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RP-HPLC Method for the Estimation of Fexofenadine and Pseudoephedrine

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ABSTRACT

A simple, accurate, rapid, reproducible HPLC method has been developed for estimation of Fexofenadine and pseudoephedrine using different mobile phases. Such as for Fexofenadine-pH-2.0 phosphate buffer, acetonitrile and triethyl amine in the ratio of 65:35:0.3v/v at a flow rate 1.5ml/min and for pseudoephedrine pH 3.0 phosphoric acid buffer and acetonitrile in the ratio of 90:10v/v at a flow rate 1.5ml/min. A zorbax SB Phenyl RP 18- 250x4.6mm column was used as a stationary phase. Quantification was performed using UV detector for Fexofenadine at 220nm and for pseudoephedrine at 210nm. The method showed good resolution of the peaks for two compounds. The result was validated by linearity studies.

Keywords: Estimation, Fexofenadine and Pseudoephedrine, HPLC, linearity, results.

INTRODUCTION

Fexofenadine hydrochloride [1,6] the major active metabolite of terfenadine, is an antihistamine with selective peripheral H_1-receptor antagonist activity. Fexofenadine hydrochloride inhibited antigen-induced bronchospasm in sensitized guinea pigs and histamine release from peritoneal mast cells in rats. In laboratory animals, no anticholinergic or alpha$_1$-adrenergic-receptor blocking effects were observed. Moreover, no sedative or other central nervous system effects were observed. Radio labeled tissue distribution studies in rats indicated that Fexofenadine does not cross the blood-brain barrier. Chemically it is designated as (±)-4-[1-hydroxy-4-[4-

(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-dimethyl benzene acetic acid hydrochloride. Soluble in water, freely soluble in alcohol, and sparingly soluble in chloroform.

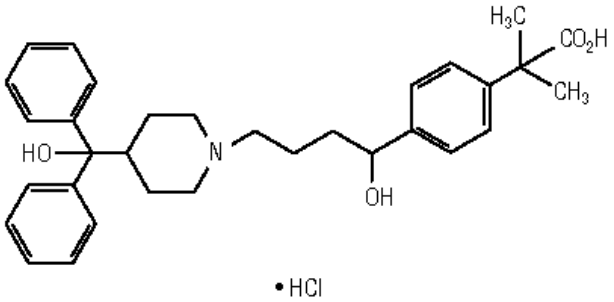

Figure.1 Structure of the Fexofenadine

Pseudoephedrine hydrochloride[6] is an orally active sympathomimetic amine and exerts a decongestant action on the nasal mucosa. Pseudoephedrine hydrochloride is recognized as an effective agent for the relief of nasal congestion due to allergic rhinitis. Pseudoephedrine produces peripheral effects similar to those of ephedrine and central effects similar to, but less intense than, amphetamines. It has the potential for excitatory side effects. At the recommended oral dose, it has little or no pressor effect in normotensive adults. Chemically it is designated as [S-(R,R)]-[1-(methyl amino)ethyl]-benzene methanol hydrochloride. Soluble in water, freely soluble in alcohol, and sparingly soluble in chloroform.

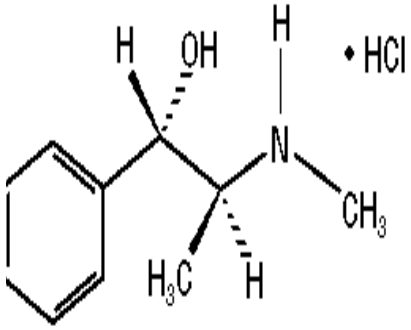

Figure.2 Structure of the pseudoephedrine

MATERIALS AND METHODS

INSTRUMENTATION:

The estimation was carried out on Agilent 1100 series HPLC system equipped with HPLC pump, an automated sample – injector and diode array detector, with empower soft ware. Sample of Fexofenadine and Pseudoephedrine was received from Dr.Reddy's lab, Hyderabad, respectively and tablets purchased from the local market, HPLC grade acetonitrile, triethanolamine and ortho phosphoric acid AR grade purchased from E.Merck and spectrochem respectively. HPLC grade water was prepared by using milli-Q purification system.

Mobile phase:

For Fexofenadine:

Preparation of pH -2.0 buffer: The phosphate buffer is prepared by dissolving 6.64g of monobasic sodium phosphate and 0.84g of sodium per chlorate in 1000ml of milli-Q water and

pH adjusted with ortho phosphoric acid and filtered through 0.45µm pall pharma nylon66 membrane filter.

Preparation of diluent: Mix buffer and acetonitrile in the ratio of 50:50 v/v respectively.

Preparation of mobile phase: the mobile phase is prepared by mixing buffer solution, acetonitrile and triethyl amine in the ratio of 65:35:0.3v/v. mix well and degas in a sonicator for about 10mins.

For pseudoephedrine:
Preparation of pH-3.0 buffer: transfer 5ml of triethyl amine into 1000ml volumetric flask and dilute to volume with milli-Q water and mix well. Adjust the pH to 3.0 with ortho phosphoric acid.

Preparation of mobile phase: the mobile phase is prepared by mixing acetonitrile and pH-3.0 buffer solution in the ratio of 90:10v/v respectively.

Standard preparation:
For Fexofenadine: stock solution of Fexofenadine[5] was prepared by dissolving 50mg of Fexofenadine in 100ml volumetric flask containing 75ml of diluent, sonicate for about 10mins and then made up to the mark with the diluent to get a concentration of 1mg/ml. this stock solution was further diluted to obtain 50,100,150,200,250,300µg/ml concentrations.

For pseudoephedrine: stock solution of Pseudoephedrine[5] was prepared by dissolving 50mg of Pseudoephedrine in 100ml volumetric flask containing 70ml of diluent, sonicate for about 10mins and then made up to the mark with the buffer solution to get a concentration of 1mg/ml. this stock solution was further diluted to obtain 50,100,150,200,250,300µg/ml concentrations.

Sample preparation:
Twenty tablets of fexofenadine [3] are weighed and powdered. A quantity of tablet powder equivalent to 50mg was taken in a 100ml volumetric flask and diluent was added up to the mark and filtered to get a concentration of 1mg/ml and sonicate for 20mins and add diluent and filtered. This solution was further diluted to obtain different concentrations.

Twenty tablets of pseudoephedrine [4] are weighed and powdered. A quantity of tablet powder equivalent to 50mg was taken in a 100ml volumetric flask and diluent was added up to the mark and filtered to get a concentration of 1mg/ml and sonicate for 20mins and add diluent and filtered. This solution was further diluted to obtain different concentrations.

Chromatographic conditions:

Table.1 chromatographic conditions for Fexofenadine

Column:	**:**	**250 mm ×4.6 mm zorbax SB phenyl RP 18, 5 µm .**
Flow Rate:	**:**	**1.5 ml/min**

Detection:	**:**	**220nm**
Injection Volume:	**:**	**20µl**
Run Time:	**:**	**15 min.**

Table.2 chromatographic conditions for pseudoephedrine

Column:	**:**	**250 mm ×4.6 mm zorbax SB phenyl RP 18, 5 µm .**
Flow Rate:	**:**	**1.5 ml/min**
Detection:	**:**	**210nm**
Programming:	**:**	**gradient**
Run Time:	**:**	**15 min.**

Procedure:

For Fexofenadine [2]**:** Into a series of 10ml volumetric flasks 0.5-3ml of above stock standard solution of Fexofenadine was transferred. The total volume in each flask was made up to 10ml with the mobile phase and filtered through 0.45µ membrane filter. Initially the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. Then ten micro liters of Fexofenadine or sample solutions were injected for 6 times. A quantitative determination of the active ingredient was made by comparison of the peak area from the sample injection to the corresponding peak area from a standard injection. The amount of Fexofenadine present in a sample was calculated through the standard calibration curve (figure-4). The retention time of Fexofenadine was found to be 11.362 minutes (figure-3).

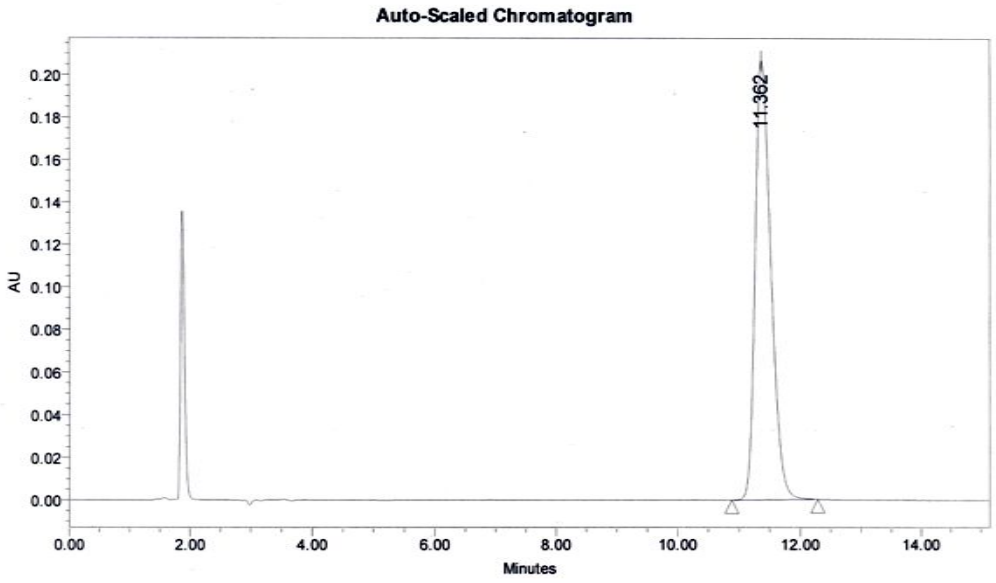

Figure.3 Chromatogram of Sample Fexofenadine

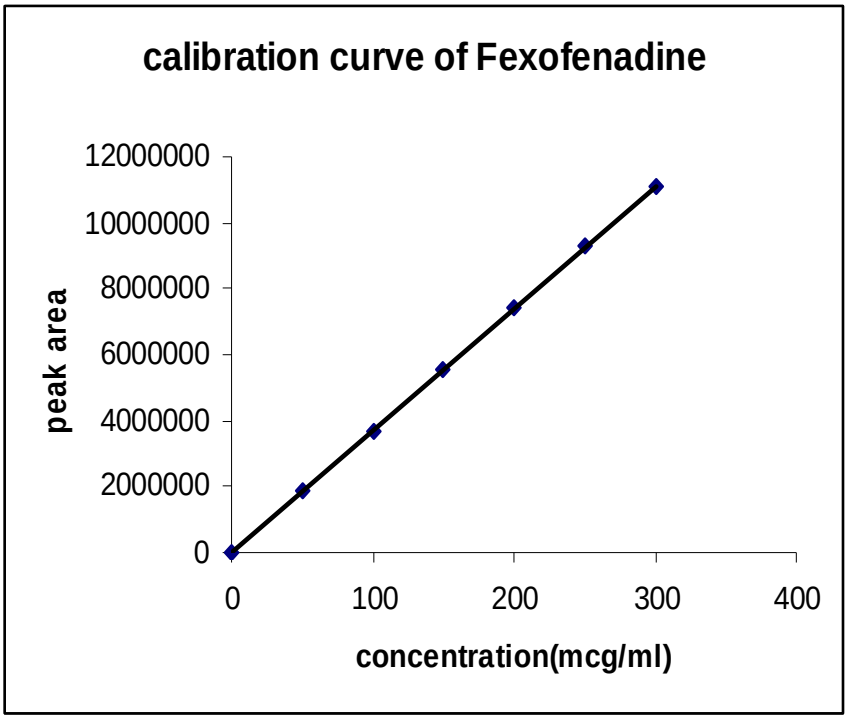

Figure.4 calibration curve of Fexofenadine

For Pseudoephedrine [7]**:** Into a series of 10ml volumetric flasks 0.5-3ml of above stock standard solution of Pseudoephedrine was transferred. The total volume in each flask was made up to 10ml with the mobile phase and filtered through 0.45µ membrane filter. Initially the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. Then ten micro liters of Pseudoephedrine or sample solutions were injected for 6 times. A quantitative determination of the active ingredient was made by comparison of the peak area from the sample injection to the corresponding peak area from a standard injection. The amount of Pseudoephedrine present in a sample was calculated through the standard calibration curve (figure-6). The retention time of Pseudoephedrine was found to be 5.799mins (figure-5).

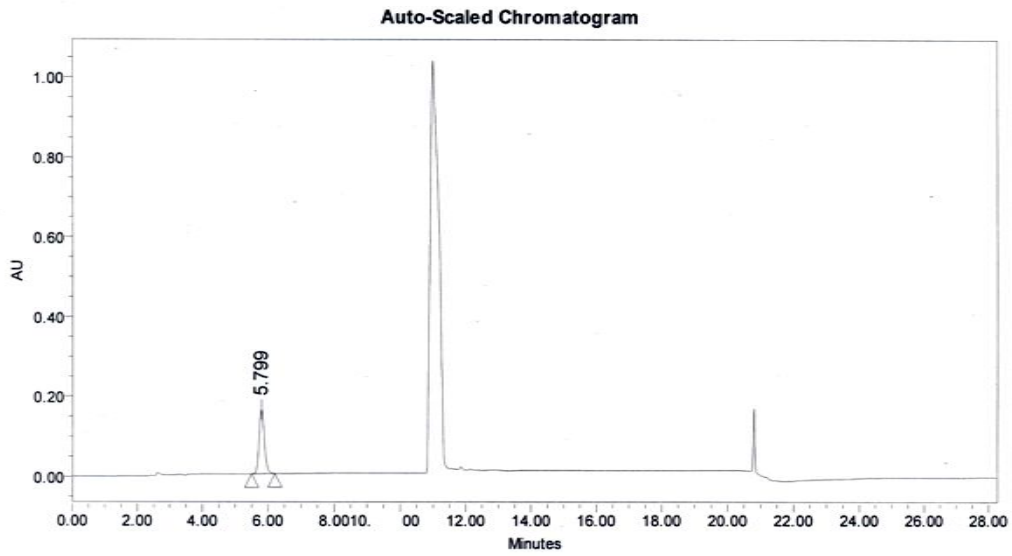

Figure.5 Chromatogram of Standard Pseudo Ephedrine

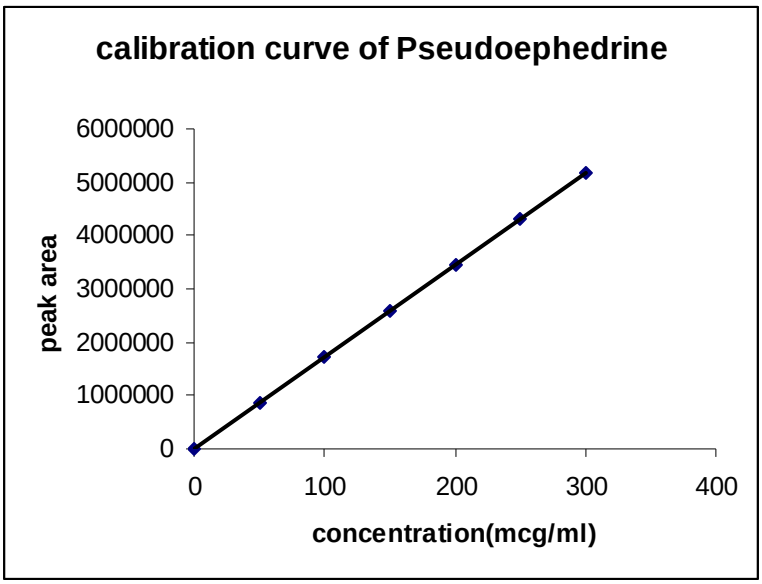

Figure.6 calibration curve of Pseudoephedrine

RESULTS AND DISCUSSION

Linearity:

Linearity was demonstrated by analyzing five different concentrations of active compound. Peak areas recorded for all the peaks and plotted peak area versus concentration of drug such as Fexofenadine and pseudoephedrine were found to be linear. Coefficient correlation for Fexofenadine was 0.999 and for pseudoephedrine was 0.999 respectively.

Table.3 Linearity for Fexofenadine

Concentration (μg/ml)	Average area	Statistical Analysis	
50	1854724	Slope	37094.4
100	3707449	Intercept	0.466
150	5544172	Correlation coefficient	0.999
200	7418896		
250	9273620		
300	11128344		

Table.4 Linearity for pseudoephedrine

Concentration (μg/ml)	Average area	Statistical Analysis	
50	864954	Slope	17299.0
100	1729909	Intercept	0.733
150	2594863	Correlation coefficient	0.999
200	3459816		
250	4324770		
300	5189724		

Accuracy:

Accuracy was done by recovery study using standard addition method. Known amount of standard Fexofenadine and pseudoephedrine was added into the pre analyzed sample and subjected to proposed HPLC method. Results are shown in the following tables.

Table.5 Accuracy for Fexofenadine

Validation Parameters		Results
Linearity	Correlation Coefficient	1.0
	Slope	37094
	Intercept	0.466
	Regression	Y= 0.466X +37094
Accuracy	Spiked Concentration	%Recovery
	50 %	102.9
	75%	102.2
	100%	101.98
Precision	%RSD	0.891
LOD	Concentration (µg / mL)	1.32.
LOQ	Concentration (µg / mL)	4.011

Table.6 Accuracy for Pseudoephedrine

Validation Parameters		Results
Linearity	Correlation Co-efficient	1.0
	Slope	1729.07
	Intercept	0.733
	Regression	Y= 0.733X+1729.07
Accuracy	Spiked Concentration	%Recovery
	50 %	99.97
	75%	99.99
	100%	99.93
Precision	%RSD	0.929
LOD	Concentration (µg / mL)	1.192
LOQ	Concentration (µg / mL)	3.6

APPLICATIONS

This method is useful for the study of the different formulations of the Fexofenadine and Pseudoephedrine.

CONCLUSION

These RP-HPLC methods of the Fexofenadine and Pseudoephedrine have the acceptable correlation co-efficient and linearity.

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