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METHOD DEVELOPMENT AND VALIDATION OF FLUPHENAZINE HCl TABLET BY RP-HPLC

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ABSTRACT

A simple, fast, accurate, sensitive, specific and accurate instrumental planar chromatographic method has been developed and validated for the quantification of Fluphenazine HCl tabletFor system development and validation the RP-HPLC Shimadzu LC Prominence-i 2030 model consisting of UV detector and auto sampler was used. Wavelength used for detection of Fluphenazine HCl was 254 nm. The calibration plots were linear with a correlation coefficient of 0.995. Intraday and interday precision, expressed as relative standard deviation (RSD), were found to be 0.25 and 0.21 respectively. Recovery of Fluphenazine HCl was between 99.5 and 100.5%, with RSD not more then 2. When the approach was used to measure Fluphenazine HCl in actual pharmaceutical samples, product content was within the approved Limits (95–110 percent of the labeled content of formulations). Because the method is sensitive, precise, accurate, and selective for the compound tested, it can be used for routine quality control testing of Fluphenazine HCl in solid dosage form.

KEYWORDS: Fluphenazine HCl, HPLC, Validation, Method Development.

INTRODUCTION

Fluphenazine hydrochloride (FPZ) is an antipsychotic drug that activates postsynaptic dopaminergic mesolimbic receptors D_1 and D_2 in the brain. It is used for the treatment of bipolar disorder and schizophrenia.[1] It is worth noting, however, that some side effects to Fluphenazine have also been documented, such as akathisia, extrapyramidal side effects, particularly in the case of overdoses or long-term therapy.[2] Fluphenazine is a piperazine side chain derivative of trifluoromethyl phenothiazine, and its hydrochloride salt is used for oral

administration. Chemically, fluphenazine is 2-(4-{3-[2-(trifluoromethyl)-10H-phenothiazine-10-yl] propyl} piperazine-1-yl) ethan-1-ol dihydrochloride (Fig. 1). It is a crystalline, odorless white powder.[3] Fluphenazine is official in both BP[4] and IP.[5]

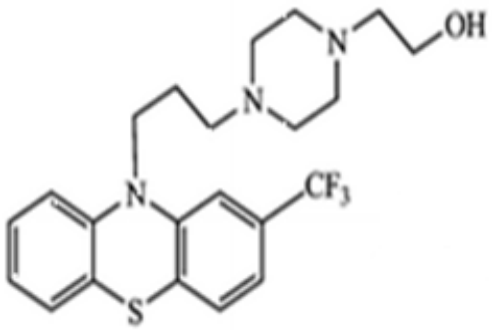

Fig. 1: Structure of fluphenazine hydrochloride.

The literature has noted numerous analytical techniques for analyzing Fluphenazine hydrochloride. These techniques include spectrophotometry,[6] spectrofluorimetry,[7] turbidmetry,[8] gas chromatography (GC),[9] HPTLC,[10,11] force degradation studies,[12] etc. A number of high performance liquid chromatography (HPLC) methods were also reported for this drug in combination with nortriptyline HCl and other fluphenazine decanoate combinations using ultraviolet (UV) detectors as well as mass (MS) detectors.[13–18] The literature study reveals that there is no reported RP-HPLC method for estimating the Fluphenazine HCl in tablet formulation. The purpose of the current study was to develop a sensitive, accurate and comparatively simple method for HPLC to quantify fluphenazine hydrochloride in pharmaceutical solid dosage form.

MATERIALS AND METHODS

Chemicals and regents

Pure fluphenazine hydrochloride drug material and drug product was obtained as a gift sample from Enaltec Pharma Research Pvt Ltd. Ambarnath, India (Mumbai). It was used without further purification. All chemicals obtained were of HPLC grade from Thermo Fisher Scientific, India Pvt. Ltd., Powai, (Mumbai).

Instrumentation and chromatographic conditions

For method development and validation RP-HPLC Shimadzu LC Prominence-i 2030 model consisting of UV detector and autosampler was used. Software used was Lab Solution. UV-

Visible spectrophotometer (UV-Visible spectrophotometer) was used to obtain maximum wavelength (π max) of compound of interest. The column of Cosmosil C18 (250 ~4.6 mm, 5µ) was used for analysis. Mobile phase A (buffer pH 2.5) and mobile phase B (Acetonitrile: Methanol 50:50) were used, Mobile phase A: Mobile phase B was used to run the experiment at a ratio of 30:70.The flow rate was 1.5 ml / min and 15 µl of injection volume was maintained. The temperature on the column was set at 30℃. Using a UV detector, fluphenazine HCl detected was at the wavelength at 254 nm. In addition, this study utilized an electronic balance, digital pH meter, sonicator, and UV-visible spectrophotometer.

Selection of wavelength

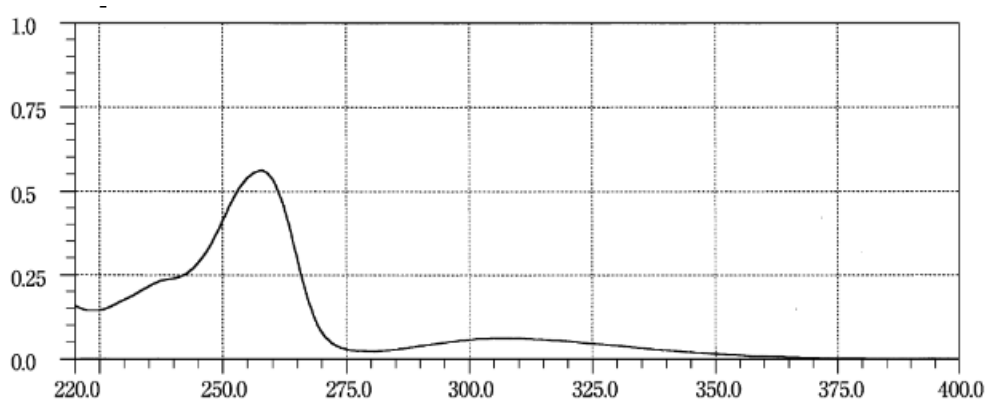

Fig. 2: Spectra of fluphenazine HCl (50ppm) in diluent.

Fluphenazine HCl standard solution, (100 ppm) was scanned at a range of 400-200 nm in 1.0 cm cell against diluent (Buffer pH 2.5, Acetonitrile and Methanol) (400:500:100 v / v / v) blank and spectrum recorded. The spectrum of standard solution Fluphenazine HCl is shown in Fig.2. The λ max of Fluphenazine HCl was found to be 254.0 nm, hence selected as detection wavelength for further experimentation.

Mobile phase preparation

The mobile phase was prepared by mixing of mobile phase A and mobile phase B in 30:70 ratio.

Preparation of mobile phase a

Approximately 1.40 g of potassium dihydrogen phosphate was weighed into 500 mL of water, 2 mL of triethylamine was added and pH adjusted to 2.50 ± 0.05 with 88% diluted ortho phosphoric acid.

Preparation of mobile phase b

The mobile phase B was prepared by mixing acetonitrile and methanol in the ratio 50:50 % v/v respectively.

Preparation of diluent

Buffer pH 2.5 and methanol mixture were prepared in the ratio 40:60 % v / v, respectively, mixed well mixed, and degassed.

Preparation of standard stock solution

Approximately 50 mg of the Fluphenazine hydrochloride standard was transferred into 50 mL volumetric flask with 35 mL diluent. It was sonicated for 5 minutes, and then volume was brought up to the mark. (Concentration of Fluphenazine hydrochloride in standard solution: 1000 ppm).

Preparation of standard solution

1.0 mL of standard stock solution was transferred was transferred into a 10 mL volumetric flask and diluted up to the mark with diluent. (Concentration of Fluphenazine hydrochloride in standard solution: 100 ppm).

Preparation of sample solution

Ten tablets were accurately weighed and transferred into a 50mL volumetric flask, 35ml of diluent was added, stired for 30 minutes with magnetic stirrer and diluted with diluent up to the mark. The sample solution had been centrifuged for 10 min with 5000 rpm. 1.0 mL of the above sample stock solution was transferred into 10 mL volumetric flask and diluted with diluent up to the mark. (Concentration of Fluphenazine hydrochloride in sample solution: 100 ppm).

Hplc method validation

The analytical method developed was validated for the parameters such as system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantification (LOQ) as per the ICH.

Specificity

It is the ability of method to reliably and precisely quantify the analyte of interest in the presence of matrix and other component likely to be present in the sample matrix and impurities, the result of degradation and other associated substances.

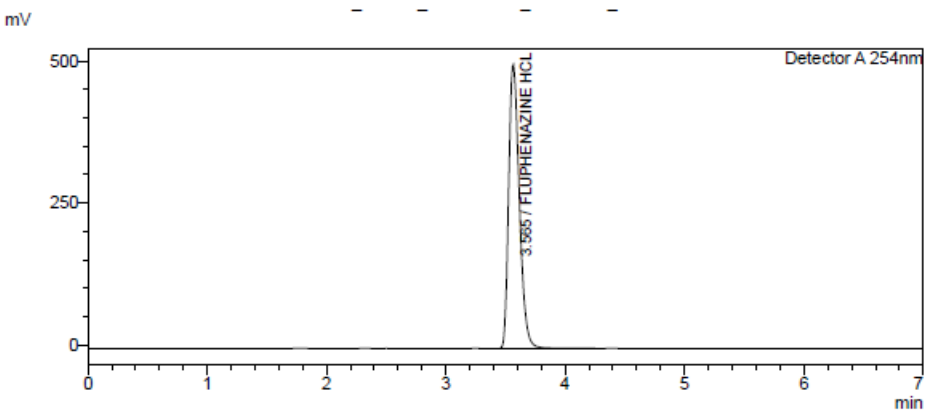

Fig. 3: Chromatogram of standard solution of fluphenazine HCl.

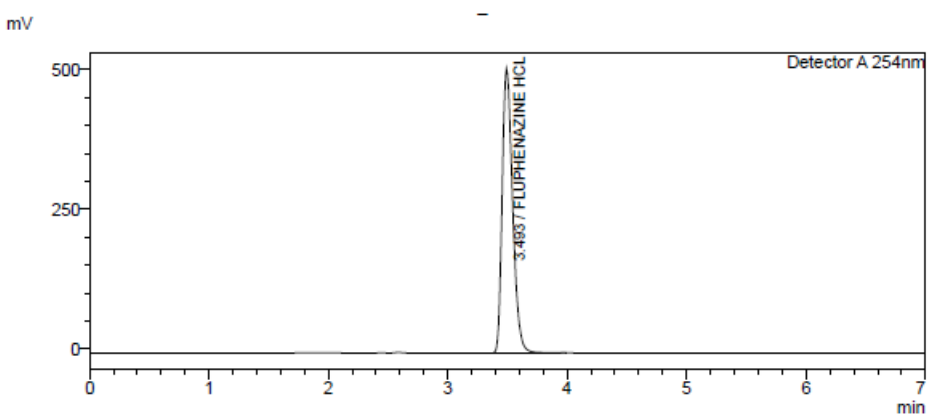

Fig. 4: Chromatogram of sample solution of fluphenazine HCl.

The accuracy of the process has been calculated by studying intervention in formulations of any ingredients encountered. The results of the tests were compared with those obtained for standard drugs. Those ingredients were shown not to interfere with the method developed. In addition, the well-shaped peaks also indicate the method specificity.

System suitability

System suitability test provides added assurance that the method gives accurate and reliable results on a specific time. System suitability testing is conducted whenever a method is used either before or during the analysis.

Table.1 Result of system suitability.

Parameters	Chromatographic conditions
Theoretical plates	6594
Tailing factor (asymmetry)	1.336
Retention time (tR)	3.565 min
Run time	7 min

The percentage of RSD for area obtained from six replicate injections of standard solution Fluphenazine HCl, tailing factor, retention time and number of theoretical plates per meter were collected, and the findings were found to be well within the acceptance criteria. Therefore parameters of suitability of the device met the chromatographic requirements.

Linearity

Analytical method linearity is the ability to obtain test results which are directly proportional to the sample concentration of an analyte.

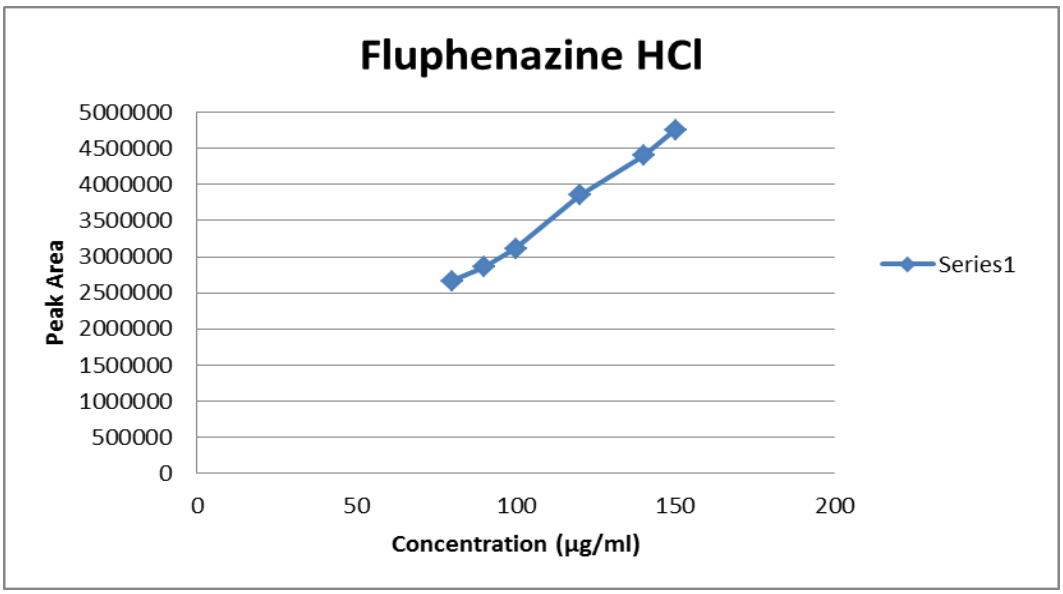

Fig. 5: Calibration curve of fluphenazine HCl obtained using HPLC.

Table 2: Linear regression data obtained from calibration curves of fluphenazine HCl.

Level in %	Concentration in ppm	Area
80	80	2664599
90	90	2856241
100	100	3112652
120	120	3848503
140	140	4397548
150	150	4760058
Co-relation coefficient (R^2)		0.9952
Equation of regression line		y = 30634x + 134783
Slope of regression line		30634
Intercept of regression line		134783

The test of linearity was conducted on a sample of Fluphenazine HCl. The results are shown in Table 2, obtained for the Fluphenazine HCl from the data plot by spiked level concentration *vs.* area. The correlation coefficient of Fluphenazine HCl was found to be

0.9952, and graphical plots are shown in Fig. 5. This indicates that the approach proposed is linear.

Precision

Precision provides a degree of agreement between the individual test results by applying the procedure or method to the homogeneous sample. Typically, it is expressed as variance, SD. In normal conditions, it is a measure of the degree of repeatability or reproducibility.

Table 3: Results of precision study (intra-day).

Injection no.	Sample	Peak area	%RSD
1	Fluphenazine HCl	3104014	0.21
2		3114445	
3		3118388	
4		3116843	
5		3116568	
6		3104024	

Table 4: Results of precision study (inter day).

Injection no.	Sample	Peak area	%RSD
1	Fluphenazine HCl	3112452	0.25
2		3106054	
3		3111752	
4		3121288	
5		3126512	
6		3110256	

Intra-day precision was examined under similar conditions by replicate applications and peak area measurements for six times on the same day. Inter day precision was obtained by repeating the assay six times on two different days from which %RSD values were obtained. The % relative standard deviation (% RSD) was determined which is not more than 2.0. The intra-day and inter-day precision results are shown in Table 3 and Table 4 respectively.

Accuracy

It is the estimate of how close the value of the experiment is to the true value. It is represented by the assay of known volume of analyte within the linearity range as % recovery.

Table 5: Result of % recovery study.

Level (%)	% Recovery	Average	Standard Deviation	% RSD
80	99.5	100	0.208	0.21
	99.9			
	99.6			
100	99.8	100	0.435	0.44
	100.5			
	99.7			
120	100.1	100	0.346	0.35
	99.5			
	99.5			

Accuracy of the proposed method was calculated on the basis of recovery studies performed using standard method of addition. Recovery analysis of Fluphenazine was found to be very similar to 100 percent reflecting the drug's accuracy and also indicates that excipients do not interfere with the estimation. The findings are listed in Table 5, according to the acceptance criteria, the mean recovery should be within the range of 98.0 % -102.0 % and found to be within the range; therefore the procedure is reliable.

Robustness

Table 6: Results of robustness study.

Parameter	Deviation n=3	Fluphenazine hcl Tablets	
		Theoretical plates	RT
Flow rate (mL/min)	1.3 ml	7545	4.010
	1.7 ml	6112	3.102
Column temperature	29^0C	6613	3.484
	31^0C	6556	3.474
Wavelength	252 nm	6482	3.468
	256 nm	6526	3.477

An analytical method's robustness is a measure of its ability to remain unaffected by minor but deliberate changes in device parameters, which provides an indicator of its efficacy during everyday use, measured when one or more operating parameters differed.

Assay of fluphenazine hcl tablet formulation

For analysis of fluphenazine HCl tablet formulation, 10 tablets were weighed and transferred to a 50 ml volumetric flask and 35 ml of diluent was added. The flask was resonated with frequent shaking for 30–45 min. Volume was adjusted up to mark with diluent and the sample solution was centrifuged at 5000 rpm for 10 min and filtered through Whatman filter paper. 1ml of above solution was taken and transferred into 10ml flask and volume was

adjusted up to the mark with diluent. The percentage assay for the marketed formulation was found to be 100.0%.

(Concentration of Fluphenazine hydrochloride in sample solution: 100 ppm).

RESULT AND DISCUSSION

The aim of this study was to establish a precise, sensitive, rapid and accurate HPLC method for the analysis of Fluphenazine HCl in bulk product and in forms of pharmaceutical dosage. Column Cosmosil C18 (250 ~4.6 mm, 5µ) was used for analysis in order to achieve phenomenal retention time and peak asymmetry, and mobile phase A (buffer pH 2.5) and phase B (Acetonitrile: Methanol 50:50) were used, mobile phase A: mobile phase B was used to run the experiment at a ratio of 30:70. The flow rate was 1.5 ml / min and 15 µl of injection volume was preserved. Column temperature was set at 30 ° C. The detected fluphenazine HCl was at a wavelength of 254 nm using a UV detector. Furthermore, an electronic balance, digital pH meter, sonicator, and UV-visible spectrophotometer were used for this analysis. Fluphenazine HCl had retention time of 3.565 min. The regression correlation coefficient (0.9952) was found to be nearly equal to 1 which means that the process was linear to concentration versus peak area responsesOn slight variation in the mobile phase ratio of up to ±5 percent, the shift in peak asymmetry, plate count and retention time is within the limits indicating that the system is robust and also suggesting lack of influence for the proposed method on the test results by operational variable. This shows that the method is having phenomenal system suitability parameters under given conditions. The precision studies were performed and the % RSD of the determinations was found to be 0.21 for intra-day precision and 0.25 for inter-day precision which are within the limits. The method's accuracy was found to be high at 80 percent, 100 percent and 120 percent were all within the limits with the overall percent RSD for recovery. Which means that the approach proposed was found to be correct. A collection of parameters was successfully established for the detection, quantification, and standardization of Fluphenazine HCl tablet formulation. The peaks of fluphenazine HCl were resolved both clearly and distinctly and were quantified from the pharmaceutical formulation. The method has been validated in linearity, specificity, precision, accuracy, detection robustness limit, and quantification limit according to the guidelines given in ICH. This novel method developed by RP-HPLC can be used to quantify hcl of Fluphenazine in solid dosage form.

CONCLUSION

The RP-PLC method developed for quantifying the Fluphenazine HCl tablet has several advantages, such as less retention time, good peak symmetry and phenomenal linearity, highly sensitive, fast, accurate and robust. The mobile phase can be prepared quickly, and diluent is inexpensive and readily available, and sample preparation with sophisticated techniques or instruments is not necessary. The proposed method can be used for routine analysis of Fluphenazine HCl in product bulk preparations and in routine application forms of pharmaceutical dose in quality control laboratories without interference of excipients.

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