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Article *in* JPC - Journal of Planar Chromatography - Modern TLC · August 2018

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Simultaneous Quantification of Chlorpheniramine, Pseudoephedrine, and Ibuprofen in Antitussive Preparation by High-Performance Liquid Chromatography and Thin-Layer Chromatography–Densitometric Methods

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Key Words:

Chlorpheniramine
Pseudoephedrine
Ibuprofen
Reversed-phase high-performance liquid chromatography
Thin-layer chromatography–densitometry

Summary

Simple, accurate, precise, sensitive, and validated high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC)–densitometric methods were developed for the simultaneous determination of chlorpheniramine maleate (CPM), pseudoephedrine HCl (PSE), and ibuprofen (IBF) in tablet dosage form. In method A, reversed-phase (RP)-HPLC analysis was performed on Zorbax C_8 column (150 mm × 4.6 mm, 5 µm particle size i.d.), using a mobile phase consisting of methanol–acetonitrile–distilled water (pH 4) using orthophosphoric acid in the ratio (80:10:10, *v/v*) and flow rate of 0.7 mL min^{-1}. Quantification was achieved with ultraviolet (UV) detection at 220 nm. In method B, TLC analysis was carried out on an aluminum-backed sheet of silica gel 60 F_{254} layer using ethyl acetate–methanol–ammonia (8:2:0.8, *v/v*) as the mobile phase. Quantification was carried out with UV detection at 262 nm. The validation of the proposed methods was applied according to the International Conference on Harmonization (ICH) guidelines. The suggested methods were successfully applied for the determination of the cited drugs in bulk powder and commercial dosage form.

1 Introduction

Chlorpheniramine maleate (CPM), 3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-pyridin-2-yl-propan-1-amine, is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria that causes a moderate degree of sedation [1]. Pseudoephedrine hydrochloride (PSE), also known as [(+)-threo-α-[1-methylamino)ethyl]benzylalcohol]hydrochloride, is a useful bronchodilator and nasal decongestant. It shrinks swollen mucosal membranes, increases nasal airway passages, reduces nasal congestion, and diminishes tissue hyperemia [2]. Ibuprofen (IBF), (2*S*)-2-(4-isobutylphenyl)propanoic acid, is a non-steroidal anti-inflammatory drug (NSAID) used for relieving pain, helping with fever and reducing inflammation [3]. CPM, PSE, and IBF are commonly combined in one formulation for symptomatic treatment of coughs and the common cold. The chemical structures for these drugs are shown in **Figure 1.**

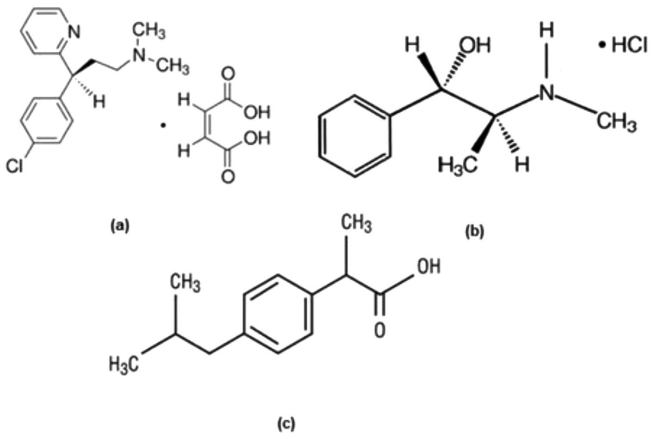

Figure 1

Chemical structure of (a) chlorpheniramine maleate, (b) pseudoephedrine HCl, and (c) ibuprofen.

Literature survey has revealed that CPM, PSE, and IBF can be determined either alone or in combination with other drugs by several methods, including spectrophotometric methods [4, 5], high-performance thin-layer chromatography (HPTLC) [6], mass spectroscopic methods [7–9], high-performance liquid chromatography (HPLC) [10], and capillary electrophoresis [11, 12]. The official pharmacopeial methods for CPM and PSE were found to be potentiometric titration methods, while for IBF a titrimetric method is described [13]. After comprehensive literature survey, one reported method was found for the analysis of the ternary mixture utilizing liquid chromatography with experimental design [14].

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DOI: 10.1556/1006.2018.31.4.2

The aim of this work was to develop validated simple, accurate, precise, and specific reversed-phase (RP)-HPLC and thin-layer chromatographic (TLC)–densitometric methods for resolving and quantification of the ternary mixture and to perform a comparison between the reported HPLC method [14] and our method regarding the chromatographic conditions to shed the light on the advantages offered by our proposed method.

2 Experimental

2.1 Apparatus and Software

2.1.1 The HPLC System

Agilent 1100 series chromatographic system equipped with quaternary pump, microvacuum degasser, thermostated column compartment, and variable wavelength ultraviolet–visible (UV–vis) detector was used. Sample injections were made through an Agilent 1100 series autosampler. Data collection and processing were performed using Agilent ChemStation software, version A.10.01. The utilized column was a Zorbax C_8 column (150 mm × 4.6 mm, 5 µm) from Agilent Technologies (Polo Alto, CA, USA). A "Jenway 3505" pH-meter (Staffordshire, United Kingdom), equipped with combined glass electrode, was used for pH adjustment.

2.1.2 The TLC–Densitometry System

A CAMAG (Muttenz, Switzerland) TLC Scanner 3 S/N 130319 operated with winCATS software, Linomat 5 autosampler, CAMAG microsyringe (100 µL) and TLC aluminum sheets (20 × 20 cm) precoated with silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used.

2.2 Materials

2.2.1 Samples

2.2.1.1 Pure Sample

Chlorpheniramine maleate (CPM) and pseudoephedrine HCl (PSE) were kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO, Cairo, Egypt). Ibuprofen (IBF) was kindly supplied by Glaxo Wellcome (Cairo, Egypt); their purities were found to be 99.72 ± 1.065, 99.45 ± 1.011, and 100.05 ± 1.357 for CPM, PSE, and IBF, respectively, according to reported method [14].

2.2.1.2 Pharmaceutical Dosage Form

Sinlerg® coated tablets (each tablet is labeled to contain 2 mg of CPM, 30 mg of PSE, and 200 mg of IBF [batch number: 212314]) were manufactured by Eva Pharma for Pharmaceuticals and Medical Appliances S.A.E. (Haram, Giza, Egypt) and were purchased from the local market.

2.2.2 Chemicals

Ethyl acetate, 33% ammonia solution, and orthophosphoric acid (85%) (Adwic – El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt) were used in this study. Acetonitrile and methanol were of HPLC grade (LabScan Limited, Dublin, Ireland), while the water used was double distilled water.

2.3 Standard Solutions

2.3.1 For HPLC

Stock solutions of concentrations 1 mg mL^{-1} for CPM and 5 mg mL^{-1} for both PSE and IBF were prepared separately using methanol as the solvent.

Working standard solutions were freshly prepared by dilution from the stock standard solutions with the mobile phase to obtain a concentration of 200 µg mL^{-1} for CPM and 2000 µg mL^{-1} for PSE and IBF. The prepared solutions were found to be stable in refrigerator for 30 days.

2.3.2 For TLC

Stock standard solutions of concentration 2 mg mL^{-1} for CPM, 8 mg mL^{-1} PSE, and 10 mg mL^{-1} for IBF were prepared using methanol as the solvent. Working standard solutions were freshly prepared by dilution from the stock standard solutions with the same solvent to obtain a concentration of 1 mg mL^{-1} for CPM, 4 mg mL^{-1} for PSE, and 5 mg mL^{-1} IBF. The prepared solutions were found to be stable in refrigerator for 30 days.

2.4 Procedure

2.4.1 Chromatographic Conditions

2.4.1.1 RP-HPLC Method

RP-HPLC was carried out at ambient temperature on Zorbax C_8 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of methanol–acetonitrile–distilled water (pH 4) using orthophosphoric acid in the ratio 80:10:10 (*v/v*). The mobile phase was filtered using 0.45 µm Millipore membrane filter (Billerica, MA, USA) and delivered at a rate of 0.7 mL min^{-1}. The injection volume was 20 µL, and the detection was carried out at 220 nm.

2.4.1.2 TLC–Densitometric Method

TLC aluminum sheets (20 × 20 cm) precoated with 0.25 mm silica gel 60 F_{254} were used. The samples were applied to the TLC plate as bands (bandwidth, 6 mm; bands were spaced 1 cm apart from each other and 1 cm from the bottom edge of the plate). The applied volume per band was 10 µL using a 100-µL syringe. The developing system was ethyl acetate–methanol–ammonia (8:2:0.8, *v/v*). Linear ascending development was performed in a chromatographic tank previously saturated with the developing system for 1 h at room temperature to a distance of approximately 8 cm from the lower edge. The developed plates were air dried and scanned at 262 nm.

The detection was done using a CAMAG TLC Scanner 3 operated in the absorbance mode, with deuterium lamp as a source of radiation; the slit dimension was kept at 3 mm × 0.45 mm, and 20 mm s^{-1} scanning speed was employed.

2.4.2 System Suitability

2.4.2.1 RP-HPLC Method

An amount of 20 µL of the working standard solutions was injected, and chromatographic conditions were applied. The system suitability parameters including retention time (t_R), tailing factor (T), theoretical plate count (N), height equivalent to of theoretical plate (HETP), and resolution were calculated according to the United States Pharmacopeia (USP) guidelines [15] as listed in **Table 1.**

Table 1

System suitability parameters for the proposed RP-HPLC and TLC–densitometric methods.

Parameter	RP-HPLC method			TLC–densitometric method			Reference value [15]
	CPM	IBF	PSE	IBF	PSE	CPM	
t_R (RP-HPLC) R_F (TLC)	2.333	3.296	8.934	0.29 ± 0.01	0.64 ± 0.01	0.81 ± 0.01	$t_R > 1$ (HPLC)
N (column efficiency)	2285	2196	4877				$N > 2000$ Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	0.065	0.068	0.030				The smaller the value, the higher the column efficiency
Selectivity factor (α)	1.525	3.016		4.153	1.996		$\alpha > 1$
T (tailing factor)	1	0.95	1.33	0.85	0.9	1	$T \leq 2$ $T = 1$ for symmetric peak
R_s (resolution)	1.75	8.054		4.348	2.484		$R_s > 1.5$

2.4.2.2 TLC–Densitometric Method

Parameters including retention factor (R_F), selectivity factor (α), tailing factor (T), and resolution (R_s) were calculated according to the USP guidelines [15] as shown in Table 1.

2.4.3 Construction of Calibration Curves

2.4.3.1 RP-HPLC Method

Separate aliquots were transferred from the working standard solution of each drug to prepare solutions of different concentrations. The corresponding chromatographic conditions were applied for these solutions, and the chromatograms were recorded. The calibration curve of each drug was constructed by plotting the relative peak area (the peak area found to that of a standard of the same drug) against the corresponding concentration, from which the regression equations were calculated. Calibration curves were constructed in the range of 10–100 μg mL^{-1} for CPM, 100–1600 μg mL^{-1} for PSE, and 100–1400 μg mL^{-1} for IBF using the average of three experiments.

2.4.3.2 TLC–Densitometric Method

Separate aliquots were accurately transferred from the working standard solutions of each drug to prepare solutions of different concentrations. The corresponding chromatographic conditions were applied, and the chromatograms were recorded. The calibration curves were constructed by plotting the relative peak area against the corresponding concentration, from which the regression equations were calculated. Calibration curves were constructed in the range of 0.2–9 μg band^{-1} for CPM, 2–30 μg band^{-1} for PSE, and 5–50 μg band^{-1} for IBF, using the average of three experiments.

2.4.4 Assay of Laboratory-Prepared Mixtures

Different aliquots of the drugs were accurately transferred from their working standard solutions and mixed to prepare solutions of different ratios. The chromatographic conditions of both methods were adopted for each laboratory-prepared mixture, and the concentrations of each drug were calculated from the corresponding regression equation. Each concentration was conducted from the average of three experiments.

2.4.5 Application to Pharmaceutical Dosage Form

Ten Sinlerg® tablets (each tablet was labeled to contain 2 mg of CPM, 30 mg of PSE, 200 mg of IBF) were accurately weighed, powdered and mixed well. An amount of the powdered tablets equivalent to one tablet was accurately weighed and transferred into a beaker, then extracted with 3 × 10 mL methanol and sonicated for 15 min (for each extraction). The solution was filtered into a 50-mL volumetric flask and completed to volume with methanol.

For the HPLC method, an appropriate dilution was made with the mobile phase to prepare three different solutions from the previously prepared tablet solution having the concentrations of 20, 300, and 2000 μg mL^{-1} of CPM, PSE, and IBF for CPM determination; 10, 150, and 1000 μg mL^{-1} of CPM, PSE, and IBF; for PSE determination; and 4, 60, and 400 μg mL^{-1} of CPM, PSE, and IBF for IBF determination. For the TLC–densitometric method, an appropriate dilution was made with methanol to prepare two different solutions from the previously prepared tablet solution having the concentrations of 40, 600, and 4000 μg mL^{-1} of CPM, PSE, and IBF for CPM determination and 20, 300, and 2000 μg mL^{-1} of CPM, PSE, and IBF for PSE and IBF determination.

The prepared working standard solutions of dosage form were filtered through a 0.45-μm Millipore syringe membrane filter. The corresponding chromatographic conditions were applied for each working standard solution. Six replicates of each experiment were done. The concentration of each drug was calculated from its corresponding regression equation. The standard addition technique was applied by adding different known concentrations of pure standard drugs to the pharmaceutical formulation before proceeding in the previously mentioned methods.

3 Results and Discussion

The UV absorption spectra of CPM, PSE, and IBF display a considerable overlap **(Figure 2).** RP-HPLC and TLC–densitometric methods were successfully applied for the determination of the ternary mixture. These methods were found to be efficient for the separation of CPM, PSE, and IBF in their laboratory mixture and were successively applied to pharmaceutical formulation without interference from the excipients.

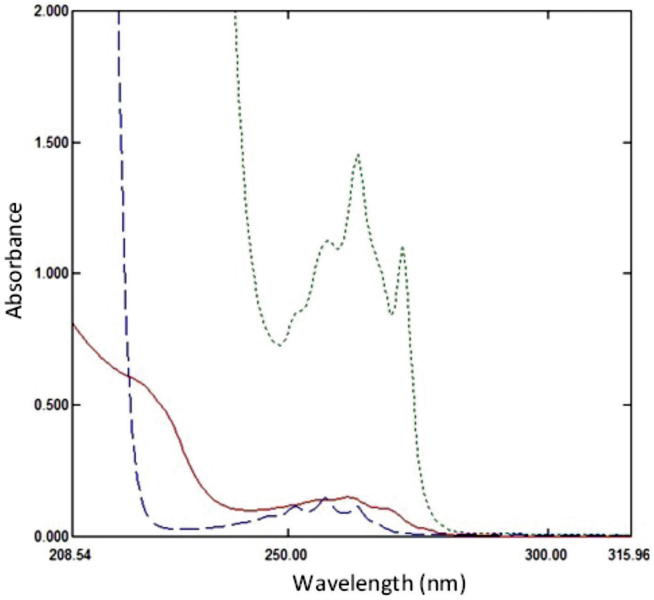

Figure 2

Zero order absorption spectra of 10, 150, and 1000 µg mL^{-1} of chlorpheniramine maleate (—), pseudoephedrine HCl (– – –), and ibuprofen (· · · ·), respectively, using methanol as a blank.

3.1 RP-HPLC Method

In this section, an RP-HPLC method is described for the separation and determination of CPM, PSE, and IBF in their pure forms, laboratory-prepared mixtures and pharmaceutical dosage form.

In order to achieve the best separation and resolution of the three drugs, different columns as well as different mobile phases were tried using different organic and inorganic solvents in different ratios and having different pH values. Using methanol and water in equal volume failed to separate the ternary mixture. Increasing the percentage of methanol caused better resolution, but broad peaks were obtained. By adding acetonitrile, sharper peaks were detected. Different flow rates (0.5–1.2 mL min^{-1}) were tested; good resolution was obtained using 0.7 mL min^{-1}. High pH values above pH 4 have caused overlapping of CPM and IBF peaks.

It was found that using C8 column and mobile phase of methanol–acetonitrile–distilled water (pH 4) using orthophosphoric acid (80:10:10, *v/v*) with a flow rate of 0.7 mL min^{-1} was the most suitable condition to get well resolved and sharp peaks. The optimum wavelength for detection and quantification was 220 nm, at which high sensitivity was obtained with symmetrical peaks.

Upon applying the previously described RP-HPLC optimum experimental conditions, good and efficient separation was observed for CPM, PSE, and IBF **(Figure 3).**

Our proposed RP-HPLC method has offered a number of advantages over the reported method [14]. The utilization of the C8 column with the dimension of (150 mm × 4.6 mm, 5 µm) in our proposed method has resulted in a short run time (9 min) in addition to very good resolution due to the small particle size. However, in the reported method, the used column was C18 with the dimension of (300 mm × 3.9 mm, 10 µm) which has resulted in a longer run time (17 min). Additionally, we have used isocratic elution which is considered to be simpler and less tedious than the gradient elution utilized in the reported method. Moreover, we have employed a lower flow rate of 0.7 mL min^{-1} compared to 1.5 mL min^{-1} which was used in the reported method; thus, our method has offered less solvent consumption which is considered to be more economical for both the industrial scale and routine work. Finally, under the utilized conditions, our proposed method has presented a wider calibration range of 10–100 µg mL^{-1} for CPM,

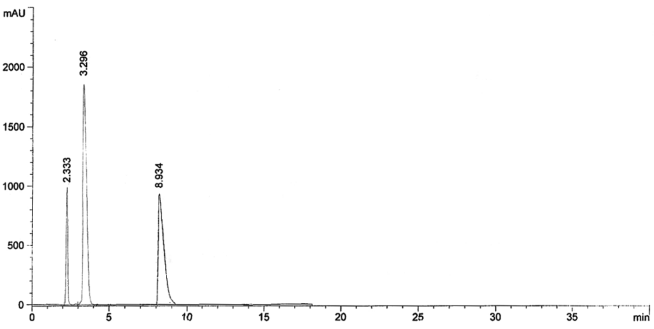

Figure 3

RP-HPLC chromatogram of 60 µg/mL (a) CPM (t_R = 2.333), 300 µg/mL (b) IBF (t_R = 3.296), and 200 µg/mL (c) PSE (t_R = 8.934), using Zorbax C_8 column and mobile phase of methanol–acetonitrile–distilled water (pH 4) in ratio (80:10:10, *v/v*), flow rate of 0.7 mL min^{-1} at 220 nm.

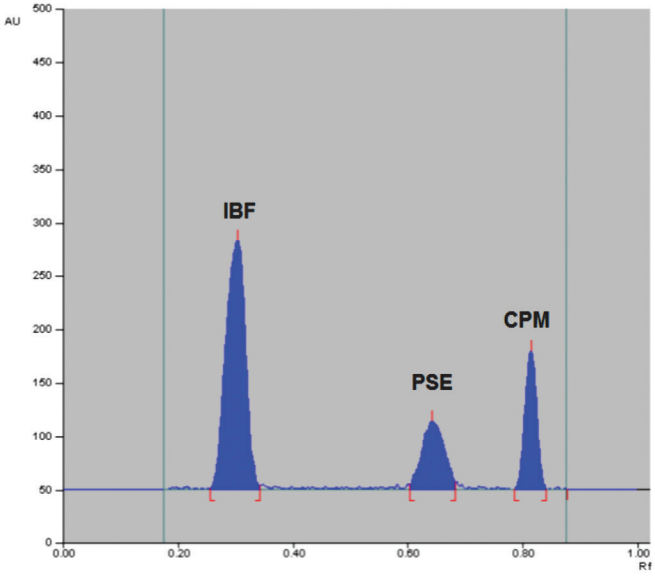

Figure 4

TLC chromatogram of 25 µg/band of IBF, 15 µg/band of PSE, and 1 µg/band of CPM using ethyl acetate–methanol–ammonia (8:2:0.8, *v/v*) as the developing system.

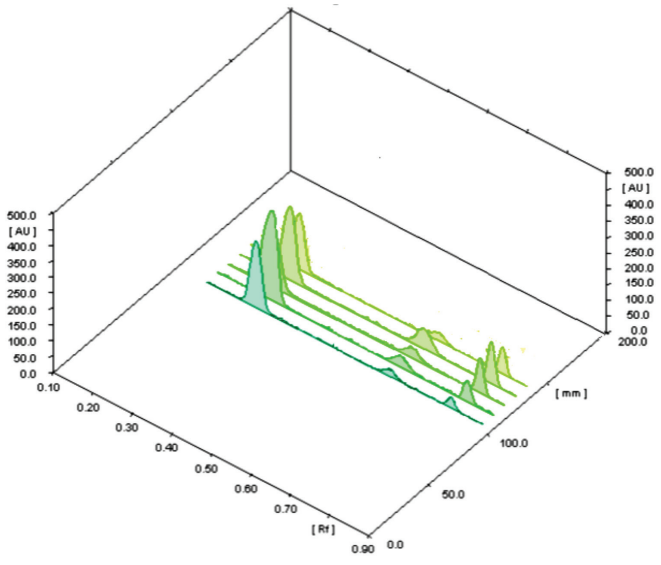

Figure 5

TLC chromatograms of different laboratory-prepared mixtures of CPM, PSE, and IBF using ethyl acetate–methanol–ammonia (8:2:0.8, *v/v*) as the developing system.

100–1600 µg mL^{-1} for PSE, and 100–1400 µg mL^{-1} for IBF compared to 6–14 µg/mL for CPM, 50–250 µg/mL for PSE, and 250–1250 µg/mL for IBF.

3.2 TLC–Densitometric Method

This method offers a simple way to quantify the drugs directly on TLC plate by measuring the optical density of the separated bands. The amounts of compounds were determined by comparing to a standard curve from reference materials chromatographed simultaneously under the same condition. Different solvent systems were tried for the separation of the ternary mixture. Satisfactory results were obtained by using a mobile phase composed of ethyl acetate–methanol–ammonia (8:2:0.8, *v/v*) which gave good resolution and sharp symmetrical peaks.

In order to minimize band diffusion, the optimum bandwidth was chosen to be 6 mm. Different scanning wavelengths were tried; on using 262 nm, the separated peaks were more sharp and symmetrical with minimum noise. The R_F values were 0.78, 0.64, and 0.29 for CPM, PSE, and IBF, respectively.

A typical chromatogram of the three components is shown in **Figures 4 and 5,** in which the separation has allowed for the determination of the three drugs without any interference from each other or from excipients.

The TLC–densitometric method has the advantages of being simple (simple developing systems with no pH adjustments), several samples can be run simultaneously using a small quantity of the mobile phase, thus lowering analysis time and cost per analysis, and having high sensitivity.

The system suitability parameters were calculated for the two proposed methods as demonstrated in Table 1. Both methods were successfully applied to determine the target drugs in laboratory-prepared mixtures without any interference from each other as abridged in **Table 2.**

3.3 Application to Pharmaceutical Dosage Form

The suggested RP-HPLC and TLC–densitometric methods were valid and applicable for the analysis of CPM, PSE, and IBF in Sinlerg® coated tablets. The validity of the proposed methods was further assessed by applying the standard addition technique, which has shown accurate results. We have compared our results for the determination of the ternary mixture with those of the reported method as shown in **Table 3.** The results have confirmed the suitability of the proposed methods for the routine determination of these components in their combined formulations.

3.4 Method Validation

Method validation was performed according to the International Conference on Harmonization (ICH) guidelines [16] for all the proposed methods as follows:

Table 2

Determination of CAR, PHL, and EPH in laboratory-prepared mixture by the proposed RP-HPLC and TLC–densitometric method.

Mixture No.	RP-HPLC method						TLC–densitometric method					
	Concentration [µg mL^{-1}]			Recovery[a] %			Concentration [µg band^{-1}]			Recovery[a] %		
	CPM	PSE	IBF	CPM	PSE	IBF	CPM	PSE	IBF	CPM	PSE	IBF
1[b]	10	150	1000	101.63	101.46	102.07	0.2	3	20	101.96	100.29	99.27
2	20	200	600	99.76	99.99	100.38	0.4	6	40	101.30	101.37	101.47
3	40	400	1100	100.26	101.25	101.84	0.8	5	10	99.18	98.44	100.93
4	100	100	100	102.16	101.06	99.39	1	15	25	102.17	99.61	99.17
5	60	300	1000	101.49	100.48	101.11	0.6	4	15	99.00	100.96	101.86
Mean ± SD				101.06 ± 1.003	100.85 ± 0.600	100.96 ± 1.100				100.72 ± 1.525	100.13 ± 1.162	100.54 ± 1.248

a)Average of three determinations
b)Ratio of CPM, PSE, and IBF in Sinlerg® tablets

Table 3

Application of standard addition technique to the analysis of CPM, PSE, and IBF in Sinlerg® tablets by the proposed RP-HPLC and TLC–densitometric methods compared to the reported method [14].

Pharmaceutical formulation	Drug	RP-HPLC method				TLC–densitometric method				Reported method[b]
		Claimed amount taken [μg mL^{-1}]	Added [μg mL^{-1}]	Found [μg mL^{-1}]	Recovery[a] %	Claimed amount taken [μg band^{-1}]	Added [μg band^{-1}]	Found [μg band^{-1}]	Recovery[a] %	
Sinlerg® tablets labeled to contain 2 mg CPM, 30 mg PSE and 200 mg IBF/tablet (batch No. 212314)	CPM[c]	20	10	10.12	101.20	0.4	0.300	0.306	102.13	99.72 ± 1.065
			20	19.84	99.19		0.400	0.395	98.83	
			30	30.41	101.38		0.800	0.809	101.09	
			Mean ± SD		100.59 ± 1.129		Mean ± SD		100.68 ± 1.683	
			Student's *t*-test 0.499 (2.26)[c]		*F* test 1.446 (6.26)[c]		Student's *t*-test 0.527 (2.26)[c]		*F* test 2.497 (6.26)[c]	
	PSE[d]	150	100	99.45	99.45	3	2	1.97	98.32	99.45 ± 1.011
			150	152.51	101.67		3	3.05	101.71	
			200	202.07	101.04		4	4.02	100.56	
			Mean ± SD		100.72 ± 1.143		Mean ± SD		100.19 ± 1.724	
			Student's *t*-test 1.119 (2.26)[c]		*F* test 1.278 (6.26)[c]		Student's *t*-test 0.392 (2.26)[c]		*F* test 2.908 (6.26)[c]	
	IBF[e]	400	200	200.25	100.13	20	10	10.11	101.14	100.05 ± 1.357
			400	398.91	99.73		20	20.18	100.91	
			500	507.27	101.45		30	29.79	99.30	
			Mean ± SD		100.44 ± 0.905		Mean ± SD		100.45 ± 0.999	
			Student's *t*-test 0.751 (2.26)[c]		*F* test 2.247 (5.19)[c]		Student's *t*-test 0.747 (2.26)[c]		*F* test 1.845 (5.19)[c]	

[a]Average of three experiments

[b]Reported HPLC method using C18 column; flow rate, 1.5 mL min^{-1}; mobile phase is a gradient elution of acetonitrile–phosphate buffer (15:85, *v/v*) for 5.5 min, (45:55, *v/v*) for 5.5–12 min, (60:40, *v/v*) for 12–17 min at pH = 3 and UV detection at 220 nm [14]

[c]First dilution (20 μg mL^{-1} CPM, 300 μg mL^{-1} PSE, and 2000 μg mL^{-1} IBF) for the standard addition for HPLC method and (0.4 μg mL^{-1} CPM, 6 μg mL^{-1} PSE, and 40 μg mL^{-1} IBF) for TLC method of CPM

[d]Second dilution (10 μg mL^{-1} CPM, 150 μg mL^{-1} PSE, and 1000 μg mL^{-1} IBF) for the standard addition for HPLC method and (0.2 μg mL^{-1} CPM, 3 μg mL^{-1} PSE, and 20 μg mL^{-1} IBF) for TLC method of PSE

[e]Third dilution (4 μg mL^{-1} CPM, 60 μg mL^{-1} PSE, and 400 μg mL^{-1} IBF) for the standard addition for HPLC method and (0.2 μg mL^{-1} CPM, 3 μg mL^{-1} PSE, and 20 μg mL^{-1} IBF) for TLC method of IBF

3.4.1 Range and Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [16].

The estimation of linearity of the proposed methods was achieved by construction of the calibration curves. The peak area ratio (drug/standard) against the concentration of CPM, PSE, and IBF was obtained. In this study, six concentrations were chosen for each drug. Each concentration was repeated three times, in order to provide information on the variation in peak area values among samples of the same concentration.

The calibration range was established through consideration of the necessary practical range, according to the ternary mixture concentration present in the pharmaceutical product, to give accurate, precise, and linear results. For RP-HPLC method, calibration curves were constructed in the range of 10–100 μg mL^{-1} for CPM, 100–1600 μg mL^{-1} for PSE, and 100–1400 μg mL^{-1} for IBF. For TLC–densitometric method, calibration curves were constructed in the range of 0.2–9 μg band^{-1} for CPM, 2–30 μg band^{-1} for PSE, and 5–50 μg band^{-1} for IBF. The corresponding assay parameters and validation sheet for the proposed methods are demonstrated in **Table 4.**

3.4.2 Limits of Detection and Quantification

According to the ICH [16] recommendations, the limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected. It was found to be 1.110 μg mL^{-1}, 17.262 μg mL^{-1}, and 26.070 μg mL^{-1} for CPM, PSE, and IBF, respectively, for HPLC method, while for TLC–densitometric method, it was found to be 0.062 μg band^{-1}, 0.285 μg band^{-1}, and 1.004 μg band^{-1} for

Table 4

Assay parameters and validation sheet obtained for the analysis of CPM, PSE, and IBF by applying the proposed RP-HPLC and TLC–densitometric methods.

Parameters	RP-HPLC method			TLC–densitometric method		
	CPM	PSE	IBF	CPM	PSE	IBF
Calibration range[a)]	10–100	100–1600	100–1400	0.2–9	2–30	5–50
Slope	0.0226	0.0013	0.001	0.4125	0.0681	0.0641
Intercept	0.0941	−0.0407	0.2064	0.0773	0.3774	0.0373
Standard error of slope	9.76×10^{-5}	5.206×10^{-6}	7.178×10^{-6}	0.0078	0.0010	0.0005
Standard error of intercept	0.0059	0.0055	0.00668	0.01215	0.00632	0.01659
Mean[b)]	100.14	99.36	98.74	99.71	99.55	100.18
SD	1.133	0.525	0.885	0.541	1.408	0.892
LOD[a)]	1.110	17.262	26.070	0.072	0.285	1.004
LOQ[a)]	3.363	52.308	79.000	0.218	0.864	3.042
Accuracy[c)] (mean ± SD)	101.34 ± 1.160	100.15 ± 1.170	100.84 ± 1.152	100.50 ± 1.441	100.90 ± 0.661	100.72 ± 1.113
Correlation coefficient (*r*)	1.00	1.00	0.9999	0.9998	0.9999	0.9999
Precision						
Intra-day precision[c)]	0.773	0.543	0.675	0.802	0.770	0.719
Inter-day precision[c)]	0.987	0.895	1.165	1.474	1.026	1.088

[a)]RP-HPLC method (in μg mL^{-1}); TLC–densitometric method (in μg $band^{-1}$)
[b)]Average of three experiments
[c)]Standard deviations (SDs) of three samples

CPM, PSE, and IBF, respectively. The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH recommendations below which the calibration graph is nonlinear. It was found to be 3.363 μg mL^{-1}, 52.308 μg mL^{-1}, and 79.00 for CPM, PSE, and IBF, respectively, for HPLC method, whereas, for TLC–densitometric method, it was found to be 0.187 μg $band^{-1}$, 0.864 μg $band^{-1}$, and 3.042 μg $band^{-1}$ for CPM, PSE, and IBF, respectively, as shown in Table 4, where:

$$LOD = 3.3\ Sa / b,\ LOQ = 10\ Sa / b$$

*S*a is the standard deviation of the intercept of the calibration curve and *b* is the slope of the calibration curve.

3.4.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found [16]. To study the accuracy of the proposed methods, procedures under linearity were repeated three times for the determination of five blind concentrations of pure CPM, PSE, and IBF. The accuracy expressed as percentage recoveries is shown in Table 4. The interference of excipients in the pharmaceutical formulations was studied by applying standard addition method. Good accuracy proved that the excipients in pharmaceutical formulations did not interfere in the analysis of these compounds, as shown in Table 3.

3.4.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility [16].

The precision of the proposed methods, expressed as relative standard deviation *(RSD),* was determined by the analysis of three different concentrations of pure drugs within the linearity range. The intra-day precision (repeatability) was performed through replicate analysis of three concentrations of the studied drugs on three successive occasions (20, 60, and 80 μg mL^{-1} for CPM; 100, 800, and 1200 μg mL^{-1} for PSE and IBF) for RP-HPLC method and (1, 5, and 7 μg $band^{-1}$ for CPM; 4, 15, and 20 μg $band^{-1}$ for PSE; and 15, 35, and 45 μg $band^{-1}$ for IBF) for TLC–densitometric method. The results are illustrated in Table 4.

3.4.5 Specificity

The specificity of a method is the extent to which it can be used for analysis of a particular analyte in a mixture or matrix without interference from other components. The specificity of the proposed methods was tested by the analysis of five laboratory-prepared mixtures containing different concentrations of CPM, PSE, and IBF within the linearity range. The laboratory-prepared mixtures were analyzed according to the previous procedures described under each of the proposed methods. The specificity was demonstrated by the chromatograms recorded

Table 5

Statistical comparison between the results obtained by the proposed methods and reported method for the determination of CPM, PSE, and IBF in pure powder form.

Parameters	RP-HPLC method			TLC–densitometric method			Reported method[b]		
	CPM	PSE	IBF	CPM	PSE	IBF	CPM	PSE	IBF
Mean	100.14	99.36	98.74	99.71	99.55	100.18	99.72	99.45	100.05
SD	1.133	0.525	0.885	0.541	1.408	0.892	1.065	1.011	1.357
Variance	1.283	0.276	0.783	0.293	1.982	0.796	1.134	1.022	1.841
No. of experiments	6	6	6	6	6	6	5	5	5
Student's *t*-test	0.628 (2.26)[a]	0.182 (2.26)[a]	1.930 (2.26)[a]	0.027 (2.26)[a]	0.133 (2.26)[a]	0.195 (2.26)[a]			
F test	1.131 (6.26)[a]	3.703 (5.19)[a]	2.351 (5.19)[a]	3.870 (5.19)[a]	1.939 (6.26)[a]	2.313 (5.19)[a]			

[a]Figures between parentheses represent the corresponding tabulated values of t and F at $P = 0.05$

[b]Reported HPLC method using C18 column; flow rate, 1.5 mL min^{-1}; mobile phase is a gradient elution of acetonitrile–phosphate buffer (15:85, *v/v*) for 5.5 min, (45:55, *v/v*) for 5.5–12 min, (60:40, *v/v*) for 12–17 min at pH = 3 and UV detection at 220 nm [14]

for mixtures of CPM, PSE, and IBF, indicating that the methods enabled highly specific analysis of the drug mixture. Well resolved peaks for CPM, PSE, and IBF were observed (Figures 3 and 4). Satisfactory results were obtained (Table 2), indicating the high selectivity of the proposed methods for simultaneous determination of CPM, PSE, and IBF.

3.5 Statistical Analysis

Results obtained by the proposed methods for the determination of pure samples of CPM, PSE, and IBF were statistically compared to those obtained by the reported method [14]. The values of the calculated t and F were less than the corresponding tabulated ones, which revealed that there was no significant difference with respect to accuracy and precision between the proposed methods and the reported ones as shown in **Table 5.** Calculations were done using GraphPad Instat software.

4 Conclusion

By applying the RP-HPLC and TLC–densitometric methods for the analysis of CPM, PSE, and IBF, it was found that the proposed methods were simple, sensitive, accurate, precise, repeatable, and specific. The methods could be applied to the analysis of the studied drugs in their synthetic mixtures and their combined dosage form without interference from common excipients, and the results were in good agreement with the reported method. The proposed RP-HPLC has presented a rapid separation technique with a simple isocratic mobile phase without the need for pre-column derivatization or complicated gradient elution. It was capable of accurate determination of the ternary mixture quantitatively in a wider concentration range as compared to the proposed TLC–densitometric method. The TLC–densitometric method had the advantages of being simple, sensitive, and economic, as it saves cost and time, as up to 20 samples could be applied to a single plate and analyzed per one development. The developed methods were quite sensitive for quantitative detection of the CPM, PSE, and IBF in their pharmaceutical preparation and can thus be used for routine analysis and in quality control.

References

[1] *S.U. Yasuda, A. Wellstein, P. Likhari, J.T. Barbey, R.L. Woosley,* Clin. Pharmacol. Ther. **58** (1995) 210–220.

[2] *L.L. Brunton, B.A. Chabner, B.C. Knollmann* (eds) Goodman and Gilman's the Pharmaceutical Basis of Therapeutics, 9th edn., Macmillan Publishing Company, New York, NY, 1996.

[3] *A. Van Esch, H.A. Van Steensel-Moll, E.W. Steyerberg, M. Offringa, J.D. Habbema, G. Derksen-Lubsen,* Arch. Pediatr. Adolesc. Med. **149** (1995) 632–637.

[4] *J.L. Murtha, T.N. Julian, G.W. Radebaugh,* J. Pharm. Sci. **77** (1988) 715–718.

[5] *I.M. Palabiyik, E. Dinç, F. Onur,* J. Pharm. Biomed. Anal. **34** (2004) 473–483.

[6] *S. Chitlange, D. Sakarkar, S. Wankhede, S. Wadodkar,* Indian J. Pharm. Sci. **70** (2008) 398–400.

[7] *X. Chen, Y. Zhang, D. Zhong,* Biomed. Chromatogr. **18** (2004) 248–253.

[8] *H.-g. Lou, H. Yuan, Z.-r. Ruan, B. Jiang,* J. Chromatogr. B **878** (2010) 682–688.

[9] *J.A. Thompson, F.H. Leffert,* J. Pharm. Sci. **69** (1980) 707–710.

[10] *M. Louhaichi, S. Jebali, M. Loueslati, N. Adhoum, L. Monser,* Talanta **78** (2009) 991–997.

[11] *Y. Dong, X. Chen, Y. Chen, X. Chen, Z. Hu,* J. Pharm. Biomed. Anal. **39** (2005) 285–289.

[12] *Y. Liu, W. Zhou,* Anal. Sci. **22** (2006) 999–1003.

[13] British Pharmacopoeia, The Stationery Office on behalf of the Medicines and Healthcare Products Regulatory Agency (MHRA)©, Crown Copyright, Vols. I and II, London, 2012.

[14] *B. Aşçi, Ö.A. Dönmmez, A. Bozdoğan, S. Sungur,* J. Anal. Chem. **65** (2010) 743–748.

[15] The United States Pharmacopeia 36, the National Formulary Official Monographs 31, U.S. Pharmacopeial Convention, Rockville, MD, 2013.

[16] ICH Q2A, Note for Guidance on Validation of Analytical Methods: Definition and Terminology. International Conference on Harmonization, IFPMA, Geneva, 1994.

Ms received: February 11, 2018
Accepted: May 25, 2018