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Method Development and Validation of Fexofenadine Hydrochloride in Bulk and Solid Dosage Form (Tablets) by UV-Visible Spectrophotometry

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ABSTRACT

A simple, accurate, and sensitive method was developed and validated for the determination of Fexofenadine hydrochloride (FEX. HCl) in bulk and tablet formulation. The method is based on the reaction of FEX. HCl with Di cyclohexyl carbodiimide (DCC) and 2-Nitrophenyl hydrazine (2-NPH) in solution, forms 2-Nitrophenyl hydrazide of Fexofenadine hydrochloride. Using ethanol as a solvent, and subsequently measured spectrophotometrically at 415nm. The reaction was extremely rapid at room temperature and absorbance values remained unchanged for at least 24hrs. Beer's law was obeyed in the concentration range of 10-60µg/Ml within the detection limit of 0.62 and 0.68 µg/mL and limit of quantification of 1.88 and 2.06 µg/mL for FEX. HCl bulk and tablet derivatives respectively. The analytical method was validated according to ICH guidelines. The correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 100.1 and 99.9, %RSD for repeatability was found to be 1.324 and 0.7824 (intraday), 1.365 and 0.7015 (interday), %RSD for intermediate precision for analyst 1 was found to be 0.435 & 0.520, for analyst 2 %RSD was found to be 0.437 & 0.522 for FEX. HCl bulk and tablet derivatives respectively. The robustness results of derivatives of FEX. HCl in bulk and tablets (20µg/mL) was performed by changing wavelengths and no noticeable changes were found on small changes in wavelength. Hence the method was found to be robust. The results obtained were compared statistically with those obtained by the official method and showed no significant differences regarding accuracy, precision & other analytical parameters.

INTRODUCTION

Fexofenadine hydrochloride (FEX. HCl) is chemically known as 2-[4-[1-hydroxy-4-[4-[hydroxy(diphenyl)methyl]piperidin-1-yl]butyl]phenyl]-2-methyl propanoic acid; hydrochloride[1-4]. The molecular formula of FEX. HCl is $C_{32}H_{40}ClNO_4$.HCl and the molecular weight is 538.1 g/mol. It occurs as a white to off-white crystalline powder, its melting point is 148-150ºC and is freely soluble in methanol & ethanol, its Half-life is 14.4 hours[5-9]. It belongs to the category of antihistamines. The mechanism of action of Fexofenadine is to selectively antagonize H1 receptors on the surface of cells on multiple different organ systems. It is a second-generation H1 receptor blocker and is non-sedating. Fexofenadine also affects inflammatory mediators[10-13].

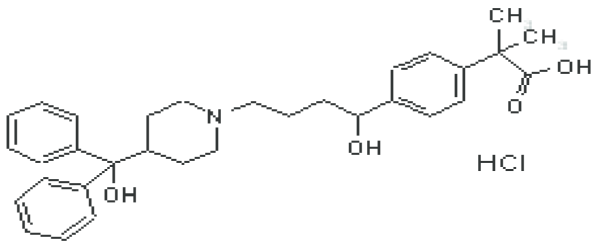

Fig. No. 1 Structure of Fexofenadine hydrochloride

Di cyclohexyl carbodiimide (DCC) is chemically known as N, N'- Di cyclohexyl carbodiimide. The molecular formula of DCC is $C_{13}H_{22}N_2$ and the molecular weight is 206.33g/mol. It occurs as a white to light ash color, its melting point and boiling points are 34-35°C & 122-124°C respectively. DCC is used as a dehydrating agent and is one of the most frequently used coupling agents, especially in organic synthesis applications. DCC is water-insoluble. DCC has been used to prepare active esters of carboxylate-containing compounds using NHS[14]. The organic soluble DCC is often used to create amide bonds, especially between water-insoluble compounds.

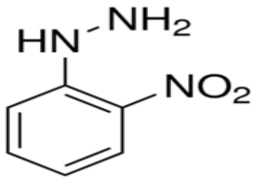

Fig. No. 2: Structure of Di cyclohexyl carbodiimide

2-NPH is chemically known as 2-Nitrophenyl hydrazine. The molecular formula of 2-NPH is $C_6H_7N_3O_2$ and the molecular weight is 153.14g/mol. It appears as a red to orange color, its melting point 91-93°C. It is soluble in acetonitrile, ethanol, and methanol. 2-NPH is widely used for the derivatization of carboxylic acids, aldehydes, and ketones. 2-NPH is a selective reagent for colorimetric determination of carboxylic acid anhydrides and chlorides. Carboxylic acid anhydrides and chlorides react with 2-NPH to form the corresponding hydrazides[15].

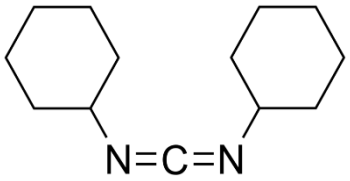

Fig. No. 3: Structure of 2-Nitrophenyl hydrazine

Literature survey reveals that there are very few reports for the analysis of Fexofenadine hydrochloride in bulk and dosage forms by UV-Visible spectroscopy. However, they suffer from some disadvantages concerning time, sensitivity, cost, etc. Hence there is a need to develop more simple, sensitive, economic, newer methods to analyze Fexofenadine hydrochloride by UV-Visible spectroscopy. A simple, fast visible spectroscopic method development by using DCC, 2-Nitrophenyl hydrazine for drugs containing carboxylic acid group was applied in the present study. The aim of this study is to develop and validate a new colorimetric method for Fexofenadine hydrochloride in bulk and tablets. The objective of this study is to develop a specific, precise, accurate, less expensive derivatization method for the estimation of antihistamines. To treat the antihistamine with N, N Di cyclohexyl carbodiimide (DCC) to produce an intermediate which upon treatment with 2-NPH gives colored derivatives for Fexofenadine hydrochloride. To develop a method that can be used for bulk and formulation.

Solubility test: Solubility of Fexofenadine hydrochloride is determined by dissolving the drugs in a different solvent like Water, Methanol, Ethanol, Acetonitrile, etc. Ethanol displayed the highest solubility for the selected drug. Hence it was selected for analysis.

MATERIALS AND METHODS

Instruments

The instruments used for the present work are weighing/electronic balance, model AUY-220, company Shimadzu and UV-VIS Spectrophotometer, model UV-1800, company Shimadzu.

Chemicals and Reagents

The chemicals and reagents needed for the work were obtained from various sources, Fexofenadine Hydrochloride (Bulk) from MSN Pharmaceuticals, Fexofenadine Hydrochloride -180mg (Formulation) purchased from SANOFI INDIA LIMITED, Distilled Water (Analytical Reagent) from VCOP, Ethanol (Laboratory Reagent) from SD Fine- chem Limited, Di cyclohexyl carbodiimide (Analytical Reagent) from Bio. chem fine Pvt. Ltd, 2-nitrophenyl hydrazine (Analytical Reagent) from Sigma Aldrich, Pyridine (Laboratory Reagent) from Fine chemicals, KOH (Pure) from SD Fine- chem limited, HCL (Laboratory Reagent) from Research lab Fine chem industries, NaOH (Extra pure) from Research lab Fine chem industries, H_2O_2(Pure)from SD Fine- chem Limited.

Preparation of standard stock solution:

This solution was prepared by dissolving 0.1gm of fexofenadine hydrochloride in 100ml of ethanol in a 100ml volumetric flask.

Preparation of Carboxylic acid hydrazide of Fexofenadine hydrochloride:

The direct conversion of a carboxylic acid to an amide is difficult because amines are basic tend to convert carboxylic acids to their highly unreactive carboxylates. In this reaction, the carboxylic acid of Fexofenadine hydrochloride, adds to the DCC molecule (activating agent) to form a good leaving group which can then be displaced by an amine (2- nitrophenyl hydrazine) during nucleophilic substitution. The DCC-induced coupling to form an amide is an important reaction in synthetic organic chemistry.

Method development

The chemical structure of Fexofenadine hydrochloride derivative is shown in Fig. No. 4. The visible spectra for the 2-nitrophenyl hydrazide of Fexofenadine hydrochloride in bulk and tablet are presented in Fig. No. 6 and 7 respectively. The revealed λ_{max} is 415nm for the bulk and tablet FEX. HCl derivatives (brown to orange colored) in ethanol. The scheme showing

the reaction of formation of the 2-Nitrophenyl hydrazide of Fexofenadine hydrochloride is shown in Fig. No. 5.

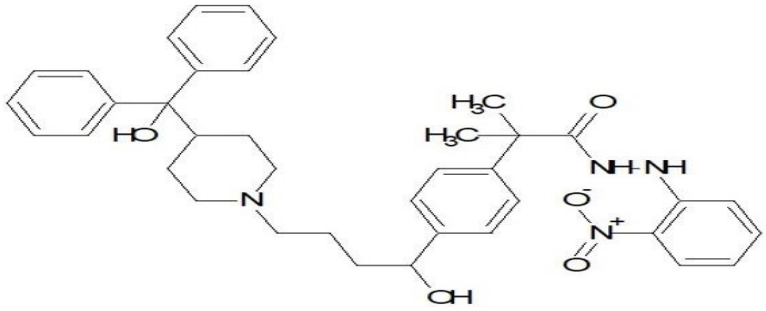

Fig. No.4: 2-Nitrophenyl hydrazide of Fexofenadine hydrochloride

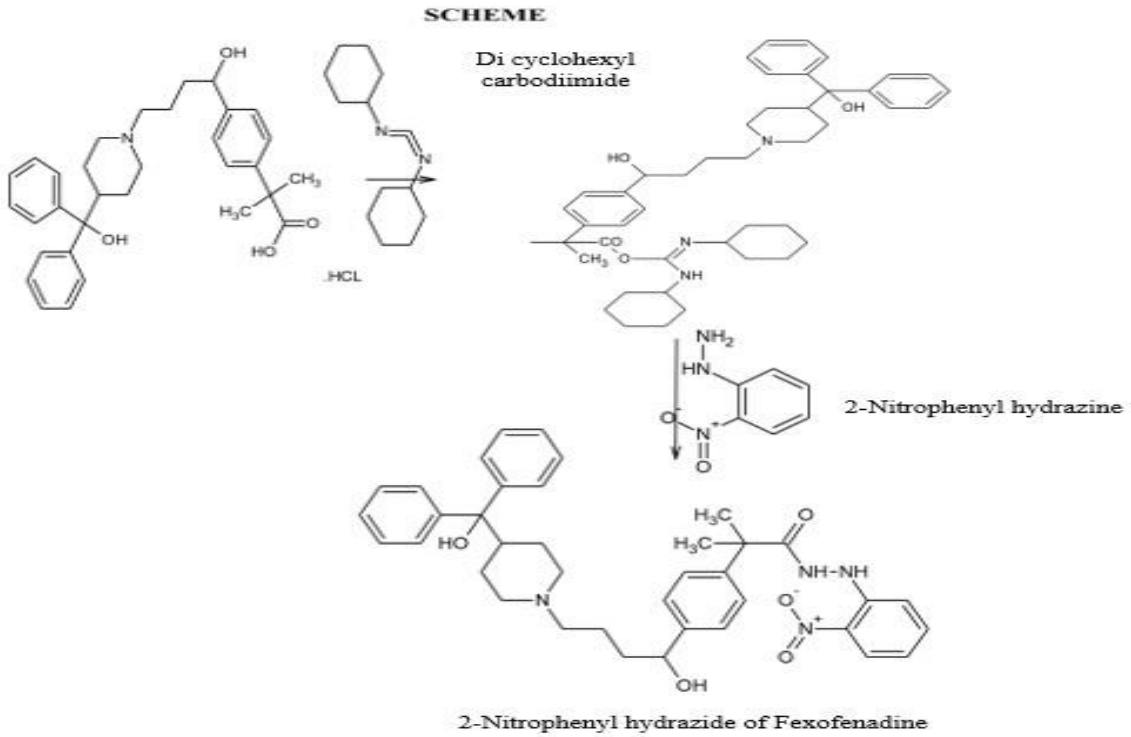

Fig. No. 5: Scheme showing the reaction of formation of the 2-Nitrophenyl hydrazide of Fexofenadine hydrochloride

Procedure for preparation of Fexofenadine hydrochloride derivative: Aliquots of the standard drug solutions, Fexofenadine hydrochloride (0.1gm) representing (10-60µg/ml) were placed in a series of 10ml volumetric flasks successively, a 0.5 ml volume each of 2-NPH, pyridine, and DCC solutions were added to each flask. The reaction mixture was heated on a

thermostat water bath for the specified time. Then 0.25 ml volume of potassium hydroxide solution was added, the flasks were capped and warmed at 60^0C for 5 min. After cooling to room temperature, the volume was made up to the mark with ethanol. The absorbance of the solution was measured in 1-cm cells, against a blank at the specific wavelength of maximum absorption.

Determination of λ max:

Fexofenadine hydrochloride derivative formed by reaction with DCC and 2-NPH (brown-orange colored chromogen) was measured in 1-cm cells against a blank and λ_{max} was scanned in UV-Vis Spectrophotometer from 400-800nm.

•λ_{max} of Fexofenadine hydrochloride derivative was found to be 415 nm.

Method validation:

Linearity:

Aliquots of the standard drug solutions, Fexofenadine hydrochloride (0.1gm) representing (10-60µg/ml) were placed in a series of 10ml volumetric flasks successively, a 0.5 ml volume each of 2-NPH, pyridine, and DCC solutions were added to each flask. The reaction mixture was heated on a thermostat water bath for the specified time. Then 0.25 ml volume of potassium hydroxide solution was added, the flasks were capped and warmed at 60^0C for 5 min. After cooling to room temperature, the volume was made up to the mark with ethanol. The absorbance of the solution (Fexofenadine hydrochloride derivative) brown-orange colored chromogen was measured in 1-cm cells against a blank and λ max was scanned in UV-Vis Spectrophotometer from 400-800 nm. **λmax** of fexofenadine hydrochloride derivative was found to be 415 nm. The amount of the sample is computed from the calibration graph.

Precision: Repeatability of the method was performed by measuring the absorbance of the sample in replicates of five.

$$\% \text{ RSD} = \text{SD/mean} * 100$$

Accuracy:

Then a recovery of standard solutions of 10µg/ml Fexofenadine hydrochloride in ethanol was prepared separately. Sample solutions of 8, 10, 12µg/ml were prepared as 80%, 100%, 120%

samples respectively. All the solutions were scanned under the visible range (400-800nm) and the % recovery was calculated from the absorbance values.

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount taken}} * 100$$

Robustness:

The robustness of the developed method is a measure of its capacity to remain unaffected, but not to affect by deliberately made changes in the method parameters, like variations in pH, solvent, $\lambda_{max,}$ etc, should be considered during the development stage. Typical variations are the stability of analytical solutions. In the present study, robustness was measured by varying the wavelengths and temperature conditions.

LOD & LOQ:

Limit of Detection (LOD): The detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated, under the stated experimental conditions.

Limit of Quantification (LOQ): It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

From the standard stock solution 0.1-0.5µg/ml FEX. HCl derivative is prepared and then the absorbance of the solution (Fexofenadine hydrochloride derivative) brown-orange colored chromogen was measured in 1-cm cells against a blank. The LOD & LOQ were calculated by using the following equations:

- LOD = 3.3 * SD deviation of response /slope of calibration curve &
- LOQ = 10* SD deviation of response /slope of calibration curve

Thin-layer chromatography

Preparation of mobile phase:

Take 1.5ml of Methanol (polar solvent) & 8.5ml of chloroform (non-polar solvent) and mix them together in a beaker and take them into the developing chamber.

Mobile phase: Chloroform 85% non-polar solvent, Methanol 15% polar solvent

Stationary phase: TLC plate (silica gel G).

Standard compounds preparation:

Bulk compound: Standard stock FEX. HCl solution (5mg of FEX. HCl is dissolved in 5ml of ethanol).

Bulk derivative compound: 1ml(1000µg/ml) of the standard stock FEX. HCl solution is taken into a volumetric flask, then we follow the above procedure to prepare the bulk derivative.

Tablet compound: Stock solution of tablet formulation (1000µg/ml).

Tablet derivative compound: 1ml of stock solution of the tablet formulation is taken into the volumetric flask (1000µg/ml), then we follow the above procedure to prepare the tablet derivative.

Procedure:

The first two TLC sheets are taken and one is spotted for bulk and bulk derivative and the other is spotted for tablet and tablet derivative.

TLC sheet is taken & and a line was drawn leaving about 1 cm space from the bottom with the help of a pencil, so that it does not touch the mobile phase and points were marked for spotting the sample, by using fused capillary tubes, the samples were spotted and air-dried, after air drying, to run the TLC sheet, it was immersed into the mobile phase in developing chamber and closed with a lid. Due to capillary action, the mobile phases move along the TLC sheet, wait for a few min till the mobile phase run, leaving about 1cm margin from the top defined as the solvent front, Wait till the development of spots. Once the spots are developed take out the TLC sheets and dry them. Place the TLC sheets under a UV light chamber then we observe spots on the TLC sheets, with reference to the spots observed on the TLC sheets Retention factor values, were calculated.

FORCED DEGRADATION STUDIES:

Force degradation is the exposure of the drug product or drug substance to different little harsh stress conditions.

Force degradation conditions applied in the present study:

Acid hydrolysis- 0.1M HCl, **Akali hydrolysis-** 0.1M NaOH

Oxidation- 30% 0.1M H_2O_2, **Thermal Dry** heat in hot air for 6 hours at 40°C, Sunlight for 6 hours.

Acid degradation studies:

From 100µg/mL Fexofenadine hydrochloride derivative solution 2mL was pipetted out into a 10mL volumetric flask. To this 1mL of 0.1M HCl was added and contents were mixed well and made up the volume to the mark with ethanol. The absorbance of the solution was measured.

Base degradation studies:

From 100µg/mL Fexofenadine hydrochloride derivative solution 2mL was pipetted out into a 10mL volumetric flask. To this 1mL of 0.1M NaOH was added and contents were mixed well and volume was made up to the mark with ethanol. The absorbance of the solution was measured.

Oxidative degradation studies:

From 100µg/mL Fexofenadine hydrochloride derivative solution 2mL was pipetted out into a 10mL volumetric flask. To this 1ml of 0.1M hydrogen peroxide was added and contents were mixed well and made up the volume to the mark with ethanol. The absorbance of the solution was measured.

Thermal degradation studies:

Hot air oven: Fexofenadine hydrochloride drug was exposed to a temperature in a hot air oven. After 6hr the required amount of drug was taken and 10µg/ml derivative solution was prepared and absorbance measured.

Sunlight: Fexofenadine hydrochloride drug was exposed to a temperature in a hot air oven. After 6hr the required amount of drug was taken and 10µg/ml derivative solution was prepared and absorbance measured.

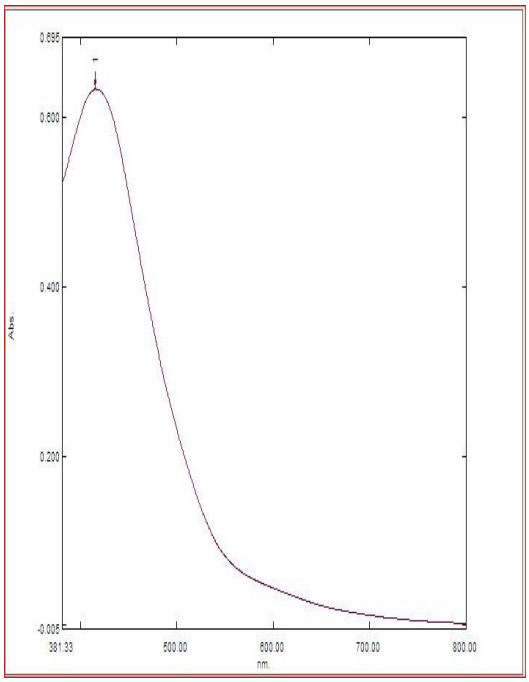

Fig. No. 6: Visible spectrum of Fexofenadine hydrochloride bulk derivative at λ max 415nm.

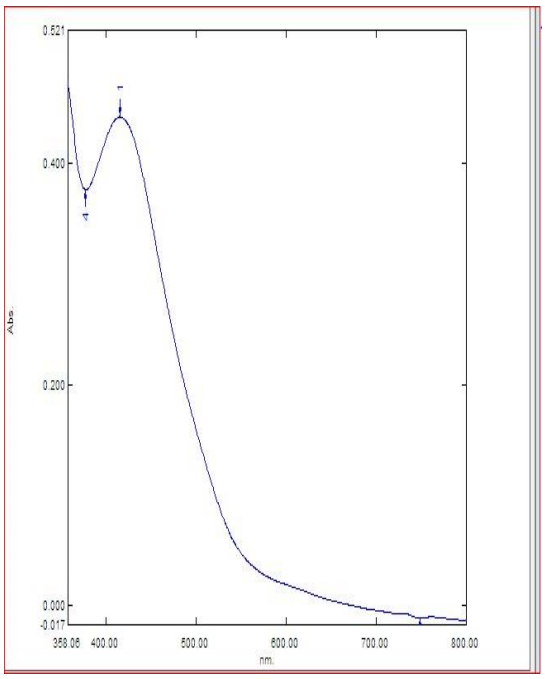

Fig. No.7: Visible spectrum of Fexofenadine hydrochloride tablet derivative at λ max 415nm.

RESULTS AND DISCUSSION

METHOD VALIDATION:

Linearity of Fexofenadine hydrochloride derivative:

Table No. 1 & 2 shows the linearity values of Fexofenadine hydrochloride derivative in both bulk and tablet formulation. The graphs pertaining to them are depicted in Fig. No. 10 &11 respectively.

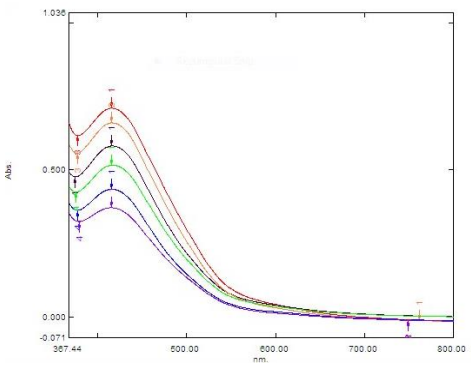

Fig. No. 8: Overlay spectrum of FEX. HCl derivative bulk at various concentrations (10-60µg/mL).

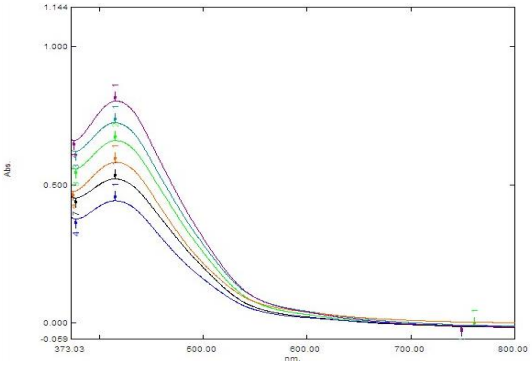

Fig. No. 9: Overlay spectrum of FEX. HCl derivative tablet at various concentrations (10-60µg/mL).

Table No. 1: Linearity of Fexofenadine hydrochloride derivative in bulk.

Concentration (µg/mL)	Absorbance
10	0.368
20	0.435
30	0.506
40	0.58
50	0.64
60	0.711

Table No. 2: Linearity of Fexofenadine hydrochloride derivative in tablet.

Concentration (µg/mL)	Absorbance
10	0.465
20	0.522
30	0.585
40	0.66
50	0.721
60	0.789

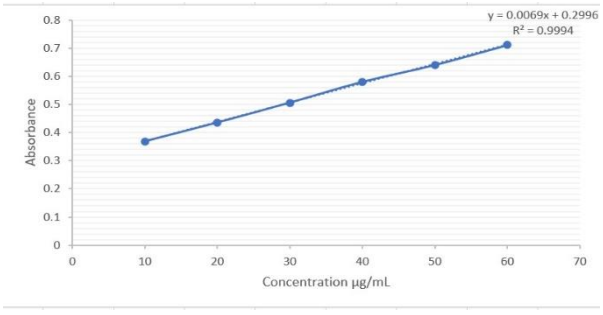

Fig. No. 10: Calibration curve of Fexofenadine hydrochloride derivative in bulk.

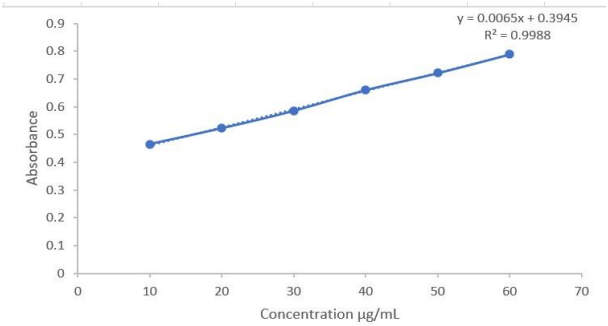

Fig. No. 11: Calibration curve of Fexofenadine hydrochloride derivative in tablet.

Linearity of Fexofenadine hydrochloride derivative in bulk and tablet in ethanol was found to be (10-60) µg/mL. The results are presented in Table No. 1 & 2 and the graph about it is depicted in Fig. No. 10 & 11. The r^2 value was found to be 0.999 & 0.998. Data from the regression line itself provide mathematical estimates of the degree of linearity (reproduce the test results which are directly proportional to the true concentration in the sample).

Precision

Table No. 3: Precision of Fexofenadine hydrochloride derivative in bulk.

Concentration (µg/ml)	**Intra day**	**Inter day**
30	0.511	0.512
30	0.517	0.519
30	0.521	0.524
30	0.524	0.522
30	0.528	0.527
30	0.532	0.533
Mean	0.5221	0.5228
Standard deviation	0.0069	0.0071
% RSD	1.324	1.365

Table No.4: Precision of Fexofenadine hydrochloride derivative in tablet.

Concentration (µg/ml)	**Intra day**	**Inter day**
30	0.572	0.574
30	0.569	0.571
30	0.574	0.575
30	0.577	0.574
30	0.579	0.576
30	0.581	0.583
Mean	0.5753	0.5755
Standard deviation	0.0045	0.0040
% RSD	0.7824	0.7015

The precision of an analytical procedure, when the procedure is applied repeatedly to multiple samples, under the same operating condition of 6 determinations. The results of precision of fexofenadine hydrochloride derivative in bulk and tablet formulation obtained are presented in Tables 3&4. The % RSD values for both the derivatives were found to be ≤2.

ACCURACY:

Table No. 5: Accuracy results of FEX.HCL derivative in bulk and tablets**.**

% Concentration (At specification level)	% Recovery of FEX. HCL derivative Bulk	% Recovery of FEX. HCL derivative Tablets	% Mean recovery of FEX. HCL derivative Bulk	% Mean recovery of FEX. HCL derivative Tablets
80%	98.99	99.42	100.1	101.04
100%	100.25	100.16		
120%	101.06	103.55		

The accuracy results of Fexofenadine hydrochloride derivative in bulk and tablets are presented in Table No.5. The % Recovery in all three concentrations of bulk and three concentrations of tablet formulation was found to be within the limits.

Robustness

Table No. 6: Precision of Fexofenadine hydrochloride derivative in bulk.

Changing Wavelengths (± 2nm)	Bulk Absorbance	Tablet Absorbance
413	0.427	0.516
414	0.430	0.519
415	0.432	0.521
416	0.435	0.524
417	0.434	0.526

Table No.7: Precision of Fexofenadine hydrochloride derivative in bulk.

Change in temperature	Bulk Absorbance	Tablets Absorbance
Room Temperature	0.435	0.491
Sunlight	0.482	0.401
Refrigerator	0.433	0.490

The robustness results of derivatives of Fexofenadine HCl in bulk and tablets (20µg/mL) were performed by changing wavelengths and the results are presented in Table No. 6. No noticeable changes were found on small changes in wavelength. Hence the method was found

to be robust. The robustness results of derivatives of Fexofenadine HCl in bulk and tablets (20µg/mL) were performed by changing its temperature and the results are presented in Table No.7 Upon changing the temperature, it was found that they were stable at room temperature & refrigerator.

Ruggedness:

Table No. 8: Ruggedness results of FEX. HCL derivative in bulk and tablets.

Variation	**Bulk**	**Tablets**
Analyst 1	0.435	0.520
Analyst 2	0.437	0.522

Ruggedness results are presented in Table No.8. The method was found to be reproducible when subjected to different analysts.

LOD & LOQ:

Table No. 9: LOD & LOQ values of Fexofenadine Hydrochloride derivatives in bulk and tablets.

Parameters	**FEX. HCL Derivative BULK**	**FEX. HCL Derivative TABLETS**
LOD (µg/ml)	0.62 µg/ml	0.68 µg/ml
LOQ (µg/ml)	1.88 µg/ml	2.06 µg/ml

The Limit of Detection and Limit of Quantification results are presented in Table No.9.

The method is relatively sensitive in bulk as it displayed lower LOD & LOQ values.

Thin-layer chromatography:

Thin-layer chromatography is performed using Chloroform (non-polar) and methanol (polar) in the ratio (8.5:1.5) as mobile phase for both bulk and formulation Fig.No.12 & 13 respectively. The Rf values are presented in Table-10.

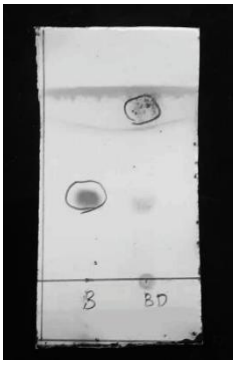

Fig. No. 12: TLC of FEX. HCl bulk & FEX. HCl bulk derivative

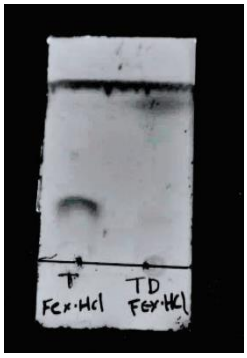

Fig. No. 13: TLC of FEX. HCl of tablet FEX. HCl tablet derivative

B- Fexofenadine HCl bulk

T- Fexofenadine HCl tablet

BD- Fexofenadine HCl bulk derivative

TD- Fexofenadine HCl tablet derivative

Table No. 10: TLC profile (Rf values) of FEX. HCl & FEX. HCl derivatives in bulk and tablet.

Thin-layer chromatography	**Bulk**		**Tablets**	
Rf value	FEX. HCL bulk	FEX. HCL bulk derivative	FEX. HCL tablet	FEX. HCL tablet derivative
	0.354	0.912	0.296	0.937

Forced degradation studies:

The results of the forced degradation studies of FEX. HCl derivatives in bulk and tablets are presented in Tables 11-12.

Table No.11: Forced degradation studies of FEX. HCl derivative in bulk at various time intervals.

Samples	**0hour Nm (Abs)**	**1hour Nm (Abs)**	**3hour nm (Abs)**	**6hour nm (Abs)**	**12hour nm (Abs)**	**24hour nm (Abs)**
HCL	415 nm (0.468)	415 nm (0.422)	415 nm (0.401)	414 nm (0.291)	411 nm (0.344)	409 nm (0.295)
NaOH	415 nm (0.449)	421 nm (0.434)	416 nm (0.373)	418 nm (0.321)	409 nm (0.314)	286 nm (0.392)
H_2O_2	429 nm (0.301)	430 nm (0.327)	431 nm (0.382)	431 nm (0.441)	441 nm (0.317)	441 nm (0.315)
Sunlight (Thermal degradation)	416 nm (0.435)	415 nm (0.434)	412 nm (0.298)	411 nm (0.290)	410 nm (0.285)	409nm (0.279)
Dry heat (Thermal degradation)	415 nm (0.228)	414 nm (0.223)	414 nm (0.198)	406 nm (0.198)	404 nm (0.197)	404 nm (0.196)

Table No. 12: Forced degradation studies of FEX. HCl derivative in tablets at various time intervals.

Samples	**0 hour nm (Abs)**	**1 hour nm (Abs)**	**3 hour nm (Abs)**	**6 hour nm (Abs)**	**12 hour nm (Abs)**	**24 hour nm (Abs)**
HCL	416 nm (0.520)	416 nm (0.452)	415 nm (0.422)	414 nm (0.398)	415 nm (0.380)	410 nm (0.337)
NaOH	415 nm (0.389)	418 nm (0.382)	420 nm (0.331)	415 nm (0.330)	415 nm (0.286)	-
H_2O_2	431 nm (0.501)	432 nm (0.411)	430 nm (0.363)	430 nm (0.298)	444 nm (0.376)	443 nm (0.375)
Sunlight (Thermal degradation)	419 nm (0.459)	419 nm (0.458)	411nm (0.253)	409 nm (0.246)	299 nm (0.669)	299 nm (0.760)
Dry heat (Thermal degradation)	415 nm (0.227)	414 nm (0.221)	414 nm (0.201)	406 nm (0.199)	404 nm (0.196)	404 nm (0.195)

Acid degradation studies of FEX. HCl bulk derivative

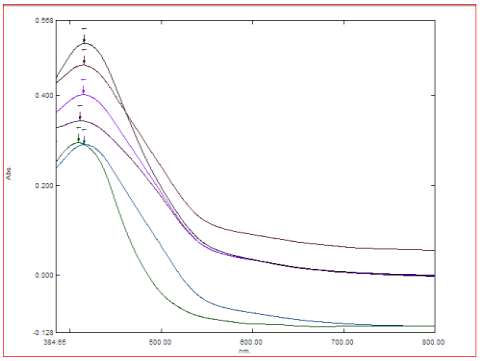

Fig. No. 14: Overlay spectrum of acid degradation studies of Fexofenadine hydrochloride derivative in bulk.

From the color derivative at 6th hour the λ_{max} is decreased, at 24th hour it shows 409 nm it was shifted to lower wavelength produced hypsochromic shift, & absorbance also gets decreased showing a hypochromic shift.

Acid degradation studies of FEX. HCl tablet derivative

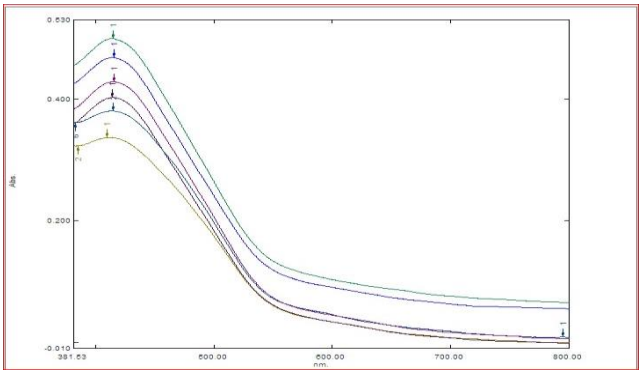

Fig. No. 15: Overlay spectrum of acid degradation studies of Fexofenadine hydrochloride derivative in tablet

From the color derivative at 6th hour the λ_{max} was decreased, at 24th hour it shows 410 nm it was shifted to lower wavelength produced hypsochromic shift, & absorbance also gets decreased showing a hypochromic shift.

Base degradation studies of FEX. HCl bulk derivative

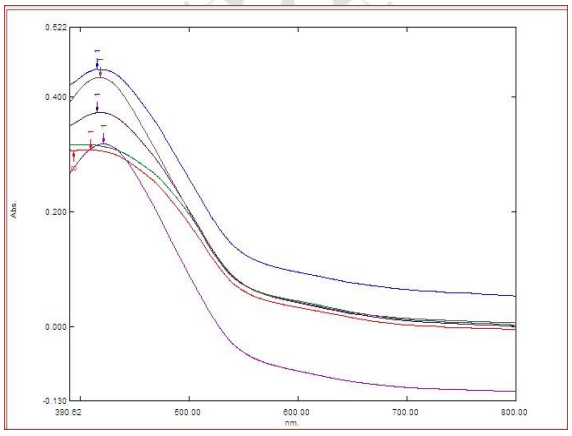

Fig. No. 16: Overlay spectrum of base degradation studies of Fexofenadine hydrochloride derivative in bulk.

From the color derivative at 6th hour the λ_{max} was decreased, at 24th hour it shows 286 nm, the λ_{max} was shifted to lower wavelength (UV region) producing hypsochromic shift. The sensitivity of the analytical method gets decreased, in basic conditions. The color intensity gets decreased.

Base degradation studies of FEX. HCl tablet derivative

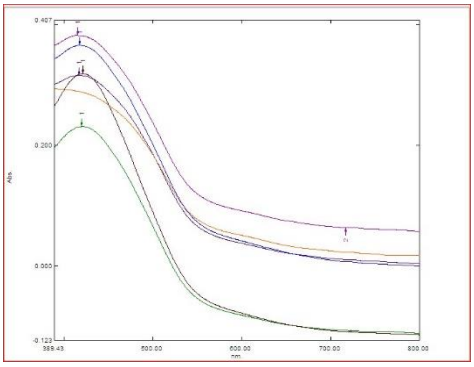

Fig. No. 17: Overlay spectrum of base degradation studies of Fexofenadine hydrochloride derivative in the tablet.

From the color derivative at 6th hour the λ_{max} was decreased, at 24th hour it was not shown any peak. The derivative gets degraded at 24thhr in basic condition because no peak gets absorbed. The color intensity also gets decreased.

Oxidation degradation studies of FEX. HCl bulk derivative

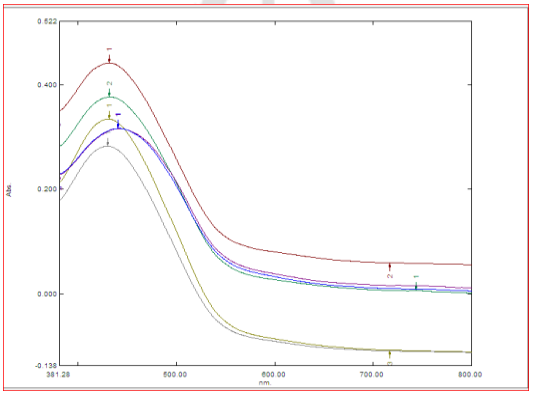

Fig. No. 18: Overlay spectrum of oxidative degradation studies of Fexofenadine hydrochloride derivative in bulk.

From the color derivative λ, the $_{max}$ shifted from 415 to 441nm at the 24th hour the shift to higher wavelength produced a bathochromic shift, in oxidative condition.

Oxidation degradation studies of FEX. HCl tablet derivative:

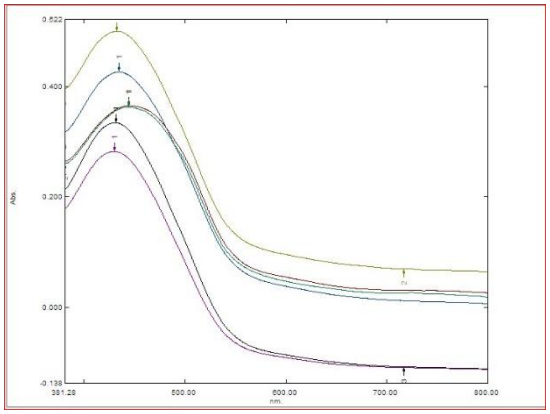

Fig. No. 19: Overlay spectrum of oxidative degradation studies of Fexofenadine hydrochloride derivative in the tablet.

From the color derivative λ, the $_{max}$ shifted from 415 to 443nm at the 24th hour the shift to higher wavelength produced a bathochromic shift, in oxidative condition.

Sunlight bulk degradation studies of FEX. HCl bulk derivative:

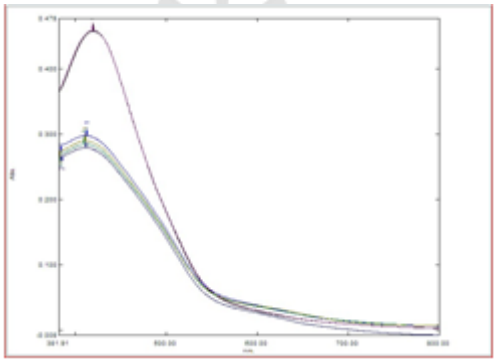

Fig. No. 20: Overlay spectrum of sunlight degradation studies of Fexofenadine hydrochloride derivative in bulk.

From the color derivative at 6th hour the λ $_{max}$ was decreased, at 24th hour it shows 409 nm it was shifted to lower wavelength produced hypsochromic shift, & absorbance also gets decreased showing a hypochromic shift. The sensitivity of the analytical method gets decreased, in sunlight conditions.

Sunlight degradation studies of FEX. HCl tablet derivative

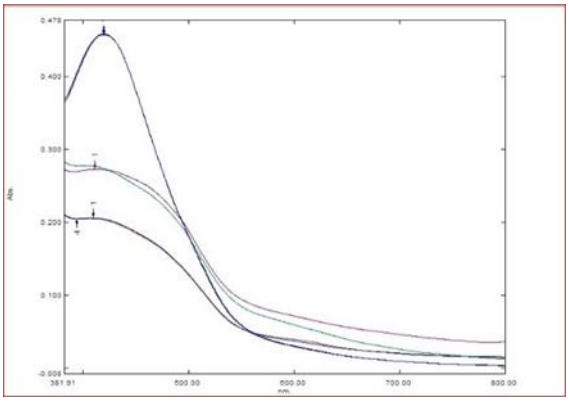

Fig. No. 21: Overlay spectrum of sunlight degradation studies of fexofenadine hydrochloride derivative in the tablet.

From the color derivative at 6th hour the λ_{max} is decreased, at 24th hour it shows 299 nm it shifts to lower wavelength (hypsochromic shift), & absorbance also gets decreased shows a hypochromic shift. The sensitivity of the analytical method gets decreased, in sunlight conditions.

Hot air oven degradation studies of FEX. HCl bulk derivative

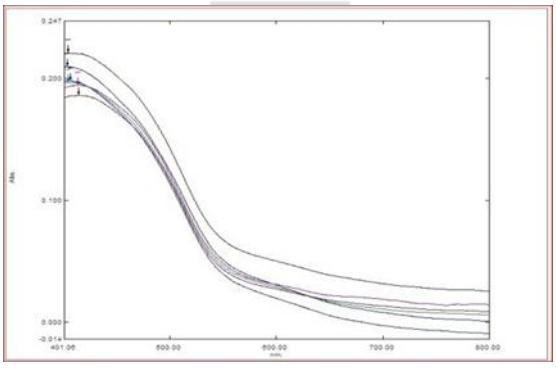

Fig. No. 22: Overlay spectrum of hot air oven degradation studies of Fexofenadine hydrochloride derivative in bulk.

From the color derivative at 6th hour the λ_{max} was decreased, at 24th hour it shows 404nm it shifts to lower wavelength produce a hypsochromic shift, Absorbance also gets decreased shows a hypochromic shift in hot air oven condition.

Hot air oven degradation studies of FEX. HCl tablet derivative

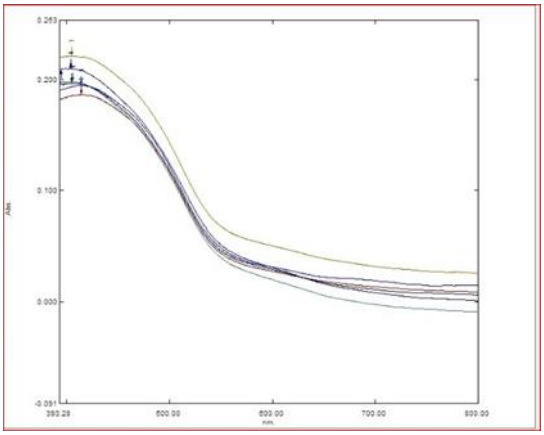

Fig. No. 23: Overlay spectrum of hot air oven degradation studies of Fexofenadine hydrochloride derivative in the tablet.

From the color derivative at 6^{th} hour the λ_{max} was decreased, at 24^{h} hour it shows 404 nm it was shifted to a lower wavelength produce a hypsochromic shift, Absorbance also gets decreased shows a hypochromic shift in hot air oven condition.

The validation parameters for Fexofenadine Hydrochloride in bulk and solid dosage form are presented in Table-17.

Table. No. 13: Summary of Validation Parameters for Fexofenadine Hydrochloride in bulk and solid dosage form.

Parameters	**FEX. HCl derivative Bulk form**	**FEX. HCl derivative Solid dosage form**
Wavelength (λ_{max})	415 nm	415 nm
Linearity (µg/mL)	10-60	10-60
Regression equation (y=mx+c)	0.0069x+0.2996	0.0065x+0.3945
Slope (m)	0.0068	0.0065
Correlation coefficient (r^2)	0.9994	0.9988
%RSD	1.32	0.78
LOD (µg/mL)	0.62	0.68
LOQ (µg/mL)	1.88	2.06
% Recovery	100.1	99.9

CONCLUSION

A simple UV-Visible Spectrophotometric method was developed for the estimation of Fexofenadine Hydrochloride in bulk and tablets by UV-Visible spectroscopy using chemical derivatization with reagents DCC and 2-NPH. The method is validated in terms of linearity, repeatability, precision, accuracy, robustness, ruggedness, LOD and LOQ, and stability studies. All the parameters' values were found to be within the limits according to ICH guidelines. The proposed spectrophotometric method was simple, accurate, precise, sensitive, robust, and economic. Hence this method can be used for routine analysis for the estimation of Fexofenadine Hydrochloride in bulk and tablets in a UV-Visible spectrophotometer.

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