

Flupentixol is an antipsychotic neuroleptic drug. It is a thioxanthene, and therefore closely related to the phenothiazines.

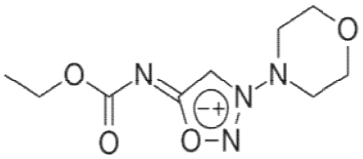


Fig 1: Chemical structure of Flupentixol

Melitracen (brand names Melixeran) is a tricyclic antidepressant (TCA), for the treatment of depression and anxiety. In addition to single drug preparations, it is also available as Deanxit, marketed by Lundbeck, a combination product containing both melitracen and flupentixol.

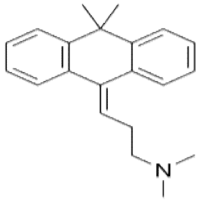


Fig 2: Chemical structure of Melitracen

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler:

Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech, U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Flupentixol and Melitracen, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized Chromatographic conditions:

Parameters	**Description**
Flow rate	: 0.8ml min^{-1}
Column	: Kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	: Phosphate buffer: ACN P^H 4.5(20:80 v/v)
Buffer	: Potassium dihydrogen orthophosphate PH 4.5 adjusted with Orthophosphoric acid
Detector	: PDA
Column temperature	: Ambient
Type of elution	: Isocratic
Wavelength	: 252 nm
Injection volume	: 20µl
Run time	: 10min

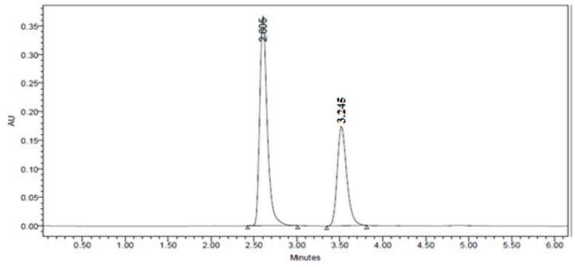


Fig 3: Optimized Chromatogram

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Flupentixol and Melitracen 10mg of working standard into a 10mL& 100ml 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 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Flupentixol and Melitracen (marketed formulation) sample 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 3 ml of Flupentixole and Melitracen of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: Results of system suitability parameters for Flupentixol and Melitracen

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Flupentixol	2.6	145508	215642	4.2	1.3	8674.6
2	Melitracen	3.8	1368498	154568	4.2	1.3	5080.2

Table 2: Results of method precession for Flupentixol

Injection	Area
Injection-1	1438726
Injection-2	1402948
Injection-3	1403823
Injection-4	1403978
Injection-5	1409659
Average	1404529.8
Standard Deviation	2971.1
%RSD	0.6

Table 3: Results of method precession for Melitracen

Injection	Area
Injection-1	135147
Injection-2	135768
Injection-3	134276
Injection-4	138697
Injection-5	134857
Average	134152.6
Standard Deviation	825.4
%RSD	0.5

Table 4: Results of Intermediate precision for Flupentixol

Injection	Area
Injection-1	1405148
Injection-2	1404528
Injection-3	1406937
Injection-4	1406472
Injection-5	140671
Average	1445071.2
Standard Deviation	3561.4
%RSD	0.4

Table 5: Results of Intermediate precision for Melitracen

Injection	Area
Injection-1	132489
Injection-2	132826
Injection-3	132635
Injection-4	132704
Injection-5	132963
Average	132681.8
Standard Deviation	184.6
%RSD	0.3

Table 6: Accuracy (recovery) data for Flupentixol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656759.5	5.0	5.035	100.7%	98.86%
100%	1308258	10.0	10.004	100.0%	
150%	1864607	14.6	14.254	98.76%	

Table 7: Accuracy (recovery) data for Melitracen

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65807	5.2	5.35	100.8%	100.41%
100%	124354	10	10.10	100.01%	

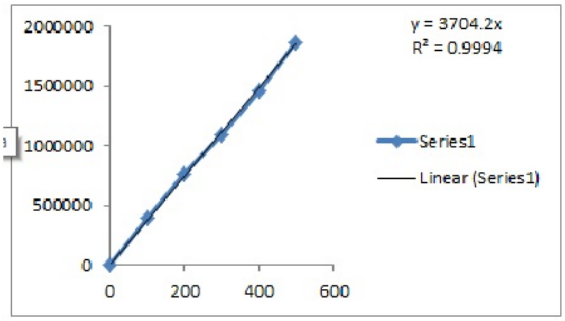
150%	178940	14.3	14.46	99.67%	

Table 8: Area of different concentration of Flupentixol

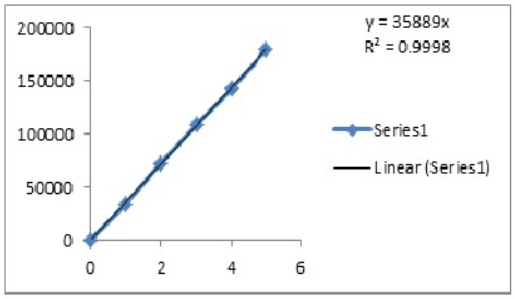
S.No.	Linearity Level	Concentration	Area
1	I	100ppm	388934
2	II	200ppm	756781
3	III	300ppm	1093873
4	IV	400ppm	1463458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 9: Area of different concentration of Melitracen

S.No	Linearity Level	Concentration	Area
1	I	1ppm	34510
2	II	2ppm	71701
3	III	3ppm	108802
4	IV	4ppm	142731
5	V	5ppm	179732
Correlation Coefficient			0.999



Calibration graph for Flupentixol



Calibration graph for Melitracen

Fig 4: Calibration graphs

Table 10: Analytical performance parameters of Flupentixol and Melitracen

Parameters	Flupentixol	Melitracen
Slope (m)	68579	15427
Intercept (c)	37042	35889
Correlation coefficient (R^2)	0.999	0.999

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Flupentixol	51	142	2.8
Melitracen	51	136	3.5

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Flupentixol	53	523	10.02
Melitracen	53	542	11.2

Table 13: Flow Rate (ml/min) data for Flupentixol

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5389.6	1.3
2	0.8	46837	1.3
3	1.0	6246.0	1.4

Table 14: Flow rate (ml/min) data for Melitracen

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7265.2	1.3
2	1.0	6080.3	1.4
3	1.2	5978.0	1.3

Table 15: Change in Organic Composition in the Mobile Phase for Flupentixol

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 16: Change in Organic Composition in the Mobile Phase for Melitracen

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

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