International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.4, pp 1493-1497, Oct-Dec 2013

Validated Spectrophotometric Method for Simultaneous Estimation of Flupenthixol and Melitracen in Combined Pharmaceutical dosage form

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Abstract: A simple, precise and cost effective spectrophotometric method have been developed for the estimation Flupenthixol and Melitracen in combination in tablet dosage form by UV spectroscopy, using multi-component mode of analysis. Methanol used as solvent. λmax of Flupenthixol and Melitracen was found to be 229.5 nm and 258.5 nm respectively. The method shows linearity in the Flupenthixol and Melitracen the Beer-Lamberts concentration range was found to be 10 – 60 µg/mL respectively. Results of analysis for bulk and tablet formulation the methods were validated statistically and by recovery studies. The proposed methods are found to be free from interference of excipients and are successfully applied to estimation of the amount of Flupenthixol and Melitracen in bulk and pharmaceutical dosage forms.
Key words: Flupenthixol and Melitracen, estimation, UV spectrometry.

Introduction

Flupenthixol is chemically (EZ)-2-[4-[3-[2-(trifluoromethyl)thioxanthen-9-ylidene]propyl] piperazin-1-yl]ethanol fig 1. Flupenthixol is Antipsychotic—psychotic conditions by blocking postsynaptic dopamine receptors in the brain. They also produce an alpha-adrenergic blocking effect and depress the release of most hypothalamic and hypophyseal hormones. Melitracen is chemically 3-(10,10-dimethylanthracen-9(10H)-ylidene)-N,N-dimethylpropan-1-Amine Hydrochloride fig 2. The main purpose of the present study was to establish a relatively simple, sensitive, validated and inexpensive spectrophotometric method for the determination of Flupenthixol and Melitracen in pure form and in pharmaceutical dosage form. Several studies for the estimation of the drug using various techniques have been carried out for Flupenthixol and Melitracen, some of them being: Spectrophotometric method for the determination of Flupenthixol dihydrochloride in bulk and pharmaceutical formulations[3]. Simultaneous determination of seven tricyclic antidepressant drugs in human plasma by direct-injection HPLC-APCI-MS-MS with an ion trap detector[4]. Validation of a sensitive LC/MS/MS method for simultaneous quantitation of flupentixol and melitracen in human plasma [5].

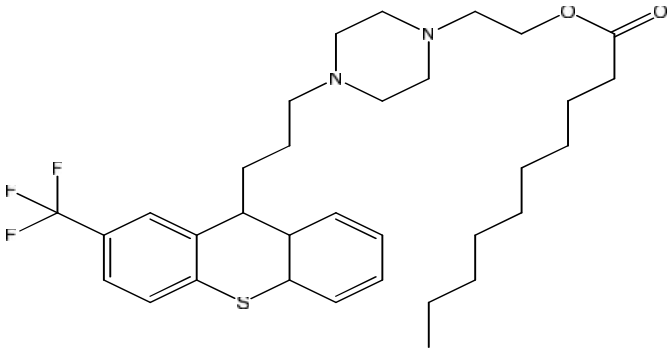

Fig 1.chemical structure of Flupenthixol[1]

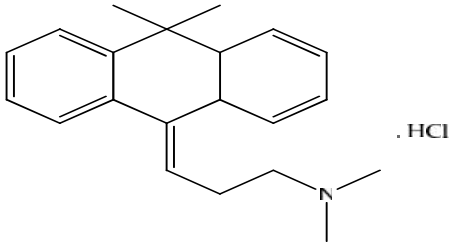

Fig 2.chemical structure of Melitracen Hydrochloride[2]

Experimental

Instrument:

A Jasco V630 double beam UV-Visible spectrophotometer equipped with 10mm matched quartz cells was used in the present study. All weights were taken on electronic balance (Denver, Germany).

Chemicals and Reagents:

Flupenthixol and Melitracen working standards were generous gifts from CapTab Biotec Ltd., Baddi, India. Combination drug products of Flupenthixol and Melitracen (Label claim: Flupenthixol equivalent to Flupenthixol 0.5 mg, and Melitracen 10 mg), **PSYFLU-M** (psycogen captab, India), purchased from local pharmacy. Methanol used was of analytical reagent grade.

Preparation of Standard Stock Solution:

Weigh 10mg each of Flupenthixol and Melitracen in 100 ml of methanol in separate volumetric flask, first dissolved in 25ml and then volume is make up to mark to obtain final concentration of 100 µg/ml of each component.

Preparation of Synthetic Mixture of Flupenthixol and Melitracen:

The standard solutions of Flupenthixol and Melitracen were prepared in the range of 10 µg/mL to 60 µg/mL in Methanol.All the mixed standard solutions were scanned over the range of 200- 400nm; using two sampling wavelengths 229.5 nm for Flupenthixol and 258.5 nm for Melitracen respectively. The spectral data from these scans were used to determine the concentration of these drugs in tablet formulation.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weight accurately and triturated to powder form and quantity of powder equivalent to 10 mg of the drug was transferred to a 100 mL volumetric flask and dissolved first in about 50 mL methanol and volume is make up to the mark. The solution is ultrasonicated for 30 minutes and then filtered through Whatman filter paper (No. 41). After suitable dilution, the spectrum of the final sample corresponding to 5 µg/ml of Flupenthixol and 10 µg/ml of Melitracen was recorded against methanol as blank.

Validation of the Method

The following validation parameters; linearity, range, accuracy, precision, LOD and LOQ were studied as per ICH guidelines[6]. The accuracy of the method was ascertained by carrying out recovery studies using Dual wave length method. The recovery study was performed to determine if there was any positive or negative interference from excipients present in the formulation. The precision of an analytical method is expressed as standard deviation and relative standard deviation of a series of measurements. It was ascertained by triplicate estimation of drug by the proposed method. LOD and LOQ were calculated by using the formula 3.3S.D/S and 10S.D/S where S.D is the standard deviation of Y-intercept and S is the slope of the calibration curve.

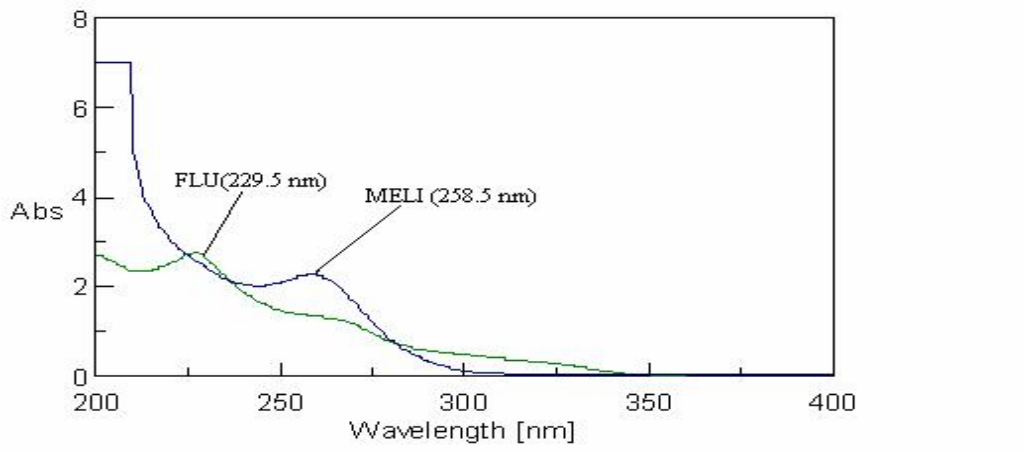

Fig 3.Overlay Spectrum of Flupenthixol and Melitracen

Results and Discussion

A UV-spectroscopic, multicomponent mode of analysis, method was developed for the simultaneous estimation of Flupenthixol and Melitracen in tablet dosage forms. Solvent used was methanol. The absorbance was recorded at 229.5 nm and 258.5 nm respectively. The UV-Visible absorption spectra of Flupenthixol and Melitracen overlay are shown in fig 3 respectively. The developed validated method is simple, rapid, precise and accurate. The newly developed method can be used for routine analysis as method for the simultaneous estimation of Flupenthixol and Melitracen in tablet dosage forms. The linearity of measurement was evaluated by analyzing different concentration of standard solution of Flupenthixol and Melitracen the Beer-Lamberts concentration range was found to be 10 – 60 µg/ml for both drugs respectively (Table 1). In accordance with the formula given by International Conference on Harmonization (ICH), LOD is defined as 3.3 s/b and LOQ is defined as 10 s/b, where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and b is the sensitivity, the slope of the calibration curve. LOD were calculated as 1.34 µg/mL for Flupenthixol, and 2.93 µg/mL for Melitracen and LOQ were calculated as 4.06 µg/ mL for Flupenthixol, and 8.8 µg/mL for Melitracen (Table 1). Analysis of commercial formulation is as shown in Table 2. In this study accuracy was determined by analyzing the recoveries of known amount of Flupenthixol and Melitracen added into preanalyzed sample of Flupenthixol and Melitracen tablet. To determine the precision of the methods, each method was studied for three levels. The percent recoveries (Accuracy) were found as 99.17 and 99.20 for Flupenthixol and Melitracen respectively and the developed methods had good precision (Table 3). Precision was calculated as repeatability, intra and inter day variations for Flupenthixol and Melitracen, RSD was found to be less than 1 (Table 4). The robustness of the proposed methods was tested by changing wavelength range and scanning speed. None of these variables significantly affect the absorbance of Flupenthixol and Melitracen that the proposed methods could be considered as robust. The ruggedness of the developed methods was tested by changing operators on different days for developed methods.

Table-1: Validation Parameters

Parameters	Flupenthixol	Melitracen
Beer's law limit (µg/ml)	10-60 µg/mL	10-60 µg/mL
λmax	229.5nm	258.5nm
Regression Equation (y = mx+c)	y = 0.032x + 0.013	y = 0.018x + 0.016
Slope (m)	0.032	0.018
Intercept ®	0.013	0.016
Correlation coefficient ®	0.998	0.997
LOD	1.34	2.93
LOQ	4.06	8.8

y= mx+c, where x is concentration in µg/mL, y is amplitude (Absorbance and ΔA) for Methods, LOD= limit of Detection, LOQ= limit of Quantitation

Table-2: Analysis of Commercial Formulation

		Flupenthixol	Melitracen
Sr. No		% Label claim	% Label claim
1		99.80	99.7
2		100.21	99.8
3		101.80	100.3
4		99.67	100.6
5		100.65	99.7
6		100.16	99.3
	MEAN	100.38	99.9
	S.D	0.77	0.46
	% RSD	0.772	0.469

n= 6, S.D. = standard deviation, R.S.D. = Relative standard deviation,

Table-3: Accuracy

	Flupenthixol			Melitracen		
	Level of % Recovery (λ_{max} = 229.5 nm)			Level of % Recovery (λ_{max} = 258.5 nm)		
	80	100	120	80	100	120
Amount present(mg)	1	1	1	20	20	20
Amount of standard added (mg)	0.8	1	1.2	16	20	24
Total amount recovered (mg)	1.79	1.98	2.18	35.97	39.27	43.8
% Recovery	99.44	99.0	99.09	99.91	98.17	99.54
% mean	99.17			99.20		
SD	0.232			0.916		
% RSD	0.234			0.924		

SD: Standard deviation, R.S.D: Relative standard derivation (n=3).

Table-4: Precision

Drug	Precision	S.D	%RSD
Flupenthixol	Intra-day	0.023	0.0231
	Inter-day	0.036	0.0363
Melitracen	Intra-day	0.0167	0.0168
	Inter-day	0.107	0.1078

Conclusion

Flupenthixol and Melitracen can be estimated by using the UV Spectrophotometric methods. All the procedures have the advantages of simplicity, precision, accuracy, and convenience. Moreover, the methods use simple reagents with minimum sample preparation, which allows them to be used for routine analysis and quality-control assays of Flupenthixol and Melitracen in bulk and tablets.

Acknowledgement

The authors thankful to the Mrs. Fatima Rafiq Zakaria Chairman Maulana Azad Educational Trust and Dr. M.H.G. Dehghan , Principal,Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431 001 (M.S.), India for providing the laboratory facility.

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