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VALIDATED SPECTROPHOTOMETRIC AND STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF FEXOFENADINE HCL IN MARKETED TABLET

Ramashankar Dubey*[1], Navneet Kumar Verma[2], Vikas Yadav[2]

[1*]NRI Institute of Pharmacy, Bhopal, Affiliated to Rajiv Gandhi Praudyogiki Vishwavdyalaya, Madhya Pradesh, India.

[2]Buddha Institute of Pharmacy, Gorakhpur, Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, Uttar Pradesh, India.

ABSTRACT

The aim and objective of the present work is to develop new simple, sensitive and validated UV spectrophotometric method for the estimation of Fexofenadine Hydrochloride in marketed formulation. The developments of analytical methods for the determination of drugs in bulk, in dosage forms or in body fluids have received a considerable attention in recent years because of their importance in quality control, bioavailability and pharmacokinetic study etc. Validation of developed Analytical methods according to ICH guidelines.

KEYWORDS

UV spectrophotometric method, Fexofenadine Hydrochloride, Bioavailability and Pharmacokinetic study.

Author for Correspondence:

Ramashankar Dubey,
NRI Institute of Pharmacy,
Bhopal, Affiliated to Rajiv Gandhi Praudyogiki Vishwavdyalaya,
Madhya Pradesh, India.

Email: rama.s.dubey@gmail.com

INTRODUCTION[1-3]

Spectroscopy could be a general term for the science that deals with the interaction of assorted kinds of radiation with matter. Qualitative analysis and qualitative analysis strategies see the measuring of the intensity of radiation with a measurement electrical device or alternative style of device. The spectrophotometric assay of medication seldom involves the measuring of absorbance of samples containing just one engrossing element. The pharmaceutical analyst oft encounters true wherever the concentration of 1 or additional substances is needed in samples noted to contain alternative

engrossing substances, that probably interfere within the assay. If the formula of the samples is thought, the identity and concentration of the interferences square measure noted and therefore the extent of interference within the assay could also be determined.

The basis of all the spectrophotometric techniques for single and multicomponent samples is that the property that in any respect wavelengths

The absorbance of an answer is that the add of absorbance of the individual elements or The measured absorbance is that the distinction between the entire absorbance of the answer within the sample cell which of the answer within the reference cell. Following Methods are most commonly Used[4-6].

Simultaneous Equation Method (Vierodt's method)

If a sample contains two absorbing drug (x and y) each of which absorbs λ_{max} of other, it may be possible to determine both drugs by the technique of simultaneous equation provided that certain criteria apply.

1. The information required is:
2. The absorptivity of x at λ_1 and λ_2, ax_1 and ax_2 respectively.
3. The absorptivity of y at λ_1 and λ_2, ay_1 and ay_2 respectively.
4. The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.
5. Cx and Cy be the concentrations of x and y respectively in the diluted sample,

So, concentration can be calculated by:

$Cx = (A_2ay_1-A_1ay_2)/ (ax_2ay_1-ax_1ay_2)$

$Cy = (A_1ax_2-A_2ax_1)/ (ax_2ay_1-ax_1ay_2)$

E.g. the B.P. assay of quinine- related alkaloids and cinchonine – related alkaloid in cinchona bark.

Absorption Ratio Method

The absorbance magnitude relation methodology could be a modification of the co occurring equation methodology. It depends on the property that for a substance that obeys Beer's law in any respect wavelengths, the magnitude relation of absorbance's at any 2 wavelengths could be a constant worth freelance of concentration or path length. For instance, 2 completely different dilutions of constant substance offer constant absorbance magnitude relation A1/A2 = two.0. Within the USP this magnitude relation is referred to as a Q worth.

The B.P. additionally uses a magnitude relation of absorbance at nominative wavelength in bound confirming check of identity.

E.g. Cynocobalamin exhibits 3 λmax at 278nm, 361nm and 550nm. The A361/ A550 square measure needed to be three.30 ± 0.15 and also the A361/A278 to be one 79 ± 0.09.

Geometric correction methodology

For elimination of the background tangential absorption that will be gift within the sample of biological origin, variety of mathematical correction procedures are developed. The best of this procedure is that the 3 -purpose geometric procedure, which can be applied if the tangential absorption is linear at the 3 wavelengths elite.

Orthogonal Polynomial methodology

Technique of orthogonal polynomials is another mathematical correction procedure that involves a lot of complicated calculations than the 3 -point correction procedure. The idea of the strategy is that Associate in nursing spectrum mat be painted in terms of orthogonal functions.

Distinction spectrophotometry

The essential options of a distinction spectrophotometric assay square measure that the measured worth is that the distinction absorbance (ΔA) between 2 equimolar solutions of the analyte {in completely different in several in numerous} chemical forms that exhibit different spectral characteristics. Once absorption spectra of 2 equimolar solutions of phenylephenerine, in both 0.1M HCl and zero.1 M NaOH at 257 and 278nm.

Both solutions have identical absorbance and consequently exhibit zero distinction absorbance. Such wavelengths of equal absorption factor of the 2 species square measure known as isobestic or is absorptive purpose. The measured worth in quantitative distinction spectrophotometric assay is that the ΔA.

$\Delta A = A_{alk} - A_{acid}$

ΔA also obeys the Beer-Lambert law and a modified equation may be written as ΔA=Δabc

Where ΔA is the difference absorptivity (e.g. ΔA 1% 1cm or ΔЄ) of the substance at the wavelength of measurement.

Derivative Spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or the higher derivative spectrum. In this spectrophotometry the normal absorption spectrum is referred to as the fundamental zero order or D0 spectrum.

The first by-product (D1) spectrum may be a plot of the speed of amendment of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of a da/ dλ Vs λ.

The second derivative (D2) spectrum is a plot of the curvature of the D0 spectrum against wavelength or a plot of dA2/dλ2 Vs λ.

Chemical Derivatization

It is an indirect spectrophotometric assay, which is based on conversion of the analyte by a chemical reagent to a derivative that has different spectral properties. When an excess of the reagent is used, to ensure complete conversion, the absorbance of the derivative is usually, but not always, proportional to the concentration of the analyte. In this the derivative of the longer λmax and/ or a higher absorptivity are made.

Experimental

Chemicals and solvent

Marketed Formulation of Fexofenadine Hydrochloride

Allegra, Mac Leod's Pharmaceutical Ltd., Himachal Pradesh, (India)

Batch No. AOJ-803

Olmesartan 20mg

Determination of Solubility of Fexofenadine Hydrochloride

Solubility of Fexofenadine Hydrochloride was performed in different solvents and result was shown in Table No.2.

Analytical method development by UV spectrophotometry

Instruments and tools (UV spectrophotometer)

A Double Beam U.V. Spectrophotometer, Model of Labindia 3000+ with 1cm. matched quartz cells was used.

EXPERIMENTAL PROCEDURE

Preparation of Standard Stock Solution

10mg of Fexofenadine Hydrochloride was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluent 0.1 NaoH, to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Fexofenadine Hydrochloride 1ml was taken and diluted up to 10ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25mcg/ml concentration.

Preparation of the Calibration Curves of the Drug

Each of the standard drug solutions take abs. 3 times and the mean absorbance of drug was calculated (Table No.3) and plotted against the concentration of the drug. The regression equation was found out by using this curve. Typical Spectra and the calibration curve were obtained.

Preparation of Analysis of Tablet formulation

Twenty tablets were accurately weighed and finely powered. Tablet powder equivalent to 10 mg of Fexofenadine Hydrochloride was taken in 10ml of volumetric flask; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent. 1ml of filtrate was taken in 10ml volumetric flask and volume was made up to 10ml with mobile phase to obtain concentration of 100µg/ml. Further 0.1ml of this solution was taken and diluted up to 10ml obtain final concentration of 10µg/ml of Fexofenadine Hydrochloride. The resulting solution was again filtered using Whatmann filter paper No.41 and then sonicated for 10 min.

Finally diluted sample was taken and absorbance was measured by using spectrophotometer at 225nm. Concentration of Fexofenadine Hydrochloride was found out by using regression equation. Result was shown in Table No.4.

VALIDATION

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to absorbance of analyte in the sample. The calibration plot was constructed after analysis of five different (from 4 to 12µg/ ml) concentrations and absorbance for each concentration was recorded three times, and mean absorbance was calculated (Table No.3). The regression equation and correlation coefficient of curve and the standard curve of the drug is shown in (Figure No.1).

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100% and 120%) was added and then its recovery was analyzed and result was shown in Table No.8 and statistical validation of recovery studies shown in (Table No.4).

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis (Table No.5). Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision

Day to Day

Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out and the data is presented in (Table No.6 and No.7).

LOD (Limit of Detection)

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table No.8.

$LOD = 3.3 (\sigma / S)$

Where, S = slope of calibration curve, σ = standard deviation of the response.

LOQ (Limit of Quantitation)

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table No.8.

$LOQ = 10 (\sigma / S)$

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

RESULTS AND DISCUSSION

Regression Equation

$Y = mx + c$,

Slope- M= 0.021, intercept -C= 0.024, r^2= 0.992.

Table No.1: Chemicals and Solvents Used

S.No	Chemicals	Manufacturer
1	Fexofenadine Hydrochloride	Working standard, MacLeod's Pharmaceutical Ltd.
2	All Chemical For U.V	Analytical Grade
3	All Chemical For HPLC	HPLC Grade

Results of Solubility Study

Table No.2: Solubility of Fexofenadine Hydrochloride

S.No	Solvent	Solubility
1	Water	Insoluble
2	0.1 N HCl	Insoluble
3	0.1 N NaOH	Soluble
4	Methanol	Freely soluble
5	Acetonitrile	Freely soluble
6	Ethanol	Soluble

Preparation of the Calibration Curves of the Drug

Table No.3: Linearity of Fexofenadine Hydrochloride

S.No	Conc. µg/mL	0	5	10	15	20	25
1	Rep.1	0	0.151	0.247	0.365	0.447	0.552
2	Rep.2	0	0.150	0.246	0.364	0.448	0.551
3	Rep.3	0	0.151	0.247	0.365	0.449	0.552
4	Mean	0	0.181	0.246	0.364	0.448	0.551
5	S.D.	00	0.001	0.001	0.001	0.001	0.001
6	R.S.D%	000	0.552	0.234	0.158	0.223	0.105

Optical parameter

Table No.4: Result of Optical Parameter of Fexofenadine Hydrochloride

S.No	Parameters	Observation
1	λ_{max}	225.0 nm
2	Beer's law limit (µg/mL)	5-25
3	Regression equation	Y = 0.021 x +0.024
4	Correlation Coefficient (r^2)	0.992
5	Molar Absorptivity (L mol^{-1} cm^{-1})	1.9 X 10^4
6	Sandell's Sensitivity µg/mL 0.001 absorbance unit	0.5 X 10^{-4}

Result of Analysis of gel formulation

Table No.5: Assay of Tablet Formulation

S.No	Brand Name	Fexofenadine Hydrochloride	
		Label Claim	% Purity
1	Alegra	20mg	99.82

Results of Validation Parameters

Results of Accuracy

Table No.6: Recovery Studies for Accuracy of Tablet formulation

S.No	Level of Recovery (%)	80	100	120
1	Amount Present	10 10 10	10 10 10	10 10 10
2	Amount of Std. Added	8 8 8	10 10 10	12 12 12
3	Amount Recovered	8.103 8.104 8.103	9.98 9.99 9.98	12.05 12.06 12.05
4	% Recovery	101.73 101.74 101.73	99.67 99.68 99.67	100.83 100.84 100.83

Table No.7: Statistical Validation of Recovery Studies

S.No	Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
1	80	Fexofenadine Hydrochloride	101.73	0.000471	0.07191
2	100	Fexofenadine Hydrochloride	99.67	0.000471	0.06624
3	120	Fexofenadine Hydrochloride	100.83	0.000471	0.060617

*Denotes average of three determinations

Result of Precision

Repeatability

Table No.8: Results of analysis Data of Tablet Formulation

S.No	Drug	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
1	Fexofenadine	20 mg	0.05	100%	0.000816	0.147117

Intermediate Precision (Inter-day and Intra-day precision)

Table No.9: Intra-day and Inter-day precision

S.No	Intra-day Precision		Inter-day Precision	
		% Label Claim		% Label Claim
1	After 1hr	98.92	First day	98.20
2	After2hr	98.56	Second day	98.02
3	After3hr	98.38	Third day	97.84
4	Mean	98.62	Mean	98.02
5	SD	0.224	SD	0.146
6	% RSD	0.227	% RSD	0.149

Analyst to Analyst

Table No.10: Result of Analyst to Analyst Precision

Analyst	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
1	20mg	19.80mg	99%	0.00081	0.14711

Result of LOD and LOQ

Table No.11: Result of LOD and LOQ

S.No	Fexofenadine Hydrochloride	
	LOD	LOQ
1	0.06µg/Ml	0.20µg/Ml

Determination of λ_{max} of Drug: 225.0 nm

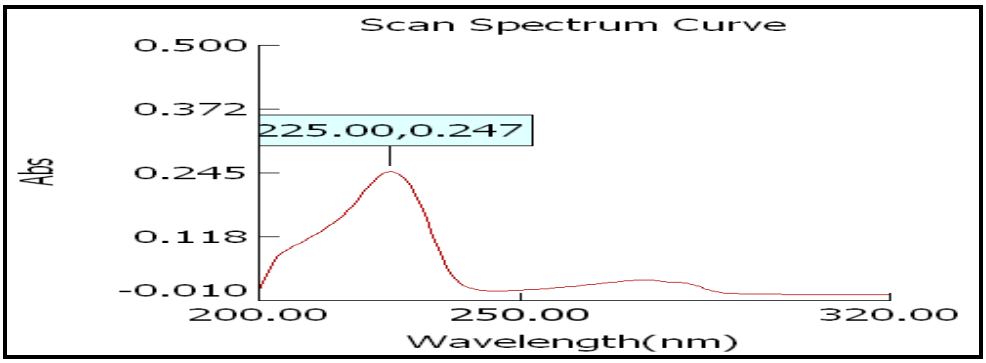

Figure No.1: Selection of λ_{max} of Fexofenadine Hydrochloride

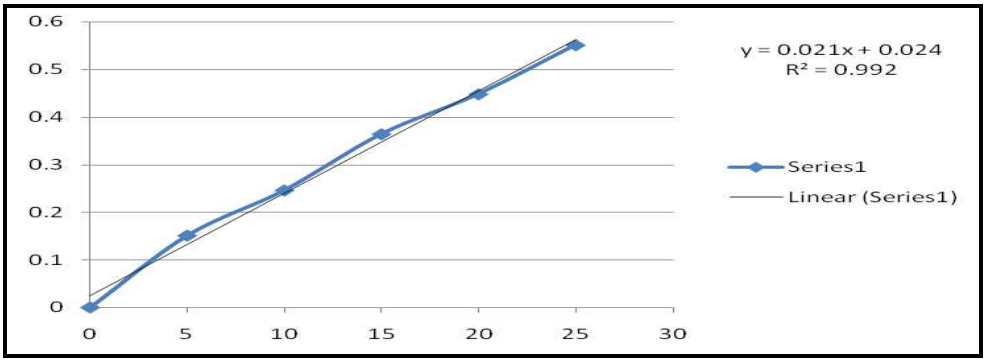

Figure No.2: Calibration Curve of Standard Fexofenadine Hydrochloride

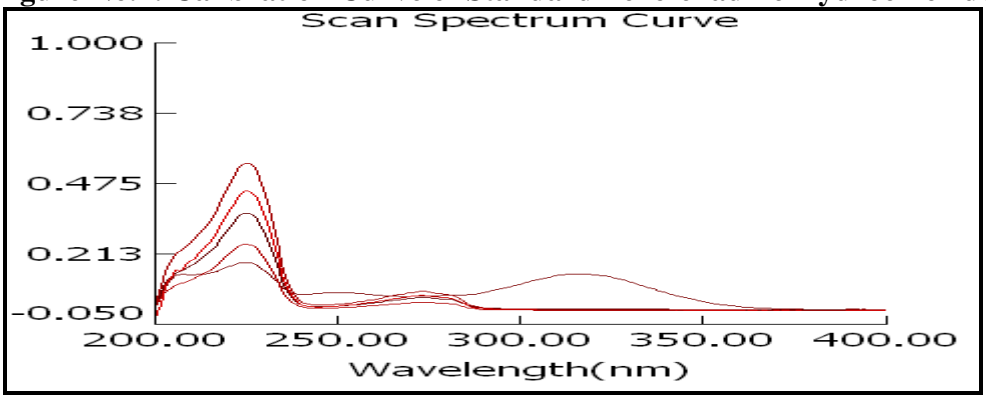

Figure No.3: Linearity of standard Fexofenadine Hydrochloride

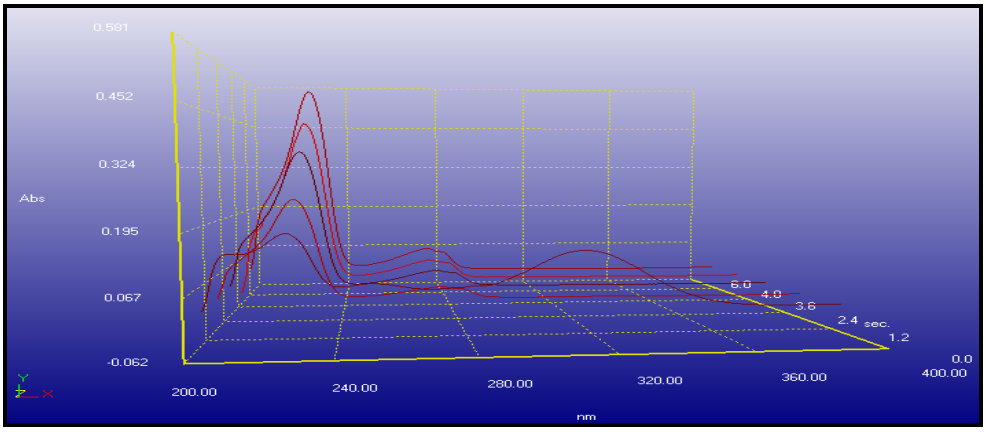

Figure No.4: Linearity of standard Fexofenadine Hydrochloride

CONCLUSION

Analytical method is an important quality control tool for estimation of percentage of drug in formulation. Fexofenadine Hydrochloride plays an important role in the maintenance of human health in case of verity of disorder like Hypersensitivity reaction, treatment of Gastric ulcer etc. This is where analytical method plays important role to estimation the content of drug in marketed formulations. In present work analytical methods was developed for estimation of Fexofenadine Hydrochloride in marketed tablet formulations can be used in routine analysis, in industry and academics.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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