Simultaneous Quantification of Pseudoephedrine Hydrochloride and Fexofenadine Hydrochloride In Tablets by Liquid Chromatography

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ABSTRACT

An accurate and precise liquid chromatographic method was developed for the simultaneous estimation of pseudoephedrine hydrochloride and fexofenadine hydrochloride in tablets. The chromatographic analysis was performed on XTerra symmetry C_8 column (250 × 4.6 mm, 5 μ particle size) with mobile phase consisting of methanol and sodium di-hydrogen ortho phosphate buffer (pH 2.8) in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min and eluents monitored at 219 nm. The calibration curves of peak area versus concentration, which was linear from 20-100 μg/mL for pseudoephedrine hydrochloride and 10-50 μg/mL for fexofenadine hydrochloride, had regression coefficient (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of paracetamol and meloxicam in tablets. The proposed method is simple, economical, accurate, and precise and could be successfully employed in routine quality control for the simultaneous analysis of pseudoephedrine hydrochloride and fexofenadine hydrochloride in tablets.

Keywords: Pseudoephedrine Hydrochloride, Fexofenadine Hydrochloride, RP-HPLC.

INTRODUCTION

Pseudoephedrine hydrochloride, chemically 2-methylamino-1-phenyl-1-propanol hydrochloride, has sympathomimetic activity. Pseudoephedrine hydrochloride is a decongestant that shrinks blood vessels in the nasal passages. It is used to relieve nasal congestion caused by colds, allergies, and fever. Pseudoephedrine occurs naturally as an alkaloid in certain plant species, the majority of pseudoephedrine produced for commercial use is derived from yeast fermentation of dextrose in the presence of benzaldehyde [1,2]. Fexofenadine hydrochloride, α, α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl] benzeneneacetic acid hydrochloride, is the most important terfenadine metabolite, prevents allergic inflammation. It is nonsedating and does not decrease performance even in extremely high doses [3,4].

A survey of pertinent literature revealed that few LC-MS/MS [5, 6] methods have been reported for the determination of these drugs in human plasma. Ion Interaction Chromatography has been reported for separation Fexofenadine, Pseudoephedrine, Potential Impurities, and Degradation Products [7]. Two HPLC methods [8-9] have been reported for the simultaneous determination of pseudoephedrine hydrochloride and

fexofenadine hydrochloride in dosage form. But the reported methods have either longer chromatographic run time or lacks in chromatographic resolution, sensitivity and peak symmetry. Present study involves development and validation of HPLC method for the simultaneous estimation of pseudoephedrine hydrochloride and fexofenadine hydrochloride in combined tablet dosage form, which is fast, sensitive with better resolution and peak symmetry.

MATERIALS AND METHODS
Materials

Pure pseudoephedrine hydrochloride (PSE) and fexofenadine hydrochloride (FEX) used as working standards, were gifts from Hetero Drugs Pvt. Ltd., Hyderabad, India. Methanol and water (HPLC-grade) were purchased from Rankem, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck, India. Extended release tablets containing 120 mg of pseudoephedrine hydrochloride and 60 mg of fexofenadine hydrochloride (Allegra-D®) were obtained from local pharmacies and used within their shelf life period.

Instrumentation

The chromatographic system comprised of Waters 2695 binary gradient pump, with in-built auto sampler, column oven and Waters 2487 dual wavelength absorbance detector (DAD). Data integration was carried out using Empower-2 software. Samples were injected into X-Terra symmetry C_8 column (250 × 4.6 mm, 5 μ particle size). A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Chromatographic conditions

The high performance liquid chromatographic (HPLC) system was operated isocratically with the column temperature was maintained at ambient, using a mobile phase composition of methanol and sodium di-hydrogen ortho phosphate buffer (pH adjusted to 6.8 with potassium hydroxide) in the ratio of 60:40 % v/v at a flow rate of 1.0 mL/min within a run time at 8 min. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a millipore vacuum filter system equipped with a 0.45 μm high vacuum filter. Both drugs were detected and quantified at 219 nm.

Preparation of standard solutions

The standard solutions were prepared by transferring 100 mg of PSE and 100 mg of FEX working standards into 100 mL volumetric flasks. To each, 30 mL methanol was added, and the mixture was sonicated to dissolve and make up the volume with methanol. Aliquots of these standard solutions were transferred using A-grade bulb pipettes into 100 mL volumetric flasks and the solutions made up to volume with mobile phase to give final concentrations of 20-100 μg/mL and 10-50 μg/mL of PSE and FEX, respectively.

Quantification of pseudoephedrine hydrochloride and fexofenadine hydrochloride from tablets

Twenty tablets were accurately weighed and crushed to a fine powder in a mortar. An amount of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask and 30 mL of methanol was added to it. The mixture was sonicated to dissolve and then made up to volume with methanol. Following 25 min of mechanical shaking, it was kept in an ultrasonic bath for 5 min, and the solution filtered through 0.45 μm filter paper. Suitable aliquots of the filtered solution were transferred to a volumetric flask and made up to volume with mobile phase to yield concentrations of PSE (60 μg/mL) and FEX (30 μg/mL). A 20 μL volume of the sample solution was injected into the chromatographic system, six times, under optimized chromatographic conditions. The peak areas were measured at 219 nm.

Method validation

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility, robustness and system suitability [10].

Linearity: Twelve different concentrations of the mixture of PSE and FEX were prepared for linearity studies and injected into chromatographic system (n = 3). The responses were measured as peak areas and construct calibration curve (concentration vs peak area).

Detection limit and quantitation limit: Limit of detection (LOD) and Limit of quantification (LOQ) were calculated based on the ICH guidelines.

Accuracy: The accuracy was carried out by adding known amounts of each standard drug corresponding to three concentration levels - 50, 100 and 150 % - of the labeled claim to the analytes. At each level, three determinations were performed and the results were recorded. The accuracy was expressed as percent analyte recovered by the proposed method.

Precision: The precision of an analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for ten determinations of peak areas of PSE (60 µg/mL) and FEX (30µg/mL), performed on the same day. For both intra- and inter-assay variation, standard solutions of PSE (40, 60 and 80 µg/mL) and FEX (20, 30 and 40 µg/mL) were injected in triplicate.

Robustness: The robustness of the proposed method was determined by carrying out the analysis, during which mobile phase composition (concentration of methanol was varied by ± 2 %), and buffer pH (varied by ± 0.1) were altered and the peak areas and retention times were noted.

System Suitability: To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like asymmetry factor, theoretical plate number (N), resolution (Rs) and retention time (t_R) were analyzed by using 20 µL of the working standard solution containing PSE (60 µg/mL) and FEX (30 µg/mL) injecting six times into HPLC system.

RESULTS AND DISCUSSIONS

The RP-HPLC method, as described, was validated and successfully employed for the simultaneous quantification of PSE and FEX in tablets. There is need to consider the successive steps for the development of RP-HPLC method. In particular, the problems relating to the standardization of sample preparations and selection of mobile phase needs to be emphasized. The optimized chromatographic conditions were selected based on sensitivity, retention time, peak shape and baseline drifts. The method was selective for the determination of PSE and FEX since no interfering peaks appeared near the retention time of the compound of interest. A typical chromatogram recorded at 219 nm is shown in Figure 1. The retention times of PSE and FEX at a flow rate of 1.0 mL/min were 3.003 and 3.851 min, respectively. The analyte peaks were well resolved and were free from tailing (< 2 for both the analytes).
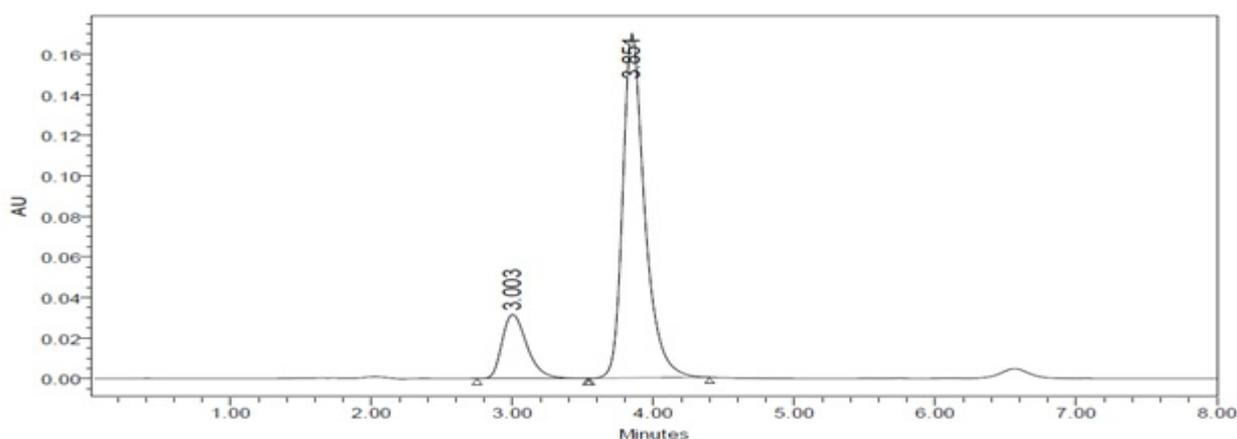

Figure 1: A typical chromatogram of pseudoephedrine (t_R: 3.003) and fexofenadine (t_R: 3.851)

To ensure the validity of a system and analytical method, system suitability test was performed. The percent relative standard deviation (%RSD) of the retention times (RT) and peak areas of PSE and FEX from the six consecutive injections of the standard solutions were 0.736 and 1.294, and 1.452 and 1.032, respectively. The tailing factor for PSE and FEX peaks were 1.32 and 1.47, respectively, thus reflecting good peak symmetry. The resolution (Rs) between PSE and FEX was 3.12, indicating good separation of both analytes from each other. The theoretical plate no. for PSE and FEX were 2448 and 3275, respectively, thus indicating good column efficiency (Table 1). The results

for linearity were shown in Table 1. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 20-100 µg/mL for PSE and 10-50 µg/mL for FEX. The regression coefficients of PSE (r^2 = 0.9994) and FEX (r^2 = 0.9991) indicate a good linear relationship between peak area versus concentration over a wide range. LOD for PSE and FEX was 0.19 and 0.04 µg/mL, respectively, while LOQ was 0.57µg/mL and 0.13µg/mL, respectively (Table 1). The mean recoveries obtained for PSE and FEX was 100.43 and 100.78 %, respectively, indicating that the developed method was accurate (Table 2).

Table 1. System suitability parameters and linearity data for proposed method

Validation parameter	Results	
	PSE	**FEX**
Linearity	20-100 µg/mL	10-50 µg/mL
Regression line	y = 8957 x + 205564	y = 83153 x + 960283
Regression coefficient (r^2)	0.9994	0.9991
Limit of detection (µg/mL)	0.19	0.04
Limit of quantitation (µg/mL)	0.57	0.13
System suitability parameter*		
Peak area (%RSD)	1.452	1.032
Retention time (%RSD)	0.736	1.294
Tailing factor	1.32	1.47
Number of theoretical plates	2448	3275
Resolution	3.12	

* Replicates of six determinations

Table 2. Results of recovery studies by standard addition method

Analyte	Amount (%) of known drug added to analyte	Theoretical content (µg/mL)	Conc. of analyte found (Mean± SD)	RSD (%)	SEM	Recovery (%)
PSE	50	30	20.19±0.2651	1.31	0.1531	100.95
	100	40	20.31±0.3204	1.58	0.185	101.57
	150	50	19.9±0.3092	1.55	0.1785	99.52
FEX	50	15	10.02±0.1709	1.71	0.0987	100.20
	100	20	10.1±0.155	1.54	0.0895	100.97
	150	25	10.07±0.1193	1.19	0.0689	100.67

Injection repeatability values (%RSD) of PSE and FEX were found to be 1.55 and 1.36, respectively. Results for intra and inter-assay precision, expressed as %RSD, results were given in Table 3. The low values of %RSD indicate that the method is precise.

Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between %RSD values, which indicates that the proposed method was reproducible.

Table 3. Precision data of the proposed method

Analyte	Analyte Conc. (µg/mL)	Intra-assay precision*	Inter-assay precision*	Reproducibility*	
				Analyst one	Analyst two
PSE	40	0.51	1.28	0.82	1.34
	60	0.85	0.58	0.54	1.49
	80	0.91	1.76	1.58	1.45
FEX	20	1.58	1.53	1.42	1.8
	30	1.53	1.26	1.21	0.59
	40	0.75	1.52	1.29	0.81

*%RSD Values

There was no significant change in the peak areas and retention times of PSE and FEX when the organic strength and pH of buffer were changed. The low values of %RSD indicate that the method was robust (Table 4).

The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and retention time.

Table 4. Results for robustness of the proposed method

Parameter	Original	Used	Analyte	Peak area		Retention time	
				Mean± SD	*RSD (%)*	*Mean± SD*	*RSD (%)*
Methanol	60	58	PSE	745227±9381	1.26	2.925±0.0199	0.68
		60		743639±5522	0.74	3.055±0.0495	1.62
		62		756613±517	0.07	3.111±0.0107	0.34
		58	FEX	3403695±11907	0.35	3.87±0.0455	1.18
		60		3445903±57624	1.67	3.868±0.0218	0.56
		62		3443463±58094	1.69	3.787±0.0093	0.25
pH (Buffer)	6.8	6.6	PSE	746652±5655	0.76	2.96±0.0086	0.29
		6.8		750129±2465	0.33	3.059±0.0841	2.75
		7.0		747509±8700	1.16	3.033±0.0051	0.17
		6.6	FEX	3381067±63171	1.87	4.041±0.0543	1.34
		6.8		3494380±3694	0.11	3.88±0.0051	0.13
		7.0		3488183±40390	1.16	3.898±0.0095	0.24

The proposed method was applied to the simultaneous determination of pseudoephedrine and fexofenadine in tablets. The results of the assay yielded 100.57% for PSE and 99.35 % for FEX, of label claim of the tablets. The

assay results show that the method was selective for the simultaneous determination of PSE and FEX without interference from the excipients used in the tablet dosage form and the results were shown in the Table 5.

Table 5. Results for robustness of the proposed method

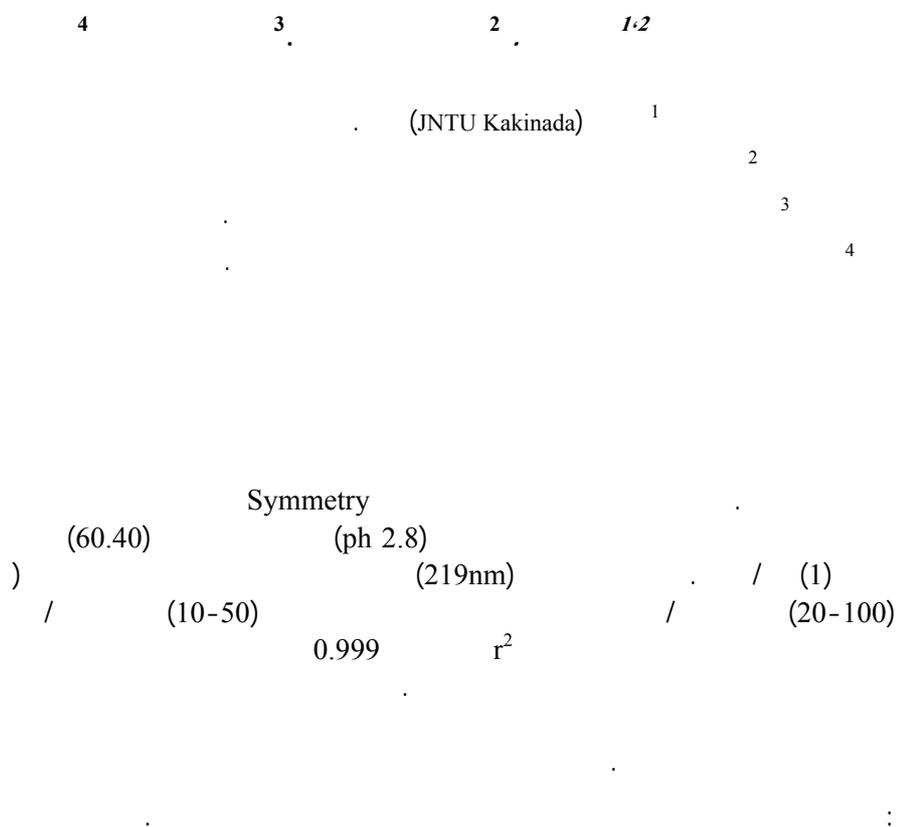| Product	Analyte	Label Claim per tablet (mg)	% Analyte estimated (mean±SD)*	RSD (%)	SEM
Allegra-D	PSE	120	100.57±1.2533	1.25	0.5116
	FEX	60	99.35±1.0504	1.06	0.4288

*n= 6; SEM= standard error of mean

CONCLUSION

The developed method for the simultaneous determination of pseudoephedrine hydrochloride and fexofenadine hydrochloride has the advantages of sensitivity, accuracy, precision and low cost. The non-interference of tablet excipients makes the method suitable for the determination of these drugs in tablets, and hence can be used for routine quality control of pseudoephedrine hydrochloride and fexofenadine hydrochloride in this dosage form.

REFERENCES

(1) Laurence L Brunton, ed. (2006). Goodman & Gilman's The Pharmacological Basis of Therapeutics (11th ed.). New York: McGraw-Hill Medical Publishing Division.

(2) Martindale (1989). In Edited by Reynolds JEF. Martindale: The complete drug reference (29th ed.). London: Pharmaceutical Press.

(3) Gelfand, E. W.; Cui, Z. H.; Takeda, K.; Kanehiro, A.; Joetham, A.; *J. Allergy. Clin. Immunol.* 2002; 110(1), 85-95.

(4) Sussman, G. L.; Mason, J.; Compton, D.; Stewart, J.; Ricard, N.; *J. Allergy. Clin. Immunol.* 1999; 104(1), 100-106.

(5) Nirogi, R. V.; Kandikere, V. N.; Shukla, M.; Mudigonda, K.; Maurya, S.; Komarneni, P.; *Rapid Commun. Mass Spectrom.* 2006; 20(20), 3030-8.

(6) Vijaya, D. B.; Kanbarpa, R.; Banda, J.; Gajjela, R.; Indu, B.; Andra, N.; Ramesh, M.; *Chromatogr.* 2008; 67(5-6), 461-6.

(7) Walker, T. A.; Schmitt, G. L.; *J. Liquid Chromatogr. Related Tech.* 2006; 29(1), 25-43.

(8) Karakus, S.; Kucukguzel, I.; Kucukguzel, S. G.; *J. Pharm. Biomed. Anal.* 2008, 46(2), 295-302

(9) Maggio, R. M.; Castellano, P. M.; Vignaduzzo, S. E.; Kaufman, T. S.; *J. Pharm. Biomed. Anal.* 2007; 45(5), 804-10.

(10) International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedure: Methodology (ICH–Q 2B), 1996; pp. 1-8.

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