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Development and validation of a rapid RP-HPLC method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms

Sevgi Karakuş, İlkay Küçükgüzel *, Ş.Güniz Küçükgüzel

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Haydarpaşa, 34668 İstanbul, Turkey

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Abstract

The objective of the current study was to develop a simple, accurate, precise and rapid reversed-phase HPLC method and subsequent validation using ICH suggested approach for the determination of antihistaminic-decongestant pharmaceutical dosage forms containing binary mixtures of pseudoephedrine hydrochloride (PSE) with fexofenadine hydrochloride (FEX) or cetirizine dihydrochloride (CET). The chromatographic separation of PSE, FEX and CET was achieved on a Zorbax C8 (150 mm × 4.6 mm; 5 μm particle size) column using UV detection at 218 and 222 nm. The optimized mobile phase was consisted of TEA solution (0.5%, pH 4.5)–methanol–acetonitrile (50:20:30, v/v/v). The retention times were 1.099, 2.714 and 3.808 min for PSE, FEX and CET, respectively. The proposed method provided linear responses within the concentration ranges 30–240 and 1.25–10 $\mu g\, ml^{-1}$ with LOD values of 1.75 and 0.10 $\mu g\, ml^{-1}$ for PSE and CET, respectively. Linearity range for PSE–FEX binary mixtures were 10–80 and 5–40 $\mu g\, ml^{-1}$ with LOD values of 0.75 and 0.27 $\mu g\, ml^{-1}$ for PSE and FEX, respectively. Correlation coefficients (r) of the regression equations were greater than 0.999 in all cases. The precision of the method was demonstrated using intra- and inter-day assay R.S.D. values which were less than 1% in all instances. No interference from any components of pharmaceutical dosage forms or degradation products was observed. According to the validation results, the proposed method was found to be specific, accurate, precise and could be applied to the quantitative analysis of these drugs in capsules containing PSE–CET or extended-release tablets containing PSE–FEX binary mixtures.
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Keywords: Cetirizine; Fexofenadine; Pseudoephedrine; Reversed-phase HPLC; Validation

1. Introduction

Cetirizine (CET), (*RS*)-2-[4-[(*p*-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxyacetic acid dihydrochloride (Fig. 1), a piperazine derivative and active metabolite of hydroxyzine, is a second generation antihistaminic drug used in symptomatic treatment of seasonal allergic rhinitis and perennial allergic rhinitis as well as chronic urticaria and pruritus [1–3]. CET selectively inhibits histamine H_1-receptors without causing sedation due to its physicochemical properties which prevents its passage through blood–brain barrier [1,4]. As an advantage compared to the first generation compounds, CET is a non-sedating derivative and it does not affect serotonergic, muscarinergic and α-adrenergic receptors [1,4]. Combined formulation of CET with pseudoephedrine (PSE), a sympatomimetic decongestant, is prescribed mainly for the treatment of seasonal allergic rhinitis [5].

CET is official in EP [6]. A literature survey reveals a variety of analytical methods for the analysis of CET including titrimetric [7], spectrophotometric [8,9] and liquid chromatographic methods [10–14]. There is also reports on stereoselective determination of CET using a chiral stationary phase and its application to pharmacokinetic studies [15] and enantioselective analysis of CET in pharmaceuticals by cyclodextrin-mediated capillary electrophoresis [16].

Use of acid–base titration [7] and HPLC [12] techniques have been reported for the determination of CET alone in raw material or pharmaceuticals. Spectrophotometric methods for the estimation of CET in dosage forms alone [7,8] or combined with pseudoephedrine [9] were also described. Several HPLC methods [8,12,14] have been reported to be applied to tablets containing

* Corresponding author. Fax: +90 216 345 29 52.
E-mail addresses: kucukguzel@hotmail.com, ikucukguzel@marmara.edu.tr (İ. Küçükgüzel).

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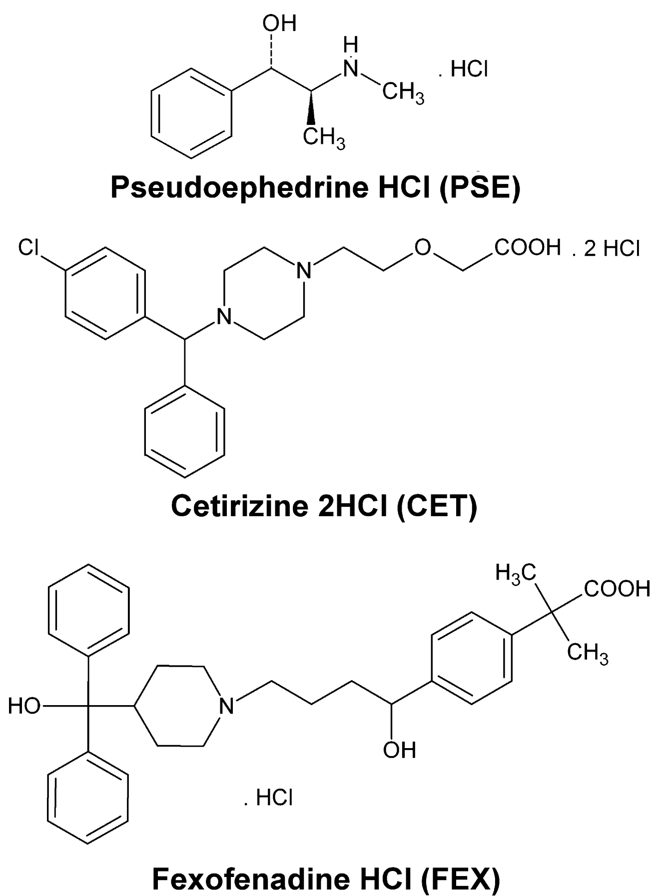

Fig. 1. Chemical structures of pseudoephedrine HCl, cetirizine 2HCl and fexofenadine HCl.

CET. Jaber et al. have reported a validated RP-HPLC method for the determination of CET and its related impurities in oral solution and tablets [17]. Another report by Nagavalli et al. describes liquid chromatographic determination of CET and paracetamol in human plasma and pharmaceutical formulations [18].

Two HPTLC procedures were reported for the quantification of CET in human plasma [19] and in pharmaceutical dosage forms [20], respectively. There is also a recent report about a validated HPLC method for dissolution test of combined tablets containing cetirizine dihydrochloride and pseudoephedrine hydrochloride [14].

Fexofenadine, α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]benzeneacetic acid (Fig. 1), a selective H_1-receptor antagonist, is the principal active metabolite of terfenadine and it bears antihistaminic properties of the parent compound. Thanks to its capability to exist in zwitter-ionic form, it cannot pass through blood–brain barrier and therefore does not cause sedation [21,22]. FEX displays some anti-inflammatory properties and it has also another advantage as it lacks the cardiotoxic side effects (fatal arrythmy) associated with terfenadine [21,22]. FEX is official in the USP [23]. There are several reports on liquid chromatographic determination of fexofenadine in biological fluids using HPLC-MS [24], HPLC with tandem mass detection [25] and fluorescence detection [26]. Literature survey revealed a few methods for the quantitative determination of fexofenadine and its related compounds in bulk or pharmaceutical dosage forms by HPLC with UV detection [27,28], as well as a spectrophotometric method in combined pharmaceutical dosage forms with pseudoephedrine [9]. Recently, another HPLC method were reported for dissolution tests for fexofenadine containing capsules and coated tablets [29]. There are also two recent reports on capillary electrophoresis methods for the determination of FEX in capsules [30] or tablets [31].

Pseudoephedrine, (1*S*,2*S*)-2-methylamino-1-phenylpropan-1-ol (Fig. 1), is formulated with several antihistaminic active substances including cetirizine [5] and fexofenadine [32,33] as antihistaminic-decongestant combination in capsule or coated tablet forms for the treatment of seasonal allergic rhinitis. A derivative spectrophotometric method have been reported for the determination of pseudoephedrine in binary mixtures with antihistamines including cetirizine, fexofenadine and loratadine [9]. There are also liquid chromatographic methods reported for the HPLC quantitation of pseudoephedrine in combined pharmaceutical dosage forms with acrivastine [34] and loratadine [35].

There is no report on a validated HPLC method which separates PSE, FEX and CET; thus providing simultaneous determination of CET–PSE or FEX–PSE in pharmaceutical dosage forms. Therefore it was aimed at developing a simple, sensitive, precise and rapid reversed-phase HPLC method and subsequent validation using ICH suggested approach for the determination of antihistaminic-decongestant pharmaceutical dosage forms containing binary mixtures of pseudoephedrine hydrochloride (PSE) with fexofenadine hydrochloride (FEX) or cetirizine dihydrochloride (CET).

2. Experimental

2.1. Materials and reagents

Cetirizine dihydrochloride (CET) and pseudoephedrine hydrochloride (PSE) were kindly provided by Deva Pharmaceuticals (Istanbul, Turkey). Fexofenadine hydrochloride (FEX) was a gift from Sanovel Pharmaceutical Company (Istanbul, Turkey). Methanol and acetonitrile were of gradient grade and purchased from Merck company (Darmstadt, Germany). Triethylamine (TEA) and orthophosphoric acid (85%) were of analytical grade and procured from Fluka and Carlo–Erba companies, respectively. Doubly distilled water was used for preparing mobile phase and other solutions. Pharmaceutical finished dosage forms utilized in the present work include: Cirrus® capsules claimed to contain 5 mg of CET and 120 mg of PSE; Allegra-D® 12 h extended release tablets claimed to contain 60 mg of FEX and 120 mg of PSE.

2.2. Instrumentation

The liquid chromatographic system, used in the present study, consisted of an Agilent technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G-13158 photodiode array detector. A Rheodyne syringe loading sample injector with a 20 μl sample

loop was used for the injection of analytes. Chromatographic data were collected and processed using Agilent Chemstation Plus software. The separation was performed at ambient temperature, on a reversed phase Zorbax Eclipse XDB-C8 column (150 mm × 4.6 mm; 5 μm particle size). A Zorbax C8 analytical guard column (12.5 mm × 4.6 mm) packed with the same sorbent was used. All experiments were employed in the isocratic mode.

2.3. Chromatographic conditions

The mobile phase was prepared by mixing methanol, acetonitrile and triethylamine (TEA) solution (0.5%) in varying proportions. Before mixing with organic solvents, the final pH of TEA solution (0.5%) was adjusted to the desired value (pH 3.0–5.0) with orthophosphoric acid. The optimum mobile phase which was used in the validation studies consisted of TEA solution (0.5%, pH 4.5)–methanol–acetonitrile (50:20:30, v/v/v). This phase was filtered through a 0.45 μm membrane and degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 1.5 ml min^{-1}. Detection of the analytes were carried out at 218 nm for FEX –PSE; and 222 nm for CET–PSE binary mixtures. Injection volume of the analytes was set to a constant volume of 20 μl using a sample loop.

2.4. Standard stock solutions and construction of calibration curves

Stock solutions of 1 mg ml^{-1} of CET, FEX and PSE were prepared by dissolving them in methanol and kept in a refrigerator until being used. Standard calibration solutions were prepared by dilution of the stock solutions using the mobile phase. These solutions were considered at seven different levels which were 25%, 50%, 75%, 100%, 125%, 150% and 200% of the test concentration. For CET–PSE binary mixtures, standard solutions of CET and PSE containing a constant concentration of 5 μg ml^{-1} of FEX (internal standard) were prepared in mobile phase by maintaining the concentrations in the range of 1.25–10 and 30–240 μg ml^{-1}, respectively. For FEX–PSE binary mixtures, standard solutions of FEX and PSE containing a constant concentration of 20 μg ml^{-1} of CET (internal standard) were prepared in mobile phase by maintaining the concentration in the range of 5–40 and 10–80 μg ml^{-1}, respectively. Six replicate injections were made for each concentration. The calibration curves for CET–PSE and FEX–PSE binary mixtures were constructed by plotting the peak area ratio of the drug to that of internal standard, against the drug concentration.

2.5. Sample preparations

Twenty tablets or capsule contents were accurately weighed, their mean weight were determined, and they were then finely powdered. An amount equivalent to one tablet or capsule content was transferred into a 50 ml volumetric flask, added 40 ml of methanol, sonicated for 30 min, diluted to 50 ml with methanol and a 10 ml sample taken from this solution was centrifuged at 3000 rpm for 15 min. A 1-ml aliquot from supernatant was then decanted to another 10-ml volumetric flask. Appropriate amounts of internal standards, FEX for CET–PSE binary mixtures and CET for FEX–PSE binary mixtures, were added to give fixed concentrations of 5 and 20 μg ml^{-1}, respectively. Test solutions were then made up to volume with the mobile phase. The amounts of CET–PSE and FEX–PSE in binary mixtures or dosage forms were individually calculated using the related linear regression equations.

2.6. Recovery studies

To demonstrate the accuracy of the proposed method and to see whether there is inferference from excipients used in the dosage forms, recovery studies were employed by the standard addition method. This was carried out by adding known amounts of CET–PSE (for Cirrus® capsules) or FEX–PSE (for Allegra-D® extended release tablets) to the powdered commercial tablets or capsule contents. HPLC samples were then prepared and the resulting mixtures were analysed as described for pharmaceutical dosage forms.

2.7. Accelerated degradation studies

All degradation experiments in solution were performed at a drug concentration of 500 μg ml^{-1}. For acid and basic degradation, 2 ml of stock solution was heated with 2 ml of 0.5 N HCl or 0.5 N NaOH at 80 °C for 4 h, and then neutralized by adjusting the pH to 7.0. For photo and thermal decomposition experiments, separate solutions of pure drug samples in the mobile phase (500 μg ml^{-1}) were prepared. These solutions were exposed to ultraviolet light (254 nm) for 10 h, direct daylight for 24 h; or, kept at 80 °C for 8 h. Another solution which was protected from light, was also kept at ambient temperature for 24 h in order to observe short term stability of the stock solutions.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

During the optimization of the separation method, three columns (Zorbax C8 5 μm, 150 mm × 4.6 mm; Kromasil C18 5 μm, 250 mm × 4.6 mm; Symmetry C18 5 μm, 150 mm × 4.6 mm), two organic solvents (acetonitrile and methanol) and five different pH values (3.0–5.0) with and without ion pairing agent (hexane sulphonate) were tested. CET and FEX exhibited a similar behaviour as they both are amphoteric due to the presence of alicyclic amine and carboxylic acid moieties. PSE did not give dramatic responses to moderate variations in chromatographic conditions as CET and FEX did; due to its very polar nature. Of the stationary phases experienced, Zorbax C8 gave the best results in terms of peak shape, resolution and analysis time. To overcome the weak retention of PSE, formation of its ion pair with hexane sulphonate was tried; but this resulted in very late elution or no peaks for CET and FEX.

After trying several mobile phases containing acetonitrile and methanol with various buffers, the one consisting of methanol–acetonitrile–TEA (0.5%) proved to be useful for bet-

ter resolution and peak symmetry. TEA not only provided the desired pH together with orthophosphoric acid, but also prevented peak tailing of our basic analytes due to its silanol masking feature [36]. To optimize this mobile phase, proportions of methanol + acetonitrile were systematically changed from 10 + 40 to 40 + 10%, whilst percentage of TEA (0.5%, pH 3.0) was always 50%. Higher acetonitrile ratio resulted in shorter retention times of all analytes whereas all three compounds tend to elute later with increasing ratio of methanol. For further optimization, methanol + acetonitrile (20 + 30%) were mixed with TEA (0.5%) of different pH values varied in the range of 3.0–5.0. As a result of pH screening, the optimum mobile phase was chosen as TEA solution (0.5%, pH 4.5)–methanol–acetonitrile (50:20:30, v/v/v). The flow rate used was set to 1.5 ml min^{-1} for all experiments. Using this mobile phase, best results were obtained in terms of peak symmetry, selectivity and analysis time for both pharmaceutical formulations.

Detection wavelengths were chosen considering the ratios of active ingredients in pharmaceutical dosage forms. Thus, it was aimed at maintaining the peak heights close to each other as much as possible. The detector was set to 218 nm for determination of the extended–release tablets which contained 120 mg of PSE and 60 mg of FEX. Using this wavelength, the peak of CET was very small in comparison to PSE peak, as the capsules contained 120 mg of PSE and only 5 mg of CET. Therefore, detection was performed at 222 nm where CET had a higher absorbance in spite of its low amount in the formulation.

Our aim was to develop a rapid and sensitive liquid chromatographic method for the quality control analysis of either fexofenadine HCl or cetirizine 2HCl binary mixtures with pseudoephedrine HCl in pharmaceutical dosage forms. As it was possible to separate these three compounds within less than 5 min, FEX was selected as internal standard for the quantitation of capsules containing CET–PSE, whereas CET was the internal standard for the analysis of FEX–PSE in extended–release tablets.

Table 1
System suitability results of the proposed method

Compound	*N*	*R*	*T*	R.S.D. of	
				t_R	Peak area
PSE	2434	–	1.13	0.24	0.70
FEX	4896	12.53	1.21	0.13	0.21
CET	6915	7.54	1.17	0.14	0.35
Required limits	$N > 2000$	$R > 2$	$T < 1.5$	R.S.D. < 1%	

N: theoretical number of plates; *R*: resolution; *T*: USP tailing factor; t_R: retention time; R.S.D.: relative standard deviation for retention time or peak areas obtained from six replicate injections (instrument precision).

3.2. Validation of the method

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2A and Q2B [37]. The described method has been extensively validated in terms of specifity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability. The precision (% relative standard deviation) was expressed with respect to the intra- and inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of the active pharmaceutical ingredients (APIs) added to the known amount of the pharmaceutical dosage forms. After validation, the developed method have been applied to pharmaceutical dosage forms containing CET–PSE (FEX used as IS) and FEX–PSE (CET used as IS), respectively.

3.2.1. System suitability

System suitability tests are an integral part of a liquid chromatographic method, and they were used to verify that the proposed method was able to produce good resolution between the peaks of interest with high reproducibility [38]. The system

Table 2
Summary of forced degradation studies

Stress conditions	Time (h)	% recovered	Purity factor[a]	RRT of degradants
Pseudoephedrine				
Acid hydrolysis (0.5 N HCl at 80 °C)	4	97.6	998.298	1.45
Basic hydrolysis (0.5 N NaOH at 80 °C)	4	99.3	998.797	1.45
Thermal decomposition (at 80 °C)	8	98.9	999.598	1.44
Photodecomposition under direct daylight	20	98.5	998.802	1.44
under UV (254 nm)	10	98.3	999.385	1.44, 2.40
Cetirizine				
Acid hydrolysis (0.5 N HCl at 80 °C)	4	98.7	999.973	0.41, 2.75
Basic hydrolysis (0.5 N NaOH at 80 °C)	4	96.3	999.934	0.41
Thermal decomposition (at 80 °C)	8	89.7	999.923	0.41, 2.75
Photodecomposition under direct daylight	20	99.6	999.846	0.41
under UV (254 nm)	10	97.2	999.931	0.41
Fexofenadine				
Acid hydrolysis (0.5 N HCl at 80 °C)	4	82.5	999.726	0.61, 1.84, 2.06
Basic hydrolysis (0.5 N NaOH at 80 °C)	4	88.6	999.220	0.59, 1.83
Thermal decomposition (at 80 °C)	8	99.5	999.958	0.59
Photodecomposition under direct daylight	20	99.6	999.970	0.59, 1.47
under UV (254 nm)	10	99.9	999.942	0.60, 1.47, 1.99

[a] Purity factors were calculated for the related peaks corresponding to pseudoephedrine, cetirizine and fexofenadine.

suitability was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (*N*), resolution (*R*) and tailing factors (*T*). System suitability requirements for CET, FEX and PSE were a R.S.D. of peak areas and retention times less than 1%, peak resolution (*R*) greater than 2.0 between two adjacent peaks for three analytes, theoretical plate numbers (*N*) at least 2000 for each peaks and USP tailing factors (*T*) less than 1.5. The results of system suitability test in comparison with the required limits can be shown in Table 1. According to the results presented, the proposed method fulfils these requirements within the accepted limits.

3.2.2. Stability/specificity

Specifity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition experiments, indicates that the proposed method was not affected by interferences from excipients used in formulations. Photodiode array detection also supported the specificity of the method and provided evidence for the homogeneity of the peaks of analytes. Peaks obtained from recovery experiments or analysis of dosage forms, were checked for uniformity using UV spectra taken from seven different points of the peak of interest. These spectra were superimposable whenever overlaid, showing that there was no other co-eluting peaks, in every instance for each of the analytes, PSE, FEX and CET.

Another approach was accelerated degradation studies which were performed to demonstrate the validity of the method. Accelerated degradation studies were performed to provide an evidence for the specificity of the proposed method. Intentional degradation experiments were designed using acid, base, heat, UV (254 nm) and direct daylight.

Peak purity of the stressed APIs was checked using an Agilent 1100 Diode array detector (DAD). The purity factor was always found above the threshold limit in all stressed samples, as a support to analyte peak homogeneity. Results obtained from stress tests have been summarized in Table 2. PSE was degraded under acidic stress conditions by 3.4%; whereas no degradation was detected when it was exposed to 0.5 N NaOH at 80 °C. The drug degradation was observed by 10.3% when CET was exposed to thermal degradation. No degradation was observed with CET under direct daylight whereas UV radiation resulted in 2.8% loss in the API. FEX was observed to be degraded more than 10% when it was subjected to acidic or basic stress conditions. However, it was not affected if thermal- or photodegradation was performed. Degradant peaks were observed to be resolved from their parent APIs showing the specificity of the proposed method. Some selected HPLC profiles representing drug degradation studies were given in Fig. 2.

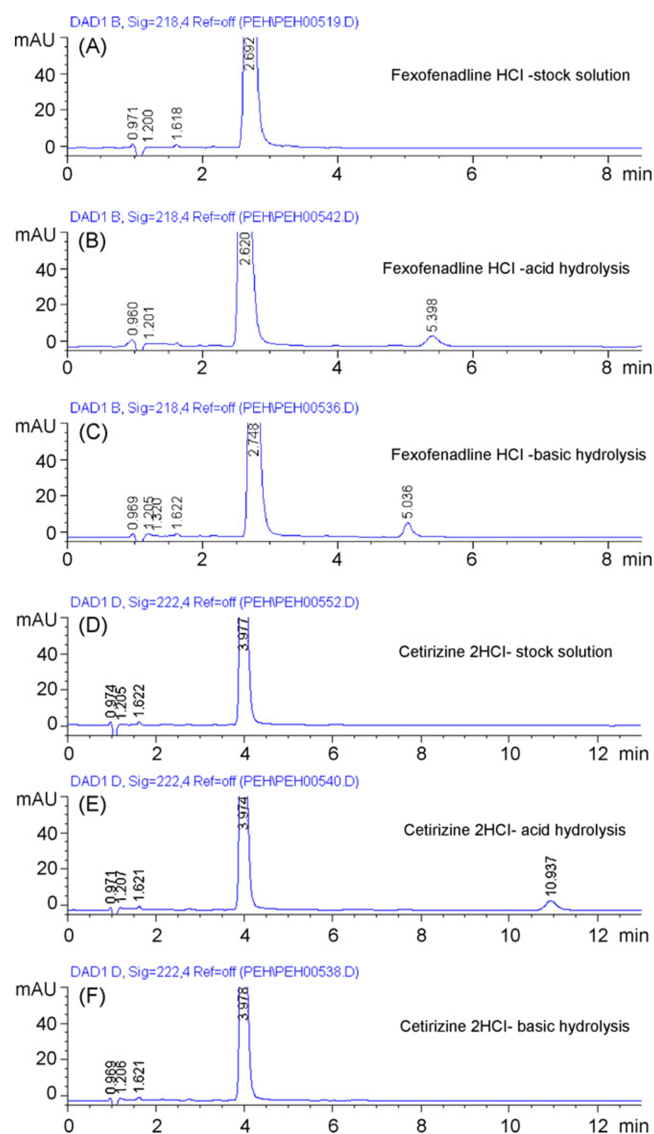

Fig. 2. HPLC profiles of FEX and CET active substances under stress conditions (A) pure FEX sample; (B) FEX sample degraded with 0.5 N HCl; (C) FEX sample degraded with 0.5 N NaOH; (D) pure CET sample; (E) CET sample degraded with 0.5 N HCl; and (F) CET sample degraded with 0.5 N NaOH.

3.2.3. Linearity

The calibration curves for FEX and PSE in binary mixtures were constructed by plotting the ratio of the peak area of FEX or PSE to peak area of internal standard (CET) against the concentration. Linearity data were obtained using standard solutions containing FEX and PSE at seven different concentrations ranging from 50 to 200% of nominal concentration, 5.0–40.0 μg ml^{-1} for FEX and 10.0–80.0 μg ml^{-1} for PSE, whilst keeping the concentration of the CET (IS) constant at 20 μg ml^{-1}.

For CET–PSE binary mixtures, linearity was checked at seven different concentration levels ranging from 50 to 200% of nominal concentration, 1.25–10 μg ml^{-1} for CET and 30–240 μg ml^{-1} for PDE, whilst keeping the concentration of FEX (IS) constant at 5 μg ml^{-1}. Triplicate injections were made for each concentration. The linearity of the calibration curves was determined on two different days for intra- and inter-day variation. The plot of peak area ratios vs. concentrations of all analytes were found to be linear within the concentration ranges stated above.

Table 3
Characteristics of PSE and CET calibration plots[a]

	PSE	CET
Linearity range (μg ml^{-1})	30–240	1.25–10
Slope	0.0146	0.1498
Intercept	−0.0492	−0.0390
Correlation coefficient (*r*)	0.9999	0.9998
R.S.D.% of slope	0.06	0.56
R.S.D.% of intercept	1.30	1.29
Limit of detection (μg ml^{-1})	1.75	0.10
Limit of quantification (μg ml^{-1})	5.31	0.30

[a] Mean of six injections.

Table 4
Characteristics of PDE and FEX calibration plots[a]

	PSE	FEX
Linearity range (μg ml^{-1})	10–80	5–40
Slope	0.0293	0.0776
Intercept	−0.0086	−0.0195
Correlation coefficient (*r*)	0.9998	0.9999
R.S.D.% of slope	0.41	0.39
R.S.D.% of intercept	0.74	0.96
Limit of detection (μg ml^{-1})	0.75	0.27
Limit of quantification (μg ml^{-1})	2.26	0.83

[a] Mean of six injections.

Table 5
Summary of intra-day (repeatability) and inter-day (intermediate precision) variability data for simultaneous determination of PSE and CET standards

Compound	Theoretical concentration (μg ml^{-1})	Intra-day measured concentration (μg ml^{-1})[a]		Inter-day measured concentration (μg ml^{-1})[b]	
		Mean	R.S.D.%	Mean	R.S.D.%
PSE	36	36.13	0.53	35.70	0.79
	120	119.18	0.51	120.31	0.57
	216	216.26	0.53	213.83	0.76
CET	1.5	1.49	0.64	1.48	0.83
	5	4.96	0.44	5.02	0.40
	9	9.04	0.35	8.95	0.66

[a] Mean values represent five different sample standards for each concentration.
[b] Inter-day precision was determined from five different runs over a 2-week period.

Table 6
Summary of intra-day (repeatability) and inter-day (intermediate precision) variability data for simultaneous determination of PSE and FEX standards

Compound	Theoretical concentration (μg ml^{-1})	Intra-day measured concentration (μg ml^{-1})[a]		Inter-day measured concentration (μg ml^{-1})[b]	
		Mean	R.S.D.%	Mean	R.S.D.%
PSE	12	11.91	0.27	11.89	0.76
	40	39.73	0.60	39.69	0.49
	72	71.91	0.71	72.88	0.35
FEX	6	6.06	0.54	5.99	0.77
	20	20.00	0.50	19.99	0.80
	36	35.61	0.69	35.56	0.41

[a] Mean values represent five different sample standards for each concentration.
[b] Inter-day precision was determined from five different runs over a 2-week period.

Table 7
Statistical analysis of assay results and recovery experiments in commercial samples

	Cirrus® Capsules		Allegra-D® 12 h extended release tablets	
	PSE	CET	PSE	FEX
Analysis of pharmaceutical dosage forms				
Label claim (mg)	120	5	120	60
Mean of amount found (mg)[a]	121.23	4.92	119.22	58.87
Confidence limits[b]	±0.68	±0.04	±1.07	±0.22
Recovery %	101.02	98.47	99.35	98.11
R.S.D.%	0.54	0.81	0.86	0.35
Recovery analysis using standard addition method				
Added (mg)	30	1.25	30	15
Mean of amount found (mg)[a]	29.26	1.23	30.29	14.85
Confidence limits[b]	±0.97	±0.03	±0.18	±0.08
Recovery %	97.53	98.40	100.98	98.97
R.S.D.%	0.62	0.53	0.55	0.54

[a] Mean values represent six determinations.
[b] Calculated value for 95 % confidence level.

A linear simple regression by the least squares method was applied. The representative linear equations were $y = 0.0146x - 0.0492$ ($n = 6$, $r^2 = 0.9999$) and $y = 0.1498x - 0.0390$ ($n = 6$, $r^2 = 0.9998$) for PSE and CET, respectively, in PSE–CET binary mixtures. In the PSE–FEX binary mixtures, the regression equations were found as $y = 0.0293x - 0.0086$ ($n = 6$, $r^2 = 0.9998$) and $y = 0.0776x - 0.0195$ ($n = 6$, $r^2 = 0.9999$) for PSE and FEX, respectively. R.S.D. of the slope at the linearity range were found to be between 0.06 and 0.59%, indicating the repeatability of all calibration curves. The correlation coefficiencies (r) were found to be greater than 0.999 in all instances. Tables 3 and 4 represent calibration characteristics for CET–PSE and FEX–PSE binary mixtures.

3.2.4. Limit of detection (LOD) and limit of quantitation (LOQ)

ICH guideline Q2B [37] describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criterions, respectively; where σ is the standard deviation of the peak area ratios and s is the slope of the corresponding calibration curve. The LOD and LOQ values of the developed method are presented in Tables 3 and 4.

3.2.5. Precision

The precision of the proposed method were assessed as repeatability and intermediate precision performing five replicate injections of three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily (Tables 5 and 6). These experiments were repeated over a 2-week period to evaluate day-to-day variability (intermediate precision). As can be seen in Tables 5 and 6, the % R.S.D. values of the measurements ranged between 0.27 and 0.71%. The % R.S.D. of assay results obtained in intermediate precision study were not greater than 0.83%, confirming good precision of the proposed method between days.

3.2.6. Accuracy

Accuracy of the proposed method was established by recovery experiments using standard addition method. This study was employed by addition of known amounts of FEX, CET and PSE onto known concentration of commercial capsules and tablets. The resulting mixtures were analyzed as described in Section 3.3. Results obtained from recovery studies are given in Table 7. The recovery experiments, using Cirrus® capsules containing PSE–CET, showed mean recoveries of 97.53 and 98.40% with R.S.D. values of 0.62 and 0.53% for PSE and CET, respectively. The recoveries obtained from Allegra-D® extended release tablets were found as 100.98 and 98.97% with R.S.D.% values of 0.55 and 0.54% for PSE and FEX, respectively.

High recovery results obtained from the proposed method for the analysis of CET–PSE capsules and FEX–PSE extended release tablets indicate that this assay procedure can be used for quantitation and routine quality control analysis of these binary mixtures in commercial samples.

3.3. Application of the validated method to pharmaceutical products

On the basis of above results, the proposed method was applied to the determination of CET and PSE in capsule dosage forms which comprised the binary mixture (5 mg CET and 120 mg PSE); as well as FEX and PSE in extended release tablets which comprised the binary mixture (60 mg FEX and 120 mg PSE).

Fig. 3 shows representative chromatograms obtained from the analysis of CET and PSE in capsules and FEX and PSE in extended release tablets. The differences between the amount

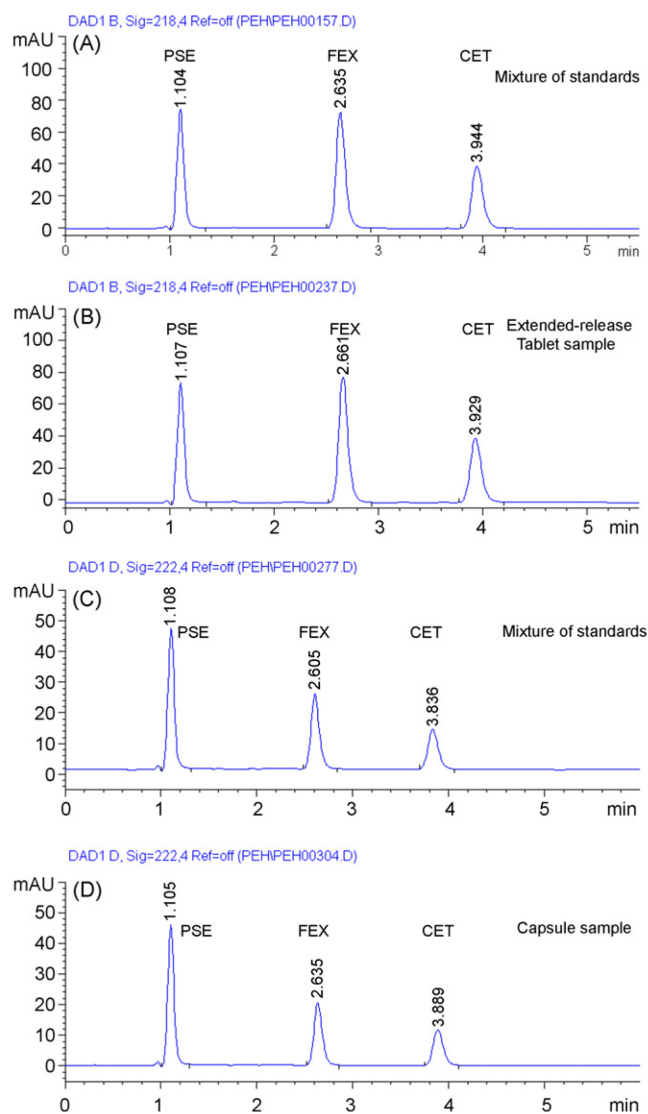

Fig. 3. Representative chromatograms of standard solutions and pharmaceutical dosage forms. (A) A standard solution containing 40 μg ml^{-1} of PSE, 20 μg ml^{-1} of FEX and 20 μg ml^{-1} of CET; (B) an assay sample solution of extended–release tablets containing PSE and FEX (CET was used as IS); (C) a standard solution containing 120 μg ml^{-1} of PSE, 5 μg ml^{-1} of FEX and 5 μg ml^{-1} of CET; and (D) an assay sample solution of capsules containing PSE and CET (FEX was used as IS).

claimed and those assayed were very low and the R.S.D. values were within the acceptable range mentioned by pharmacopoeias. The mean values of 121.23 and 4.92 mg with R.S.D.% of 0.54 and 0.81 were obtained for PSE and CET (Cirrus® capsules), respectively ($n=6$). Analysis of Allegra-D® 12 h extended–release tablets gave the assay results of 119.22 and 58.87 mg with R.S.D.% values of 0.86 and 0.35 for PSE and FEX, respectively.

Since the dosage forms of PSE with CET or FEX is not pharmacopoeial, recovery of the procedure was determined by standard addition method. The already analyzed samples of capsules (Cirrus®) or extended–release tablets (Allegra-D® 12 h) were spiked with the known amounts of standard PSE and CET or FEX. The mean percentage recoveries obtained after six repeated experiments were found between 97.53 and 100.98 (Table 7), indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

4. Conclusion

The validated HPLC method has been proved to be simple, precise, rapid and reliable. The proposed method provides a good resolution between PSE, FEX and CET. Using this single procedure, it is possible to perform quantitative analysis of two different pharmaceutical dosage forms within a short analysis time less than 5 min. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH-Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies.

Using FEX (for PSE–CET binary mixtures) or CET (for PSE–FEX binary mixtures) as internal standard for the quantitative determination, reduced the possible analytical errors due to the sample dilution and injection procedures and improved the sensitivity of the method. The proposed method has the advantages of simplicity, repeatability, sensitivity and requires less expensive reagent than the other methods. Compared to the other reported ones, the developed method offers a short analysis time of PSE, FEX and CET which is essential in routine analysis of pharmaceutical dosage forms. Thus the proposed method is suitable for the screening of formulated samples in routine quality control applications.

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References

[1] D.M. Campoli-Richards, M.M. Buckley, A. Fitton, Drugs 40 (1990) 762–781.
[2] M.P. Curran, L.J. Scott, C.M. Perry, Drugs 64 (2004) 523–561.
[3] C.M. Spencer, D. Faulds, D.H. Peters, Drugs 46 (1993) 1055–1080.
[4] A. Pagliara, B. Testa, P.A. Carrupt, P. Jolliet, C. Morin, D. Morin, S. Urien, J.P. Tillement, J.P. Rihoux, J. Med. Chem. 41 (1998) 853–863.
[5] K. Wellington, B. Jarvis, Drugs 61 (2001) 2231–2240.
[6] European Pharmacopeia 5.0, 5th edition, Council of Europe—European Directorate for the Quality of Medicines, 2005, pp.1237–1239.
[7] K. Basavaiah, V.S. Charan, Farmaco 57 (2002) 9–17.
[8] A.F.M. El Walily, M.A. Korany, A. El Gindy, M.F. Bedair, J. Pharm. Biomed. Anal. 17 (1998) 435–442.
[9] H. Mahgoub, A.A. Gazy, F.A. El-Yazbi, M.A. El-Sayed, R.M. Youssef, J. Pharm. Biomed. Anal. 31 (2003) 801–809.
[10] J. Macek, P. Ptáček, J. Klíma, J. Chromatogr. B 736 (1999) 231–235.
[11] M.F. Zaater, Y.R. Tahboub, N.M. Najib, J. Pharm. Biomed. Anal. 22 (2000) 739–744.
[12] B. Paw, G. Misztal, H. Hopkala, J. Drozd, Pharmazie 57 (2002) 313–315.
[13] Q. Song, H. Junga, Y. Tang, A.C. Li, T. Addison, M. McCort-Tipton, B. Beato, W. Naidong, J. Chromatogr. B 814 (2005) 105–114.
[14] M.D. Likar, H.L. Mansour, J.W. Harwood, J. Pharm. Biomed. Anal. 39 (2005) 543–551.
[15] S.O. Choi, S.H. Lee, H.S. Kong, E.J. Kim, H.Y.P. Choo, J. Chromatogr. B 744 (2000) 201–206.
[16] P. Mikuš, I. Valášková, E. Havránek, J. Sep. Sci. 28 (2005) 1278–1284.
[17] A.M. Jaber, H.A. Al Sherife, M.M. Al Omari, A.A. Badwan, J. Pharm. Biomed. Anal. 36 (2004) 341–350.
[18] B.S. Nagaralli, J. Seetharamappa, B.G. Gowda, M.B. Melwanki, J. Chromatogr. B 798 (2003) 49–54.
[19] K.K. Pandya, R.A. Bangaru, T.P. Gandhi, I.A. Modi, R.I. Modi, B.K. Chakravarthy, J. Pharm. Pharmacol. 48 (1996) 510–513.
[20] S.N. Makhija, P.R. Vavia, J. Pharm. Biomed. Anal. 25 (2001) 663–667.
[21] K. Simpson, B. Jarvis, Drugs 59 (2000) 301–321.
[22] S.G. Meeves, S. Appajosyula, J. Allergy Clin. Immunol. 112 (2003) S69–S77.
[23] United States Pharmacopeia, 29th revision, US Pharmacopeial Convention, Rockville, MD, 2006, pp. 905–906.
[24] U. Hofmann, M. Seiler, S. Drescher, M.F. Fromm, J. Chromatogr. B 766 (2002) 227–233.
[25] W. Naidong, W.Z. Shou, T. Addison, S. Maleki, X. Jiang, Rapid Commun. Mass Spectrom. 16 (2002) 1965–1975.
[26] T. Uno, N. Yasui-Furukori, T. Takahata, K. Sugawara, T. Tateishi, J. Pharm. Biomed. Anal. 35 (2004) 937–942.
[27] T. Radhakrishna, G.O. Reddy, J. Pharm. Biomed. Anal. 29 (2002) 681–690.
[28] T.A. Walker, G.L. Schmitt, J. Liq. Chromatogr. Rel. Technol. 29 (2006) 25–43.
[29] A.R. Breier, C.S. Paim, M. Steppe, E.E. Schapoval, J. Pharm. Pharm. Sci. 8 (2005) 289–298.
[30] A.R. Breier, S.S. Garcia, A. Jablonski, M. Steppe, E.E. Schapoval, J. AOAC Int. 88 (2005) 1059–1063.
[31] P. Mikuš, I. Valášková, E. Havránek, Drug Dev. Ind. Pharm. 31 (2005) 795–801.
[32] G.L. Sussman, J. Mason, D. Compton, J. Stewart, N. Ricard, J. Allergy Clin. Immunol. 104 (1999) 100–106.
[33] R.B. Berkowitz, G.G. Woodworth, C. Lutz, K. Weiler, J. Weiler, M. Moss, S. Meeves, Ann. Allergy Asthma Immunol. 89 (2002) 38–45.
[34] T.G. Altuntas, S.S. Zanooz, D. Nebioglu, J. Pharm. Biomed. Anal. 17 (1998) 103–109.
[35] M.M. Mabrouk, H.M. El-Fatatry, S. Hammad, A.A.M. Wahbi, J. Pharm. Biomed. Anal. 33 (2003) 597–604.
[36] L.R. Snyder, J.L. Glajch, J.J. Kirkland, Practical HPLC method development, John Wiley & Sons Inc., New York, 1988.
[37] ICH-Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 1996.
[38] I. Krull, M. Swartz, LCGC N. Am. 17 (1999) 244–246.