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Validated chromatographic methods for determination of paracetamol, pseudoephedrin and cetirizin in pharmaceutical formulation

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ABSTRACT

Two specific, sensitive and precise stability indicating chromatographic methods had been developed, optimized and validated for determination of Paracetamol (PAR), Pseudoephedrin (PS) and Cetirizin (CET) in their laboratory prepared mixtures and pharmaceutical formulatios. The first method was based on thin layer chromatographic densitometry (TLC). The optimum separation was achieved using silica gel 60 F_{254} Aluminum TLC plates and ethyl acetate: methanol: ammonia (75: 20: 5 by volume) as a developing system. Good correlations were obtained in the ranges of (1-12mg ml^{-1}) for PAR and PS and (3-15 mg ml^{-1}) for CET.

The second method was based on high performance liquid chromatography with ultraviolet detection (HPLC-UV), at which the proposed components were separated on a reversed phase C18 column using phosphate buffer(pH 3.6): acetonitrile (1: 1.2 by volume) as the mobile phase, maintaining constant flow rate of 1mLmin^{-1} and detection at 210 nm. Linear regressions were obtained in the ranges of 1.0-60, 1.0-50 and 0.1-60 µgmL^{-1} for PAR, PS and CET, respectively. Different parameters affecting the suggested methods had been optimized in order to obtain maximum separation of the cited components. System suitability parameters of the two developed methods were also tested. The suggested methods were successfully applied for determination of PAR, PS and CET in their commercial capsules. Both methods were validated in compliance with the ICH guidelines with satisfactory results. Also they were statistically compared to each other and to the reported method, no significant difference was found, providing their accuracy and precision.

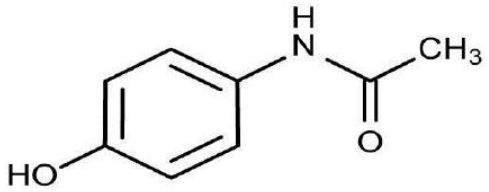
KEYWORDS

Paracetamol;
Pseudoephedrin;
Cetirizin;
Thin layer chromatography;
High performance liquid chromatography.

INTRODUCTION

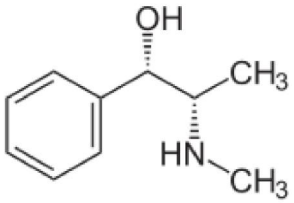
Paracetamol (PAR), Figure 1, is chemically designated as (*N*-(4-hydroxyphenyl)acetamide)[1]. It is commonly used for the relief of headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol could be used also in the management of more severe pain (such as in advanced cancer[2]. Pseudoephedrin (PS), Figure 2, is chemically designated as ((R*,R*)-2-methylamino-1-phenylpropan-1-ol)[3]. It is a sympathomimetic amine, Its principal mechanism of action relies on its indirect action on the adrenergic receptor system. Pseudoephe-

drine is also indicated for vasomotor rhinitis, and as an adjunct to other agents in the optimum treatment of allergic rhinitis, croup, sinusitis, otitis media, and tracheobronchitis[4].



Mol. Formula $C_8H_9NO_2$
Mol.Wt. 151.17

Figure 1 : Chemical structure and molecular formula of PAR



Mol. Formula $C_{10}H_{15}NO$
Mol.Wt. 165.23

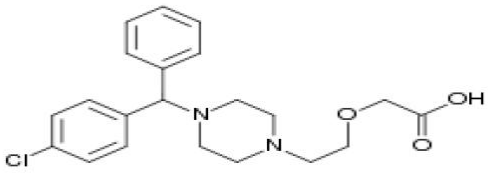
Figure 2 : Chemical structure and molecular formula of PS

The salts pseudoephedrine hydrochloride and pseudoephedrine sulfate are found in many over-the-counter preparations either as single-ingredient preparations, or more commonly in combination with antihistamines, guaifenesin, dextromethorphan, paracetamol (acetaminophen), and/or NSAIDs (e.g., aspirin, ibuprofen, etc).

Cetirizin (CET), Figure 3, is chemically designated as ((±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid)[3]. Cetirizin crosses the blood-brain barrier only slightly, eliminating the sedative side-effect common with older antihistamines; however it still causes mild drowsiness[5]. It has also been shown to inhibit eosinophil chemotaxis and LTB4 release. At a dosage of 20 mg, Boone *et al.* found that it inhibited the expression of VCAM-1 in patients with atopic dermatitis .Combination of PAR, PS and CET is used to improve urine output and lung function in infants with broncho-pulmonary dysplasia[6]. These necessities the development of validated analytical methods for the simultaneous determination of PAR, PS and CET which is more often useful in quality control laboratories.

A comprehensive literature search revealed the lack of any method for determination of the studied drugs in their ternary mixture and in pharmaceutical formulations.

So, the present work deals with the determination of the three active compounds as bulk drugs and in pharmaceutical dosage forms using simple TLC and a RP-HPLC method.



Mol. Formula $C_{21}H_{25}ClN_2O_3$
Mol.Wt. 388.89

Figure 3 : Chemical structure and molecular formula of CET

EXPERIMENTAL

Instruments

- TLC Scanner 3 densitometer (Camage, Muttenz, Switzerland).
- Sample applicator for TLC linomat V with 100µl syringe (Camage, Muttenz, Switzerland).
- TLC plates (20×20cm) coated with silica gel 60 F_{254} (Merck KgaA, Darmstad, Germany).
- The LC system (Perkin- Elmer, USA) consisted of Series 200 Vacuum degasser, Series 200 LC pump, Series 200 variable- wavelength UV-VIS detector and Series 200 autosampler fitted with a 200µL sample loop. LC separations were performed on RP-C18 Zorbax (22.5 cm×4.6mm i.d. 5µm particle size) analytical column.

Samples

(a) Authentic samples

- Paracetamol (PAR) was kindly supplied by MISR PHARM. IND., Cairo, Egypt. It was certified to contain 100.19 % according to the USP method[3].
- Psudoephedrin (PS) was kindly supplied by El KAHIRA PHARM. & CHEM. IND. CO, Cairo, Egypt. It was certified to contain 99.79 % according to United States Pharmacopeia convention Inc. 2007[3].
- Cetirizin (CET) was supplied by GLOBAL NAPI PHARMACEUTICAL, 6 October, GIZA, Egypt .Its purity was labeled to be 100% United States Pharmacopeia convention Inc. 2007[3].

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(b) Commercial products

Allercet cold® capsules (Batches No. 0611108 and 820805) are labeled to contain 400 mg of PAR, 30 mg of PS and 10 mg of CET, manufactured by GLOBAL NAPI PHARMACEUTICALS and were obtained from the local market.

Chemicals and solvents

All chemicals and solvents used through out this work were of analytical grade, ethyl acetate, ammonia, methanol, deionized water, potassium dihydrogen orthophosphate and disodium hydrogen phosphate (El-NASR Pharmaceutical Chemicals Co., Abu- Zaabal, Cairo, Egypt) and Acetonitrile HPLC grade (SDS, France).

Solutions

(a) Stock solutions

- Stock standard solutions of each PAR, PS and CET (1 mg mL^{-1}).

Standard stock solutions of *each PAR, PS and CET* of 1mg mL^{-1} concentration were prepared in either methanol (for TLC method) or in the previously mentioned mobile phase (for HPLC method).

(b) Working solutions

- Working standard solutions of each PAR, PS and CET (0.1 mg mL^{-1}).

They were prepared by diluting 10 ml from their respective Stock solutions (1mg mL^{-1}) in 100-ml volumetric flask and the volume was completed with the mobile phase

Procedure

(a) Chromatographic conditions

(A) TLC-densitometry

Was performed on 20 x 10 cm TLC aluminum sheets precoated with 0.25 mm silica gel 60 F_{254}, the plates were prewashed with methanol prior to use. The samples were applied as bands of bandwidth 6 mm and they were spaced 8.9 mm apart from each other and 15 mm apart from the bottom edge of the plate. Linear ascending development was done in a chromatographic tank previously saturated with ethyl acetate: methanol: ammonia (75: 20: 5 by volume) for one hour at room temperature to a distance of about 80 mm from the lower edge. The developed plates were air dried and scanned at 254 nm .

(B) RP-HPLC method

- Was carried out at ambient temperature on RP-C18 Zorbax (22.5 cm×4.6mm i.d. 5µm particle size) analytical column.

The mobile phase consisted of Phosphate buffer (pH 3.6): acetonitrile (50: 60, v/v). The mobile phase was filtered using 0.45µm millipore membrane filter and was delivered at rate 1mL min^{-1}. The injection volume was 15 µl and the effluent was detected at 210 nm.

(b) Linearity

(A) TLC method

Accurate aliquots of volume 10 µL from stock standard solutions of concentrations ranges (1-12 *mg* mL^{-1}) of *PAR, PS* and (3-15 *mg* mL^{-1}) *of CET*, respectively were separately applied to TLC plates, chromatographed and scanned. The calibration curves relating the integrated area under the peak*10^{-4} in triplicates to the corresponding concentrations of *each PAR, PS and CET* as µg/band were constructed, respectively.

(B) RP-HPlC method

Working standard solutions (0.1mg mL^{-1}) of *each PAR, PS and CET* were further diluted with the mobile phase to obtain dilutions in the range of (1-60, 1-50 and 1-60 µg mL^{-1}) of each of *PAR, PS and CET* respectively. Triplicate 15µL injections were made for each prepared solution and chromatographed. The peak area ratios of *PAR, PS and CET* were plotted against the corresponding concentrations to obtain the calibration graph for each component.

(c) Application to commercial products

20 capsules were accurately Weighed after evacuation. An amount of the powder equivalent to 400 mg PAR, 30mg PS and 10mg CET was accurately weighed in a 50-ml beaker; 5 ml methanol and 2 drops ammonia were added, Stirred for 10 minutes using a magnetic stirrer and cover the beaker with a watch glass. The solution was filtered into 10 ml-volumetric flask. The residue washed three times each with 1 ml methanol then completed to the mark with the same solvent (solution 1). 1, 2 and3 µl of the prepared solution were

apllied in triplicate using Linomat applicator onto a TLC plate and the procedure was completed as under linearity. The concentration of both CET and PS were calculated from the regression equations.

The previously prepared Solution (solution 1) was then diluted to1/40 of its original concentration (solution2, equivalent to10 mg PAR) 2,4 and 6 µl of the diluted solution were applied in triplicate using Linomat applicator onto a TLC plate, the procedure was completed as under linearity .The concentration of PAR was calculated from the regression equation.

Twenty capsules of Allercet cold® were evacuated. An accurately weighted portion of the powder equivalent to 0.1 gm of each of *PAR, PS and CET* was separately transferred into 100-ml volumetric flask and then 75 ml mobile phase was added. The prepared solution was sonicated for 45 minutes and the volume was completed with the same solvent to get 1mg mL^{-1} of each drug, and then the prepared solution was filtered. An appropriate dilution was made to prepare the working solution and then proposed methods were applied, standard addition technique was also performed.

RESULTS AND DISCUSSION

Today, analytical techniques based on chromatographic separation have become the procedures of choice for determining drugs either in pharmaceutical formulations or biological fluids[7]. Although several chromatographic methods had been reported to assay the studied drugs, they generally deal with each of them alone or with other drugs. In developing new conditions and methods capable of eluting wide range of compounds of different polarities, TLC densitometry and RP-HPLC methods had been investigated. These methods were described for simultaneous determination of *PAR, PS and CET* in their bulk powder and pharmaceutical preparations. They were validated according to (ICH) guidelines[8], and they were statistically compared to each other and to the reported method.

TLC method

This technique offers a simple way to quantify directly on TLC plates by measuring the optical density of the separated bands. The amounts of compounds are determined by comparing the peak height or area of the unknown band to a standard curve from reference materials chromatographed simultaneously under the same condition[9].

A sensitive TLC method was suggested for the determination of *PAR, PS and CET* in ternary mixtures. This method is based on the difference of R_f values of the five compounds using ethyl acetate: methanol: ammonia (75: 20: 5 by volume) as a developing system. It was necessary to test the effect of different variables to optimize this method:

Mobile phase

Different developing systems of different composition were tested in order to obtain optimum separation such as, chloroform: methanol: ammonia (5:5:1 by volume), ethyl acetate: methanol: ammonia (100:8:2 by volume), ethyl acetate: methanol: ammonia (90:8:2 by volume). Upon using the first two systems, CET remained on the base line. The last system gave good separation of the three components with good R_f values, but PAR and PS had close Rf values.

Certain modifications were applied to the last system to achieve separation of the three drugs, satisfactory separation was achieved upon using ethyl acetate: methanol: ammonia (75: 20: 5 by volume) as a developing system. This system was found to give compact sharp symmetrical spots for the three cited compounds with suitable R_f values at 254 nm. Figure 4 showed typical chromatogram of the three components.

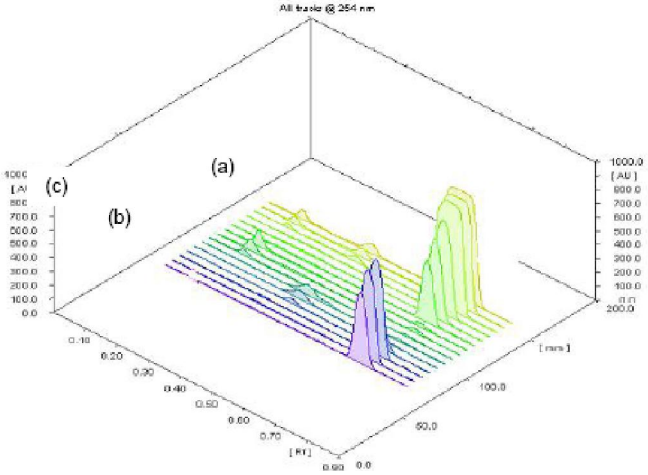

Figure 4 : Thin layer chromatogram of PAR (a), PS (b), CET (c), using ethyl acetate: methanol:ammonia (75: 20: 5 by volume)

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Spot stability

The time the sample is left to stand on the solvent prior to chromatographic development can influence the stability of separated spots which are required to be investigated for validation[10-11]. Two dimensional chromatography with the same solvent system was used to find out any decomposition occurring during spotting and development. Since no additional spots were detected, this indicated the stability of drugs in the solutions.

Detection of the related impurities

In order to detect any related impurities, large volumes of the three drugs standard stock solutions were applied on TLC plate and the chromatogram was run as described under chromatographic condition. No additional spots were observed.

RP-HPLC method

The great utility of RP-HPLC arises from the fact that the wide range of available solvents provides much scope for easily altering the separation selectivity. One of the major reasons of the wide spread success of HPLC as an analytical technique lies in the variety of separation mechanisms that may be exploited[7].

A simple, accurate and selective RP- HPLC had been investigated and validated for quantitative analysis of *PAR, PS and CET*. The LC procedure was optimized with a view to develop a quantitative method in a convenient time analysis and with high quality separation of the three proposed components. The chromatographic operational conditions were selected by considering the peak resolution and retention times of the first and the last eluted components.

Parameters affecting the efficiency of the chromatographic separation had been tested and optimized in a trial to obtain the maximum separation of the cited components. At the first attempt, an isocratic mobile phase of deionized water: methanol: acetonitrile (50:20:30, v/v) was used to separate the three compounds, unfortunately, CET was eluted after very long time (> 45 min) with tailed peak. However, increasing the organic modifier more than 10% resulting in very bad resolution of PAR and PS (eluted together as one peak without separation) and also with very long time of analysis for elution of CET. Several trials were tried by using different ratios and combinations of different mobile phases. None of these trials successes in separation of the proposed components.

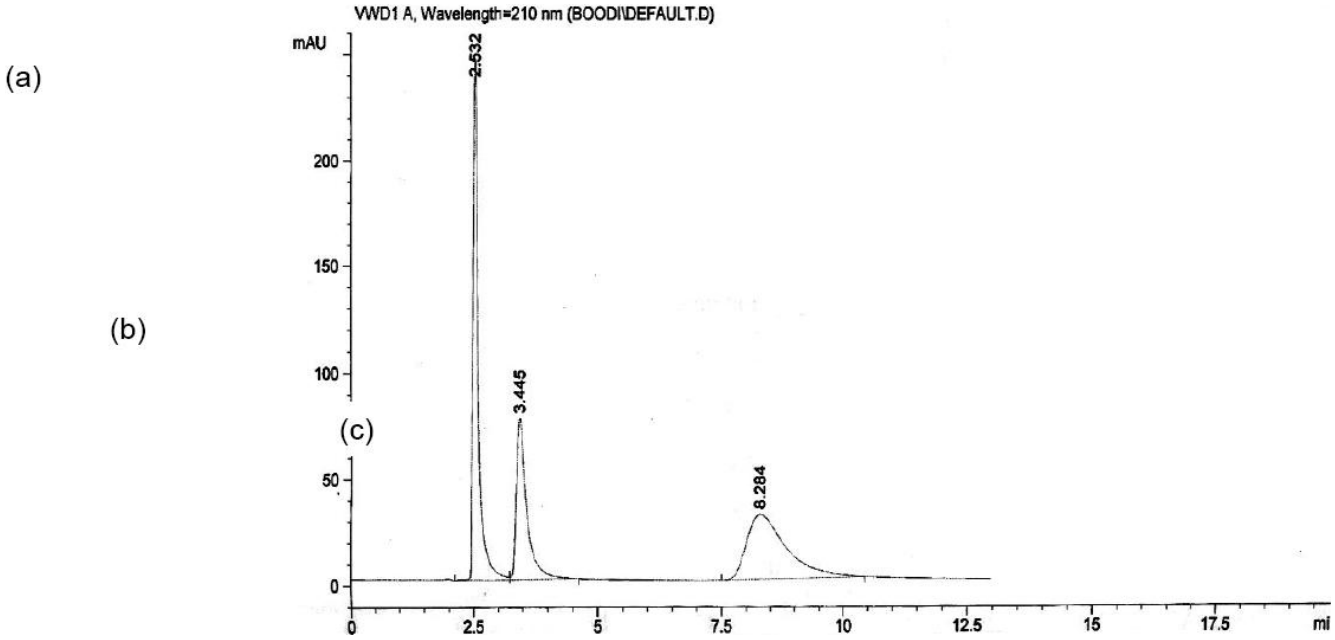

Figure 5 : HPLC chromatogram of the mixture of PAR (a), PS (b), CET (c), at 210 nm

The effect of the mobile phase pH on the separation was tested by using phosphate buffer of different pH values; phosphoric acid and sodium hydroxide were used to adjust the pH values under study. Changing the pH values had no effect on the separation efficiency. Several wavelengths were tested (254,230, and 218). The most suitable wavelength for detection of the active drugs was 210 nm at which reasonable sensitivity

of each was obtained.

Different stationary phases (C_8 and C_{18}) had been tested to optimize the resolution and enhance the peaks. The shape of peaks had been enhanced on C18 column. The mobile phase was delivered at different rates (0.8, 1 and 2 mL/min), best separation with good resolution and suitable analysis time was obtained upon using flow rate of 1mL/min. Under the optimum chromatographic conditions, satisfactory separation of the three proposed components was obtained and *PAR, PS and CET* were eluted at 2.63, 3.44 and 8.28 min respectively as shown in Figure 5.

ICH guidelines for method validation[12] were followed for validation of the suggested methods.

Linearity and ranges

Under the above described experimental conditions, linear relationships were obtained by plotting the drug concentrations against peak areas for each drug, for both chromatographic methods. The corresponding concentration ranges, calibration equations, LOD and LOQ and other statistical parameters are listed in TABLE 1.

TABLE 1 : Results of regression and assay validation parameters of the proposed chromatographic methods for determination of HCT and SPR

Parameters	TLC method			RP- HPLC		
	PAR	PS	CET	PAR	PS	CET
Range	1-12µg /band	1-12µg /band	1-10µg /band	1-60µg/mL	1-50µg/mL	1-60µg/mL
			Linearity			
Slope	0.015	0.002	0.004	0.096	0.103	0.107
Intercept	0.394	2.903	0.118	0.000	0.012	0.009
Correlation coefficient (r)	0.9998	0.9998	0.9997	0.9999	0.9997	0.9998
Standard error of the slope	0.0059	0.0028	0.0150	0.0007	0.0007	0.0009
Standard error of the intercept	0.0371	0.0222	0.1154	0.0202	0.0183	0.0269
Accuracy	100.13±1.854	99.52±0.193	100.25±0.986	101.26 ±1.020	100.55 ± 0.911	99.85 ± 0.960
Specificity and selectivity	The method showed good separation of the three drugs.			The method showed good separation of the three drugs.		
			Precision (RSD %)			
Repeatability *	1.264-1.107-0.989	0.971-0.654-0.507	1.012-0.879-0.779	0.972-1.102-0.830	1.069-0.890-1.110	1.385-1.620-1.150
Intermediate precision*	1.698-1.502-1.002	1.002-0.991-0.972	1.416-1.500-1.416	1.221-1.455-0.987	0.984-1.006-0.962	1.047-1.096-0.998
LOD**	0.33(µg\band)	0.182(µg\band)	0.250(µg\band)	0.175 µg/mL	0.245 µg/mL	0.257 µg/mL
LOQ**	1.003(µg\band)	0.552(µg\band)	0.781(µg\band)	0.58 µg/mL	0.81 µg/mL	0.85 µg/mL

***The intra- day and inter- day relative standard deviations of 0.5, 1 and 1. 20, 30 and 40 µg /mL of the three drugs; **Limit of detection and quantitation are determined via calculations; LOD = (SD of the response/ slope) × 3.3 LOQ = (SD of the response/ slope) × 10**

Accuracy

The accuracy of the investigated methods was validated by analyzing pure samples of each *PAR, PS and CET* in triplicate. The concentrations of the active drugs were calculated from the calculated regression equations or by comparison with standard applied on the same plate (in TLC method), Good results are shown in TABLE 1.

Precision

It was evaluated by calculating intra and inter- day precision. By repeating the assay of three different concentrations three times in the same day and assaying the same samples in triplicate on three successive days using the developed chromatographic methods and calculating the recovery% and RSD%. Results in TABLE 1 indicated satisfactory precision of the proposed methods.

Specificity

Specificity was ascertained by analyzing different mixtures containing the three proposed components in different ratios. The spots of the active drugs in the prepared mixtures were confirmed by comparing their R_f values and spectra of the spot with that of a standard drugs solutions (in TLC method). Other param-

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eters such as the resolution, capacity factor and selectivity for the separated spots and peaks were then calculated.

Application to commercial tablets

The suggested methods were successfully applied for determination of *PAR, PS and CET* in Allercet cold® capsules. The results shown in TABLE 2 were satisfactory and with good agreement with the labeled amounts. Applying the standard addition technique, no interference due to excipients was observed as shown from the results in TABLE 2.

In order to validate the suggested chromatographic methods, an overall system suitability testing was done to determine if the operating systems are performing properly. Good results were obtained and shown in TABLES 3,4.

TABLE 2 : Determination of the studied drugs in tablets by the proposed chromatographic methods

Sample	TLC method			RP- HPLC method		
	PAR	PS	CET	PAR	PS	CET
Allerce cold Capsules **(B.No.)	101.798 ±1.834	102.3 ±0.403	101.39 ±2.085	98.89 ± 0.76	102.3 ±0.403	101.39 ±2.085
Standard addition*	101.407 ±0.529	99.63 ±1.566	100.97 ±1.0944	100.45 ±1.002	98.69 ±0.570	99.13 ±0.435

****Average of 6 experiments; *Average of 3 experiments.**

TABLE 3 : Statistical analysis of parameters required for system suitability testing of TLC method

Parameters	Obtained value		
	PAR	PS	CET
Symmetry factor	1	1	1.15
Resolution (Rs)	5.562	9.153	
Capacity factor (k')	1.75	4	
Selectivity (α)	1.41	2.3	

TABLE 4 : Statistical analysis of parameters required for system suitability testing of RP- HPLC method

parameters	Obtained value	Obtained value	Obtained value	Reference value
	PAR	PS	CET	
Resolution (Rs)	5.562	9.153		>0.8
Relative retention (α)	2.532	3.445	8.284	>1
Tailing factor (T)	1.005	1.030	1.240	T = 1, for a typical symmetrical peak
Capacity factor (k')	1.75	4	6.8	1-10 acceptable
Number of theoretical plates (n)	8033	4097	2095	Increase with the efficiency of the separation
HETP [a]	0.01867	0.03661	0.07159	The smaller the value the higher the column efficiency

HETP [a] = height equivalent to theoretical plates (cm/ plate).

TABLE 5 : Statistical comparison of the results obtained by the proposed methods and the reported one for determination of PAR,PS and CET in pure form.

Items	TLC method			RP- HPLC			Official method[3]**		
	PAR	PS	CET	PAR	PS	CET	PAR	PS	CET
Mean	100.13	99.51	100.25	101.26	100.55	99.85	100.19	99.79	100
SD	1.854	0.193	0.986	1.020	0.910	0.960	0.840	0.572	0.971
RSD%	1.852	0.194	0.983	1.007	0.905	0.961	1.128	0.232	1.348
n	3	3	3	5	5	5	5	5	5
Student's t- test	0.0457 (2.353)	1.383 (2.015)	1.202 (2.131)	1.202 (2.13)*	0.184 (2.31)*	0.877 (2.31)*			
F- value	1.860 (6.944)	1.433 (6.944)	1.183 (6.944)	1.860 (6.944)*	2.96 (6.3382)*	2.08 (6.3882)*			

***Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.**

When results obtained by applying the proposed methods for analysis of pure *PAR, PS and CET* compared to those obtained by applying the official method[3], they showed no significance difference regarding accuracy and precision, and results were given in TABLE 5.

CONCLUSION

PAR, PS and CET are coformulated together in rhinitis and common cold formulations and being widely

used drugs, it is seriously to found simple, rapid and inexpensive methods of their analysis especially in quality control laboratories. The suggested chromatographic methods provide simple, accurate and reproducible stability indicating methods for quantitative analysis of *PAR, PS and CET* in ternary mixtures. The developed TLC method is highly sensitive and so it may be used for analysis of the suggested drugs in biological fluids. It has the advantages of short run time, large sample capacity and use of minimal volume of solvents. HPLC method gives a good resolution between the three proposed components within suitable analysis time, it is highly specific but more expensive. The proposed methods have advantage than other published methods of being analyzing the ternary mixture without the need of gradient ellution.

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