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Development & Validation of Simultaneous Equation Spectrophotometric Method for Estimation of Flupentixol & Melitracen in Combined Dosage Form

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ABSTRACT

A simple, accurate, precise, reproducible and economical UV spectroscopic methods for simultaneous estimation of Flupentixol and Melitracen in tablet dosage form have been developed. simultaneous equation method employs formation and solving of mathematical simultaneous equation using 254 nm and 230 nm as the λmax of Melitracen and Flupentixol respectively in 0.1N HCl. These methods were validated as per ICH norms. Calibration curves were linear over the concentration ranges of 1-10 µg/ml for Flupentixol and 10-100 µg/ml for Melitracen with mean recovery of 100.71 ± 1.53 & 100.36 ± 0.66 for Flupentixol & Melitracen respectively by Simultaneous Equation method. The validation study is statistically significant as all the statistical parameters are within the acceptance range (% RSD < 2.0 and S.D. < 2.0) for both accuracy and precision. The methods are successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. The proposed methods are simple, rapid, economic and accurate for routine simultaneous estimation of Flupentixol and Melitracen.

Keywords: Flupentixol, Melitracen, Simultaneous Equation Method, UV Spectrophotometer.

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INTRODUCTION

Chemically, Flupentixol (FLU) is 2-[4-[3-[2-(trifluoromethyl) thioxanthene-9-ylidene] propyl] piperazin-1-yl] ethanol[1]. It is slight soluble in Water. Soluble in ethanol, free soluble in Chloroform & Ether[2]. Flupentixol (FLU) is an anxiolytic, antidepressive agent and a mood stabilizer. It inhibits the central monoamine receptors, particularly the dopamine D1 and D2 receptors. Therefore, it increases the amount of serotonin and noradrenaline that control mood, thinking, and improves mood FLU is not official in IP and USP but official in BP. Chemically, Melitracen (MEL) is 3-(10,10-dimethylanthracen- 9(10*H*) -ylidene) -*N*,*N*-dimethyl propan-1-amine. It is soluble in Water & Methanol[3]. It is a tricyclic antidepressant. Melitracen is an antidepressant agent. It increases the amount of noradrenaline that control mood and thinking, and improves mood[4]. MEL was usually coadministered with FLU in order to decrease the side effects. The combination has low drug dosage (10 mg MEL and 0.5 mg FLU per tablet) Literature survey revealed that HPLC, UV and HPTLC methods have been reported for the estimation of FLU and MEL individually and with other drugs in pharmaceutical dosage forms. FLU and MEL are formulated together in the form of a tablet[5,10]. There have been no published reports about the simultaneous estimation of FLU and MEL by UV spectrophotometer in standard drug and in pharmaceutical dosage forms. This present study reports for the simultaneous UV spectrophotometric estimation of FLU & MEL by Simultaneous Equation method in std drug and in pharmaceutical dosage forms. The proposed method was optimized and validated as per the International conference on harmonization (ICH) guidelines**.**

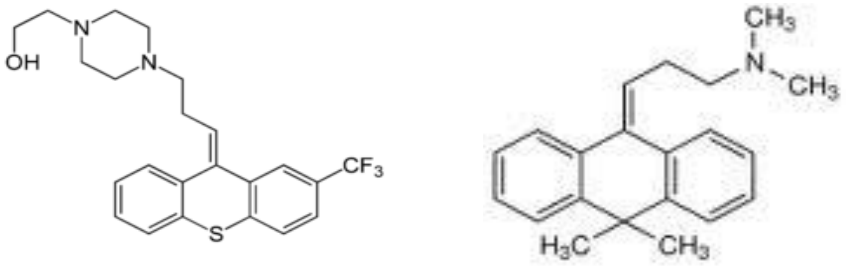

Figure 1 Structure of Flupentixol & Melitracen

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu -1800 double beam UV - Visible spectrophotometer attached to a computer software UV probe 3.22, with pair of 10 mm matched quartz cells. 'A' grade Glassware's were used of and were soaked overnight in a mixture of

chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven. All weighing were done on electronic Analytical balance (CP224S, Sartorius, Germany).

Reagents & Chemicals

Pharmaceutically pure sample of Flupentixol (FLU) and Melitracen (MEL) were obtained with 99.96% purity as a gift samples from reputed Pharmaceutical Company, Gujarat, India. The commercial fixed dose combination product containing 10 mg MEL and 0.5 mg FLU was procured from the local pharmacy. All solvents were of AR grade obtained from S.D. Fine Chemical Ltd., Mumbai, India. Calibrated glasswares were employed throughout the work.

Experimental Condition

According to the solubility characteristics, the common solvent for both the drugs were found to be methanol. Hence, the stock solution was prepared in 0.1N HCl and further dilutions were made up with distilled water.

Preparation of Standard Stock Solution

Accurately weighed FLU (10 mg) and MEL (100 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with 0.1N HCl to obtain a standard solutions having concentration FLU (100 µg/ml) and MEL (1000 µg/ml).

Preparation of Sample Solution

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 0.5 mg of FLU and 10 mg of MEL was transferred to a 100 ml volumetric flask. The content was mixed with 0.1N HCl (50 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was filtered through a whatman filter paper No. 41. The volume was adjusted up to the mark with 0.1N HCl. An aliquot of this solution (4.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with 0.1N HCl.

Determination of Analytical wavelengths

The standard solutions of FLU (6 µg/ml) and MEL (40 µg/ml) were scanned separately in the UV range of 200-400 nm. Data were recorded at an interval of 1 nm. Overlain spectra show 254 nm as the λ_{max} of MEL and 230 nm as the λ_{max} of FLU.

Method Validation[11,12]

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 1-10 µg/ml for FLU (at 230nm)

and 10-100 µg/ml MEL (at 254nm). Accurately measured standard working solutions of FLU (0.1, 0.2, 0.4, 0.6, 0.8 & 1.0ml) and MEL (0.1, 0.2, 0.4, 0.6, 0.8 & 1.0ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with 0.1N HCl, and absorbances were measured at 230 nm for FLU and 254 nm for FLU & MEL. The calibration curves were constructed by plotting absorbances Vs concentrations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of FLU and MEL by the standard addition method. Known amounts of standard solutions of FLU and MEL were added at 50, 100 and 150 % level to prequantified sample solutions of FLU and MEL (2 + 40 µg/ml). The amounts of FLU and MEL were estimated by using the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instrument was checked by measurement of absorbance of solution of (n=6) of FLU (4µg/ml) & MEL (60µg/ml) without changing the parameter.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on 6 different days over a period of 1 week for 3 different concentrations of standard solutions of FLU (2, 4 and 6 µg/ml) and MEL (20, 40 and 60 µg/ml). The results were reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

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

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

Analysis RIS and THP In Combined Dosage Forms

The response of the sample solution was measured at 230nm and at 254 nm for quantitation of both FLU & MEL. The amounts of the FLU and MEL present in the sample solution were calculated by fitting the responses into the regression equation for FLU and MEL in the proposed method.

RESULTS & DISCUSSION

Simultaneous Equation Method (ICH guideline)

10µg/ml solutions of FLU and MEL were prepared separately in 0.1N HCl and the solutions were scanned against blank in the entire UV range to determine the λmax values. Clear peaks were observed at 230nm for FLU and 254nm for MEL. Hence these wavelengths were chosen as the λmax values for each drug respectively (Fig 1).Standard solutions of FLU and MEL in the concentration range of 1-10µg/ml and 10-100µg/ml respectively were prepared in 0.1N HCl and the absorbance of these solutions was measured at 230nm and 254nm. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values, A (1%, 1cm).

$$Cx = \frac{ay_1A_2 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \quad \text{and} \quad Cy = \frac{ax_2A_1 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Where, CX and CY are the concentrations of FLU and MEL measured in gm/100ml in sample solutions. A1 and A2 are the absorbances of mixture at selected wavelengths 230nm and 254nm respectively. (Figure.1).

Validation of the Proposed Method

Linearity: Linear correlation was obtained between absorbances versus concentrations of FLU in range of 1 - 10 µg/ml and MEL in the ranges of 10 – 100 µg/ml. Regression parameters are mentioned in Table 1 and the calibration curves of FLU at 254 nm, FLU at 230 nm, MEL at 230 nm and MEL at 254 nm are shown in Figure 2,3, 4,&.5.

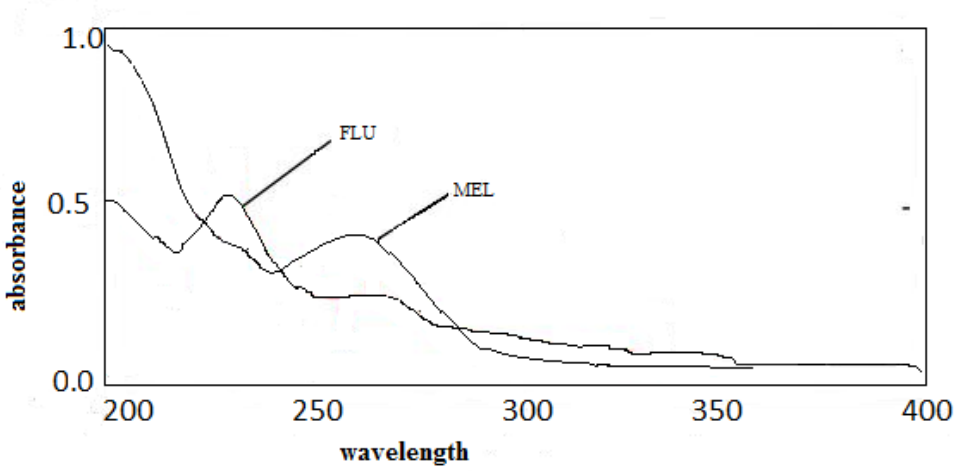

Figure 2: Overlain Absorption Spectra of Standard FLU and MEL in 0.1N HCl

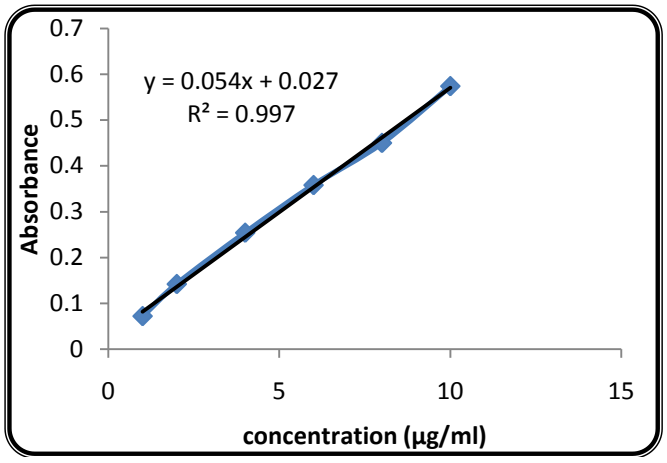

Figure 3: Calibration Curve of FLU at 254 nm

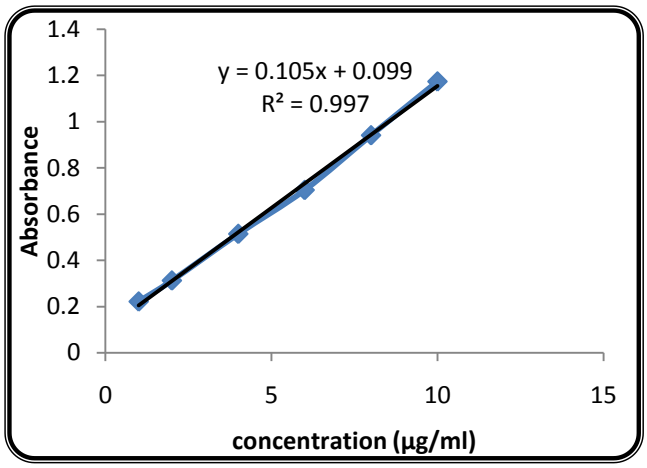

Figure 4: Calibration Curve of FLU at 230 nm

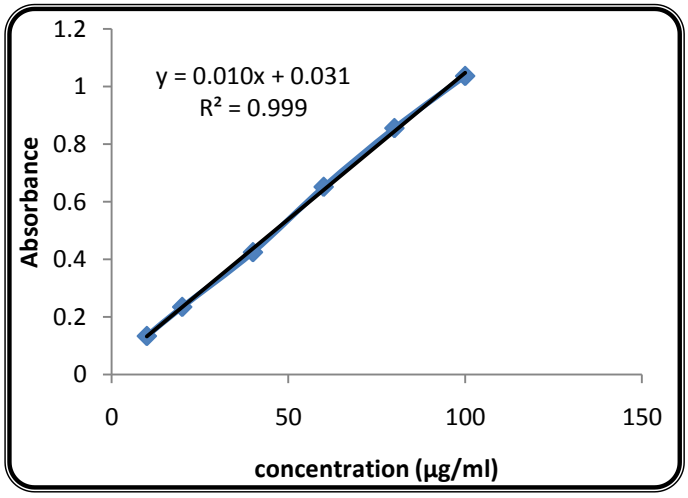

Figure 5: Calibration Curve of MEL at 230nm

Accuracy

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.71 ± 1.53 and 100.36 ± 0.66 % for FLU and MEL, respectively (Table 1). The low

value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

Method Precision (% Repeatability)

The RSD values for FLU and MEL were found to be 1.37 and 1.35%, respectively (Table- 1 & 3). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable.

Intermediate Precision (Reproducibility)

The low RSD values of interday (0.139-0.839, 0.458-1.313, 0.209-0.970 & 0.311 – 0.945) and intraday (0.234 – 0.836, 0.925-1.080, 0.626-0.888 & 0.581 –0.863) variations for FLU at 230 & 254nm, MEL at 230 & 254nm respectively, reveal that the proposed method is precise (Table 1).

LOD and LOQ

LOD values for FLU & MEL at 230nm & 254nm were found to be 0.058, 0.091, 0.975 and 0.958 µg/ml, respectively and LOQ values for FLU & MEL at 230nm & 254nm were found to be 0.291, 0.457, 4.874 & 4.789 µg/ml, respectively (Table 1). These data show that the proposed method is sensitive for the determination of FLU and MEL.

Table 1: Regression Analysis Data and Summary of Validation Parameter for the proposed Method

Parameters	Absorption correction UV Spectrophotometry method			
	FLU at 230 nm	**FLU at 254 nm**	**MEL at 230 nm**	**MEL at 254 nm**
Concentration range (µg/ml)	1 – 10	1 – 10	10-100	10– 100
Sandell's sensitivity (µg/cm^2 absorbance unit)	0.0074	0.0159	0.0893	0.0843
Slope	0.01055	0.0543	0.0102	0.0089
Intercept	0.0992	0.0279	0.0314	0.0991
Correlation coefficient (r^2)	0.9978	0.9978	0.9992	0.9978
LOD (µg/ml)	0.096	0.151	1.608	1.580
LOQ (µg/ml)	0.291	0.457	4.874	4.789
% Recovery (Accuracy, n = 6)	100.71 ± 1.53		100.36 ± 0.66	
Repeatability (RSD, n = 6), %				
Precision (RSD), %	0.511	1.504	0.579	0.435
Interday (n = 6)	0.139-0.839	0.458-1.313	0.209-0.970	0.311 – 0.945
Intraday (n = 6)	0.234 – 0.836	0.925-1.080	0.626-0.888	0.581 –0.863

Assay of the Pharmaceutical Formulation

The proposed validated method was successfully applied to determine FLU and MEL in their combined dosage form. The result obtained for FLU and MEL was comparable with the corresponding labeled amount (Table 4). The spectrum for FLU and MEL in sample was recorded and is shown in Figure. 6.

Table 2: Recovery Data for the proposed Method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± SD*
FLU	I	2	50 %	100.51 ± 1.02
	II	2	100 %	100.18 ± 1.58
	III	2	150 %	100.47 ± 1.15
MEL	I	40	50 %	99.70 ± 1.36
	II	40	100 %	99.65 ± 1.33
	III	40	150 %	99.91 ± 0.62

* Mean % Recovery ± SD of six observations.

Table 3: Precision Data for FLU and MEL

FLU (4 µg/ml) & MEL (60 µg/ml)	Absorbance at 230 nm (FLU)	Absorbance at 254 nm (FLU)	Absorbance at 230 nm (MEL)	Absorbance at 254 nm (MEL)
1	0.517	0.251	0.647	0.630
2	0.520	0.249	0.654	0.624
3	0.518	0.255	0.652	0.628
4	0.515	0.252	0.657	0.632
5	0.514	0.257	0.655	0.629
6	0.513	0.259	0.649	0.627
Mean	0.516	0.254	0.652	0.628
S.D.	0.0026	0.0038	0.0038	0.0027
% CV	0.511	1.504	0.579	0.435

* Mean % Recovery ± SD of six observations.

Table 2: Assay Results for the Combined Dosage Form (n = 5)

Sample No.	Label Claim		Amount Found		% Label Claim	
	FLU (mg/tab)	MEL (mg/tab)	FLU (mg/tab)	MEL (mg/tab)	FLU (mg/tab)	MEL (mg/tab)
1	0.5	10	0.50	9.99	100.07	99.92
2	0.5	10	0.49	9.95	98.69	99.47
3	0.5	10	0.50	10.07	100.30	100.66
4	0.5	10	0.51	10.06	102.15	100.59
5	0.5	10	0.51	10.11	102.32	101.15
Mean			0.50	10.04	100.71	100.36
S.D.			0.008	0.07	1.53	0.66

* Mean % Recovery ± SD of six observations.

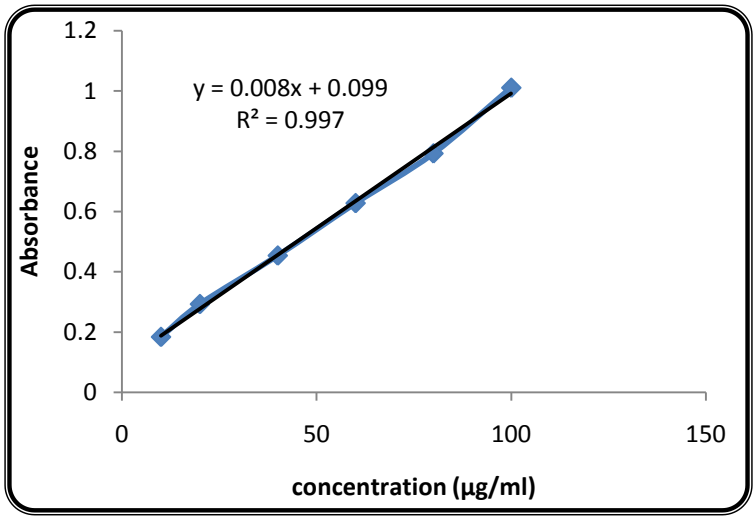

Figure 6: Calibration Curve of MEL at 254 nm

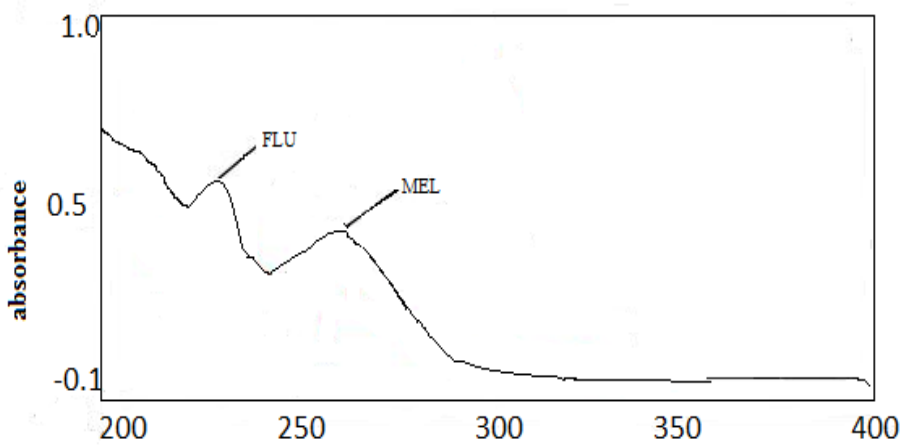

Figure 7: Overlain Absorption Spectra of FLU and MEL from Std Solution in 0.1N HCl

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 1 – 10 µg/ml with co-efficient of correlation, (r^2) = 0.9978 for FLU (at 230nm), (r^2) = 0.9978 for FLU (at 254nm) & 1-100 µg/ml with co-efficient of correlation, (r^2) = 0.9992 for MEL (at 230nm), (r^2) = 0.9978 for MEL (at 254nm). The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the FLU and MEL in combined dosage form without any interference of the excipients.

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