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## A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FLUPENTHIXOL DIHYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS

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### ABSTRACT

**Keywords:**

Flupenthixol,  
Absorbance,  
Validation,  
Detection Limit

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A simple and cost effective spectrophotometric method is described for the determination of flupenthixol dihydrochloride in pure form and in pharmaceutical formulations. The drug was highly soluble in methanol so it was selected as the solvent system for the drug. This ensured adequate drug solubility and maximum assay sensitivity. The linearity range for flupenthixol dihydrochloride at its wavelength of detection of 230 nm was obtained as 1-15 µg/ml. The linear regression equation obtained by least square regression method, were  $Y = 0.0639X + 0.0013$ , where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution. The absorbance was found to increase linearly with increasing concentration of flupenthixol dihydrochloride, which is corroborated by the calculated correlation coefficient value of 0.9997. The limit of detection and limit of quantification was found to be 0.324 µg/ml & 0.982 µg/ml. The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of flupenthixol dihydrochloride in pharmaceutical formulations without any interference from common excipients.

**INTRODUCTION:** Flupenthixol, (Z) - 4- [3- [2-(Trifluoro methyl) - 9H-thioxanthen- 9- ylidene] propyl] -1-piperazin ethanol dihydrochloride (Figure 1). It is very soluble in water, soluble in alcohol and practically insoluble in methylene chloride<sup>1</sup>. FPD is not official in IP and USP but official in BP. It is a typical antipsychotic drug of the thioxanthen class. Flupenthixol acts as an antagonist at various dopamine (D<sub>1</sub>-D<sub>5</sub>), serotonin (5-HT<sub>2</sub>), adrenaline (α<sub>1</sub>), and histamine (H<sub>1</sub>) receptors, without affecting the muscarinic acetylcholine receptors<sup>2</sup>. Its antipsychotic effects are likely caused by D<sub>2</sub> and/or 5-HT<sub>2A</sub> antagonism, whereas its antidepressant effects at lower doses may be mediated by preferential D<sub>2</sub>/D<sub>3</sub> autoreceptor blockade, resulting in increased postsynaptic activation.

Review of literature revealed only few analytical methods for the estimation of flupenthixol alone and in combination of other drugs in formulation and in human plasma<sup>3-8</sup>. However survey of the literature did not reveal any UV spectrophotometric method for the analysis of flupenthixol dihydrochloride. The aim of the study was to develop a simple, sensitive, accurate and precise method for determination of Flupenthixol.

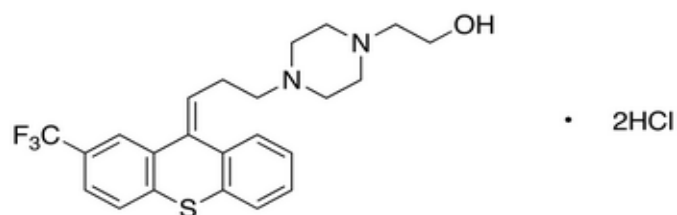


FIG. 1: STRUCTURE OF FLUPENTHIXOL DIHYDROCHLORIDE

## Experimental:

**Instrumentation:** Spectral and absorbance measurements were made on UV visible Spectrophotometer. Shimadzu AUX-220 balance was used for weighing the samples. Commercially available tablets of flupenthixol were procured from the local market and estimated.

### CHEMICALS AND REAGENTS

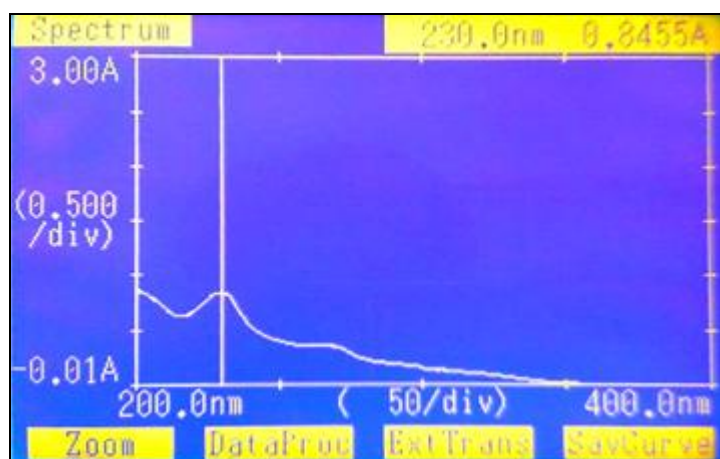
Solvents & Reagents	Manufacturers
Methanol(AR Grade)	Rankem
Water (Milli Q grade)	Millipore water filter
Flupenthixol	Centaur Pharmaceuticals,
Dihydrochloride (99.8%)	Mumbai

### APPARATUS/INSTRUMENTS

Name	Model/Code	Manufacturer/Supplier
UV-Visible Spectrophotometer	UV-1700	Shimadzu, Japan
Micropipettes	FAA-1000	Microlit, Lucknow
Micro-pipette tips	521020	Tarsons
Double Distillation Unit		Borosil-
Centrifuge Machine	DB-5586	Decibel

## Optimization:

**Scanning and determination of maximum wavelength ( $\lambda_{max}$ ):** In order to ascertain the wavelength of maximum absorption ( $\lambda_{max}$ ) of the drug, qualitative solution of the drug was prepared in methanol and scanned using UV spectrophotometer within the wavelength region of 200- 400 nm against methanol as blank. The resulting spectra are shown below (**Fig. 2**) and the absorption curve showed characteristic absorption maxima at 230 nm for Flupenthixol.



**FIG. 2: SPECTRUM OF FLUPENTHIXOL DIHYDROCHLORIDE (200-400nm)**

## Method:

**Preparation of Standard Stock Solutions:** Standard stock solution (primary) was prepared by dissolving 10 mg of flupenthixol in 10 ml of methanol to get concentration of 1mg/ml (1000 $\mu$ g/ml) and was stored at +4 $^{\circ}$ C during the study. Secondary stock solution was prepared daily by diluting 1ml of the primary stock solution to final volume of 10 ml using methanol to get concentration of 0.1mg/ml (100 $\mu$ g/ml).

**Preparation of calibration standard solutions:** The calibration standard solutions were prepared daily by diluting secondary stock solution with methanol to get calibration standard solutions of 1, 2, 4, 6, 8, 10 and 15  $\mu$ g/ml of flupenthixol to construct Beer's law plot for pure drug, the absorbance was measured at  $\lambda_{max}$  230 nm, against methanol as blank.

**Procedure for formulations:** Twenty tablets of flupenthixol were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to 10mg of flupenthixol was transferred into a 10 ml volumetric flask and 3mL of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with methanol to give final concentration (10 $\mu$ g/ml). Then the absorbance of these solutions was measured at 230nm. The amount of flupenthixol per tablet was calculated using the calibration curve.

## RESULTS AND DISCUSSION:

### Validation:

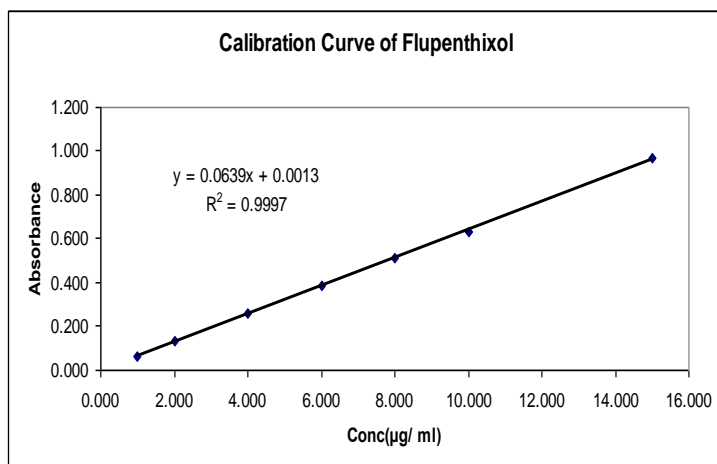
**Linearity range:** Under the experimental conditions, the linearity range was found to be 1-15  $\mu$ g/ml for flupenthixol at 230 nm. The statistical analysis (ICH guidelines 1996; USP 2000) of data obtained for the estimation of flupenthixol in pure solution indicated high level of accuracy for the proposed methods as evidenced by the low values of standard deviation and coefficient of variation (**Table 1**). The linear regression equation obtained was  $Y = 0.0369.X + 0.0013$ , where Y is the absorbance and X is the concentration (in  $\mu$ g/ml)

of pure drug solution (**Figure 3**). Linearity of the regression equation and negligible scatter of points for the two drugs by the proposed methods were demonstrated from the highly significant ( $p > 0.05$ ) correlation coefficient value. The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of flupenthixol solutions in methanol did not deviate from the origin as the above-obtained values fall within the confidence limits (**Table 2**).

**TABLE 1: LINEARITY TABLE OF FLUPENTHIXOL IN WORKING STANDARD**

Conc( $\mu\text{g/ml}$ )	Mean absorbance*	%CV
1	0.066 $\pm$ .001	1.515
2	0.132 $\pm$ .001	0.758
4	0.257 $\pm$ .002	0.778
6	0.384 $\pm$ .004	1.042
8	0.514 $\pm$ .001	0.195
10	0.629 $\pm$ .001	0.159
15	0.967 $\pm$ .005	0.517

\*Average of three determinations with standard deviations; CV – Coefficient of variation



**FIG. 3: LINEARITY CURVE OF FLUPENTHIXOL DIHYDROCHLORIDE**

**TABLE 2: REGRESSION ANALYSIS OF DATA FOR THE ESTIMATION FROM STANDARD SOLUTION BY THE PROPOSED METHOD**

Statistical Parameters	Flupenthixol dihydrochloride
Regression equation	0.0639X+.0013
Correlation coefficient	0.9997
Standard error of slope	5.24 $\times 10^{-4}$
Standard error of intercept on ordinate	4.18 $\times 10^{-3}$
Standard error of estimate	6.28 $\times 10^{-3}$
95% confidence interval of slope	6.26 $\times 10^{-2}$ , 6.53 $\times 10^{-2}$
95% confidence interval of intercept	-9.44 $\times 10^{-3}$ , 1.2 $\times 10^{-2}$
Slope without intercept	0.0641X

**Accuracy:** To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of flupenthixol within the linearity range were taken and added to the pre-analyzed formulation of concentration 5 $\mu\text{g/ml}$ . From that percentage recovery values were calculated. The results were shown in **Table 3**.

**TABLE 3: ACCURACY READINGS**

Sample ID	Concentrations in $\mu\text{g/ml}$		%Recovery	Statistical analysis
	Pure drug	Formulation		
S1:80%	4	5	101.9	Mean=100.67 SD=1.60
S2:80%	4	5	102.6	
S3:80%	4	5	98.4	
S4:80%	4	5	99.2	
S5:80%	4	5	100.7	
S6:80%	4	5	101.2	
S7:100%	5	5	99.1	Mean=99.82 SD=1.09
S8:100%	5	5	99.7	
S9:100%	5	5	98.2	
S10:100%	5	5	100.1	
S11:100%	5	5	100.5	
S12:100%	5	5	101.3	
S13:120%	6	5	97.4	Mean=98.92 SD=1.67
S14:120%	6	5	98.9	
S15:120%	6	5	96.8	
S16:120%	6	5	99.1	
S17:120%	6	5	99.9	
S18:120%	6	5	101.4	

% Recovery = (amount recovered/amount introduced) X 100

**Precision:** The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of Flupenthixol in the linear range (3, 9 and 12  $\mu\text{g/ml}$ ) were analyzed in 5 independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). The precision of the analysis was determined by calculating the relative standard deviation (RSD %). The results are shown in **Table 4 & 5**.

**TABLE 4: INTRA DAY PRECISION READINGS**

	Conc. ( $\mu\text{g/ml}$ )	Mean $\pm$ SD	%RSD
Day1	3	0.192 $\pm$ 0.003	1.62%
Day2		0.193 $\pm$ 0.003	1.67%
Day3		0.194 $\pm$ 0.004	1.91%
Day1	9	0.572 $\pm$ 0.007	1.14%
Day2		0.579 $\pm$ 0.004	0.77%
Day3		0.578 $\pm$ 0.003	0.54%
Day1	12	0.765 $\pm$ 0.003	0.38%
Day2		0.765 $\pm$ 0.003	0.44%
Day3		0.779 $\pm$ 0.004	0.57%

(n=5)

**TABLE 5: INTER DAY PRECISION READINGS**

Conc. ( $\mu\text{g/ml}$ )	Inter day	Precision
	Mean $\pm$ SD	%RSD
3	0.193 $\pm$ 0.003	1.669
9	0.576 $\pm$ 0.005	0.945
12	0.77 $\pm$ 0.007	0.961

(n=5)

**Detection Limit & Quantitation Limit:** 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. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by using the relation  $3.3 \sigma/S$  and  $10 \sigma/S$  respectively, where  $\sigma$  is the standard error of estimate and S is the slope. Calculated values of limit of detection (LOD) and quantitation (LOQ) for flupentixol were found to be 0.324 and 0.982  $\mu\text{g/ml}$  respectively.

**Analysis of Pharmaceutical Formulations:** The optimized spectrophotometric method was applied to the direct determination of Flupentixol in tablet using calibration curve method without any sample extraction or filtration. From the absorbance value, the drug content per tablet (on an average weight basis) was calculated. The results are shown in **Table 6**.

**TABLE 6: ANALYSIS OF PHARMACEUTICAL FORMULATION**

UV Spectrophotometric Method				
Formulation	Labelled Amount (mg)	Amount Recovered* (mg)	% Drug Recovered	%RSD
Fluanxol <sup>®</sup> Tablets	0.5mg	0.496 $\pm$ 0.004	99.150	0.800

\*Mean of six determinations

**CONCLUSION:** In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of Flupentixol in bulk drug & pharmaceutical formulations. This method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation such as extraction steps prior to the drug analysis. As this proposed method has the lowest LOD value and wider linear range is more sensitive method. From the results obtained, we concluded that the suggested method showed high sensitivity, accuracy and precision. Moreover, this method is simple and inexpensive and it can be employed for the routine quality control of Flupentixol in pharmaceutical formulations.

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