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A Validated Reverse Phase LC Method for the Simultaneous Estimation of Fexofenadine HCl and Pseudoephedrine HCl in Pharmaceutical Dosage form Using a Monolithic Silica Column

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A simple, rapid, accurate, precise and sensitive isocratic reverse phase high performance liquid chromatographic method (RP-LC) has been developed for the simultaneous estimation of fexofenadine HCl (FEX) and pseudoephedrine HCl (PSE) in pharmaceutical dosage forms from their combination formulation. The proposed method utilized Chromolith ® Performance, RP-18e, 100 mm × 4.6 mm column and the separation was achieved with mobile phase consisted of phosphate buffer pH 4.5:acetonitrile:methanol (65:25:10 v/v), delivered at a flow rate of 2.4 mL/min and wavelength of detection at 258 nm. Losartan was chosen as the internal standard which elution is between the two tested active ingredients to guarantee a high level of quantitative performance. The method has shown adequate separation using losartan with good resolution to both of the active ingredients, in which contest the retention time of pseudoephedrine HCl, losartan and Fexofenadine HCl were 0.7, 2.1 and 2.7 min, respectively. The described method was linear over the range of 0.8-100 μg mL^{-1} (r = 0.9995) for fexofenadine HCl and 2.0-200 μg mL^{-1} (r = 0.9999) for pseudoephedrine HCl. Intra- and inter-day % RSD (n = 6) was ≤ 1.0 %. The percentage recovery of the two drugs from their tablet formulation were 99.5 ± 0.3 (FEX) and 99.6 ± 0.4 (PSE). The developed method was validated according to ICH guidelines. The method can be used for rapid and accurate quantitative determination of these drugs in combined dosage forms.

Key Words: Fexofenadine hydrochloride, Pseudoephedrine hydrochloride, RP-LC, Monolithic column, Tablets.

INTRODUCTION

Fexofenadine hydrochloride (FEX), is chemically (RS)-2-[4-[1-hydroxy-4-[4-(hydroxy-diphenyl-methyl)-1-piperidyl]butyl] phenyl]- 2-methyl- propanoic acid and is an antihistamine drug used in the treatment of hayfever and similar allergy symptoms. It was developed as a successor of and alternative to terfenadine, an antihistamine which caused potentially leading to cardiac arrhythmia. Fexofenadine hydrochloride, like other second and third-generation antihistamines, does not readily cross the blood-brain barrier and so causes less drowsiness than first-generation histamine-receptor antagonists. It has been described as both a second-generation and third-generation antihistamine[1].

Pseudoephedrine hydrochloride (PSE) is chemically (S,S)-2-methylamino-1-phenylpropan-1-ol is a sympathomimetic drug of the phenethylamine and amphetamine chemical classes. It is used as a nasal/sinus decongestant and stimulant or as a wakefulness-promoting agent[1].

Pseudoephedrine hydrochloride is formulated with several antihistaminic active substances including cetrizine[2] and fexofenadine[3,4] as antihistaminic-decongestant combination in capsules or tablets. Literature survey revealed different methods for the analysis of PSE in combined pharmaceutical dosage forms including, derivative spectrophotometry[5] and liquid chromatography[6-9].

Several methods have also been reported on liquid chromatographic determination of FEX in biological fluids using LC-MS[10,11] and fluorescence detection[12,13]. In pharmaceutical dosage forms, FEX was also determined using spectrophotometry[5], LC[14-16], capillary electrophoresis[17,18].

Pharmaceutical combinations containing FEX/PSE have been previously analyzed using spectrophotometry[5] and LC[6,8].

The efficient use of time in the pharmaceuticals, increasing need for speed and efficiency places a demand for the development of faster throughout analytical procedure. Very fast results with optimum column efficiency monolithic stationary phase have attracted considerable attention in LC due to their simple preparation procedure, unique properties and excellent performance, especially in separation of drug in pharmaceutical preparation[19]. The theoretical advantages for small packing particles include shallower slopes in the high velocity region

of plate height *versus* linear velocity curve[20] as well as higher optimum linear velocity. In addition monolithic column are cast as continuous homogenous phase. They represent an approach that provides high rates of mass transfer at lower pressure drops as well as high efficiencies even at elevated flow rate. Faster separation are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude as compared with traditional chromatographic columns packed with porous particles. This reduces back pressure, enhances the speed of the separation process and unspecific binding without sacrificing resolution[21,22].

To our best of knowledge, there is no report on a validated LC method which separates PSE and FEX by using monolithic column. Therefore our objective was aimed at developing a simple, sensitive, precise and rapid RP-LC with monolithic column and which would serve as a stability indicating assay method for the combination drug product of FEX and PSE. The proposed method was fully validated as per ICH guidelines[23-25].

EXPERIMENTAL

Fexofenadine hydrochloride and Pseudoephedrine hydrochloride were obtained as gift samples from Drug control centre, Riyadh, Saudi Arabia. TELFAST® tablets were purchased from KSA market, manufactured by Sanofi Aventis Co. Ltd. Methanol, LC grade were obtained from Panreac Quimica SA and acetonitrile, LC grade obtained from Prolabo, used without further purification. Sodium dihydrogen orthophosphate anhydrous was obtained from Merck (Darmstadt, Germany). Analytical-grade sodium hydroxide was purchased from WINLAB (UK). Deionized water was used throughout the experiment.

Instrumentation and chromatographic conditions: The employed LC system, WATERS (Milford, MA 01757, USA) Instrument equipped with Waters 2489 dual wavelength Ultraviolet-VIS detector (UV-VIS), Waters 1525 Binary LC Pump and Waters 2707 Autosampler and a data handling system comprised of a Dell personal computer, Empower 2 software. Ultra pure water of 18 MΩ/cm was obtained from Milli-Q Plus purification system, Millipore Waters (Milford, MA, USA). Detection was performed at 258 nm. The column used for separation was Chromolith, 100 mm × 4.6 mm, RP 18e. The mobile phase consisted of phosphate buffer (12.5 mM, pH = 4.5):acetonitrile:methanol (65:25:10 v/v/v), delivered at a flow rate of 2.4 mL/min. The mobile phase was filtered through a 0.22 μm nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature. Before sample injection, the column was conditioned with mobile phase for 20 min. The injection volume was 50 μL.

Preparation of standard solutions and construction of calibration graphs: Stock solutions containing 2000 μg/mL of FEX and PSE were prepared by dissolving the active ingredients in 1 mL of methanol and completing the volume to 10 mL with solvent (mobile phase without ions). Stock solution of internal standard containing 1000 μg/mL Losartan (LOS) was prepared in deionized water. The solutions were stable for at least three weeks if kept at room temperature. Aliquots of the stock solutions of FEX and PSE were further diluted with solvent, to yield final concentrations 0.8, 2, 8, 20, 40, 80 and 100 μg/mL for FEX and concentration of 2, 8, 20, 50, 100 and 200 μg/mL for PSE so that each solution contains 2.0 μg/mL losartan (internal standard). Triplicate injection (n = 3) of each concentration were performed. The peak area ratio of each concentration to the IS (internal standard) against the corresponding standard concentration were plotted, to obtain the calibration graphs. Alternatively, the corresponding regression equation was delivered.

Preparation of laboratory-made mixtures: Laboratory-made mixtures of FEX/PSE at three concentration levels (within the linearity range of each compound) were prepared so that each solution contains 2.0 μg/mL losartan (internal standard). Six replicate injections from each solution were made. The peak area ratio of each concentration to the internal standard was calculated. The concentration of each drug was obtained using the calibration curve of the corresponding regression equations.

Sample preparations: Ten tablets of Telfast® 120 mg were weighed and ground to homogenous powder. To the resulting powder, PSE was added to yield the corresponding ratios FEX/PSE (1:2), (2:3) for analysis of tablet formulation in its several different strength combination including 60(FEX)/120(PSE) mg and 120(FEX)/180(PSE) ratios available in the market. An accurately weighed portion of the mixed powdered tablet content equivalent to 25 mg FEX was transferred into each of 25 mL volumetric flask. Five mL of methanol was added to each flask to perform the extraction of the active ingredients and the resulting solution was shaked well for about 20 min. The solutions were made up to volume with solvent and mixed well. Further dilution was made and internal standard was added so that each solution contains 2.0 μg/ mL losartan (internal standard). The peak area ratio of each concentration to the internal standard was calculated. The concentrations of each drug in commercial tablets were obtained using the calibration curve of the corresponding regression equations. The solution was filtered through a Millipore membrane filter (0.22 μm) Nihon, Millipore (Yonezawa, Japan) before injection.

Accelerated degradation studies: Stock solutions containing 1000 μg/mL of each PSE and FEX were prepared and used for forced degradation studies to provide an indication of specificity of the method. For acid and base degradation, 0.5 mL of stock solution was heated with 0.5 mL of 0.5 M HCl or 0.5 M NaOH at 80 ºC for 4 h and then neutralized by adjusting the pH to 7.0. For thermal decomposition, 0.5 mL of stock solution was heated at 80 ºC for 4 h. Photodecomposition was also checked by exposing each drug to direct daylight for 24 h. In addition, drug powders were kept in an oven at 60 ºC for 3 h to test for the effect of dry heat.

In all degradation studies (acid, base-induced degradation, thermal decomposition, photodecomposition under day light, dry head degradation), the average peak area of PSE and FEX after three replicate injections (50 μg/mL) were obtained.

RESULTS AND DISCUSSION

Optimization of the chromatographic condition: In the current study, a monolithic column (Chromolith, 100 mm × 4.6 mm, RP 18e) was evaluated for the purpose of separation among the active drugs FEX and PSE. A wavelength of 258

nm was selected for the simultaneous determination of the two drugs with high sensitivity (Fig. 1).

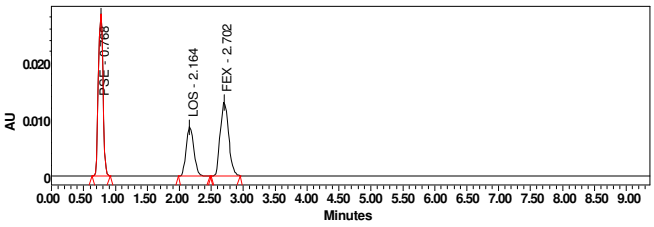


Fig. 1. A typical chromatogram of fexofenadine HCl (FEX), 100 µg/mL, pseudoephedrine HCl (PSE), 150 µg/mL and internal standard Losartan (LOS), 2 µg/mL

It is reported that FEX is an amphoteric compound due to the presence of alicyclic amine and carboxylic acid moieties[8]. However, PSE did not give dramatic responses to moderate variations in chromatographic conditions as FEX did; due to its polar nature[8].

To optimize the LC separation, two organic solvents (acetonitrile and methanol) and five different pH values (3.0-6.0) were tested. Proportions of methanol and acetonitrile were systematically changed. Higher acetonitrile ratio resulted in shorter retention times of FEX peaks whereas methanol had a significant effect on the resolution between FEX and LOS. For further optimization, methanol + acetonitrile (10 + 25 %) were mixed with phosphate buffer of different pH values varied in the range of 3.0-6.0. Best resolution were obtained with a mobile phase ratio of buffer: acetonitrile: methanol (65:25:10, v/v/v). pH of the aqueous phase had a significant effect on the resolution between PSE and FEX peaks where pH values 5.0 or more resulted in bad resolution between the two peaks. Moreover, phosphate buffer strength (10-100) had a significant effect on the sharpness and symmetry of FEX and losartan peaks and increased buffer strength above 50 mM resulted in a decreased resolution between FEX and losartan. As a result, the optimum mobile phase was chosen as phosphate buffer (12.5 mM, pH = 4.5):acetonitrile:methanol (65:25:10 v/v/v), delivered at a flow rate of 2.4 mL/min. Using this mobile phase, best results were obtained in terms of peak symmetry, selectivity and analysis time for both drugs.

Method validation

System suitability: The adequate resolution and repeatability of the proposed method, system suitability parameters including retention factor, selectivity, resolution and tailing factor were investigated (Table-1). The chromatographic characteristics of the mixture summarized indicate that the proposed LC method permitted adequate resolution of the mixture's components (good resolution and selectivity values) within reasonable run-time (suitable capacity factors). The degree of peak asymmetry was also evaluated using the tailing factor which did not exceed the critical value (1.2) indicating acceptable degree of peak asymmetry.

Specificity: The specificity of the analytical method may be defined as the ability to obviously determine the analyte in the presence of the additional components such as impurities, degradation products and matrix[25-27]. A solution of analytical placebo (containing all the tablet excipients except PSE and FEX) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients (placebo), standard solution and the commercial pharmaceutical preparation were analyzed by the developed method.

The specificity of the method was also evaluated to ensure that there were no interference products resulting from forced degradation.

TABLE-1
SYSTEM SUITABILITY TEST PARAMETERS FOR PSE, FEX AND LOS BY THE PROPOSED METHOD

System suitability test parameter	PSE	LOS	FEX
Retention time (min)(mean ± SD, n = 10)	0.75 ± 0.0013	2.00 ± 0.0005	2.65 ± 0.0022
Repeatability of retention time, RSD % [a], (n = 10)	0.05	0.21	0.08
Repeatability of peak area, RSD % = (SD/mean) × 100	0.022	0.050	0.088
[b]Resolution (R_s)	–	2.6	1.6
Tailing factor	1.11	0.88	1.21
Capacity factor (k')[c]	1.6	6.1	8.0
Selectivity factor (α)[d]	–	3.8	1.3

[a]RSD % = (SD/mean) × 100. [b]Resolution factor, calculated as $R_s = (t_2-t_1)/0.5(w_{1+}w_2)$. [c]$k' = (t_r-t_0)/t_0$, were t_r is the retention time of analyte and t_0 is the column dead-time. [d]Separation factor, calculated as k_2/k_2.

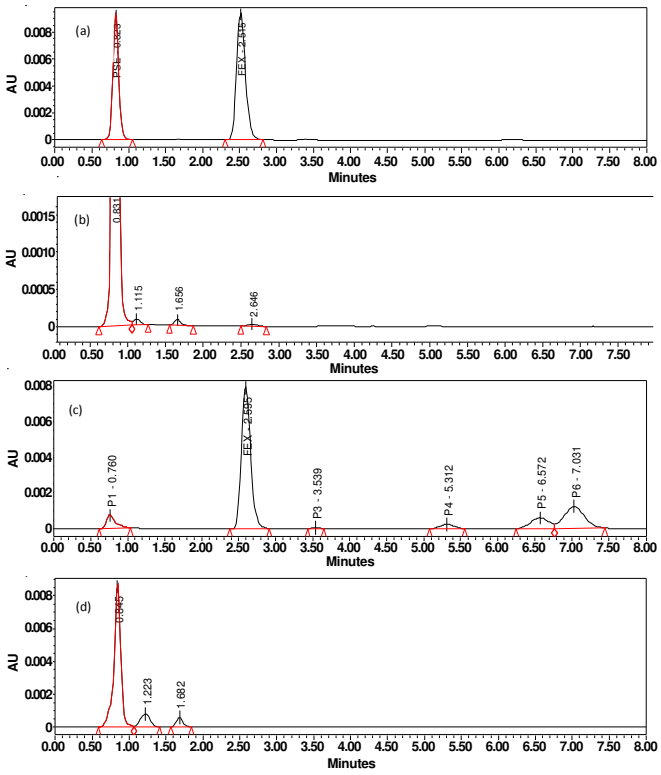
TABLE-2
SUMMARY OF FORCED DEGRADATION STUDIES

Stressed condition	Time (h)	Recovery (%)	RRT degradants
Pseudoephedrine hydrochloride			
Acid hydrolysis (0.5 M) HCl at 80°C	4	98.9	1.12, 1.68
Basic hydrolysis (0.5 M) HCl at 80°C	4	99.7	1.22, 1.68
Thermal decomposition (at 80°C)	4	98.9	1.70, 2.10
Photodecomposition under day light	24	99.2	–
Dry heat, at 60 ºC	3	98.7	–
Fexofenadine hydrochloride			
Acid hydrolysis (0.5 M) HCl at 80°C	4	83.5	3.54, 5.31, 6.57, 7.03
Basic hydrolysis (0.5 M) HCl at 80°C	4	86.2	–
Thermal decomposition (at 80 ºC)	4	99.1	1.70
Photodecomposition under day light	24	99.5	–
Dry heat, at 60 ºC	3	99.0	–

Forced degradation studies: In the present study, forced degradation studies were performed to demonstrate the validity of the method and to provide an evidence for the specificity of the proposed method according to ICH guidelines[20,21].

Degradation experiments were designed using acid, base, heat, direct daylight and dry heat and stress degradation samples were evaluated.

Results obtained from stress tests have been summarized in Table-2. Pseudoephedrine hydrochloride showed slight degradation under all stressed conditions used in the study. However, FEX showed considerable degradation when exposed to acidic (% recovery 83.5) or basic (% recovery 86.2) stress conditions. Fexofenadine hydrochloride was nearly not affected when exposed to thermal, dry heat-induced degradation or photodecomposition. Retention time of degradant peaks are shown in Table-2 and they were well separated from the major peaks of FEX, PSE showing the specificity of the proposed method. Some selected LC profiles showing degradation studies are shown in Fig. 2.



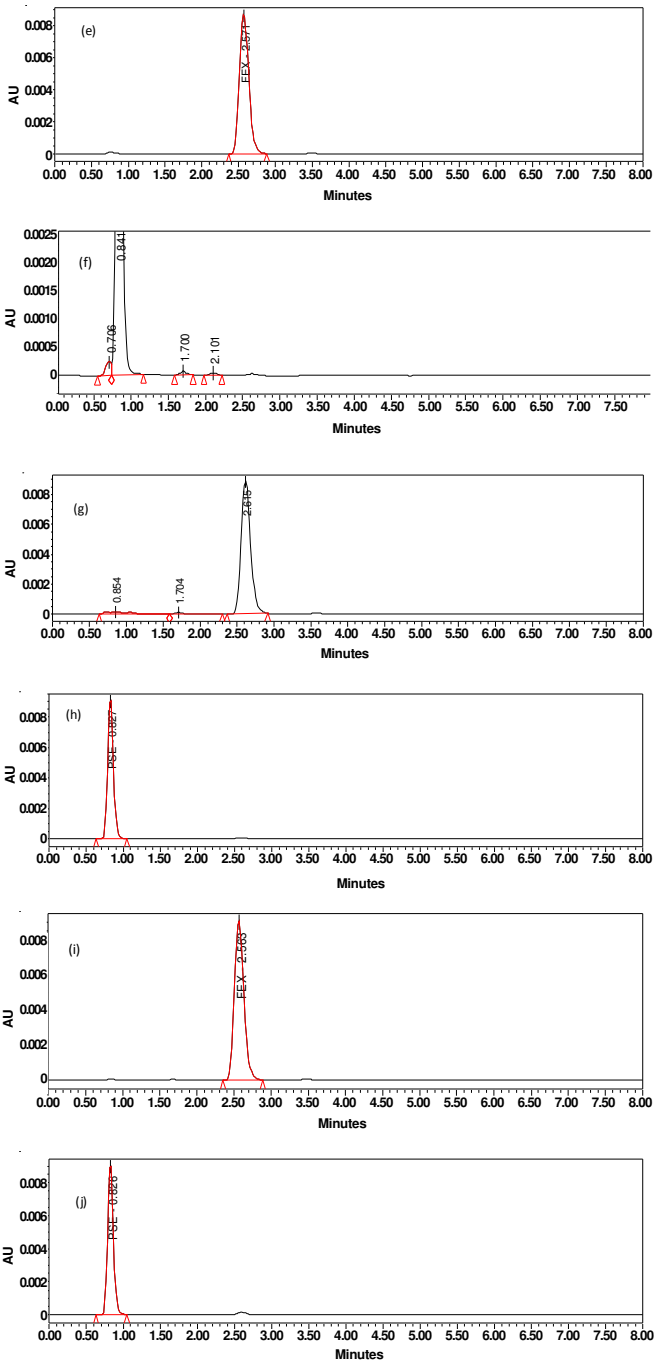


TABLE-4
ACCURACY AND PRECISION DATA FOR FEXOFENADINE HCl AND PSEUDOEPHEDRINE HCl USING THE PROPOSED METHOD

Analyte	Actual ratio conc. (µg/mL)	Experimental ratio conc. (µg/mL)	Recovery (%)	RSD (%)	Error (%)
Within-day[a] PSE	10.00	10.02	100.22	0.21	0.22
	40.00	40.44	101.10	0.26	1.10
	75.00	76.39	101.85	0.99	1.85
FEX	10.00	10.06	100.60	0.18	0.60
	20.00	20.01	100.05	0.12	0.05
	40.00	39.94	99.85	0.18	-0.15
Between-day[b] PSE	10.00	10.04	100.40	0.21	0.40
	40.00	40.03	100.08	0.82	0.08
	75.00	76.22	101.66	0.31	1.66
FEX	10.00	10.07	100.70	0.81	0.70
	20.00	20.08	100.40	0.14	0.40
	40.00	40.09	100.23	0.42	0.23

[a]Mean RSD on n = 6. [b]Mean RSD on n = 6.

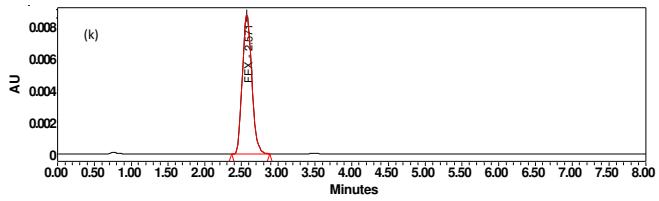


Fig. 2. Overlaid chromatograms obtained from: (a) pseudoephedrine HCl (PSE) standard, fexofenadine HCl (FEX) standard, (b) PSE treated with 0.5 M HCl, (c) FEX treated with 0.5M HCl , (d) PSE treated with 0.5 M NaOH, (e) FEX treated with 0.5 M NaOH, (f) PSE thermal decomposition (at 80 ºC), (g) FEX thermal decomposition (at 80 ºC), (h) PSE photodecomposition under direct day light, (i) FEX photodecomposition under direct day light, (j) PSE powder treated under dry heat, (k) FEX powder treated under dry heat. (50 µg/mL final concentration of intact PSE and FEX)

Limit of detection (LOD) and limit of quantitation (LOQ): The limit of quantitation (LOQ) and limit of detection LOD were determined according with ICH guidance Q2B[23]. The LOD was calculated using the equations $y\text{-}\alpha = 3.3 \times S\alpha$ and $y\text{-}\alpha = b \times LOD$, while the LOQ using the equation $y\text{-}\alpha = 10 \times S\alpha$ and $y\text{-}\alpha = b \times LOQ$ (where b is the slope of the corresponding calibration curve and Sα is the standard deviation of the intercept of the regression line) matrix[28]. The LOD obtained was 0.7 µg/mL for FEX and 0.9 µg/mL for PSE. The LOQ was 0.8 µg/mL for FEX and 2.0 µg/mL for PSE. The good linearity of the calibration graphs and the negligible scatter of experimental points are evident by the values of the correlation coefficient and standard deviation. The LOD and LOQ values of the developed method are presented in Table-3.

TABLE-3
VALIDATION PARAMETERS FOR THE DETERMINATION OF PSE AND FEX USING THE PROPOSED METHOD

Parameters	Pseudoephedrine HCl (PSE)	Fexofenadine HCl (FEX)
Concentration range (µg/mL)	2.0-200.0	0.8-100.0
Intercept (a)	0.00817	0.0117
Slope (b)	0.0281	0.0467
Correlation coefficient (r)	0.9999	0.9995
Retention time	0.7	2.6
$S_{y/x}$ (residual standard deviation)	0.015	0.029
S_a	0.552	0.38
S_b	0.000176	0.00057
LOQ (µg/mL)	2.0	0.8
LOD (µg/mL)	0.9	0.7

Linearity: The linearity of detector response for both FEX and PSE were determined by plotting the ratio response (ratio peak area of the drug) to the internal standard *versus* concentration of drug. The analytical data for the calibration graphs are listed in Table-3. The calibration curve were linear in the range of 0.8-100 µg/mL for FEX and 2.0-200.0 µg/mL for PSE, with correlation coefficient r = 0.9995 for FEX and 0.9999 for PSE.

A typical calibration curve has the regression equation of y = 0.00817 + 0.0281x, (n = 6), for PSE and y = 0.0117 + 0.0467x (n = 7), for FEX.

Precision: System repeatability was determined by six replicate applications and measurements of peak area for each active compound. Method repeatability was obtained from RSD % values obtained by repeating the assay six times on the same day (intra-day precision). Intermediate precision was assessed by the assay of sample sets on three different days (inter-day precision). The intra-and inter-day precision studies for the determination of both PSE and FEX were carried out at three different concentration levels of 10, 40, 75 µg/mL for PSE and 10, 20, 40 µg/mL for FEX (n = 6). The calculated RSD % for both drugs using the proposed LC method is mentioned in Table-4. The results indicated high degree of repeatability and reproducibility of the proposed methods.

Accuracy: Accuracy was evaluated as percentage relative error between the found and added concentrations for PSE and FEX. The results obtained are shown in Table-4, from which it is clear that accuracy is excellent for both active ingredients.

Selectivity: Selectivity is described as the ability of the method to discriminate the analyte from all potential interfering substances. Excipients commonly formulated with the studied drug did not interfere with the determination of FEX and PSE, indicating the high selectivity of the proposed method. Otherwise, there are no peaks detected at the retention time of individual drugs and of internal standard at the level of LOQ or less.

Robustness: Robustness of the method was checked by making slight deliberate change in chromatographic conditions like strength of phosphate buffer concentration (10, 12.5, 15 mM), pH of the buffer solution (4.3, 4.5, 4.7), mobile phase composition ratios (phosphate buffer:acetonitrile:methanol (65:25:10, 65:27:8, 65:23:12 v/v/v) and flow rate (2.2, 2.4, 2.6 mL/min). It was observed that there were no marked changes on RSD of peak areas or the retention times in chromatograms.

Solution stability: The stability of standard solutions in the solvent used were analyzed over a period of 8 h at room temperature. The results showed that, the retention time and the peak area of PSE and FEX remained unchanged and no significant degradation was observed.

Application of the LC method to pharmaceutical products: The application of the proposed method was examined by analyzing the % recovery of both drugs PSE/FEX in ratio (1:2), (2:3), respectively, ratios available in the market. The results of analysis showed that the amount of drug in the formulation was in good agreement with the labeled claim of formulation. All the obtained data fully met the criteria from the ICH guideline[23-25] and the proposed method was reliable for quantification of PSE and FEX in pharmaceutical formulations. The detailed analytical data are shown in Table-5.

Conclusion

A simple, rapid and accurate LC method was developed for the simultaneous determination of PSE and FEX in pharmaceutical tablets by isocratic mode using monolithic column and a very simple mobile phase. The analytical conditions developed provided good resolution for the analytes (PSE, FEX) when using LOS an internal standard. This method has provided good accuracy and precision and excellent reproducibility within a run time less than 3 min. Thus the developed LC method can be proposed for routine analysis laboratories

TABLE-5
APPLICATION OF THE PROPOSED METHOD TO THE ANALYSIS OF PSE AND FEX IN TABLET FORMULATIONS

Ratio FEX/PSE	Label claim (mg) FEX/PSE	Label (%) claim estimated FEX	Mean (%)*	Label (%) claim estimated PSE	Mean (%)*
1:2	60:120	99.85 99.33 99.51	99.55 (RSD (%) = 0.2 %)	99.73 98.59 99.15	99.16 (RSD % = 0.4 %)
2 : 3	120 : 180	100.06 99.86 99.34	99.55 (RSD % = 0.3 %)	100.39 100.10 99.60	100.03 (RSD % = 0.2 %)

*Percentage of relative standard deviation RSD % ≤ 1.0 %, (n = 5).

and quality control purposes because of the speed of analysis and simple extraction procedure. Owing to use of monolithic column, which has lower separation impedence compared to the particulate packing, much faster separations are possible and thus much less solvent consumption. In addition, the proposed method was based on the use of losartan as the internal standard which elution is between the two tested active ingredients to guarantee a high level of quantitative performance.

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