CHAPTER –V

PART–A: Assay of Fexofenadine in bulk and dosage forms by visible spectrophotometry

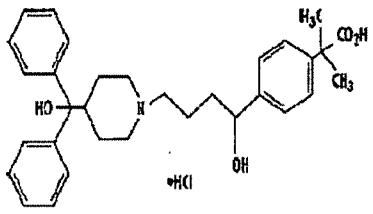
&

PART–B: RP-HPLC method for the determination of fexofenadine [FEFD] in bulk and pharmaceutical dosage forms

Part - A: Assay of Fexofenadine in bulk and dosage forms by visible spectrophotometry

5.01.A: Drug Profile:

Fexofenadine HCL (**FEFD**), chemically designated as **(±)-4-[1-hydroxy-4-(4-hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-∝,∝-dimethyl benzeneacetic acid hydrochloride**[1-2] is a histamine H1 receptor antagonist used in patients with allergic rhinitis. The structural features, category; certain characteristics, therapeutic importance and commercially available formulations of FEFD are compiled in **Table 5.01(P. 257).**



Literature survey reveals only few analytical methods has been described for its determination in pharmaceutical dosage forms and in biological fluids. In pharmaceutical dosage forms, it was quantified by ion - complex reactions [3-6], capillary electrophoresis [7-9], anodic voltammetry [10], new polymeric membrane [11,12] and HPLC with ultraviolet detection [13-15]. In biological fluids, fexofenadine hydrochloride has been determined employing HPLC with different detections, including ultraviolet [16], mass spectrometry [17-19] fluorescence [20] and spectrophotometric method [21-23]. Existing analytical methods reveal that little attention was paid in developing visible spectrophotometric methods by exploring the analytically useful functional groups in FEFD, which prompted the author to

carry out experiments in this accord. The author developed three simple and sensitive spectrophotometric methods for the determination of FEFD in pure or pharmaceutical formulations. The proposed methods will be useful in the field of drug analysis due to their simplicity, low cost and relatively short analysis time when compared with other techniques.

5.02.A: Experimental:

a. Instruments used: An Elico, UV - Visible digital spectrophotometer [SL - 159] with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

b. Preparation of standard drug solutions: Accurately weighed quantity of FEFD (100mg) was dissolved in methanol (10.0mL) in a volumetric flask (100mL) and volume was made upto the mark with distilled water and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 50µg/mL(M_{2b} & M_{2c}) and 100µg/mL(M_{18}) respectively.

c. Sample Solution: Twenty tablets were weighed and finely powdered. A quantity of tablet powder equivalent to 100mg of FEFD taken in volumetric flask (100mL) was shaken with methanol (10.0mL) for 10min and the volume was made upto the mark with distilled water. The solution was

then filtered through whatman filter paper and the aliquot portion of the filtrate was diluted to 100.0mL with distilled water to get sample solution of concentration of 50µg/mL(M_{2b} & M_{2c}) and 100µg/mL(M_{18}) respectively.

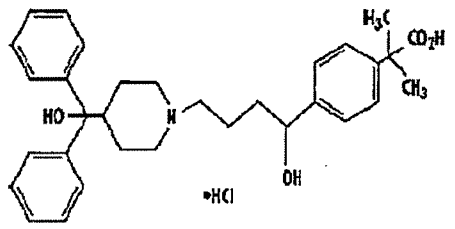
d. Preparation of reagents: All the chemicals and reagents used were of analytical grade and solutions were prepared in triply distilled water, isopropyl alcohol or chloroform.

Method M_{2b}: Solution of various reagents such as MB solution, (Fluka; 0.2%, 6.25×10^{-3}M), Buffer solution (pH - 9.4) were prepared in the same way as described under **ACFN** in **Chapter - III (P. 157)**.

Method M_{2c}: Solution of various reagents such as MV solution, (Fluka; 0.2%, 5.24×10^{-3}M), Buffer solution (pH - 9.4) were prepared in the same way as described under optimum conditions.

Method M_{18}: Solution of various reagents such as CTC solution (2.5×10^{-1}M), Buffer solution (pH 2.0) were prepared in the same way as described under ACFN in **Chapter - III (P. 159)**.

Table - 5.01
Pharmaceutical Information

Proper Name	**Fexofenadine hydrochloride**
Chemical Name	(±)-4-[1 nylmethyl)-1-piperidinyl]-butyl]- α, α-dimethylhydroxy-4-benzeneacetic acid hydrochloride
Structure	
Molecular Formulae	$C_{32}H_{39}NO_4 \cdot HCl$
Molecular Weight	538.13
Description	Fexofenadine hydrochloride is a white to off-white crystalline powder. It is freely soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane.
Melting point	--------
Commercially available formulations	ALLEGRA is formulated as a tablet for oral administration. Each tablet contains 30, 60, or 180 mg fexofenadine hydrochloride
Analytically useful functional groups	Tertiary Amine, Hydroxyl and Carboxylic group.

5.03.A: Proposed procedures:

After systematic and detailed study of the various parameters involved, the following procedures {M_{2b}[MB], M_{2c}[MV] and M_{18}[CTC]} were recommended for the assay of **FEFD** in bulk samples and pharmaceutical formulations.

Method - M_{2b} [MB]: Similar procedure described above was followed for the accurately measured portion of standard solution of FEFD (0.5 - 3.0mL,50μg.mL^{-1}) using MB dye solution (1.0mL) and buffer solution (1.0mL, pH 9.8). The absorbance of each organic layer was measured in 1.0cm cell at 610nm against blank. The amount of drug (FEFD) was computed from the Beer's Lambert plot. (**Fig.5.05,P.262** for M_{2b}).

Method - M_{2c} [MV]: Aliquots of FEFD solution (0.5 - 3.0mL,50μg.mL^{-1}) were transferred separately in a series of 125mL separating funnels, then added 1.0mL of pH-9.8 buffer solution, 1.0mL of Methylene Violet (for method M_{2c}) separately to the above separating funnels respectively. The total volume of aqueous phase in each funnel was adjusted to 10.0mL with distilled water. Then 10.0mL of chloroform was added in each separating funnel and the contents were shaken for 20min and allowed to separate. The separated layers were collected in dry test tubes containing anhydrous sodium sulphate. The absorbance of each organic layer was

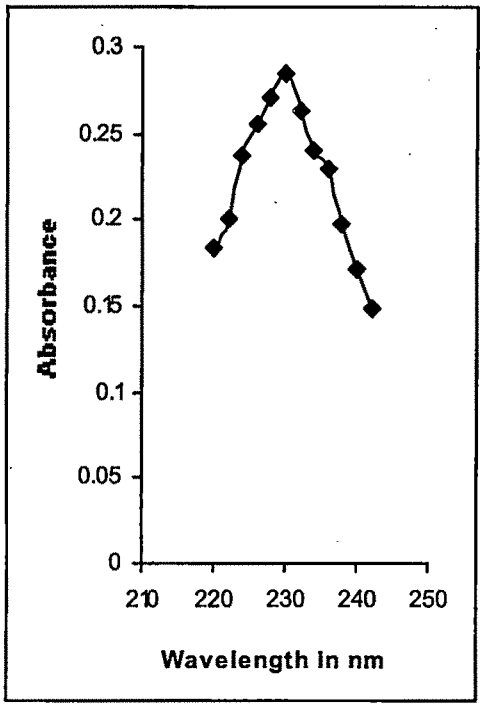
measured in 1.0 cm cell at 480nm against blank. The colored species were stable for 2 hours. The amount of drug in a sample was obtained from the Beer's Lambert plot. **(Fig.5.05,P.262).**

Method - M_{18} [CTC]: Into a series of 125mL separating funnels, aliquots of standard **FEFD** solution (0.5 -3.0mL, 100µg.mL^{-1}) were taken. Then 2.0mL of buffer (pH 2.0) and 5.0mL (2.5 x 10^{-1}M) of CTC solutions were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0mL with distilled water. To each separating funnel,10.0mL of nitrobenzene was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured at 620nm against a similar reagent blank. The amount of **FEFD** was computed from its calibration graph **(Fig.5.07,P.262).**

Procedure for the assay of FEFD in formulations

An accurately weighed portion of powdered tablets equivalent to 100mg of FEFD was dissolved in 20mL of methanol (MeOH), shaken well and filtererd, the filtrate was diluted to 100ml with MeOH to get 1mg/mL of drug in formulations.0.5mL of this solution was diluted to 100ml to get 5.0µg/mL.The absorbance of the solution was determined at λ_{max} 230nm.The quantity of was computed from Beers law of standard drug in MeOH.

Absorption spectra of FEFD in CH_3OH (UV reference method)



Beer's law plot of FEFD in CH_3OH (UV reference method)

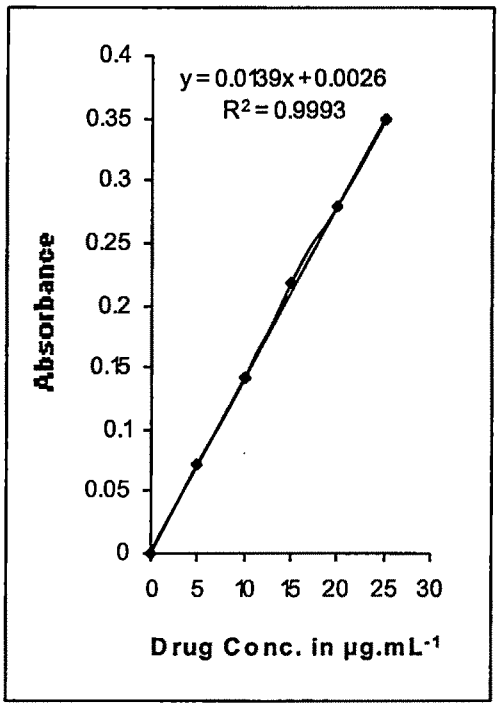


Fig.5.02: Absorption spectrum of FEFD with MB (M_{2b})

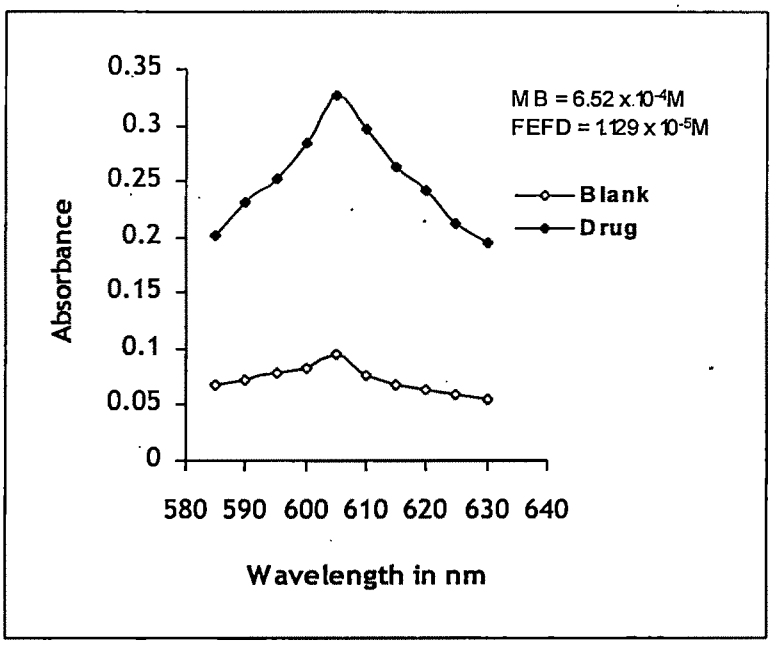


Fig.5.03: Absorption spectrum of FEFD with MV (M_{2c})

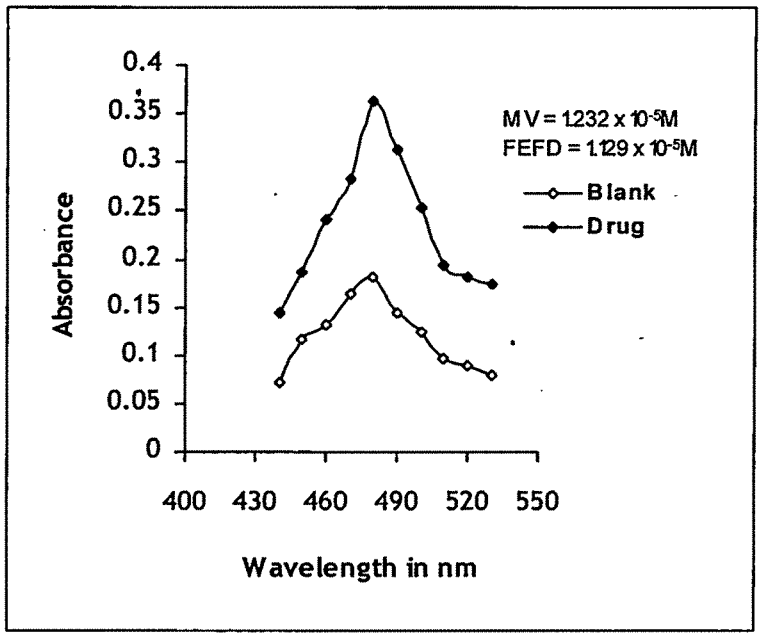


Fig.5.04: Absorption spectrum of FEFD with CTC (M_{18})

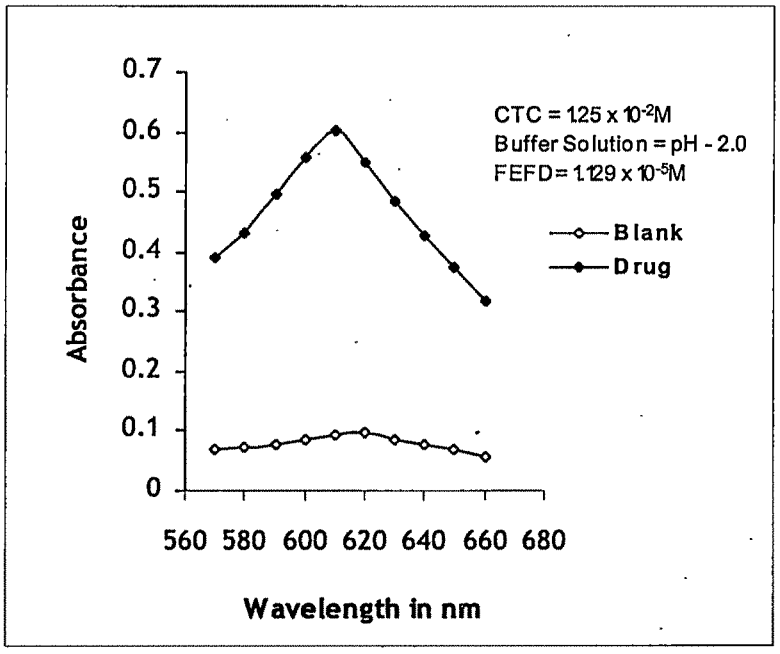


Fig. 5.05: Beer's Law plot of FEFD with MB (M_{2b})

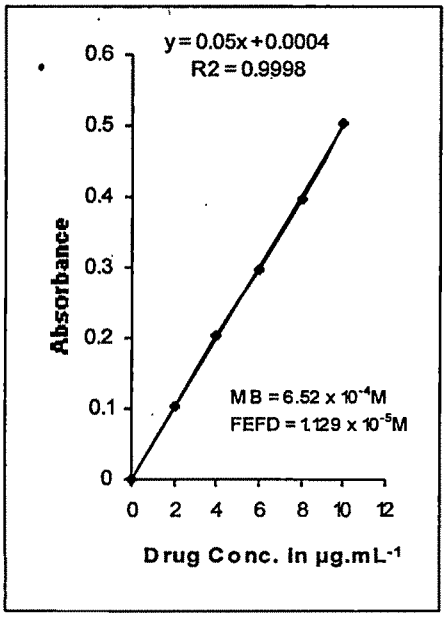


Fig. 5.06: Beer's Law plot of FEFD with MV (M_{2c})

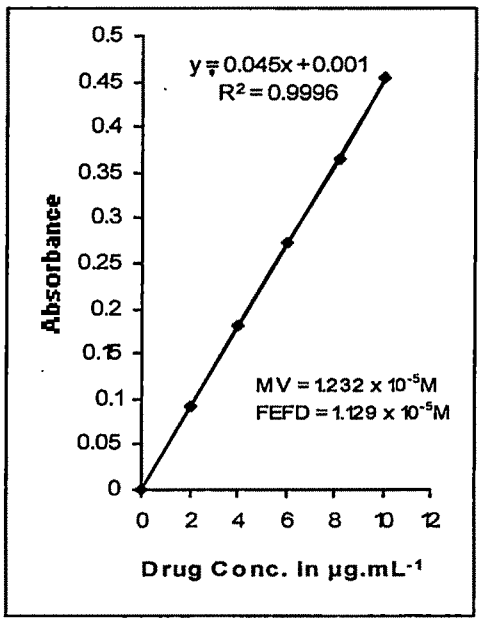


Fig. 5.07: Beer's Law plot of FEFD with CTC (M_{18})

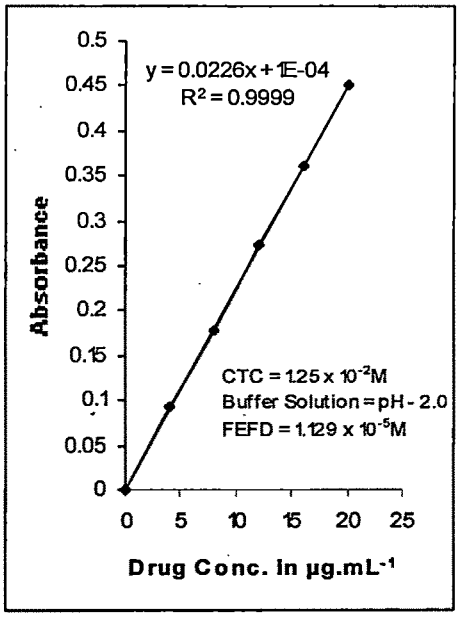


Fig. 5.08: Ringbom plot of FEFD with MB (M_{2b})

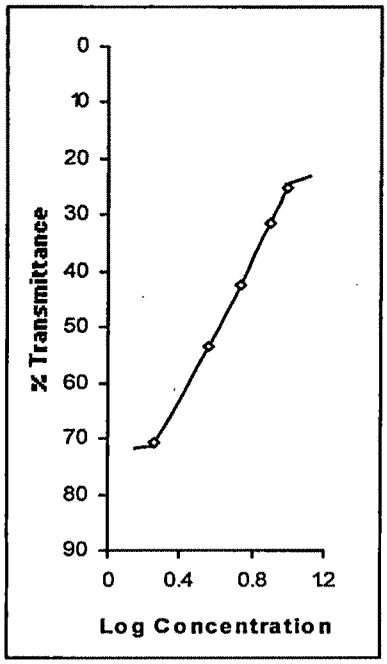


Fig.5.09: Ringbom plot of FEFD with MV (M_{2c})

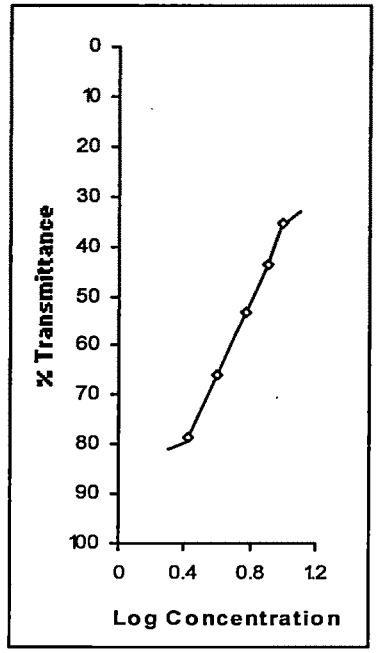
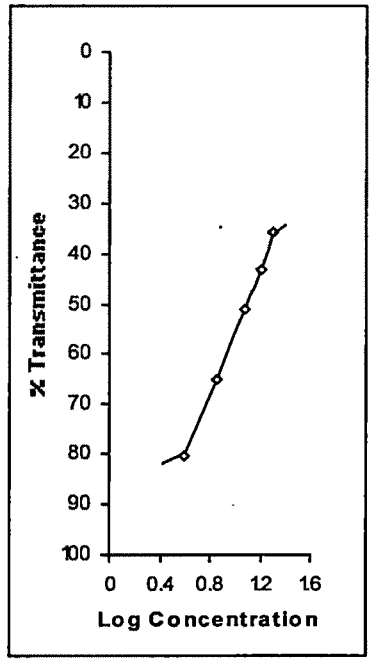


Fig. 5.10: Ringbom plot of FEFD with CTC (M_{18})



5.04.A: Results and Discussions:

i) Spectral Characteristics: In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of FEFD were taken and colors were developed separately by following the above procedures. The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in **Fig.5.01** to **5.03,P. 260 - 261.** The absorption curves of the colored species in each method show characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

ii) Optimum conditions fixation in procedures: The optimum conditions for the color development of methods (M_{2b}, M_{2c} and M_{18}) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following studies were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Method - M_{2b} [MB]:

The optimum conditions established for method M_{2b} were found to be same as described in **(Chapter - III, Table.3.02, P.174).**

Method - M_{2c} [MV]:

The optimum conditions established for method M_{2c} were found to be same as Methylene blue {**Method - M_{2b}**} and described in **Table.5.02, P.270**).

Method - M_{18} [CTC];

The optimum conditions established for method M_{18} were found to be same as described in (**Chapter - III, Table 3.09, P.180**).

iii) Optical Characteristics: In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbances at appropriate wave lengths of a set of solutions containing varying amounts of **FEFD** and specified amounts of reagents (as given in the proposed procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blank. (**Figs.5.05** to **5.07,P.262**).The optimum photometric ranges of these systems are recorded graphically (**Figs.5.08** to **5.10,P.263**). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for **FEFD** in each method developed with mentioned reagents were calculated and are tabulated in (**Table.5.03,P.271**). Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values. (**Table.5.03,P.271**).

iv.Precision: The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of FEFD' solution. The percent relative standard deviation and percent range error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table.5.03, P. 271**).

v.Accuracy: To determine the accuracy of each proposed method, different amounts of bulk samples of FEFD within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in (**Table.5.03, P.271**).

vi. Interference studies: The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of FEFD in methods (M_{2b}, M_{2c} and M_{18}) under optimum conditions were investigated. The commonly used execipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

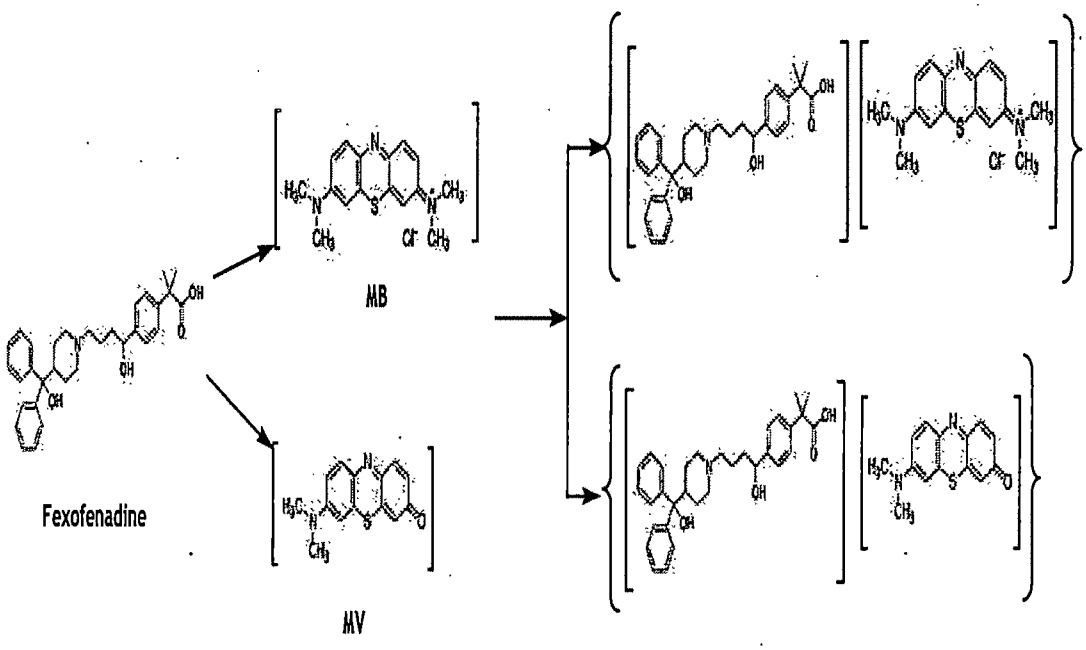
vii. Analysis of formulations: Commercial formulations (tablets) containing FEFD were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found to be not different

significantly. The results are summarized in (**Table.5.04,P.272**). Percent recoveries were determined by adding standard drug to preanalysed formulations. The results of the recovery experiments by the proposed methods are also listed in (**Table.5.04, P.272**).

viii. Recovery studies: Recovery studies were conducted by analyzing each pharmaceutical formulation in the instance for the active ingredient by the proposed methods. Known amount of pure drug was added to each previously analyzed formulation and the total amount of the drug was once again determined by all proposed methods after bringing the active ingredient concentration within the Beer's law limits. The results are reported in **Table.5.04, P.272.**

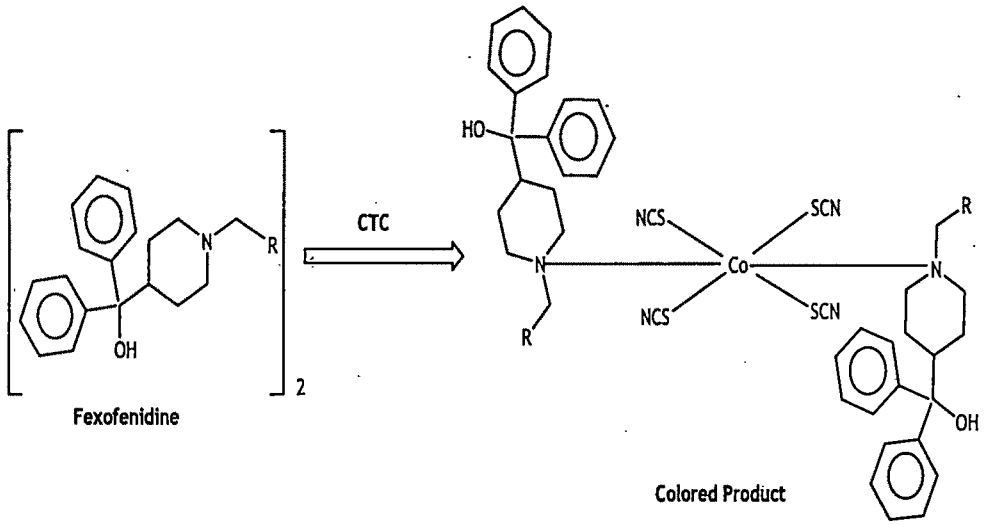
ix. Nature of color reactions: An attempt has been made to indicate the nature of colored species in each proposed method for FEFD tentatively based on analogy (reactive functional moiety in drug, reagents nature) and probable relative reactvities of functional moieties one over the other.

Method - M_{2b} and M_{2c} [MB & MV]: As FEFD possesses carboxyl group (acidic) is responsible for color formation in ion association complex with basic dyes (Methylene blue and Methylene Violet), which is extractable into chloroform from aqueous phase. The carboxylate anion (negative charge) of FEFD is expected to attract the oppositely charged part of the dye [positive charge[**Methylene blue (M_{2b}) and Methylene violet (M_{2c})**] and behave as single unit being held together by electrostatic attraction. The formation of ion association complex may be postulated as shown in **Scheme 5.01.**



Method - M_{18} [CTC or $(Co[SCN]_2]$]: Cobalt thiocynate (CTC) (formed by combination of ammonium thiocyante and cobalt nitrate) has been proved to be valuable reagent for the detection and determination of amino

compound. The colored species formed is the coordination complex of the drug (electron donor) and the central atom of cobalt thiocyanite, which is extractable into nitrobenzene from aqueous solution. Formation of the colored complex is obtained when FEFD is treated with CTC due to the presence of teritary amine group is the basis in the present investigation and is presented in **Scheme - 5.02.**



Fexofenidine

Colored Product

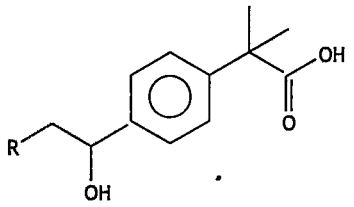


Table.5.02

Optimum conditions established for method M_{2c} for FEFD

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm) M_{2c}	650 - 660nm	655nm	--
Effect of buffer on color development	9.0 - 10.0	pH-9.8	Variations of the pH<6 and >11 resulted in low absorbance values
Volume of buffer required for maximum intensity of color (mL)	0.5 - 1.5	1.0	Optimum volume of 1.0mL of buffer was sufficient for maximum color development
Effect of vol of dye MV (M_{2c})	0.1 - 1.0	0.5	0.5mL of MB (for M_{2b}) dye was necessary for covering the broad range of beer's law limits.
Choice of organic solvent for extraction of colored complex	Chloroform	Chloroform	Water immiscible solvents tested for the extraction of the colored complex into organic phase and $CHCl_3$ was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of the ratio of organic to aqueous phase on extraction	1:1	1:1	The extraction of the colored species in to Chloroform layer was in complete when the ratio of chloroform to aqueous phase was more than the specified ratio in each case.
Effect of shaking time (min)	1 - 5	2	Constant absorbance values were obtained for the shaking period of 1-5 min.
Effect of temperature on the colored species (C^0)	Lab-Temp (28±5)	Lab-Temp (28±5)	At low temperature (<20^{0}C) and at high temperature (>35^{0}C) the extraction of the colored species was found to be improper and the stability of the colored species was found to be very less.
Stability of the colored species	Immediate to 60min	10min	The colored species after separation from organic phase was stable for 60 min, after wards the absorbance gradually decreases.

Table.5.03

Optical and regression characteristics, precision and accuracy of the proposed methods for FEFD

Parameter	M_{2b}	M_{2c}	M_{18}
λ_{max} (nm)	610	480	525
Beer's law limits (µg/mL)	2 - 10	2 - 10	5 - 25
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	9.90×10^4	8.32×10^4	9.94×10^4
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.1223	0.3936	0.1222
Optimum photometric range (µg/mL)	4.5 - 9.0	3.5 - 8.5	4.5- 20.5
Regression equation (Y=a+bc);slope (b)	0.005	0.0045	0.0226
Intercept (a)	0.0004	0.001	0.0004
Correlation coefficient (r)	0.9998	0.9996	0.9999
Relative standard deviation (%)	0.6349	0.7154	0.3314
% Range of error (confidence limits)			
0.05 level	0.6666	0.7510	0.3479
0.01 level	1.0454	1.1778	0.5456

*Average of six determinations considered

Table.5.04

Assay of FEFD in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**			Reference method	Percentage recovery by proposed methods***		
		M_{10}	M_{13}			M_{10}		M_{13}
Tablet I	30	29.71±0.10 F=3.61 t=1.79	29.74±0.12 F=2.51 t=1.34	29.81±0.14 F=1.84 t=0.525	29.86±0.19	100.16±0.35	99.49±0.09	99.59±0.12

* Tablets from four different pharmaceutical companies

** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

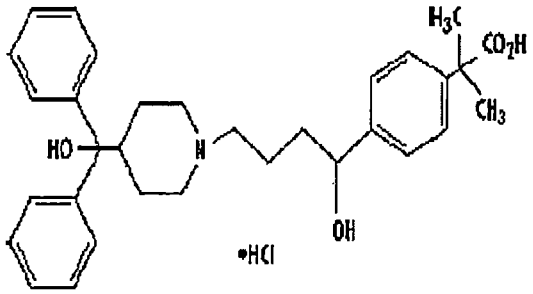
5.05.A:Conclusions:

It is concluded from the statistical results that the proposed spectrophotometric methods were found to be most sensitive, accurate and precise. However, proposed methods which are validated seem to be quite sensitive and can be successfully applied for the routine analysis of **Fexofenadine.HCL** in its tablet formulation. The proposed methods were found to be made most accurate by the result of recovery study shown in Table

Part - B:RP-HPLC method [Method M_{24}] for the determination of Fexofenadine in bulk and pharmaceutical dosage forms

5.01.B:Introduction:

Fexofenadine HCL (**FEFD**), chemically designated as **(±)-4-[1-hydroxy-4-(4-hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α-α- dimethyl benzeneacetic acid hydrochloride**[1] is a histamine H1 receptor antagonist used in patients with allergic rhinitis. The structural features, category; certain characteristics, therapeutic importance and commercially available formulations of BIC are compiled in **Table 5.01(P. 242).**



Some HPLC [14-20]. methods appeared in the literature for the assay of fexofenidine in biological fluids and this made the author to develop to a simple, precise and accurate with high sensitivity and selectivity reversed-phase HPLC method for the estimation of Fexofenadine in bulk and in pharmaceutical formulations.

5.02.B:Experimental

a) Instrumentation:

Quantitative HPLC was performed on a **Waters/Agilent HPLC**, auto sampler equipped with a 20µmL sample loop, with SPD10AVP dual absorbance detector. The output signal was monitored and integrated using Shimadzu CLASS - VP - version 6.12, SP-1 software.

b) Reagents used:

1) Water HPLC grade (Triple distilled water) [Qualigens].

2) Methanol HPLC grade [E.Merck].

3) Ammoniu Acetate [E.Merck]

c)Preparation of Standard drug solution (stock):

Weigh and transfer accurately about 50mg of Fexofenadine standard in to a 50mL volumetric flask. Dissolve and make upto the mark with diluent. Transfer 10.0mL of this solution into a 50mL volumetric flask and make up to the volume with diluent.

d) Preparation of Sample preparation:

Weigh and transfer accurately about 50mg of Fexofenadine sample in to a 50mL volumetric flask. Dissolve and make upto the mark with diluent. Transfer 10.0mL of this solution into a 50mL volumetric flask and make up to the volume with diluent.

Table - 5.05

Chromatographic conditions

Parameters	Method
Buffer	Prepare 0.05M Ammonium Acetate buffer and filter through 0.45 µm porosity membrane filter.
Mobile phase	**Solvent Mixture:** Methanol . **Mobile phase:** Prepare the mixture of buffer and solvent mixture in the ratio of 40:60 v/v.
Diluent	A mixture of buffer and Acetonitrile in the ration of 70:30v/v.
Stationary phase (Column)	Inertsil ODS 3V (C_8, 250 x 4.6 mm, 5µm).
Injection volume	20µL
Flow rate	1.0mL/minute.
Column temperature (°C)	Ambient.
Column Pressure(psi)	1450
Detection wavelength(run)	230nm
Run time	30.0 min
Drug retention time	12.120 min

5.03.B:Method development:

The contents of the mobile phase were filtered before use through 0.45µm membrane filter, degassed with a vaccum pump and pumped from the respective solvent reservoir to the column at a specified flow rate

prior to the injection of the drug solution; the column was equilibrated for at least 30minutes with the appropriate mobile phase flowing through the systems. The prepared dilutions containing concentrations of fexofenadine in the range of 1 - 5µg/mL were injected in to the chromatograph. The peak area ratios of the standard were calculated. The developed method was validated and the results obtained are tabulated in **Table -2, P.196.**

5.04.B: Results and Discussion:

i) Preparation of standard curve:

Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase Buffer (pH 2.5): Methanol (40:60) was passed through the 0.45µm membrane filter using Millipore HPLC solvent filtration assembly, was delivered at 1.0mL/min for column stabilization. During this period, the base line was continuously monitored. The wavelength of detection was selected at 230nm. The prepared dilutions containing concentrations of fexofenadine in the range 1 - 5µg/mL were injected into the chromatograph. The peak area ratios were calculated and the results are tabulated in **Table.5.06, P.281.** The stability of the solution of fexofenadine during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration. Chromatogram parameters, retention time and asymmetry factor were standardized. The amount of the drug present in

each pharmaceutical formulation was calculated through peak area ratio of component by making use of the standard calibration curve.

ii) Method validation:

a) Linearity: Five separate series of solutions of the drug 1 - 5µg/mL were prepared from the stock and analyzed. The linear regression equation obtained for the proposed method was **Y = 80.115 + 2527.7 x** .The developed method was validated according to the standard procedures and the results are tabulated in **Table.5.06, P.281.**

b) Specificity: Series of five solutions of drug in 5µg/mL were prepared from stock solution meant for the method validation and analyzed.

c) Accuracy and Precision: Five separate solutions of fexofenadine (5µg/mL) standard and the test solution were prepared in duplicate from freshly prepared stock and analyzed as per the procedure and the results are given in the **Table.5.07,P.282.**

d) Recovery studies: The method was evaluated by estimation of fexofenadine in pharmaceutical formulations by the proposed method and analysis of pure drug solution as reference. The results are presented in **Table.5.07,P.282.** The estimated drug content with low values of standard deviation has established the precision method. The accuracy of results of estimation was further tested by recovery experiments by

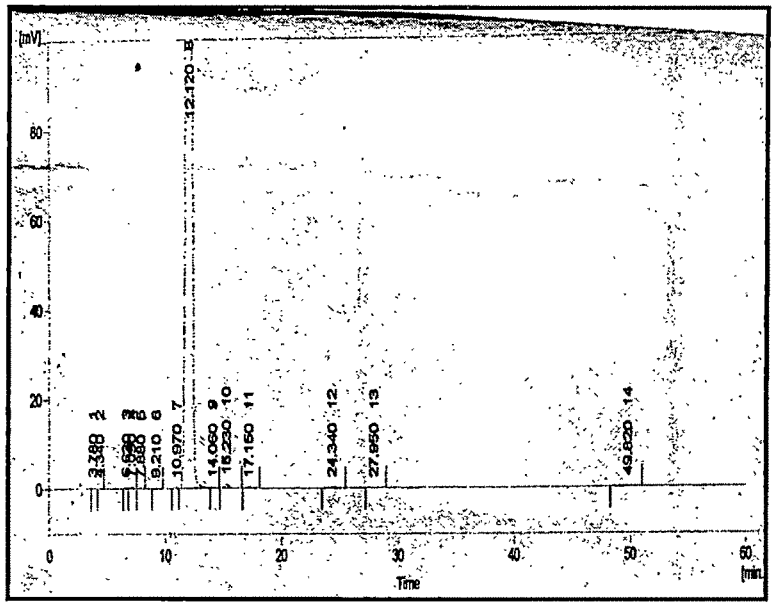
adding known amount of pure drug to pre-analyzed samples of formulations. Common formulation exciepients in the concentration normally did not effect. Recovery experiments using the developed assay procedures further indicated the absence of interference from commonly encountered pharmaceutical exciepients used in the selected formulations

e) Interference studies: The effect of wide range of exciepients and other additives usually present in the formulation of fexofenadine in the determinations under optimum conditions were investigated. The common exciepients like glycolte starch, magnesium steartate, microcrystalline and starch have been added to the sample and injected. They have not disturbed the elution of quantification of drug. In fact many have no absorption at this UV maximum.

Estimation of fexofenadine from commercial formulations by the proposed method:

Ten tablets are weighed to get the average weight and puliverized. The sample powder, claimed to contain 50mg of active ingredient was transferred into 50mL volumetric flask and dilute to volume with diluent by sonicating. This solution was further diluted stepwise with diluent as under preparation of standard solutions to get different required. The area under the curve, the drug content per each tablet was calculated.

Fig.5.11: Model chromatogram for Fexofenadine



5.12:Standard calibration graph of Fexofenadine

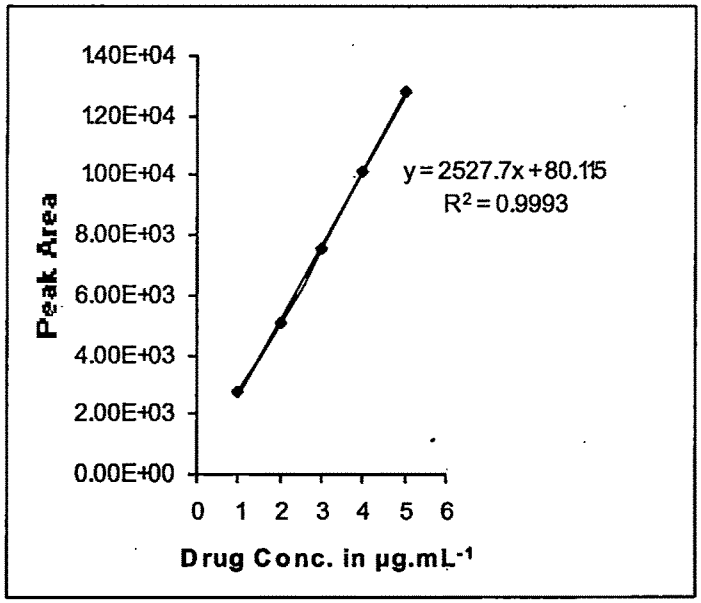


Table - 5.06

Optical and Regression Characteristics of the proposed HPLC Method(M_{24})

Parameter	Method
Detection wavelength (nm)	230
Linearity range(µg/mL)	1 - 5
Detection limits	0.016
Regression equation (Y=a+bc) Slope (b)	2527.75
Intercept (a)	80.115
Correlation coefficient (r)	0.9993

Estimation of fexofenadine from commercial formulations by the proposed method:

Ten tablets are weighed to get the average weight and puliverized. The sample powder, claimed to contain 50mg of active ingredient was transferred into 50mL volumetric flask and dilute to volume with diluent by sonicating. This solution was further diluted stepwise with diluent as under preparation of standard solutions to get different required. The area under the curve, the drug content per each tablet was calculated.

Table - 5.07

Assay and Recovery of fexofenadine

Pharmaceutical formulation	Label Claim	% Label • Claim found	Standard Deviation	Standard error	% Recovery of proposed method
Tablet	100mg	99.98	0.2905	0.1677	99.98

5.05.B:Conclusions:

The analytical method for the determination of fexofenadine in pharmaceutical formulations demonstrated linearity, precision and accuracy under the analytical conditions of the process, which include the evaluation of the active principle by high performance liquid chromatography with ultraviolet detector and addition of standard conditions non reported in literature until the moment, what means an important development for its application in bioavailability and bioequivalence studies of fexofenadine products.

References

1. **United State Pharmacopeial Formulation** (1999) Vol 25, No 23, 8050.

2. **European pharmacopoeia** (2005) 5th edn vol 2, 1321.

3.Gazy AA, Mahgoub H, El-Yazbi FA, El-Sayed MA, Youssef RM (2002). **J Pharm Biomed Ana,** 30: 859-867.

4. L.D.SRINIVAS, P.RAVI KUMAR and B.S.SASTRY, **E-Journal of Chemistry** Vol. 2, No. 8, pp 199-202, 2005.

5. PV Polawar, UD Shivhare, KP Bhusari and VB Mathur, **Research J. Pharm. and Tech.** 1(4): Oct.-Dec. 2008.

6.B..Narayana and Veena, **Indian journal of Chemical Technology,** Vol. 17, No. 8, pp 386-39., 2009.

7. Mikus P, Valaskova I, Havranek E (2005) , **Drug Dev Ind Pharm,** 31: 795-801.

8. Breier AR, Garcia SS, Jablonski A, Steppe M, Schapoval EE (2005). **J AOAC Int,** 88: 1059-1063.

9. Breier AR, Paim CS, Menegola J, Steppe M, Schapoval EE (2004). **J AOAC Int,** 87: 1093-1097.

10. Golcu A, Dogan B, Ozkan SA (2005). **Anal Lett 38**: 1913-1931.

11. Cherkaoui S, Veuthy JL (2002). **Electrophoresis,** 23: 442-445.

12. Abbas MN, Abdel Fattah AA, Zahran E (2004). **Anal Sci,** 20: 1137-1142.

13. Maggioa RM, Castellano PM, Vignaduzzo SE, Kaufman TS (2007), J **Pharm Biomed Anal,** 45: 804-810.

14. Radhakrishna T, Om Reddy G (2002). **J Pharm Biomed Anal,** 29: 681-690.

15. Breier AR, Paim CS, Steppe M, Schapoval EE (2005) , **J Pharm Pharm Sci,** 8: 289-298.

16. Yu HW, Ching CB (2001). **Chromatographia,** 54: 697-702.

17. Miura M, Uno T, Tateishi T, Suzuki T (2007) ,**J Pharm Biomed Anal,** 43: 741-745.

18. Hofmann U, Seiler M, Drescher S, Fromm MF (2002). **J Chromatogr B Analyt Technol Biomed Life Sci,** 766: 227-233.

19. Gergov M, Robson JN, Ojanpera I, Heinonen OP, Vuori E (2001). **Forensic Sci In,** 121: 108-115.

20. Fu I, Woolf EJ, Matuszewski BK (2004) , **J Pharm Biomed Anal,** 35: 837-846.

21. Uno T, Yasui-Furukori N, Takahata T, Sugawara K, Tateishi T (2004), **J Pharm Biomed Anal,** 35: 937-942.

22. Kumar KS, Ravichandran V, Raja MKMM, Thyagu R, Dharamsi A (2006), **Indian J Pharm Sci,** 68: 841-842.

23. Maggio RM, Castellano PM, Vignaduzzo SE, Kaufman TS (2007). **J Pharm Biomed Anal,** 45: 804-810.