Biotechnological Communication

Method Development and Validation for Fluphenazine in Bulk and Pharmaceutical dosage form using High Performance Liquid Chromatography –Ultraviolet Detection

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ABSTRACT

The present work was carried out to develop and validate a High-Performance Liquid Chromatography (HPLC) technique for the quantification of Fluphenazine HCl injections. The Waters 2965 RPHPLC equipment with PDA detector and autosampler was utilized for both the development and validation of the technique. A Phenomenex column (250 mm x 4. mm, 5 μm) was employed for the detection of Fluphenazine HCl, with a wavelength of 256 nm. The calibration graph exhibited linearity, with a correlation coefficient of 0. 99. The intraday accuracy, expressed as the relative standard deviation (RSD), was determined to be 0. 2 percent. The recovery of Fluphenazine HCl ranged from 99. to 100. percent, with an RSD not exceeding 2 percent. When actual pharmaceutical samples were analyzed using this method, the content of Fluphenazine HCl fell within the permissible limits. Considering its sensitivity, precision, accuracy, and selectivity for the test chemical, this method can be effectively employed for routine quality control testing of Fluphenazine HCl in liquid dosage form. The suggested approach can be employed in quality control laboratories for routine analysis of Fluphenazine HCl in product bulk drugs and in routine application forms of pharmaceutical dose without excipient interference.

KEY WORDS: FLUPHENAZINE HCL, HPLC, METHOD DEVELOPMENT, METHOD VALIDATION, PDA DETECTOR.

INTRODUCTION

Analytical technique is that the process of choosing an accurate assay procedure to work out on a pharmaceutical product. It's the method of proving that an analytical method is suitable to be used in laboratory to live the chemical analysis of latter samples. Analytical techniques must be established utilising the procedures and acceptance criteria outlined in the ICH guidelines and utilised in GMP and GLP environments (Charles et al., 1985). Methodology development encompasses numerous stages and can take months to complete, depending on the complexity and pretensions of the methodology. The literature has noted multidinous chemical analysis methods for assaying Fluphenazine hydrochloride. These approaches include spectrophotometry, (Reusch 2013), spectrofluorimetry, (El-Houssini and Zawilla 2014) turbidmetry, gas chromatography (GC), HPTLC, force degradation studies (Walash and Wahba 2014   Sistik et al., 2017).

A Numerous high performance liquid chromatography (HPLC) methods using mass (MS) (Belal et al.,2008),(Davis and Fenimore 1983), (Javaid et al.,1981) ,(Sistik et al., 2017), (Sa'sa and Jalal 1988) , (Thummar et al., 2015) and ultraviolet (UV) sensors were also described for this pharmaceutical chemical in conjunction w th Nortriptyline HCl and other Fluphenazine combinations (El-Houssini, and Zawilla 2014),(Mennickent et al.,2010). The literature study reveals that there is no reported simple HPLC-UV (Ashour and Kattan 2012) quantitative analysis methodology for estimating the Fluphenazine HCl in Injections. The goal of the current work was to create a robust, easy, and accurate method for HPLC to measure the amount of Fluphenazine hydrochloride in injection formulation.

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Drug profile: This is the salt of the phenothiazine Fluphenazine hydrochloride, which has antipsychotic and perhaps antineoplastic effects. The antipsychotic drug Fluphenazine 2[4[3[2(trifluoromethyl)phenothiazine10yl] propyl]piperazine1yl]ethanol; Fluphenazinedihydrochloride reduces hallucinations and delusions associated with schizophrenia. It does this by blocking postsynaptic dopamine D2 receptors in the visceral brain, cortical system, and basal ganglia. Additionally, by blocking 5-hydroxytryptamine type 1B (5HT type 1B) serotonin receptors, this drug can stop the growth of lymphocytes and myeloma cells.

Figure1: Structure of Fluphenazine HCl
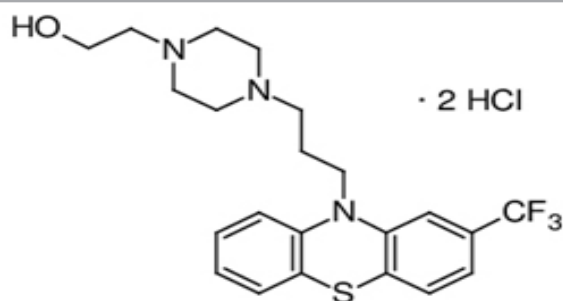
MATERIAL AND METHODS

Diluent: The diluent is often chosen based on the solubility of the medicines. Fluphenazine hydrochloride's solubility in distilled water, methanol, and acetonitrile were tested to determine the best diluent. water and methanol were selected as the diluent (40:60).

Preparation of standard stock solutions: 10 mg of Fluphenazine were carefully weighed into 10 ml volumetric flasks, then 3/4 of the diluent was added, and the mixture was then sonicated for 10 minutes. The volume was made using diluents, and it was labelled as normal stock solution (1000 g/ml Fluphenazine).

Preparation of working standard solution: From each stock solution, 1 ml of Fluphenazine was pipetted into a 10-ml volumetric flask and the remaining volume was filled with the diluent. Fluphenazine, 100 g/ml.

Preparation of standard stock solutions: To 10 ml volumetric flasks, 10 mg of Fluphenazine was accurately weighed, added 3/4 of the diluent, and then sonicated for 10 minutes. Diluents were used to create the volume, which was then marked as standard stock solution (1000 g/ml Fluphenazine).

Preparation of working sample solutions: A 10 ml volumetric flask was constructed with diluent after 1 ml of filtered stock sample solution was placed there. (Fluphenazine, 100 g/ml)

Selection of detection wavelength: Fluphenazine standard solution 10ppm was scanned in the UV range (200400)

and from the overlay spectrum, 256nm was selected as λ (lambda) max.

Selection of detection wavelength: Fluphenazine standard solution 10ppm was scanned in the UV range (200400) and from the overlay spectrum, 256nm was selected as λ (lambda) max.
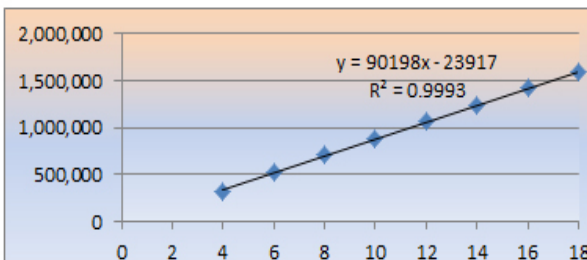
Figure 1.1 Calibration plot of Fluphenazine

$$y = 90198x - 23917$$
$$R^2 = 0.9993$$

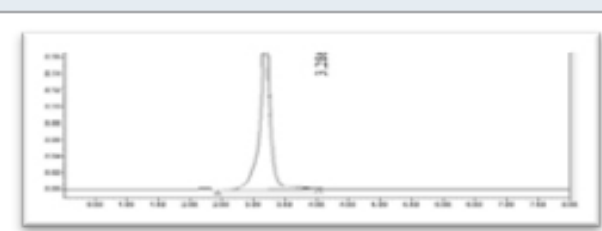**Figure 1.2: Linearity 4% Chromatogram**

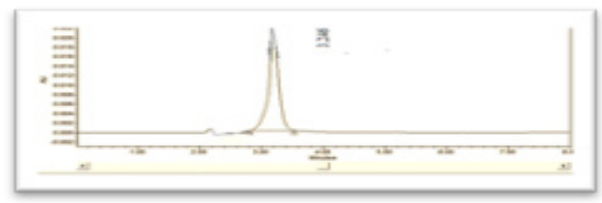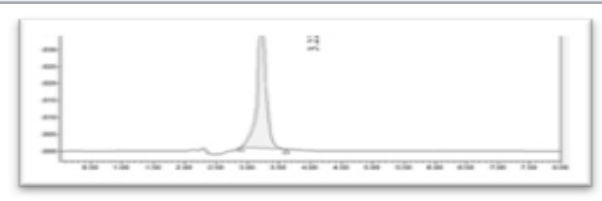
Figure 1.3: Linearity 6% Chromatogram

Figure 1.4: Linearity 8% Chromatogram

Method Validation Parameters: System suitability: The preparation of Fluphenazine standard solutions (100 ppm) and injection of the solutions six times were used to establish the system suitability parameters, which included peak tailing, resolution, and USP plate numbers. The RSD percentage for data from six standard injections shouldn't be higher than 2%

Specificity: Check the optimal approach for interferences, to be specific. The retention durations of these medications

using this method shouldn't have any conflicting peaks in the blank or placebo. As a result, this approach was deemed specific.
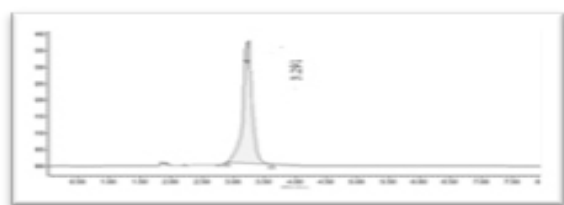
Figure1.5: Linearity 10% Chromatogram
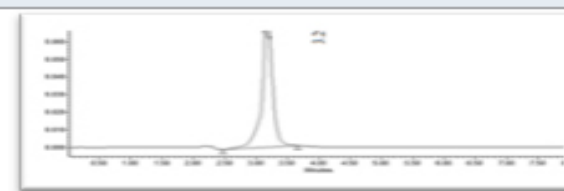

Figure 1.6: Linearity 12% Chromatogram

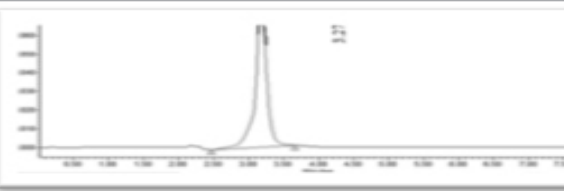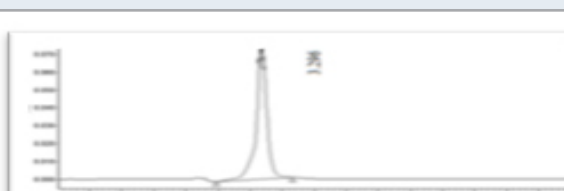**Figure 1.7: Linearity 14% Chromatogram**

Figure 1.8: Linearity 16% Chromatogram

Accuracy: The technique's accuracy: From a single homogenous mixture, six injections of Fluphenazine sample solutions at 10 ppm were created. Calculated was the peak areas' percent RSD for the injections. The percent RSD of the area of six standard injection results cannot be higher than 2%.

Linearity: Standard stock solutions should be made by precisely weighing 10 mg of Fluphenazine into 10-ml volumetric flasks, adding 3/4° of diluent, and sonicating for 10 minutes. Diluting agents were used to create the volume, which was labelled as Standard stock solution (1000 g/ml of Fluphenazine).

Preparation of Standard solution:1 ml of Fluphenazine was pipetted into a separate 10 ml volumetric flask, where the volume was then filled with diluent. (Fluphenazine, 100

g/ml)4% percent Standard solution: In a 10mL volumetric flask, 0.4ml of the working standard solutions was pipetted out and diluted to 10mL. Similar standard solutions of 6 % ,8%, 10 %,12 %,14 %, 16 %,and 18 % were created using 4g/ml of Fluphenazine.

Accuracy: Preparation of standard solution:10mg of Fluphenazine were accurately weighed and then put to individual 10ml volumetric flasks. 3/4 of the diluents were then added, and the flasks were shaken briefly. Standard stock solution (1000 g/ml of Fluphenazine) was prepared in flasks with diluents.

Figure 1.9:Linearity 18% Chromatogram
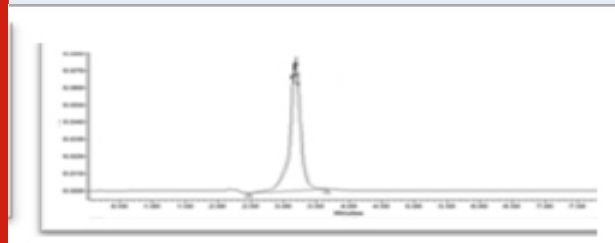
Preparation of 50% Spiked Solution:1.0 ml of each standard stock solution was pipetted out and diluted to the correct concentration before being added to a 10 ml volumetric flask along with 0.5 ml of the sample stock solution.

Preparation of 100% Spiked Solution: A 10 ml volumetric flask holding 1 mL of standard stock solution and 1 mL of sample stock solution was pipetted together to dilute the volume to the required concentration.

Preparation of 150% Spiked Solution: The solution was made in a 10 ml volumetric flask with 1.5 ml of sample stock solution.

Acceptance Criteria: Each level's recovery percentage should range from 98.0 to 102%.

LOD and LOQ sample Preparation: Preparation: Diluents were added to 1 ml of standard stock solution before being pipetted into 10 ml volumetric flasks. Fluphenazine 0.1 mg was transferred to 10 ml volumetric flasks from the aforementioned solution, and the volume was then added using the same diluents.

Assay methodology: By injecting a sample with an equivalent weight into an HPLC machine, the commercial formulation was tested. Furthermore, % To diluted the volume to the appropriate concentration, a 10 ml volumetric flask containing 1 mL of standard stock solution and 1 mL of sample stock solution was pipetted together. Formulas were used to determine purity.

Use the following formula to determine the amount of Fluphenazine that is pure in the injection:

$$\% \text{ of content} = \frac{\text{Peak Area of Sample} \times \text{Concentration of Standard}}{\text{Peak Area of Standard} \times \text{Concentration of Sample}} \times 100$$

RESULTS AND DISCUSSION

Specificity: It is the method to accurately and dependably quantify the target analyte in the presence of contaminants, degradation products, and other chemicals that are expected to be present in the sample matrix and other components. When a blank was injected, no peaks at the Rt of Fluphenazine were seen, demonstrating the method's specificity.

System suitability parameters: System suitability test provides added assurance that the method gives accurate and reliable results on a specific time. System suitability tests are performed each time a method is used before or during analysis. The system suitability parameters evaluated in this study include theoretical plates, tail factor, retention factor, run time and the values are shown in Table 1.1.

Table 1.1: System suitability parameters of Fluphenazine

Parameters	Chromatographic Conditions
Theoretical plates	7280
Tailing factor (Asymmetry)	1.345
Retention Time (Rt)	3.268
Run time	10min

Table 1.2: Method precision of Fluphenazine

Parameters	10 ppm Peak area (HPLC)
1	8,85,532
2	8,87,623
3	8,85,653
4	8,87,324
5	8,83,236
6	8,86,563
STDEV	1592.634
%RSD	0.18 %

Precision:Method precision: Five 10 ppm working sample solutions are injected the day after sample preparation. The standard deviation was calculated and the %RSD was found to be 0.18, which is not more than 2.0. The precision values of the method are presented in the following table 1.2

Linearity:Linearity: Inject 6 standard solutions containing Fluphenazine at concentrations ranging from 4 ppm to 18 ppm to show the linearity of the analytical procedure. Plot a graph showing peak area versus concentration. 90198 was the obtained slope. The correlation coefficient was found to be 0.999 and the intercept was 23917. The various concentrations and associated peak areas examined during the investigation are listed in Table 1.3 below.

Accuracy: Three concentrations of 50%, 100%, 150% are injected tripled and the average percent recovery was calculated as 100.04. The results are listed in Table 1.4, according to the acceptance criteria, the average recovery should be within the range of 98.0% to 102.0% and be within the range; therefore, the procedure is reliable.

Robustness: Methodological tweaks like Minus Flow, Plus Flow, Minus Mobile Phase, and Plus Mobile Phase are little but intentional. Calculated is the above conditions'% RSD. The parameters assessed during the robustness studies are shown in the table below. The findings show that the procedure is reproducible even when the parameters are intentionally changed slightly, there is no deviation.

Table 1.3 Linearity data of Fluphenazine

Concentration (ppm)	Peak Area\
0	0
4	3,12,958
6	5,30,937
8	7,07,916
10	8,84,896
12	10,61,875
14	12,38,854
16	14,15,833
18	15,92,812

Assay of marketed formulation: The system was given separate injections of the standard solution and sample solution. Chromatograms were then taken, and the earlier approach was used to calculate the amount of drug present in the sample.

In order to achieve outstanding retention duration and peak asymmetry, the Phenomenex C18 column (250 mm * 4.6 mm 5.) was employed for the study. Mobile phase A and phase B were also used (methanol: water 40:60). 20 μl of the injection volume were saved while the flow rate was kept at 1.0 mL/min. The temperature in the column was fixed at 30 C. Using a UV detector, the detection wavelength was 257 nm. Fluphenazine HCl had a retention time of 3.286 min. The regression correlation coefficient was found to be 0.9995, indicating that the process was linear in concentration to peak area responses. After conducting precision investigations, it was discovered that the %RSD of the determinations was 0.18%, indicating that the method's precision was within acceptable bounds.

When compared to the overall percentage RSD for recovery, the accuracy of the approach was determined to be high at 80, 100, and 150 percent. This demonstrates that the suggested strategy is the right one. For the detection and quantification of Fluphenazine HCl injectable formulation, all parameters were effectively established. According to the guidelines in the ICH Q2R1 guidelines, the approach has been validated. The HPLC method designed for both

bulk drug and Pharmaceutical Injection Formulation of Fluphenazine HCl has a number of benefits, including reduced retention time, remarkable linearity, good peak symmetry, and speed, accuracy, and robustness. The sample does not need to be prepared using time-consuming processes because the mobile phase may be made rapidly and the diluent is easily accessible.

Table 1.4: Accuracy data of Fluphenazine (HPLC)					
% Recovery	Sample Amount (µg/mL)	Standard Amount Spiked (µg/mL)	Peak area	% Recovery	Mean % Recovery
50%	4*	2*	5,29,578*	100.11*	
					100.04%*
100%	4*	4*	6,98,829*	99.94*	
150%	4*	8*	10,10,572*	99.67*	

Table 1.5: Robustness Data of Fluphenazine	
Parameter	%RSD
Parameter	%RSD
Flow Minus (0.8mL)	0.4
Flow Plus (1.2mL)	0.5
Mobile phase Minus (30:70)	0.8
Mobile phase Plus (50:50)	0.9

$$\% \ of \ drug \ content = \frac{Peak \ Area \ of \ Sample \ \times Concentration \ of \ Standard}{Peak \ Area \ of \ Sample \ \times Concentration \ of \ Standard} \times 100$$

$$= \frac{2,37,324 \ X \ 10 \mu g/ml}{2,38,432 X 10 \mu g/ml} \times 100$$

% Assay of content = 99.53%

Acceptance limit: 90-110% (as per IP)

CONCLUSION

When actual pharmaceutical samples were analyzed using this method, the content of Fluphenazine HCl fell within the permissible limits. Considering its sensitivity, precision, accuracy, and selectivity for the test chemical, this method can be effectively employed for routine quality control testing of Fluphenazine HCl in liquid dosage form. The presently suggested approach can be employed in quality control laboratories for routine analysis of Fluphenazine HCl in product bulk drugs and in routine application forms of pharmaceutical dose without excipient interference.

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Data Availability: All data are available and can be supplied on reasonable request to the corresponding author

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