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Simultaneous estimation method development as analytical method for flupentixol dihydrochloride and melitracen hydrochloride from their combine pharmaceutical dosage forms by RP-HPLC

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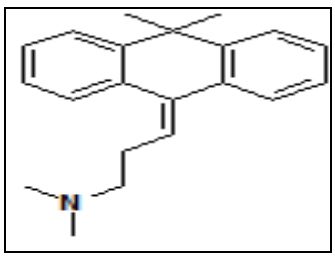
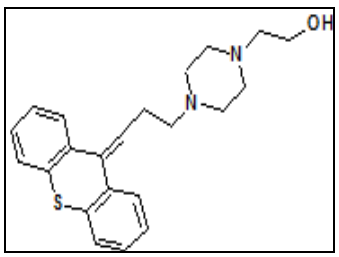
Abstract
A simple, precise and accurate method has been described for the estimation of Flupentixol and Melitracen in the formulation using SP Thermo Separation Products HPLC system and Thermo scientific BDS C_8 column (150×4.6 mm) in isocratic mode consisting of LC-10AT pumps and UV detector with mobile phase potassium dihydrogen phosphate buffer: methanol: ACN (3:6:1), at flow rate of 1.5 ml/min. The effluent is monitored at 230 nm. The retention times of Melitracen and Flupentixol were 3.16 and 5.31 min respectively. The linearity range for Melitracen and Flupentixol were found to be 80- 120 µg/ml. The correlation co-efficient were closed in 1 proving the good linearity between the concentration of drug and response. The % RSD values of 3 precision were less than 2, which indicate that the method has good reproducibility. The method validation parameters like theoretical plates, resolution, tailing factor, LOD and LOQ were found to be within the USP standards. As the chromatogram for Melitracen and Flupentixol in formulation is free from any other peaks except at the retention time corresponding to drugs, it was revealed that excipients used in the formulation were not interfering in the method. Thus the proposed method is suitable for routine analysis, formulations containing of Melitracen and Flupentixol.

Keywords: Flupentixol Melitracen, High performance liquid chromatography (HPLC).

1. Introduction
Melitracen hydrochloride (MEL) is a white to off white powder and amorphous in nature. Chemically, it is 3- (10, 10-dimethyl anthracen-9-ylidene)-N, N-dimethylpropan-1-amine) [1-2]. It is a Tricyclic Antidepressant and work by inhibiting uptake of the neurotransmitters norepinephrine and serotonin by neurons. Flupentixol dihydrochloride (FLU) is white or almost white powder. Chemically, it is (Z)-4-[3- [2 (Trifluoromethyl)-9H thioxanthen- 9-ylidene] propyl]-1-piperazin ethanol dihydrochloride [3]. It is very soluble in water, soluble in alcohol and practically insoluble in methylene chloride [4]. FLU acts by blocking the dopamine (a neurotransmitter) receptors in brain cells. Excess amount of dopamine receptors normally act to modify behavior and overstimulation resulting in psychotic illness [5]. FLU blocks these receptors to control psychotic illness. Thus, it is neuroleptic with anxiolytic and antidepressant properties. The combination of FLU and MEL is indicated in the treatment of trigeminal neuralgia [6]. Literature survey revealed that MEL hydrochloride can be estimated by spectrophotometry and by liquid chromatographic methods individually or in combination with other drugs, and FLU dihydrochloride can be estimated by liquid chromatographic methods individually or in combination with other drugs. [7-8] LC/MS/MS method has been reported for simultaneous estimation of both MEL and FLU human plasma. A literature survey revealed no method reported for the determination of MEL and FLU in combined dosage form. Present study involves the development of a stability liquid chromatographic method for the determination of MEL and FLU in combination dosage form [9].



MEL



FLU

2. Materials and Methods

Working standard of FLU and MEL were prepared in house. All solvents and chemicals used were of analytical grade, purchased from Merck specialist Pvt. Ltd. India. Marketed tablet formulation used in this study were procured from local market, Denxol Tablet from LA Pharma containing labeled amount of 10 mg of MEL hydrochloride and 0.5 mg of FLU dihydrochloride. The High Performance liquid chromatographic system (SP Thermo Separation Products) containing pump and UV Detector, Balance system (Shimadzu) which is daily calibrated, pH Meter (Systronic), BDS C_8 Column (150 x 4.6 mm, 5 µm, Thermo scientific) and Inertsil C_8 Column (150 x 4.6 mm, 5 µm, Inertsil G.L.Science), Filter (0.45 µ Nylon syring filter, Diameter 25 mm, Millipore), potassium dihydrogen phosphate and Disodium hydrogen phosphate (E. Merck, Mumbai, India) were used.

2.1 Preparation of Standard solution

A standard stock solution of FLU was prepared by transferring an accurately weighed quantity of about 14.0 mg FLU dihydrochloride working standard into 50 mL volumetric flask. Add about 25 mL of diluent to dissolve and dilute to volume with diluent; mix well. For MEL transfer an accurately weighed quantity of about 56 mg MEL hydrochloride working standard into 50 mL volumetric flask. Add about 30 mL of diluent to dissolve and dilute to volume with diluents, mix well and than transfer 2.0 ml of standard stock preparation for FLU and 10.0 ml standard stock preparation for MEL into 100 ml volumetric flask. Dilute to volume with diluent and mix.

2.2 Chromatographic Conditions

A Column (BDS C_8) equilibrated with mobile phase (30:60:10 v/v/v comprising of Buffer: Methanol: Acetonitrile) was used. Mobile phase flow rate was maintained at 1.5 mL/min, and eluent was monitored at 230 nm. A 20 μL of sample was injected, the total run time was 7.0 min and Operating pressure was 118 kgf. All the chromatographic separations were carried out at controlled room temperature.

2.3 Validation

The method was validated for Specificity, Stability of analyte in solution, Filter study, Linearity and Range, Precision (System precision, Method precision), Intermediate precision, Accuracy, Robustness.

2.4 Linearity and Range

Linear relationship between peak area and concentration of all three drugs were evaluated by making five replicate measurements for all the concentrations 4, 4.5, 5, 5.5, 6 (µg/mL) for FLU, and for MEL at the concentrations of 80, 90, 100, 110, 120 (µg/mL) as in **(Table 1 and 2)**. Calibration plots were constructed by plotting the area versus the concentration of the drug and treated using the method of ordinary regression analysis.

Table 1: Linearity of FLU

Linearity level	Volume of Linearity Stock solution to be taken (mL)	Dilute to volume with Diluent (mL)	Final concentration of FLU (µg/mL)
80%	0.8	50	4.00
90%	0.9	50	4.50
100%	1.0	50	5.00
110%	1.1	50	5.50
120%	1.2	50	6.00

Table 2: Linearity of MEL

Linearity level	Volume of Linearity Stock solution to be taken (mL)	Dilute to volume with Diluent (mL)	Final concentration of MEL (µg/mL)
80%	4.0	50	80.00
90%	4.5	50	90.00
100%	5.0	50	100.00
110%	5.5	50	110.00
120%	6.0	50	120.00

2.5 Precision

System Precision

Prepare Standard preparation as per test method described in 3.0. Inject five replicate injections from the same analytical vial into the liquid chromatographic system and record the chromatograms. Determine the difference in retention time with respect to mean retention time of replicate injections of Flupentixol and Melitracen peak. Record tailing factor and theoretical plates for the Flupentixol and Melitracen peak.

Method Precision

Prepare six sets of Assay preparation for assay as per test method described in 3.1. Separately inject duplicate injection of Assay preparations Sample preparations into liquid chromatographic system and record the chromatograms.

Calculate the Assay of in Flupentixol and Melitracen (in terms of % L.C.).

Determine the Mean and Relative standard deviation of Assay results.

Intermediate Precision

To demonstrate intermediate precision of test method, perform the precision study as mentioned in method precision by using a different column that of method precision study. Determine the Mean and Relative standard deviation of assay results. Also calculate the percentage difference against mean of method precision results and similarity factor between both the results.

Accuracy

To demonstrate the accuracy of test methods, prepare recovery samples as mentioned below in triplicate for 80%, 100% and 120% level of nominal Assay concentration for FLU and MEL tablet **(Table 3 and 4)**

Table 3: Accuracy for FLU

Accuracy Level	Weight of API to be taken (mg)	Weight of Placebo to be taken (mg)	Theoretical Amount of (mg/tablet)	Theoretical Amount of in final Assay preparation (µg/mL)
80%	8	2832	0.400	4.000
100%	10	2830	0.500	5.000
120%	12	2828	0.600	6.000

Table 4: Accuracy for MEL

Accuracy Level	Weight of API to be taken (mg)	Weight of Placebo to be taken (mg)	Theoretical Amount of (mg/tablet)	Theoretical Amount of in final Assay preparation (µg/mL)
80%	160	2680	8.000	80.00
100%	200	2640	10.000	100.00
120%	240	2600	12.000	120.00

Accuracy sample: Take eq. to 5 mg tablet powder into 100 ml volumetric flask. Add 50 mL of diluent and shake for 15 minute. Dilute to volume with diluent and mix well. Filter through 0.45 µ nylon filter. Discard first few mL of the filtrate. Dilute 5.0 mL of filtrate solution to 25 mL with diluent and mix well. Inject duplicate injection of all triplicate set into the liquid chromatographic system and record the chromatograms. Calculate the % recovery for each level. Calculate mean, relative standard deviation of the results at each level and overall results.

Robustness

Prepare Standard preparation as per test method described in 3.0. Inject five replicate injections from the same analytical vial into the liquid chromatographic system and record the chromatograms. Determine the mean retention time and relative standard deviation of replicate injections with respect to FLU and MEL peak. Record tailing factor and theoretical plates for the FLU and MEL peak **(Table 5)**.

Table 5: Robustness Parameter

Parameter
Variations in the flow rate (±0.2 mL/min) ➢ Flow rate: 1.6 mL/min ➢ Flow rate: 2.0 mL/min

In addition to the above-mentioned parameters perform assay on a single set of Assay preparation as per method precision by altering the method parameter as below:

Calculate the assay of FLU and MEL in FLU and MEL tablet (in terms of % L.C.) against Standard preparation.

Also calculate the percentage difference against mean of Method precision results.

3. Result and Discussion

The FLU and MEL showed a good correlation coefficient (r2=0.9999 for FLU, r^2=0.9998 for MEL) in the given concentration range 4-6 (µg/mL) for FLU, 80-120 (µg/mL) for MEL. Precision of developed method was evaluated by System precision, Method precision and intermediate precision, and was expressed as %RSD of peak area. System precision, Method precision and intermediate precision was showed %RSD less than 2%, Accuracy study was performed by "Standard addition" at three concentration level 80, 100 and 120%, by spiking with standard. The percentage recovery on all three levels was found in the range of 95% to 105% suggesting suitability of method to perform routine drug analysis. The marketed formulation using the developed method, showed two peaks at Retention time of 5.3 min for FLU, 3.1 min for MEL. All experiments in robustness testing were performed in a randomized fashion in order to minimize the effects of uncontrolled factors that may introduce bias to the response. The developed method was found to be simple, accurate and precise, for the simultaneous estimation of FLU and MEL in bulk and tablet formulations **(Table 6).**

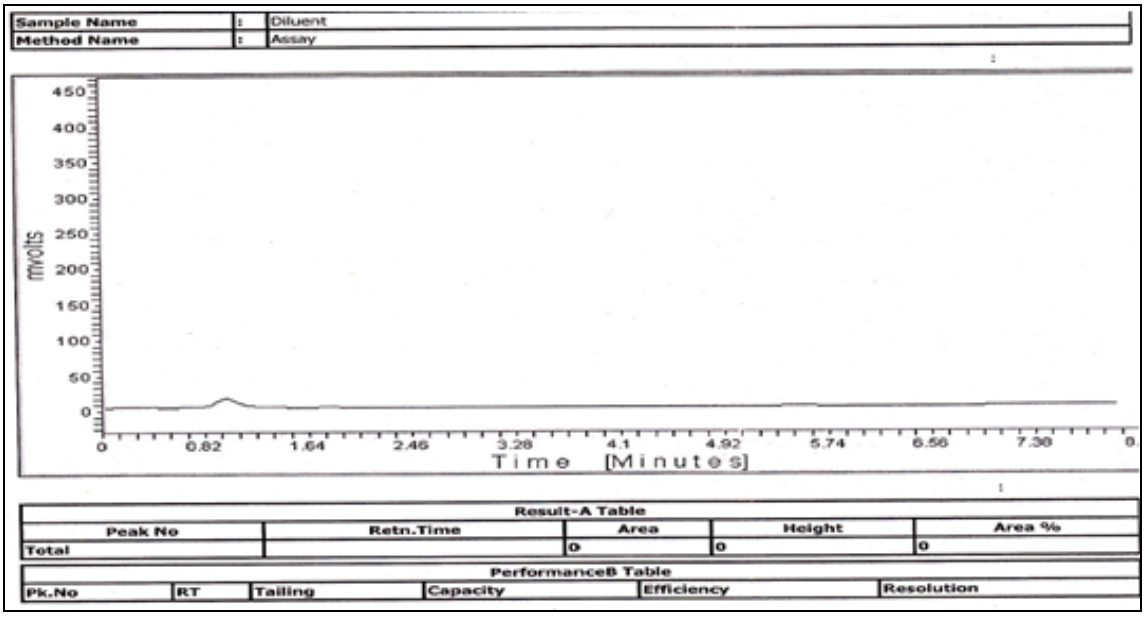
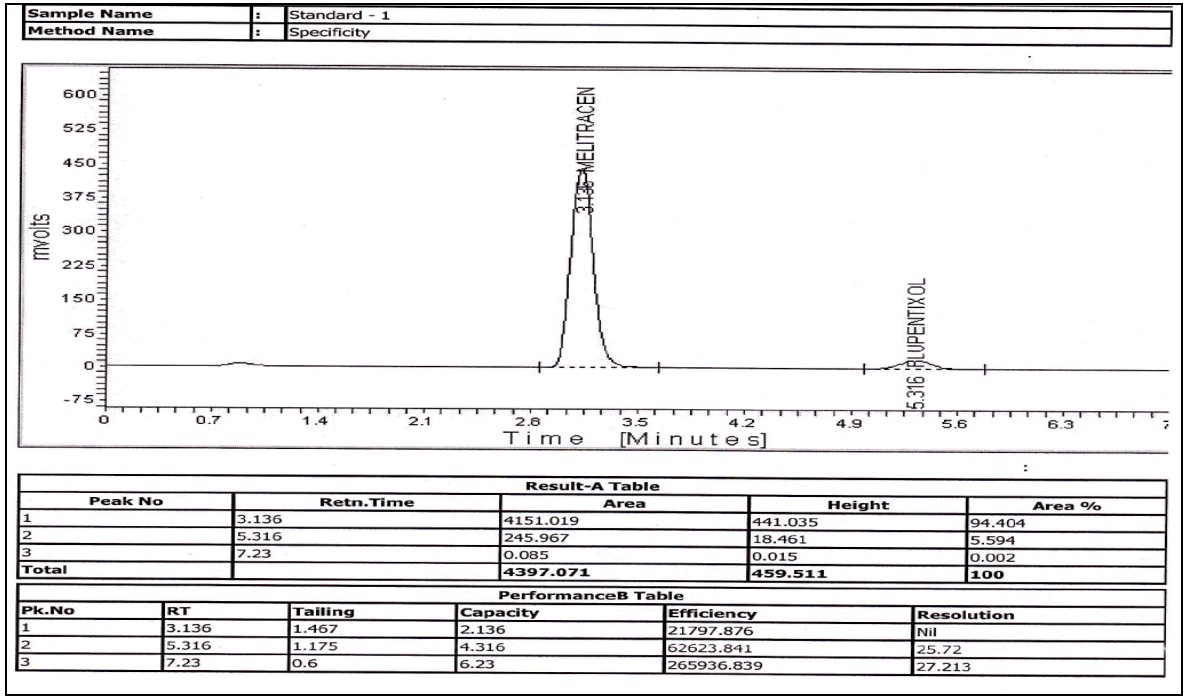


Fig 1: Chromatogram for System suitability

Table 6: Analytical parameters of HPLC method for simultaneous estimation of FLU and MEL

Sr. No.	Validation Parameter		Obtained Result				Acceptance Criteria
			MEL		FLU		
1	Specificity						
	System Suitability	RSD	0.14%		0.60%		NMT 2.00%
		Tailing Factor	1.47		1.17		NMT 2.00%
		Theoretical Plates	21798		6.2624		NLT 500
		Average Area Of Replicate Injection	4147.499		246.928		NA
2.	Stability of analytical solution	Std Prep % Difference At 24 hrs	0.22		1.58		NMT 3.00%
3.	Filter Study	Centrifuge	Assay (% LC)	% Difference	Assay (% LC)	% Difference	0.45 µ nylon are compatible but 0.45 µ Nylon syringe filter 25 mm (Millipore) is recommended for routine analysis.
			98.9	NA	99.1	NA	
		0.45 µ Nylon Syringe Filter 25 mm	Assay (% LC)	% Difference	Assay (% LC)	% Difference	
			99.4	0.51	100.5	1.41	
	Filter Compatibility	% Difference	0.99919		0.99949		NMT 2.00%
4.	Linearity	Co-relation Co-efficient	80.030 µg/mL To120.050 µg/mL.		4.000 µg/mL to 6.000 µg/mL.		NLT O.999
	Range	In µg/mL.	80.030-120.050		4.0-6.0		NA
5.	Precision		0.14%		0.60%		
	System Precision	% RSD	1.47		1.17		NMT 2.00%
		Tailing factor	21798		62624		NMT 2.00
		Theoretical Plates	4147.499		246.928		NLT 500
		Average area of Replicate Injection	0.87		1.01		NA
	Method Precision	% RSD	0.87		1.01		NMT 2.00%
6.	Intermediate Precision	% RSD	0.01%		0.39%		NMT 2.00%
	System Precision	% RSD	1.21		1.12		NMT 2.00%
		Tailing Factor	49511		61533		NMT 2.00
		Theoretical Plates	4153.800		247.948		NLT 500

		Average Area Of Replicate Injection	0.69		0.95		NA
	Intermediate Precision (For Assay)	% RSD	0.0		0.20		NMT 2.00%
	Comparison Between Method Precision & Intermediate Precision	% Difference	100		100.3		
7.	Accuracy	% Recovery	0.73		1.45		95.0% To 105.0%
		%RSD	3.136		5.316		NMT 5.00%
8.	Robustness		RT	TF	RT	TF	NA
	Without Variation (System Precision)		3.13	1.47	5.31	1.18	
	Variation in Flow rate (-0.2): 1.3 mL/min		3.818	2.031	6.67	1.083	
	Variation in Flow rate (+0.2): 1.7mL/min		3.10	0.93	5.83	0.63	



Result-A Table

Peak No	Retn.Time	Area	Height	Area %
1	3.136	4151.019	441.035	94.404
2	5.316	245.967	18.461	5.594
3	7.23	0.085	0.015	0.002
Total		**4397.071**	**459.511**	**100**

PerformanceB Table

Pk.No	RT	Tailing	Capacity	Efficiency	Resolution
1	3.136	1.467	2.136	21797.876	Nil
2	5.316	1.175	4.316	62623.841	25.72
3	7.23	0.6	6.23	265936.839	27.213

Fig 2: Chromatogram for FLU and MEL.

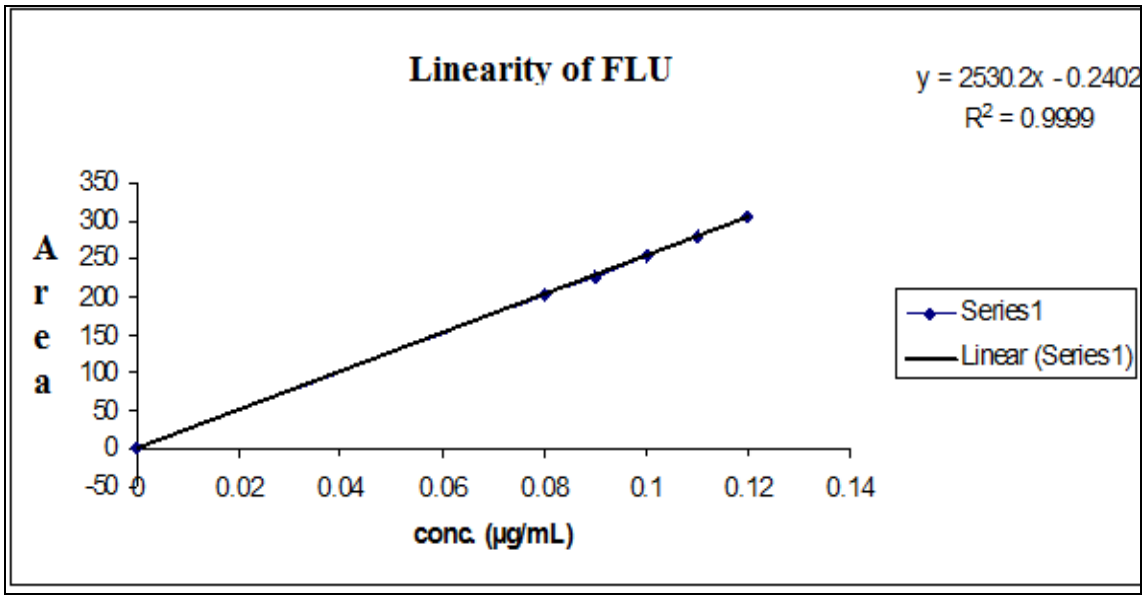


Fig 3: Linearity of FLU

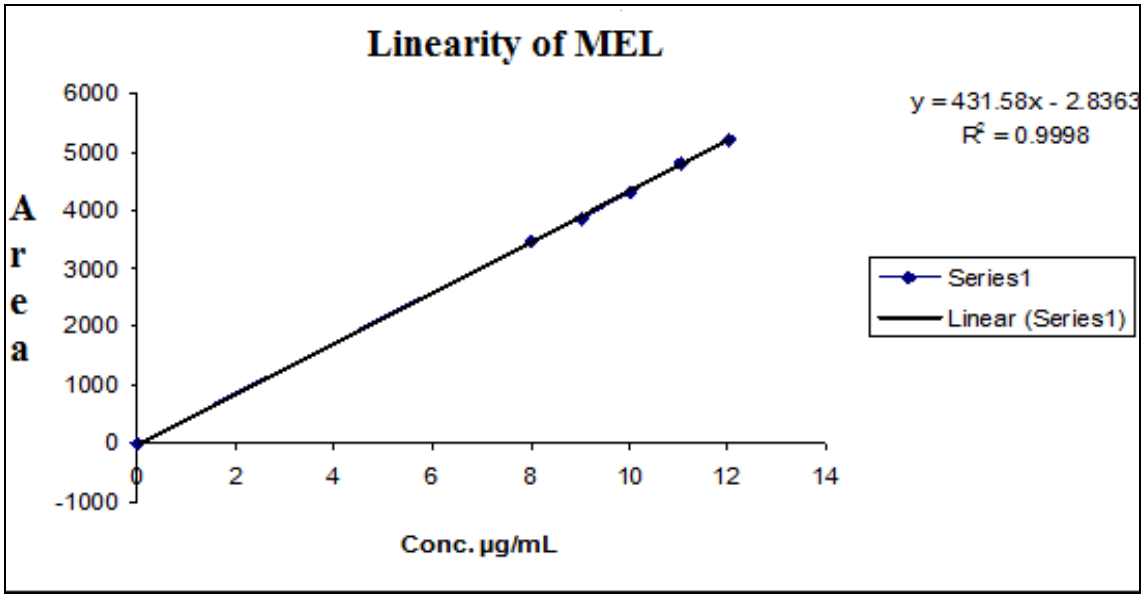


Fig 4: Linearity of MEL

4. Acknowledgements

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