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Method Development, Validation and Rapid Determination of Fexofenadine Hydrochloride Drug By Chromatographic Technique.

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ABSTRACT:

A simple, sensitive,precise and accurate Reverse phase liquid chromatographic method for the analysis of Fexofenadine Hydrochloride has been developed and validate, This method is used for the determination of compounds in commercial pharmaceutical products.The compounds were well separated on a C18 column [Use Inertsil C18, 5µm , 150 x 4.6 mm] utilizing a mobile phase consisting of acetonitrile: phosphate buffer (45:55, v/v, pH 7.0) at a flow rate of 1.0 ml/min with UV detection at 220 nm.The retention time of fexofenadine hydrochloridewas found to be 2.05 min.,With this method linearity was observed (Correlation coefficient = 0.999).According to the validation results, the proposed method was found to be specific, accurate, precise and rapid. Hence the same can be applied to the quantitative analysis of tablets containing fexofenadine hydrochloride .

Keywords: Fexofenadine Hydrochloride, RP-HPLC, C18 column, Method validation.

I .INTRODUCTION:

Fexofenadine Hydrochloride , The Active Ingredient of Telfast And Allegra, Is a Second – Generation Histamine H1 Receptor Antagonist With The Chemical Name α,α- Dimethyl-4-[1-Hydroxy-4-[4-(Hydroxydiphenyl-Methyl)-1- piperidinyl] butyl]-Benzen acetic acid.It is Non Sedating Antihistamine. Fexofenadine Hydrochloride is used as The Hydrochloride Salt in the Symptomatic relief of allergic Conditions including Seasonal allergic rhinitis and urticaria.(1,2)Fexofenadine (tradenames Allegra, Fexidine, Telfast, Fastofen, Tilfur, Vifas, Te lfexo, Allerfexo) is an antihistamine pharmaceutical drug used in the treatment of allergy symptoms, such as hay fever, nasal congestion, and urticaria.[3] Fexofenadine is sometimes called a third-generation antihistamine because it is less able to pass the blood-brain barrier and cause sedation, compared to first-generation antihistamines.[4] Fexofenadine has been demonstrated to be safe and effective for children ages 2–5 years old and 6–11 years old in treatment of seasonal allergic rhinitis.[5,6] The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [7-9].

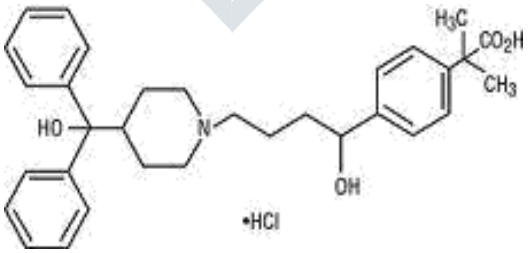


Fig.1. fexofenadine hydrochloride

I. MATERIALS AND METHODS:

Chemicals and reagents:

Active pharmaceutical ingredient of fexofenadine Hydrochloride was procured from torrent pharma ltd. Allerfex table dosage form was procured from the pharmacy. HPLC grade Acetonitrile Rankem ltd, mili -q water used, Phospate buffer was purchased from loba Chem AR grade..

Instrumentation and chromatographic conditions:

The chromatographic separation was performed on the Agilent Technologies 1200 series liquid chromatographic system equipped with G1311A quaternary pump and G1315D DAD detector.A Rheodyne syringe loading auto injector with a 20 µl sample loop was used for the injection of analyte. The system was controlled, data was collected and processed by EZ ChromEliet softwear . An Inertsil C18 column (150mm x 4.6mm i.d., 5µm particle size) was used for the separation.

Preparation of buffer:

10mM phosphate buffer was prepared in water and pH was adjusted to 7.0 with NaOH solution

Preparation of mobile phase :

Mix buffer and acetonitrile in the ratio of 45:55 v/v, mobile phase is degassed before use.

Preparation of Standard Solutions:

The stock solution of Fexofenadine hydrochlorid (100µg mL-1) was prepared by dissolving 10 mg of Fexofinadine Hydrochloride (99.9 %) in a 100 mL volumetric flask (stock solution) make up with diluent up to the mark.

Transferred (10,15,10,5, 15mL) of each stock solution to a diferrent volumetric flask and diluted up to the mark with diluents. This is working standard solution containing (10,15,20,25 and 30µg/ml) of Fexofinadine Hydrochloride.Refer-Table no.2 Linearity of Fexofinadine Hydrochloride

Preparation of test solution:

Accurately 20 Tablets were weighed to determine average weight of tablets. Then tablets were finely crushed and powder equivalent to 10 mg Fexofinadine Hydrochloride was transferred into 100 ml volumetric flask. Added 70 ml diluent and sonicated for 5 minute with intermittent shaking. Make up volume up to the mark to 100ml. From that solution take 10ml of Sample Solution was used and make up volume up to the mark 50 ml using Diluent. This solution was filtered through 0.45µm nylon syringe filter and the final concentration of test sample solution had concentration of Fexofinadine Hydrochloride 20µg/ml respectively.

Method devlopment :

1. Optimization of the chromatographic conditions: Mobile phase containing Acetonitrile and phosphate buffer was initially used. In this view Acetonitrile with phosphatebuffer of different ratios was tried as a mobile phase at a different pH and ratios were tried along with change in column temperatures. All the times peak shape was not proper and that for Fexofinadine retention time was also too long. Mobile phase

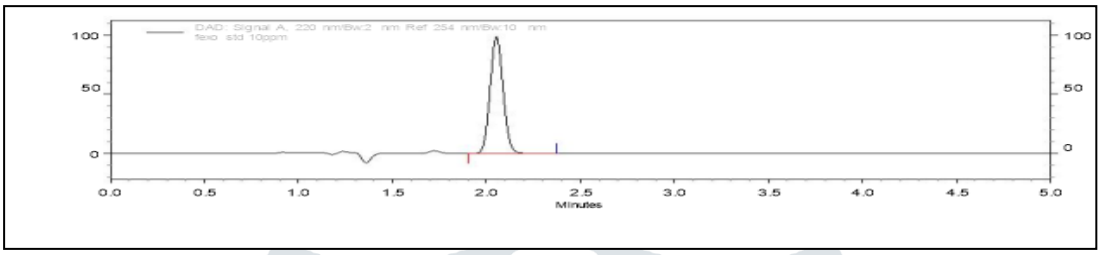
containing (45:55 v/v) Acetonitrile and phosphate buffer was tried. In this mobile phase drug showed the peak shape with reduced tailing. [Inertsil C18, 5µm, 150 mm x 4.6 mm] the column was the most suitable one since it produced symmetrical peak shape,improved the quality of peak and earlier retention time for Fexofinadine. Flow rate was set to1ml/min and UV detection was carried out at 220nm. Retention time of Fexofinadine was 2.05 min. The mobile phase was filtered through 0.45µm nylon membrane filter paper and sample solutions were filtered using 0.45µm nylon syringe filter prior to use

System Suitability:
System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time(RT) were determined. It indicates good performance.

Table no.1 system suitability parameters

Parameter	Fexofenadine Hcl
Retention time	2.05
Tailing factor	1.09
%RSD	0.08

Figure No.2 Representive chromatoram of test solution



II. METHOD VALIDATION:
The objective of the method is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristic of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Specificity:
spectral purities of Fexofenadine Hcl peaks were evaluated for the interference of the tablet excipents, degradation components or due to the presence of impurity as per the methodology. In the work,a solution of the tablet excipients were prepared using the sample prepration to evaluate possible interfearing peakes.

Linearity:
Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph.The calibration curves were plotted over a concentration range of 10-30 µg/ml for Fexofenadine. The stock solution of Fexofenadine hydrochlorid (100µg mL-1) was prepared by dissolving 10 mg of Fexofinadine Hydrochloride (99.9 %) in a 100 mL volumetric flask (stock solution) make up with diluent up to the mark. Transferred (10,15,10,5, 15mL) of each stock solution to a diferrent volumetric flask and diluted up to the mark with diluents. This is working standard solution containing (10,15,20,25 and 30µg/ml) of Fexofinadine Hydrochloride.Refer-Table no.2 Linearity of Fexofinadine Hydrochloride The absorbance of solution was then measured at 220nm curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Table .2 linearity concentration level of Fexofenadine Hcl

Standard weight	**Diluted to**	**ml taken**	**Diluted to**	**PPM**	**% Level**
10	100	10	100	10	50
10	100	15	100	15	75
10	100	10	50	20	100
10	100	5	20	25	125
10	100	15	50	30	150

Accuracy:
The accuracey of the method was detrmined by recovery experiments known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample.precent recovery was calculated by comparing the area with pre-analyzed sample.for both the druges, recovery was performed at 50%,100%,150% level and percentage recovery was calculated . data from the linearity was considered for accuacy.

Precision:
The precision of an analytical procedure expresses the closness of agreement between a series of measurment obtained from multiple sampling of the same homogenous sample under prescribed conditions. repeatability of the method was checked by injecting replicate injection of 20µg/ml of solution for 6 times.the meean area and % reative standard deviation (RSD) was

calculated. %RSD should not be more then(NMT) 2%.

Intermediate Precision:

The intermediate precison of the assay method is establised by comparison of two independent repeatability experiment on 2 diferent days .the data of the 1st day is taken from the analysis of "repeatability''.the second set of experiment is performed by a different analyst and HPLC system as well. The standard deviation , relative standard deviation and meen value difference is calculated from the results obtained on each day.

Robustness:

The robustness of analytical procedure is a measure of its capacity to remain unaffected by samll ,but delibrate variations in method parameter and provides an indication of its reliability during normal usage.it was observed that the variation like flow rate & mobile phase composition ,Variation in wavelength by ± 2nm.etc.

III. Result and Discussion:

Fexofenadine hydrochloride showed maximum absorbance at 220 nm. The praposed method for estimation of Fexofenadine Hcl drug was validated as per the ICH guidelines.

Specificity :

By comparing the chromatogram of blank, placebo solution, reference solution & test solution is it observed that there is no interference of any peaks at the retention time of Fexofenadine Hcl. The retention time of the main peaks in the chromatogram obtained with the reference solution & test solution are matching .this confirmed the specificity of the method .

Fig no.3 chromatogram of blank solution

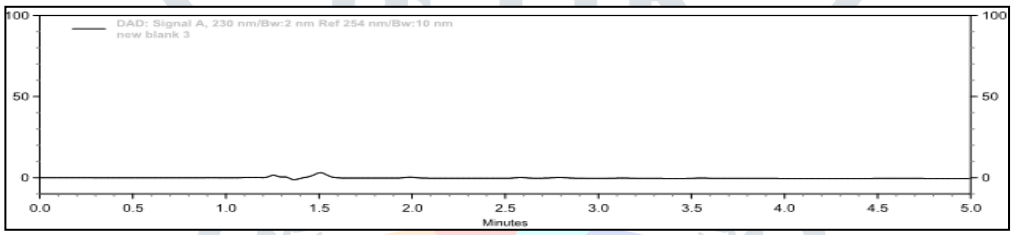


Fig no.4 chromatogram of standard solution

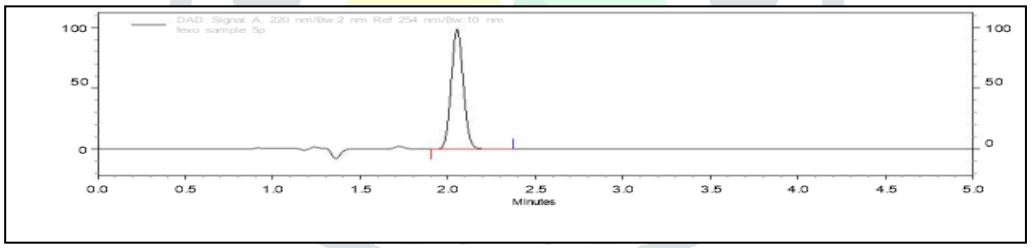


Fig no.5 chromatogram of sample solution

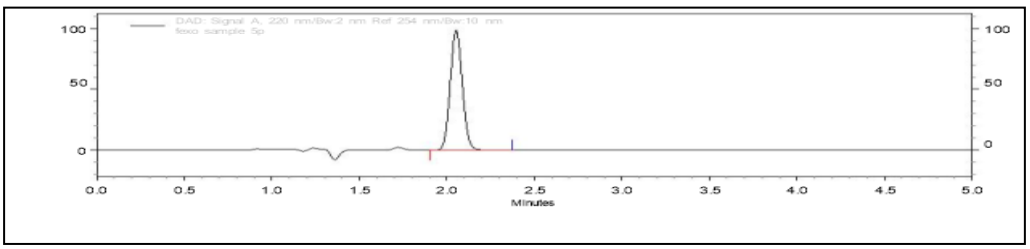
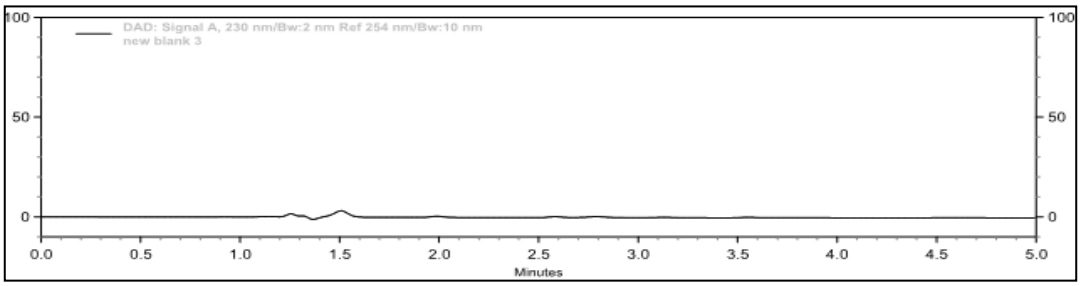


Fig no.6 chromatogram of placebo solution



Linearity :

Five concentration such as 10,15.20,25,30 µg/ml for fexofenadine Hcl and linearity graph was plotted using concetration verses peak area and shown in figures no(7). A linear relationship was obtained between peak area and quantity analyzed in the range of 50 to 150 %.

Fig no. 7 Linearity plot of Fexofenadine Hcl

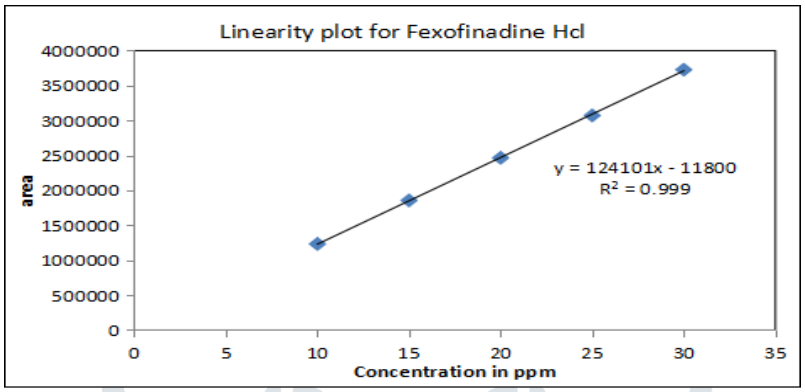


Table no .3 linearity of solution

Parameter of linearity	Value	Acceptance criteria
Correlation coefficent R	0.999	Not less then 0.997

The method is considered to be linear in the range on 10-30 µg/ml for Fexofinadine Hcll and correlation coefficent & y- axis intercept should be within the limit.

Accuracy :

The percentage recovery of Fexofenadine Hcl is 99.83 %. which shows the accuracy of the method . ref table no. (4)

For recovery at different concentration levels. The recovery values between prescribed limit of 98-102 % shown that method is free from interference of excipients present in formulaton.

Table no. 4 Accuracey of method

Accuracey level	% recovery of Fexofenadine Hcl
50%	99.5
100%	100.5
150%	99.5
Meen recovery	99.83

precision :

The exactness of the method as defined by precision and method is considered to be precised as since the relative standard from 6 determination is well within the acceptance limit. Refer table.5

Table no .5 precision

Sample no.	% assay of Fexofinadine Hcl
Sample 01	100.1
Sample 02	99.9
Sample 03	99.8
Sample 04	99.9
Sample 05	100.1
Sample 06	100.2

Meen	100.0
STD DEV	0.15
%RSD	0.16

Intermediate precision :

The intermediate precision of the assay method is established by comparison of two independent repeatability experiments on 2 different days. Refer table no.6

Table no.6 Intermediate precision

Sample no.	% assay of Fexofenadine Hcl
Sample 01	100.1
Sample 02	99.8
Sample 03	99.9
Sample 04	100.2
Sample 05	99.7
Sample 06	99.8
Mean	99.92
Std dev	0.19
% RSD	0.19

Robustness :

Robustness result of Fexofenadine Hcl Method is found to be robust as system suitability criteria is achieved for all the robusteness parameter tested. Deliberate change in parameter does not have any significant effect on the method performance , which demonstrated that the developed RP-HPLC method is Robust. The results were shown in table no.7

Table no.7 Robustness result for Fexofenadine Hcl

Sr. No.	Robustness parameter (flow)	% RSD	Tailing factor	Assay
1	0.8 ml/min	0.11	1.04	100.1
2	1.2 ml/min	0.13	1.01	99.8

Sr. No.	Robustness parameter (column oven temperature)	% RSD	Tailing factor	Assay
1	30 ^{0}C	0.11	1.01	100.2
2	40 ^{0}C	0.09	1.03	99.9

IV. CONCLUSION:

In this present work a new simple, selective,linear,pricise,accurate and robust HPLC method was developed and validate for estimation of Fexofenadine Hcl in pharmacutical tablet dosage form in accordance with the ICH guidelines. This method gives short analysis time , reproducible and showed data for all the method validation parameter . thus, The method is very simple and all peaks are well separated and there is no interference by excipients peaks with total run time , which makes it especially suitable for routine quality control analysis work. The method can be used for individual analysis of the titled drugs or their binary combination.

REFERENCES:

1] Sweetman S C (Ed) , Martindale : The Complete Drug Reference, 33rd edn (Pharmaceutical Prees, London) 2002.
2] physicians Desk Reference (PDR) Thomson Medical Economics Company Inc., Electronic Verison: Montvale, NJ (2003).
3] Lappin G, Shishikura Y, Jochemsen R, Weaver RJ, Gesson C, Houston B, Oosterhuis B, Bjerrum OJ, Rowland M, Garner C (May 2010). "Pharmacokinetics of fexofenadine: evaluation of a microdose and assessment of absolute oral bioavailability". Eur J Pharm Sci40 (2): 125–31.
4]Smith, SM; Gums, JG (July 2009). "Fexofenadine: biochemical, pharmacokinetic and pharmacodynamic properties and its unique role in allergic disorders.". Expert Opinion on Drug Metabolism & Toxicology 5 (7): 813–22.
5]Segall, N; Grubbe RE; Levy AL; Maloney MJ; Nayak AS; Kittner B; Quesada JT. (Jul–Aug 2008). "Pharmacokinetics, Safety and Tolerability of an Oral Suspension of Fexofenadine for Children with Allergic Rhinitis". Allergy Asthma Proc. 29 (4): 380–5.
6]Phan, H; Moeller, Nahata (2009). "Treatment of Allergic Rhinitis in Infants and Children: Efficacy and Safety of Second-Generation Antihistamines and the Leukotriene Receptor Antagonist Montelukast". Drugs 69 (18): 2541–76.
7]. ICH, Q2 (A). Validation of analytical procedures: text and methodology International Conference on Harmonization. Geneva: 2005:1- 13.
8]. Text book of High performance liquid chromatography by Dr. P.D Sethi Quantitative Analysis of pharmaceutical formulations. First edition 2001.
9]. Guidance for Industry Process Validation: General Principles and Practices, US FDA 2008