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METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF FLUPHENAZINE USINGSIMPLE UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION IN BULK AND MARKETED DOSAGE FORMS

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ABSTRACT

The study is focused to develop and validate a UV-Spectrophotometric method for estimation of Fluphenazine HCL from bulk and their dosage form. Based on spectrophotometric characteristic of method are described for the determination of Fluphenazine at 256 nm was found adequate for quantification. The method was validated for linearity, accuracy, precision and robustness as per International Conference on Harmonization guidelines. The linearity signal and concentration of Fluphenazine in the range of 2–22 µg/ml in aqueous solution present a correlation coefficient (r^2) of 0.9939 for simple UV spectrum. The limit of detection (LOD) and limit of quantitation (LOQ) were 1.87 and 5.68 µg/ml, respectively. The method was found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Fluphenazine bulk and marketed dosage form.

Keywords: UV-Spectrophotometric, Fluphenazine HCL, bulk and dosage form

INTRODUCTION

Fluphenazine Hydrochloride is the hydrochloride salt of fluphenazine, a phenothiazine with antipsychotic activity and potential antineoplastic activity. Fluphenazine blocks postsynaptic dopamine D2 receptors in the limbic system, cortical system and basal ganglia, resulting in a reduction of schizophrenia-associated hallucinations and delusions. In addition, as a serotonin antagonist, this agent may inhibit lymphocyte and myeloma cell proliferation by blocking 5-hydroxytrptamine type 1B (5-HT type 1B) receptors for serotonin. There is also a long acting injectable version that may last for up to four weeks **[1-5]**. The injectable form is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system **[6-10]**. It appears to be about equal in effectiveness to low-potency antipsychotics like chlorpromazine. Fluphenazine decanoate, the depot injection form of fluphenazine, should not be used by people with severe depression. Several methods are reported in UV spectrophotometric determination of fluphenazine simultaneously with other drugs and using commercial solvents, the method developed using water as solvent ,most economic method for routine analysis has not been reported earlier based on thorough literature search performed before Initiating the study. The method developed is also validated for linearity, accuracy, precision and robustness. The results indicated reliability of the method **[11-19]**.

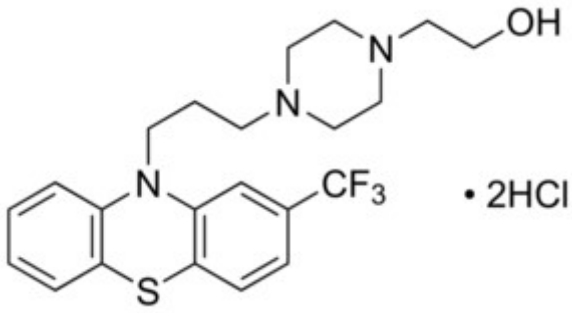

Figure 1: Structure of fluphenazine HCl

MATERIALS AND METHODS

Apparatus:

The proposed work was carried out on a ELICO UV visible spectrophotometer (model SL 210), which possesses a silicon photodiode detector with a 1 cm quartz matched cell. All weighing was done on electronic balance **(Wensar HPB 220)**.

Materials:

Fluphenazine HCL were obtained as gift sample from pharmaceutical Laboratories Ltd. Water, methanol water used were of analytical grade (FINAR limited, Hyderabad, India). All other chemicals and reagents used were analytical grade unless otherwise indicated.

Determination of wavelength of maximum Absorption

A standard stock solution of fluphenazine (1000µg/mL) was prepared using the diluent and 0.1ml standard stock was then diluted to 10ml to obtain 10µg/mL fluphenazine reference solution. An UV spectroscopic scanning (200-400nm) was carried out to determine the λmax for detection of fluphenazine using water as diluent for blank **(Figure 3)**.

Determination of wavelength of maximum Absorption using methanol

A standard stock solution of fluphenazine (1000µg/mL) was prepared using the diluent and 0.1ml standard stock was then to 10ml to obtain 10µg/mL fluphenazine reference solution .An UV spectroscopic scanning (200-400nm) was carried out to determine the λmax for detection of fluphenazine using methanol as diluent for blank **(Figure 4)**.

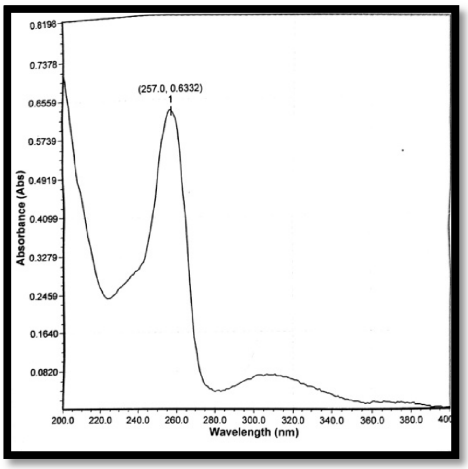

Figure 2: Determination of maximum absorption in water

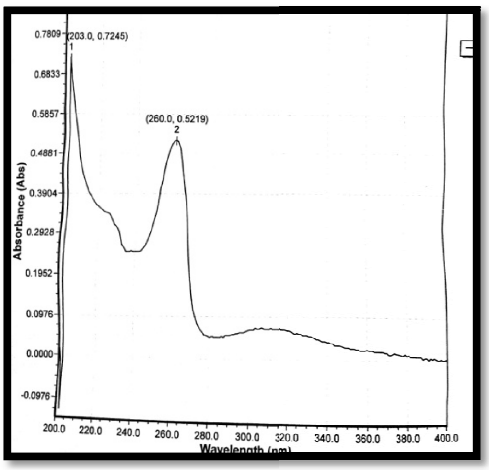

Figure 3: Determination of maximum absorption in methanol

Method Validation

Validation is a procedure of building up narrative proof showing that a system, procedure, or movement did underway or testing keeps up the desired level of consistence at all stages. The scientific strategy approval is vital for diagnostic technique improvement and tried widely for specificity, linearity, exactness, accuracy, range, detection limit, quantization cutoff. In synopsis, systematic strategy advancement and acceptance permits to affirm that a precise and dependable intensity estimation of a pharmaceutical product can be performed.

Validation parameters

The goal of the diagnostic strategy ought to be plainly comprehended since this will administer the approval attributes which need to be assessed. Average approval attributes

which ought to be considered are recorded underneath

- Linearity
- Precision
- Repeatability
- Accuracy
- Specificity
- Detection Limit
- Quantitation Limit
- Range

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the active substance (by dilution of a standard stock solution) and/or on separate weighings of synthetic mixtures of the product components, using the proposed procedure. The correlation coefficient, y-intercept and slope of the regression line should be submitted. For the establishment of linearity, a minimum of 5 concentrations is recommended.

Accuracy

Accuracy should be established across the specified range of the analytical procedure. Application of the analytical procedure to synthetic mixtures of the product components to which known quantities of the substance to be analysed to be added. Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/ 3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Limit of Detection (LOQ) and Limit of Quantification (LOQ):

Determined based on the Standard Deviation of the Response and the Slope.

The detection limit (DL) may be expressed as:

DL = 3.3 σ/ S

where σ = the standard deviation of the response

S = the slope of the calibration curve

The quantitation limit (QL) may be expressed as:

QL = 10 σ /S

where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Forced Degradation studies (Figure 4, Table 1)

Acidic Degradation:

From 100 ppm fluphenazine standard solution 1mL was pipetted out in a 10mL volumetric flask. To this 1mL of 0.1NHCL was added and allowed to stand for 24 hours. Then the solution was neutralized by adding 1mL of 0.1N NaOH. Absorbance of the solution was measured at 256 nm and percent degradants were calculated.

Basic Degradation

From 100 ppm fluphenazine standard solution 1mL was pipetted out in a 10mL volumetric flask. To this 1mL of 0.1N NaOH was added and allowed to stand for 24 hours. Then the solution was neutralized by adding 1mL of 0.1N HCl. Absorbance of the solution was measured at 256 nm and percent degradants were calculated.

Oxidative Degradation

From 100 ppm fluphenazine standard solution 1mL was pipetted out in a 10mL volumetric flask. To this 3mL of H_2O_2 was added and allowed to stand for 24 hours. Then the solution was neutralized by adding 1mL of 0.1N HCl. Absorbance of the solution was measured at 256 nm and percent degradants were calculated.

Thermal Degradation

Fluphenazine Drug was exposed to 40° temperature in a hot air oven. After 24 hours required amount of drug was taken and 10 ppm solution was prepared. Absorbance of the solution was measured at 256 nm and percent degradants were calculated.

Photolytic Degradation

Fluphenazine drug was exposed to UV light for 24 hours, then required amount of drug was taken and 10 ppm solution was prepared. Absorbance of the solution was measured at 256 nm and percent degradants were calculated.

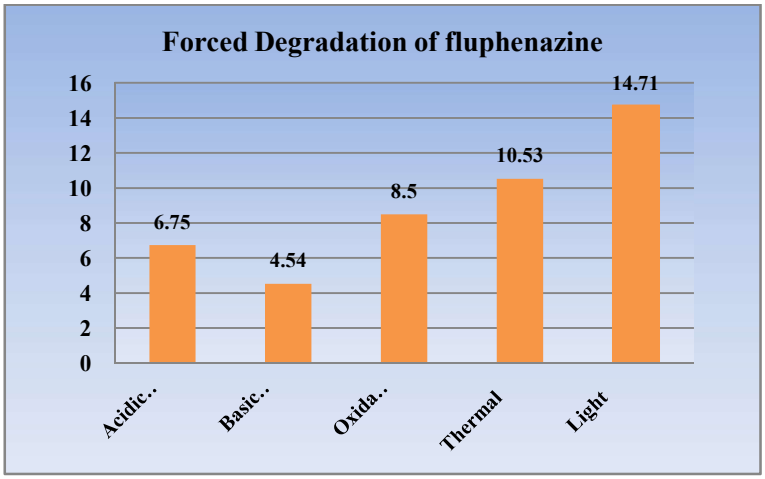

Figure 4: Forced Degradation Data of Fluphenazine

Table 1: Forced Degradation Data of Fluphenazine

Degradation Condition	% drug Degraded
Acidic Hydrolysis(0.1N HCL)	6.75
Basic Hydrolysis(0.1NnaOH)	4.54
Oxidation	8.5
Thermal	10.53
Light	14.71

RESULTS AND DISCUSSION

All validation parameters were carried out under the conditions as mentioned in the ICH Q_2 R(1) guidelines**.**

Linearity and range:

For linearity study, ten solutions at different concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22µg/mL) were prepared using ten different aliquots of reference solution and the obtained data were used for linearity calibration plot, limit of detection (LOD), limit of Quantitation (LOQ) for the Assay were also calculated.

Precision study

Fluphenazine injection were were diluted with water and the sample stock solution was prepared following same dilution pattern as standard stock solution. Six aliquots were prepared of 10µg/mL and to obtain the concentration six times over a short interval of time **(Table 1)**.

Accuracy study

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the sample to make final concentrations in the range of 50%, 100% and 150% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula. **% Recovery = [A - B/ C] X 100**

Where, A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

The results of Accuracy studies of Fluphenazine HCl are reported in the **Table 2**.

Robustness

The robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrometric condition for determination Fluphenazine HCL in marketed formulation.

Assay of marketed formulation

Six Injection formulation containing Fluphenazine HCL were procured from market. Volume of the injection containing equivalent volume to 10mg were calculated and transferred to 10mL volumetric flask the volume was made up with water (primary stock). From this stock 0.1mL was pipetted out and transferred to another 10mL volumetric flask, volume was made with water, to make 10µg/mL concentration. Absorbance was determined by scanning at 256nm. % Assay was calculated from the calibration curve equation.

y=mx+c

%assay = (obtainedconc/original conc.)*100
%assay = (9.850/10)*100
%assay = 98.50%

Sample absorbance at 10 ppm = 0.6542

y=0.059 x + 0.0073**.**

obtained absorbance (y) = 0.6542

0.6542 = 0.059 x + 0.073

0.059 x = 0.6542-0.073

0.059 x = 0.5812

X = 0.5812/0.059

X = 9.850

Method development and optimization:

Fluphenazine HCl is very soluble in chloroform, ether, cyclohexane, methanol, ethanol and water. The solvent used in earlier reported methods was acetone, methanol, Formic acid. The solvent was optimized to water .The predetermined wavelength of maximum absorption λmax was 260nm.

Method Validation

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of samples in the range of (2-22µg/mL) was linear with correlation coefficient value of R^2=0.993.

LOD and LOQ

The limit of detection and limit of quantitation were calculated from the calibration plot and was found to be 1.87 and 5.68µg/mL respectively.

Precision

The precision study performed of the developed method confirmed adequate

sample stability and method reliability where all the RSD were <2%.

Accuracy/recovery studies

Results were within the range of 99-100% ensure an accurate method as well as non interference with the excipient of formulation.

Robustness

The Robustness data for variations in wavelength of detections (±5nm) and the absorbance and its analytical performance parameters of fluphenazine HCL were shown in **Table 4**.

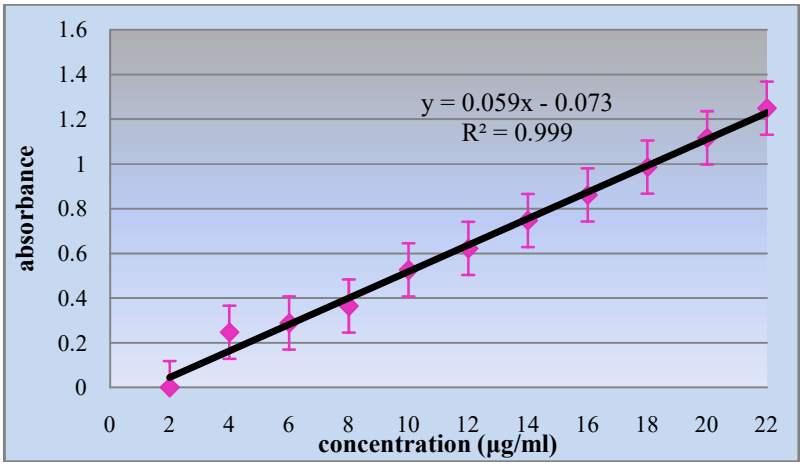

Table 1: Precision studies data of Fluphenazine HCl

Repeatability (10µg/mL)	Absorbance
1	0.9383
2	0.9379
3	0.9386
4	0.9376
5	0.9351
6	0.9360
mean	0.9371
SD	0.001167617
RSD	0.124%

Table 2: Accuracy studies of Fluphenazine HCl

% level	Concentration(µg/mL)		Absorbance	%Recovery	Mean % Recovery	Std.dev	%RSD
	Taken	Spiked					
50%	6	3	0.5064 0.5061 0.5059	99.26% 99.70% 100.54%	99.83%	0.0053	0.5319%
100%	6	6	0.7261 0.7259 0.7254	99.54% 99.60% 101%	100.05%	0.0067	0.6742%
150%	6	9	0.9981 0.9979 0.9978	98 % 99.70 % 100.20 %	99%	0.0080	0.7998%

Table 4: Robustness Study of Fluphenazine HCL

Wavelength in nm	Absorbance found in 10µg/mL mean S.D(n=3)	% RSD
255	0.9053±0.001	0.0040
256	0.9807±0.0012	0.0043
257	0.9376±0.0019	0.0049

CONCLUSION

The results and statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, accurate and precise. Therefore this method can be used for determination of fluphenazine and percentage degradants through forced degradaion studies in bulk or in the dosage forms.

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REFERENCES

[1] World Health Organization model list of essential medicines: 21st list 2019. Geneva: World Health Organization.

[2] Tardy M, Huhn M, Engel RR, Leucht S (August 2014). "Fluphenazine versus low-potency first-generation antipsychotic drugs for schizophrenia". *The Cochrane Database of Systematic Review.*

[3] Matar, Hosam E; Almerie, Muhammad Qutayba; Sampson, Stephanie J (12 June 2018). "Fluphenazine (oral) versus placebo for schizophrenia"

[4] S.I. Sa'sa, I. Jalal, Determination of nortriptyline hydrochloride and fluphenazine hydrochloride in commercial tablets by reverse phase high-performance liquid chromato-graphy, Microchem. J. 38 (1988) 181–187.

[5] Haddad, Peter; Haddad, Peter M.; Dursun, Serdar; Deakin, Bill (2004). *Adverse Syndromes and Psychiatric Drugs: A Clinical Guide*

[6] N.A. El-Ragehy, A.M. El-Kosasy, S.S. Abbas, *et al*., Polymeric membrane electrodes for selective determination of the central nervous system acting drugs fluphenazine hydrochloride and nortriptyline hydrochloride, Anal. Chim. Acta 418 (2000) 93–100.

[7] M.I. Walash, A. El-Brashy, N. El-Enany, *et al*., Second-derivative synchronous fluorescence spectroscopy for the simultaneous determination of fluphenazine hydrochloride and nortriptyline hydrochloride in

pharmaceutical preparations, J. Fluoresc. 19 (2009) 891–904

[8] W.M. Glazer, L.T. Friedhof, S.R. Marder and W.A. Brown, Schizophrenia Res., 8 (1992) i11.

[9] S. Mennickent, J. Contreras, C. Reyes, *et al*., Validated instrumental planar chromatographic method for quantification of fluphenazine hydrochloride in injections, J. Planar Chromatogr. 23 (2010) 75–78.

[10] N.A. El-Ragehy, A.M. El-Kosasy, S.S. Abbas, *et al*., Polymeric membrane electrodes for selective determination of the central nervous system acting drugs fluphenazine hydrochloride and nortriptyline hydrochloride, Anal. Chim. Acta 418 (2000) 93–100.

[11] H. Kirchherr, W.N. Kuhn-Velten, Quantitative determination of ¨forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach, J. Chromatogr. B 843 (2006) 100–113.

[12] PubChem. *"Fluphenazine"*. pubche m.ncbi.nlm.nih.gov

[13] United Kingdom: Royal Pharmaceutical Society of Great Britain. p. 192. ISBN 978-0-85369-845-6. Withdrawal of antipsychotic drugs after long-term therapy should always be gradual and closely monitored to avoid the risk of acute withdrawal syndromes or rapid relapse.

[14] S.R. Marder, K.K. Midha, T. Van Putten, M. Aravagiri, E.M. Hawes, J.W. Hubbard, G. McKay and J. Minz, Br. J.Psychiatry, 158 (1991) 658.

[15] T. Aaes-Jorgensen, Progress in Neuropsycho-pharmacology and biological psychiatry, 13 (1989) 77.

[16] A. Amdisen, T. Aaes-Jorgensen, N. Thomsen, V. Madsen and M. Nielsen, Psycho-pharmacoiogy, 90 (1986) 412.

[17] M.W. Jann, L. Ereshefsky and S.R. Saklad, Clin. Pharmacokinet., 10 (1985) 315.

[18] J. Kane, J. Clin. Psychopharmacol., 6 (1986)