Research Article

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Experimental Design Optimized Chromatographic Determination of Pseudoephedrine and Cetirizine in Bulk and Tablets

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ABSTRACT

Chromatographic separation of cetirizine and pseudoephedrine was established using cyanopropyl column and mobile phase composed of aqueous phase (20 mM ammonium acetate buffer, pH 5.5) and acetonitrile (44:56v/v) pumped at 1 mL/minute, analytes were detected at 240 nm. The linearity of the method was between 25- 135 µg/mL and 1.5 –7.5 µg/mL for pseudoephedrine and cetirizine, respectively. Pseudoephedrine and cetirizine detection limits were 5.34 and 0.13 µg/mL, respectively, whereas their quantitation limits were 16.17 µg/mL and 0.40 µg/mL for cetirizine. The method's recoveries were (99.81% ± 0.60 for pseudoephedrine and 100.08 ± 0.52 for cetirizine), indicating consistent good and consistent recoveries. Also low relative standard deviations (< 2%) for repeatability and intermediate precision were obtained supporting method's high precision. Quantification of pseudoephedrine and cetirizine in bulk and combined tablet formulation was successfully carried out using the validated method.

Keywords: Pseudoephedrine; Cetirizine; HPLC; Experimental design.

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1. Introduction

Cetirizine (CET) chemically is 2-[4-[(4-Chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid (Fig. 1a). It is a long-acting non-sedating antihistamine with low drowsiness potential. CET is commonly used for the symptomatic relief of allergic conditions such as rhinitis and chronic urticaria [1].

Pseudoephedrine (PSE) chemically is (1S, 2S)-2-(Methyl-amino)-1-phenylpropan-1-ol (Fig. 1b). PSE has both direct and indirect sympathomimetic actions. Pseudoephedrine orally used for the symptomatic relief of nasal congestion. Its combination with other ingredients are used for the relief of cough and cold symptoms [1].

The two drugs combination is indicated to relieve symptoms of allergic rhinitis [1]. The two analytes in marketed products exist in mass ratio of (24:1 w/w, PSE: CET) which presents an important analytical challenge.

The United States Pharmacoepia [2], empolyes two independent chromatographic methods for the determination of PSE: CET combined dosage form, literature review revealed that some reversed-phase liquids chromatographic methods were also developed for the determination of the two analytes combination in tablets and biological fluids [3-11], other analytical techniques such as spectrophotometry [12,13], high performance thin layer chromatography [14] and capillary zone electrophoresis [15], were also reported.

Cetirizine is very polar molecule, zwitterioic in character while pseudoephedrine is charged depending on pH, this difference in physicochemical properties of the two molecules in addition to their mass difference in the combination dosage form; present an interesting analytical challenge. The objective of this work was to develop, optimize and validate isocratic; stability indicating high-performance liquid chromatographic methodology for the simultaneous determination of CET and PSE in tablets, without the use of ion pairing agents, micellar liquid chromatography or expensive specialized column.

2. Materials and Methods

2.1. Apparatus and software

Experiments were performed using Shimadzu Prominence chromatographic system consisted of: degasser (Model DGU-20A5), pump (Model LC-20AD), Rheodyne manual injector fitted with 20 µl loop, time programmable variable wavelength UV–Vis detector (Model SPD-20A) was used to perform the experiments.

Design Expert v. 8.0.6 (Stat-Ease Inc., Minneapolis, MN, USA) was used to analyze the experimental design data analysis and response surface. The rest of data analysis was carried out in Microsoft Excel 2013 software (Microsoft, USA).

2.2. Materials and Reagents

Materials

Ammonium acetate and glacial acetic acid (Sd Fine Chem. Ltd., India), acetonitrile HPLC grade (Scharlau Chemie, Spain). Laboratory prepared double-distilled water was used throughout the analysis.

Cetirizine dihydrochloride and pseudoephedrine hydrochloride working standards were obtained from Blue Nile Pharmaceutical Industries, Sudan. Cirrus tablets manufactured by UCB, Switzerland, labeled to contain: Cetirizine dihydrochloride 5 mg and pseudoephedrine hydrochloride 120 mg per tablet, were purchased from local pharmacies.

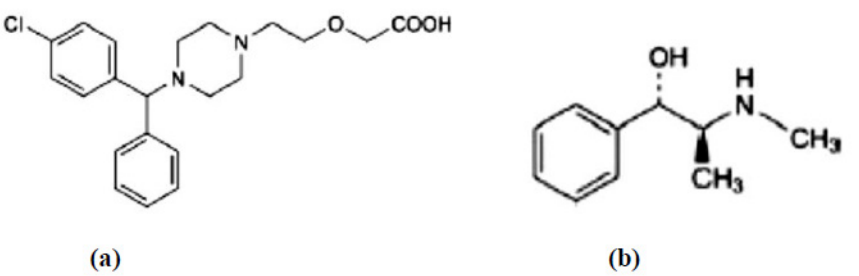

Figure 1. Chemical structure of (a) cetirizine and (b) pseudoephedrine

Acetate buffers preparation

The required amount of ammonium acetate needed to prepare the each concentration of the buffer was weighed and dissolved in 500 mL distilled water, the final pH values of the prepared buffers were adjusted using dilute glacial acetic acid.

2.3. Preparation of solutions

Optimization standard

Standard solution containing mixture of 120 µg/mL PSE and 5 µg/mL CET in 60% v/v aqueous acetonitrile was prepared by dilution from their corresponding stock solutions.

Standard stock solution

Cetirizine dihydrochloride working standard (10 mg) and pseudoephedrine hydrochloride working standard (170 mg) were accurately weighed and transferred into a 25 mL volumetric flask, dissolved and made to volume with water, 5 mL of the resulting solution were further diluted to 50 mL with water.

Construction of the calibration curve

Aliquot volumes (1-5 mL) from the standard stock solution were transferred into five separate 25 mL volumetric flasks; the volumes of the flasks were then made to mark using 60% v/v aqueous acetonitrile.

Sample preparation

Twenty tablets were weighed and powdered, an amount of the powdered tablets equivalent to 60 mg PSE were transferred into a 50 mL volumetric flask, 30 mL of water were added and sonicated for 10 minutes, the volume was then made to mark with water and filtered through 0.45 µm nylon filter, 2 ml of the clear filtrate were diluted to 20 ml using 60% v/v aqueous acetonitrile and injected into the chromatographic system.

2.4. Chromatographic procedure

Zorbax SB- Cyanopropyl column from Agilent Technologies—USA (250 mm × 4.6 mm, 5 µm particle size) was used in method development and optimization studies. Ammonium acetate buffer having various ionic strengths and pH values blended with acetonitrile in different volume ratios were used as mobile phases. The mobile phase was delivered at 1.0 mL/min. and the analytes detection was carried at 240 nm.

2.5. Method development and optimization

From the preliminary investigations; three factors were identified as independent variables; the pH (A), the ionic strength (B) and the volume percentage of acetonitrile in the mobile phase (C). The three factors were coded as +1 (high), 0 (intermediate) and −1 (low) as shown in Table 1. The effect of varying the mobile phase pH over the range of (5.5-6.5), the ionic strength (20-30 mM) and acetonitrile volume ratio (55-65%) on the the resolution between the two analytes was studied using 2^3 full factorial design (8 experiments) with three replicate runs at the intermediate levels. The optimization standard (20 µL) were injected every time and resolution between CET-PSE peaks pair was measured.

The experiments were randomized to avoid the bias. The conducted experiments and their corresponding responses are summarized in Table 2.

2.6. Method validation

The linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) of developed method were validated according to the ICH guidelines requirements [16].

Table 1. Variables and their levels

	Factors	Factors levels		
		(-)	(+)	(0)
A	pH	5.5	6.5	6.0
B	Ionic strength (mM)	20	30	25
C	% Acetonitrile	55	65	60

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Table 2. Factorial design matrix and responses

Standard order	Run order	A: pH	B: Ionic strength	C: %Acetonitrile	Resolution
1	5	5.5	20.0	55.0	4.15
2	8	6.5	20.0	55.0	10.46
3	1	5.5	30.0	55.0	2.31
4	4	6.5	30.0	55.0	8.39
5	7	5.5	20.0	65.0	10.46
6	6	6.5	20.0	65.0	15.07
7	2	5.5	30.0	65.0	8.34
8	3	6.5	30.0	65.0	12.85
9	9	6.0	25.0	60.0	8.97
10	10	6.0	25.0	60.0	8.95
11	11	6.0	25.0	60.0	8.94

Linearity

The linearity was tested in five concentration levels corresponding to the range of 25- 135 µg/mL and 1.5 –7.5 µg/mL for PSE and CET, respectively. The straight lines regression parameters were from the analytes concentration versus their corresponding peak areas.

Precision

The repeatability of the method was tested using six independent sample solutions containing analytes at 100% concentrations of their corresponding expected in the pharmaceutical product. The same process was repeated on a different day using fresh reagents and samples to determine the method's intermediate precision.

Accuracy

The method of standard addition was used to evaluate the analytes recoveries. Pre-assayed sample containing 60% of the declared drugs contents were spiked with known amounts of the two analytes to obtain final concentrations 60–120% of the expected drug concentrations in the pharmaceutical dosage form.

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were calculated from the slope and the standard deviation of the responses of calibration analysis using the following formulae (16).

LOD = 3 × SD/slope of the calibration curve.

LOQ = 10 × SD/slope of the calibration curve.

where SD = average standard deviation of the response obtained in the linearity determination.

System suitability parameters

The standard solution was injected five times under the optimized conditions, to obtain the system suitability parameters.

3. Results and Discussion

3.1. Method development and optimization

The reversed-phase chromatographic methods [3-10], were not sufficiently capable of giving optimum separation resolution of the two analytes, since PSE is very polar it tends to elute near the column void indicating insignificant retention and possible interference with the formulation additives and polar im-

purities; at the expense of preventing late elution of CET. Ion-pair chromatography has been employed to achieve optimum separation between the two analytes and avoid this shortcoming [11,12], however using ion-pairing approach in chromatography is generally not encouraged; for its deleterious effect on the chromatogrphic column by changing the chemical nature of the stantionary phase, hence limiting its future use to ion-pair separations only [17].

Our past experience of achieving optimum resolution between pseudoephedrine and loratadine using cyanopropyl column and a mobile phase composed of ammonium acetate buffer and acetonitrile [18], directed our efforts to use the same approach in the preliminary investigations for this combination.

The effect of the mobile phase pH over the range of (5.5-6.5), the ionic strength (20-30 mM) and acetonitrile percent (55-65%) on the the resolution between the two analytes was investigated using two levels full factorial design. Statistical analysis of the experimental data using analysis of variance (ANOVA) indicated that the three studied variables were having significant effect on the resolution between the two analytes (Table 3). The three center points added to the factorial runs revealed absence of significant curvature in the relation between the independent variables and the resolution, this finding together with the non significant lack of fit to the linear model strongly indicated that the resolution between the two analytes can be described by a simple first order model [19].

The first order relation between the independent variables and the dependent variable (R) was established from multiple linear regressions and the least-squares method of approximation. The linear model equation obtained using the coded factor values was as follows

The linear model equation indicated that the acetonitrile volume ratio (A) and pH (C) were having the largest positive influence on the resolution; increas-

Table 3. ANOVA for the selected factorial model

Parameter	SS	df	MSS	F-value	P-value	comment
Model	125.01	6	20.84	24235.48	< 0.0001	S
A:pH	57.84	1	57.84	67271.41	< 0.0001	S
B:ionic stregth	8.51	1	8.51	9896.00	< 0.0001	S
C:%Acetonitrile	57.30	1	57.30	66647.70	< 0.0001	S
AB	0.014	1	0.014	15.83	0.0248	S
AC	1.34	1	1.34	1554.70	< 0.0001	S
BC	0.023	1	0.023	26.88	0.0139	S
curvature	$5.5*10^{-3}$	1	$5.5*10^{-3}$	6.45	0.0847	NS
Residual	$2.58*10^{-3}$	3	$8.6*10^{-4}$			
Lack of fit	$2.11*10^{-3}$	1	$2.11*10^{-3}$	9.05	0.095	NS
R^2				0.9999		
$R^2_{adjusted}$				0.9998		
$R^2_{prediction}$				0.9988		
Adequacy of precision				355.81		

SS: sum of squares, MSS: mean sum of squares, S: significant, NS: not significant

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ing either of them increases the resolution; while the ionic strength (B) was found to have a small negative influence, the two factors interactions AB, AC and BC were also significant with much smaller negative effects on the resolution.

The high regression coefficient (R^2) and the reasonale agreement between the prediction regression cofficient ($R^2_{prediction}$) and the adjusted regression coefficient ($R^2_{adjusted}$) and the high adequacy of precision > 4 [19] supported the validity of the proposed model as shown in Table 3. The high predictability of the proposed method is indicated by the high value of the prediction regression coefficient ($R^2_{prediction}$). The close agreement between the experimental and predicted responses further confirmed the high predictability, as shown in Table 4.

Design Expert Software numerical optimization; suggested a mobile phase composed of 56% acetonitrile and 44% ammonium acetate buffer (20 mM) adjusted to pH 5.5 to obtain 5 units resolution between the two analytes. 3D figure portraying the resolution between CET and PSE as a fuction of mobile phase pH , ion strength concentration and percent acetoniotrile content is given in Fig 2.

Under the optimized conditions PSE seems to be retained according to normal phase mechanism [18] since it is fully protonated at pH 5.5, while the retention of the zwitterionic CET follows reversed-phase mechanism as its lipophilicity is enhanced through the interaction of its carboxylate group with the protonated nitrogen on the piperazine ring via folded conformers, the prontonation of one of the piperazine ring nitrogens greatly reduces the bascity of the molecule and hence the extent of ionization [20].

The retention of the polar compounds increases by increasing the ionic strength as this in effect increases the polarity of the medium and the dissociation of compounds i.e. the acidic compounds will become are more negatively charged and the basic ones are more positively charged resulting in stronger retention for the basic drugs by electrostatic attraction and less retention for the acidic drugs by repulsion [18].

Simultaneous determination of CET and PSE in tablet formulation was successfully achieved using the optimized method. Typical chromatograms for the separation of CET and PSE are shown in Figures 3 and 4.

3.2. Method validation

Linearity

Excellent linearity was obtained over the range of 25- 135 µg/mL and 1.5 –7.5 µg/mL for PSE and CET, respectively with the selected mobile phase. The linear regression analysis data is shown in Table 5. The residuals were normally distributed around the mean with uniform variance across all concentrations; indicating good correlation between the concentrations and the responses.

Limits of detection (LOD) and quantification (LOQ)

The LOD for PSE and CET were 5.34 and 0.13 µg/mL, respectively, whereas the LOQ was 16.17 µg/mL for PSE and 0.40 µg/mL for CET. According to the LOQ results obtained it is possible to determine PSE and CET with high precision and accuracy at concentrations above their LOQ values as shown in Table 5.

Recovery

Using the standard additions; consistent and high absolute recovery at all concentrations method with a mean absolute recovery of 99.81% ± 0.60 for PSE and 100.08 ± 0.52 for CET was obtained, indicating the accuracy of the optimized method and its suitability for simultaneous determination of the two analytes in combined tablet dosage (Table 6).

Table 4. Comparison of observed and predicted resolution under optimal conditions

pH 5.5	Ionic strength (mM) 20.0	% Acetonitrile 56.0	Resolution
	Experimental value		5.19
	Predicted value		4.90
	Average error %		5.87

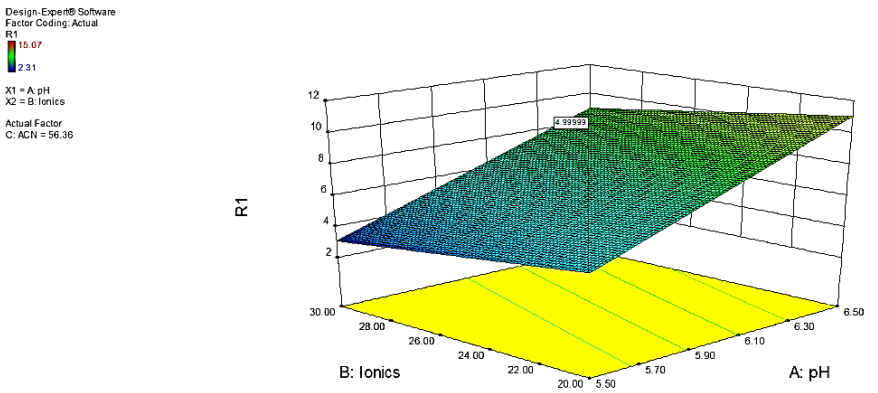

Figure 2. Graphical representation of the resolution between CET and PSE as a function of mobile phase (56% acetonitrile and 44% ammonium acetate buffer, (20 mM) adjusted to pH 5.5)

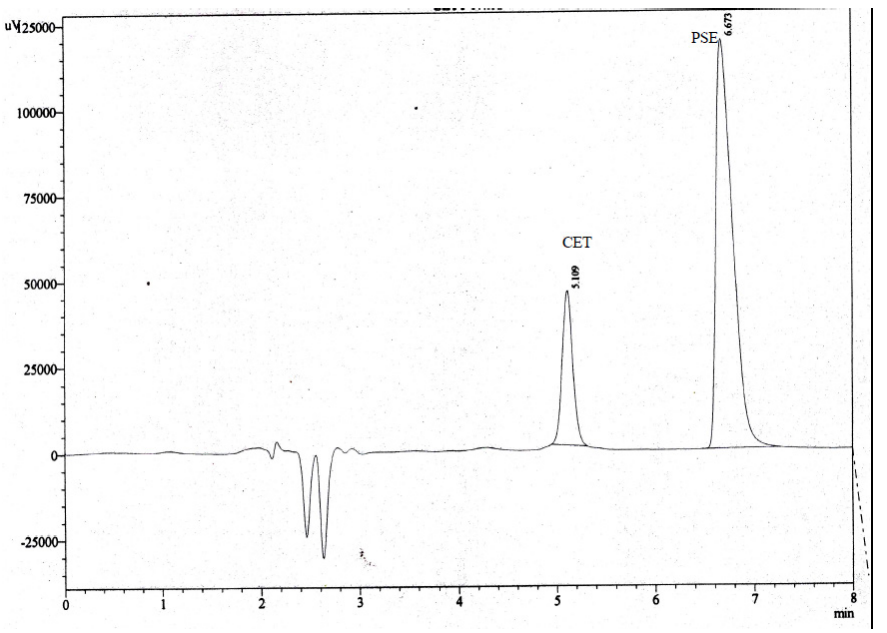

Figure 3. Typical chromatogram of the standard solution, CET (=5.11 min.) and PSE (= 6.67 min).

Precision

Repeatability and intermediate precision percent relative standard deviation (%RSD) were < 2% for the two analytes in studies. The overall %RSD of the assays calculated using the data from the day-to-day analysis (12 samples) was also < 2%, and the difference between the individual assay results was less than < 2% (Table 6). These results give sufficient evidence that the outcome of the determination was statistically similar regardless the day of the assay and reagent preparation in the determination.

Specificity and selectivity

Under the optimized conditions the method was capable of resolving the degradation product developed in Cirrus tablet stored in its original pack at room temperature for nine years after expiration. The significant drop in the assay of PSE suggests strongly that the developed degradation product was originating from PSE. Typical chromatogram is shown in Fig. 5. The stability indicating capability of the proposed method was strongly supported by this finding.

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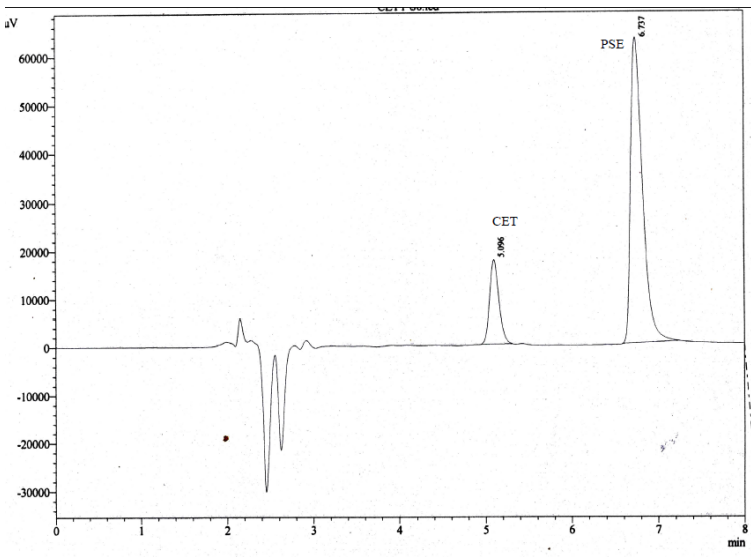

Figure 4. Typical chromatogram of the sample, CET (=5.10 min.) and PSE (= 6.77 min).

Table 5. Linear regression data.

Parameter	CET	PSE
Concentration range (µg/mL)	1.5-7.5	25-135
Linearity – Regression equation		
Slope (b)	28725.75	7049.32
Intercept (a)	1807.25	28250.25
Correlation coefficient (r)	0.9999	0.9993
Standard deviation of the slope (s_b)	236.17	7.11
Standard deviation of the intercept (s_a)	1184.75	13816.29
Limit of detection (µg/mL)	0.13	5.35
Limit of quantitation (µg/mL)	0.40	16.17

Table 6. Accuracy and precision data

Parameter	PSE % LC ± SD	CET % LC ± SD
Precision (recovery% ± SD)[a]		
Repeatability	100.29 ± 0.46	100.49 ± 0.80
Intermediate precision	99.87 ± 0.28	99.93 ± 0.69
Accuracy (recovery% ± SD)[b]	99.81 ± 0.60	100.08 ± 0.52
Commercial sample(recovery% ± SD)[b]		
Cirrus Tablets	100.08 ± 0.43	100.21 ± 0.77
Cirrus Tablets (expired)	85.70 ± 0.32	99.08 ± 0.53

[a] Six samples at 100% level. b Triplicate determination

System suitability parameters

The asymmetry factor, resolution and number of theoretical plates were above acceptable limits for both analytes [21], indicating that the optimized method is suitable in term of system performance (Table 7).

Conclusion

Despite their widely different physicochemical properties and the great mass difference in combined pharmaceutical formulation (PSE: CET 24:1, w/w), the use of very simple, relatively cheap, and easy-to-prepare mobile phase with cyanpropyl column enabled optimum separation of PSE and CET within less than 7 minutes.

The method proposed method was simple and rapid with the required linearity, precision, accuracy, selectivity, LOD and LOQ needed for the simultaneous estimation of PSE and CET in bulk and pharmaceutical formulations.

The use of thermally volatile ammonium acetate buffer as component of the mobile phase, suggests that the method use can be extended for the analysis of two analytes in biological fluids using mass spectrometric detection.

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Conflict of interests

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of Contribution of Researchers

IA, carried out the experimental work, data analysis and manuscript writing.

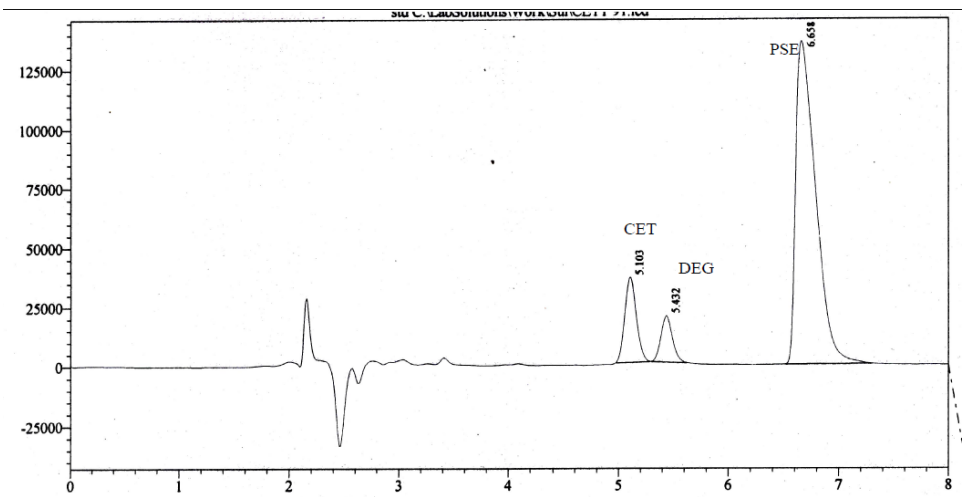

Figure 5. Typical chromatogram of the expired sample, CET (=5.10 min.), degradation product (=5.43 min.) and PSE (= 6.66 min).

Table 7. System suitability parameters

Analyte	Resolution	Asymmetry factor	Theoretical plates number
Cetirizine	-	1.34 ± 0.26	9267.67 ± 0.24
Pseudoephedrine	5.20 ± 0.39	1.94 ± 0.45	7606.67 ± 0.29

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