EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 11
153-158
Year: 2023

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF FLUPENTIXOL AND MELITRACEN IN COMBINED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Article Received on 04/09/2023 **Article Revised on 24/09/2023** **Article Accepted on 14/10/2023**

ABSTRACT
A simple, specific, precise, and efficient method for the Simultaneous estimation of Flupentixol and Melitracen in pure and pharmaceutical dosage forms by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase was in a combination of Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v). Optimized column is a Develosil C_{18} (4.6mm×250mm) 5 µm particle size and at a flow rate of 1.0 mL/min with detection wavelength at 238 nm for Flupentixol and Melitracen. In our study, the validation of analytical method for determination of Flupentixol and Melitracen in pure and pharmaceutical dosage forms was performed in accordance the parameters including system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines. In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30 µg/ml for Flupentixol and 30-90 µg/ml for Melitracen with percentage recoveries within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore proposed method can be used for routine analysis of Flupentixol and Melitracen in the pure form as well as in combined pharmaceutical dosage form.

KEYWORDS: Flupentixol, Melitracen, RP-HPLC, Method Development, Validation.

INTRODUCTION
Flupentixol: Flupentixol [$C_{23}H_{25}F_3N_2OS$] is a typical thioxanthene antipsychotic and it is a white powder[1]. Chemically it is (EZ)-2-[4-[3-[2-(trifluoromethyl) thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethanol. It is easily soluble in water and alcohol and the water solubility of the drug is 0.000346 mg/ml. Flupentixol is a powerful antagonist of a neurotransmitter called Dopamine in both D_1 and D_2 receptors, there by resulting in psychotic illness. The drug flupentixol (Fig. No. 1) is marketed under the brand name of Depixol and Fluanxol. The drug is mainly used to treat the schizophrenia as a long acting injection and also used to treat the Depression in low doses.[2] Flupentixol in combination with melitracen given in case of trigeminal neuralgia and mild to moderate mental disorders.[3]

Melitracen: Melitracen [$C_{21}H_{25}N$] is a Tricyclic antidepressent and it is available as white to off white powder in amorphous form.[1] Chemically it is 3-(10,10-dimethylanthracen-9(10H)-ylidene)-N,N-dimethylpropan-1-amine. The water solubility of the drug melitracen (Fig. No. 2) is 0.00318 mg/mL. As a tricyclic antidepressent it inhibits the uptake of neurotransmitters called norepinephrine and serotonin by neurons thereby increase in the levels of chemical messengers in the brain which helps in regulating the mood and treat depression. The drug is mainly used to treat depression and anxiety.[4]

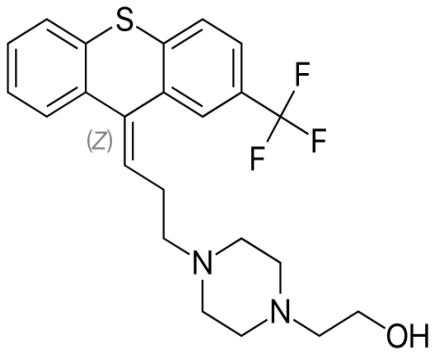

Fig. No. 1: Structure of Flupentixol.

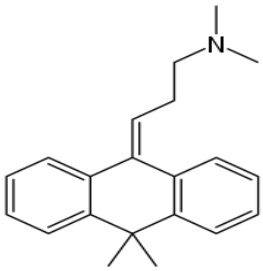

Fig. No. 2: Structure of Melitracen.

Literature survey revealed that there are many methods developed for the estimation of flupentixol individually or in combination with other drugs by HPTLC, LC and Spectroscopy and for melitracen estimated by spectroscopy and HPTLC methods individually or in combination with other drugs.[5-10] It is observed that there are less methods reported for the determination of Flupentixol and Melitracen in combined dosage form. Present study involves in development and validation of a new simple and economical method for the estimation of Flupentixol and Melitracen according to ICH guidelines and it can be used for the routine determination of Flupentixol and Melitracen in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrument: The chromatography was performed with WATERS Alliance 2695 separation module, Software: Empower 2, coupled with 996 PDA detector.

Table No. 1: List of Equipments Used.

S. No.	Name of Instrument	Name of the Manufacturer
1	HPLC	WATERS Alliance
2	Digital ultra sonicator	Labman
3	pH meter	Lab India

Reagents and Maretials: The mobile phase chosen was Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) and methanol was used as solvent. All the chemicals and reagents used in the method were obtained from Merck (India) Ltd and are of HPLC grade only. Standard drugs and samples were obtained from Sura labs and the tablet formulation was purchased from local pharmacy.

Table No. 2: List of materials and chemicals used.

S. No	Names	Manufacturer/ Supplier
1	Flupentixol	Sura labs, Hyderabad
2	Melitracen	Sura labs, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

Preparation of mobile phase: Accurately measured 350 ml of Acetonitrile (35%) and 650 ml of Acetate buffer (65%) were mixed and degassed in a digital ultra sonicator for 20 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard stock solution

Accurately weighed 10 mg of Flupentixol and Melitracen working standards were transferred into a 10 ml of clean dry volumetric flasks, added about 7 ml of Methanol and sonicated to dissolve and removal of air completely and volume was made up to the mark with the same Methanol.

Standard solution: The 100% mixed standard soliution of Flupentixol and Melitracen was prepared by transferring 0.2 ml of Flupentixol and 0.6 ml of Melitracen from the above stock solutions into a 10 ml volumetric flask and diluted up to the mark with Methanol.

Preparation of sample stock solution: 10 tablet contents were weighed and average weights of the tablets were taken then triturated to fine powder. An accurately weighed 10 mg equivalent weight of Flupentixol and Melitracen sample was taken into a 10 ml clean dry volumetric flask and added about 7 ml of Diluent and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. The sample solution was filtered by using injection filter which contains 0.45 µ pore size.

Sample solution: From the stock solution pipetted out 0.2ml of Flupentixol and 0.6ml of Melitracen into a 10ml volumetric flask and diluted up to the mark with Diluent.

VALIDATION

The developed method of analysis was validated as per ICH guidelines for the parameters like Linearity, Precision, Accuracy, Specificity, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness.

Accuracy: The accuracy of measurement is the closeness of the measured value to the true value. Typically, accuracy is represented and determined by recovery studies. Here the three concentrations were used to test the recovery studies. Then the standard deviations were calculated after each level of injection.

Precision: Precision is "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample". According to ICH guidelines precision is divided as repeatability, intermediate precision and reproducibility.

Linearity: The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. It is a measure of how well a calibration plot of response vs. concentration approximates a straight line. A total of five series of standard solutions were selected for the assessment of linearity spectrum. The calibration curve was plotted and correlation coefficient was calculated.

Specificity: Specificity is the ability to assess that the analyte in the presence of components which may be expected to be present, these include impurities, degradants and matrix etc. Blank standard and those with flupentixol and melitracen was tested.

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope the limit of detection may be expressed as;

$$LOD=3.3\ \sigma/S$$

Limit of Quantitation: The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Based on the standard deviation of the response and the slope the limit of Quantitation may be expressed as:

$$LOQ=10\sigma/S$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve.

Robustness: It is a measure of it's capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The typical variations include variations in pH of a mobile phase, different columns, mobile phase composition, temperature and flow rate etc.

RESULTS AND DISCUSSION

The developed method of analysis was validated as per ICH guidelines for the parameters like, Linearity, Precision, Accuracy, Limit of detection(LOD), Limit of quantification(LOQ) and Robustness.

Chromatographic conditions: A column (Develosil C18 (4.6mm×250mm) 5µm particle size) equilibrated with mobile phase comprising of Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) was used. Mobile phase flow rate maintained at 1ml/min and eluent was monitered at 238 nm. Run time was 6 min & here the peaks were seperated and showed better resolution, theoritical plates and symmetry. All the chromatographic separation were carried at ambient tempereture. Conditions of optimized chromatography are shown in table No. 3.

Table No.3: Optimized Chromatographic Conditions.

Mobile phase	Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)
Wavelength	238 nm
Flow rate	1 ml/min
Run time	6 min
Temperature of the column	Ambient temperature
Injection volume	20 µl
Column	Develosil C18 (4.6 mm×250 mm) 5µm particle size

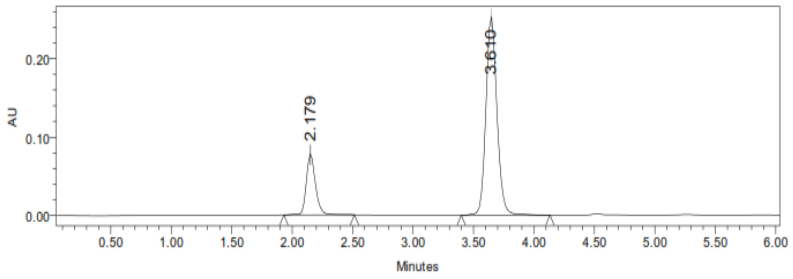

Fig. No. 3: Optimized chromatogram of Flupentixol (RT=2.179min) & Melitracen (RT=3.610 min).

Specificity: There was no participation from Flupentixol and Melitracaen at the elution time. As seen on figure -4, the blank chromatogram is present.

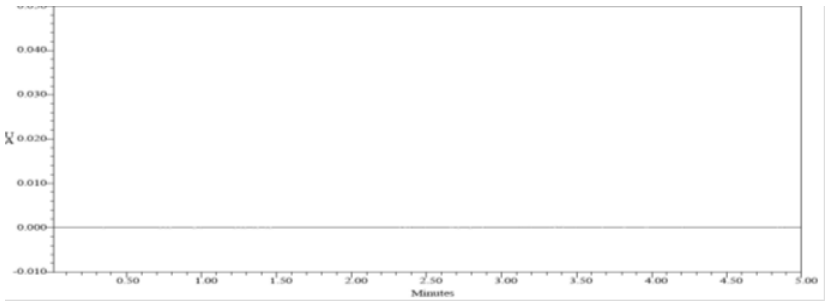

Fig. No. 4: Chromatogram of blank.

Linearity: The linearity range was found to be 10-30 µg/ml for flupentixol and 30-90 µg/ml for Melitracen and had a straight line. Correlation modules have been identified as 0.9994 & 0.9995 respectively. Linearity results were demonstrated in table no-4.

Table No. 4: Linearity Data of Flupentixol and Melitracen.

S. No.	Flupentixol		Melitracen	
	Working conc. (µg/ml)	Peak Area	Working conc. (µg/ml)	Peak Area
1	10	245899	30	863094
2	15	365687	45	1249397
3	20	481526	60	1678592
4	25	589854	75	2050412
5	30	705882	90	2468444
Correlation coefficient(r)		0.9994	0.9995	
Slope(m)		23457	27290	
Intercept(c)		7184.4	20465	

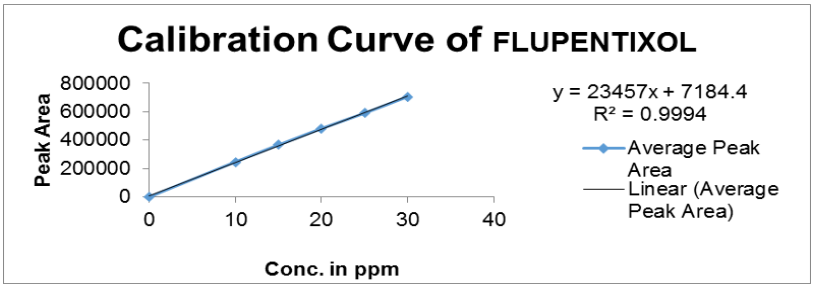

Fig. No. 5: Calibration plot of Flupentixol.

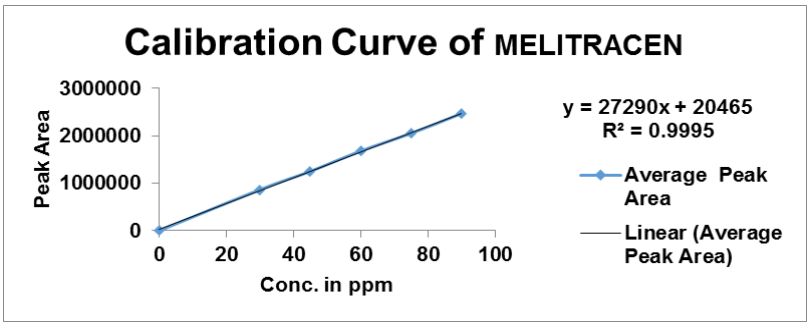

Fig. No. 6: Calibration plot of Melitracen.

Precision: To check intra and interday variation of the method, standard concentration was subjected to the proposed HPLC method of analysis. The precision of the proposed method variations in the peak area of the drug solutions was calculated in terms of the percent RSD. The relative standard deviations of the drugs at different concentration levels for five injections was less than 2.0, it proves that the process is precise.The results of method precision was shown in table no-5.

Table No.5: Results of method precision.

S.No	Flupentixol		Melitracen	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.157	513568	3.603	1635625
2	2.159	513685	3.608	1658744
3	2.186	513659	3.600	1652985
4	2.160	513254	3.696	1645898
5	2.170	513647	3.629	1652364
Mean		513562.6		1649123
Std dev		177.9475		8811.631
%RSD		0.03465		0.534322

Intermediate precision (Ruggedness): Intermediate precision was also known as Ruggedness. The intermediate precision of the method was performed by maintaining the same conditions at different days.

Table No.6: Results of intermediate precision.

S. No.	Flupentixol		Melitracen	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.198	514658	3.611	1638732
2	2.196	514895	3.623	1637438
3	2.178	514658	3.684	1638474
4	2.142	514784	3.697	1634273
5	2.177	515268	3.684	1636372
6	2.177	514598	3.684	1639283
Mean		514810.2		1637429
Std dev		248.5224		1860.366
%RSD		0.048275		0.113615

Accuracy: The recovery studies were carried out for the accuracy parameter. Accuracy at different concentrations (50%, 100%, and 150%) were prepared and calculated. The percentage recovery was found to be within the limit i.e. 98-102%. Hence method is accurate. The results for accuracy were presented in Table No. 7.

Table No. 7: Accuracy data for Flupentixol and Melitracen.

Accuracy level	Flupentixol			Melitracen		
	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery
50%	10	10.179	101.79%	30	30.114	100.38%
100%	20	20.316	101.58%	60	60.068	100.113%
150%	30	30	100.72%	90	90.268	100.297%
Mean % Recovery	101.36%			100.26%		

Limit of Detection and Limit of Quantification (LOD & LOQ): The LOQ & LOD were determined by using the formula based on the standard deviation of the response and the standard deviation of the response of the slope. The of LOD & LOQ data were presented in table 8.

Table No. 8: LOD & LOQ data for Flupentixol and Melitracen.

Drug	LOD(µg/ml)	LOQ(µg/ml)
Flupentixol	1.0 µg/ml	3.1 µg/ml
Melitracen	11.0 µg/ml	3.2 µg/ml

Robustness: The robustness of the present chromatographic technique was evaluated by changing the flow rate and mobile phase ratio and changing the phase. The chromatograms of Flupentixol & Melitracen were recorded and mean retention time and relative standard deviation was determined and are within the acceptable limits. The robustness data was shown the Table No. 9.

Table No. 9: Robustness data for Flupentixol and Melitracen.

Parameter used for sample analysis	Flupentixol		Melitracen	
	Retention Time	Tailing factor	Retention Time	Tailing factor
Actual Flow rate of 1.0 mL/min	2.179	1.2	3.610	1.1
Less Flow rate of 0.9 mL/min	2.210	0.9	4.498	0.9
More Flow rate of 1.1 mL/min	2.184	1.0	3.505	0.8
Less organic phase	2.200	0.9	4.504	0.9
More Organic phase	2.172	0.8	3.512	0.9

CONCLUSION

In this study a novel, sensitive, quick and easy HPLC method have been developed and validated for simultaneous estimation of Flupentixol and Melitracen. The characteristic benefits are low cost, short retention time then the validation parameters are verified and are found to be within the limits including system suitability parameters, Accuracy, Precision, Linearity, Limit of detection(LOD), Limit of quantification (LOQ). It can be concluded that the peaks of Flupentixol and Melitacen indicates that the developed method is specific for the estimation of Flupentixol and Melitracen. Therefore it is possible to use the current study in routine analysis of Flupentixol and Melitracen.

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