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Simultaneous determination of carbinoxamine maleate and pseudoephedrine HCl in their pure form and in their pharmaceutical formulation by HPTLC-densitometric method

Maha Mohamed Abdelrahman, Eglal Abdelhameed Abdelaleem, Nouruddin Wageih Ali and Raghda Abdelmoneim Emam *

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni- Suef University, 62514, Beni-Suef, Egypt

** Corresponding author at: Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni- Suef University, 62514, Beni-Suef, Egypt. Tel.: +2.010.4408208. Fax: +2.082.2317950. E-mail address: raghdaemam88@gmail.com (R.A. Emam).*

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ABSTRACT

An accurate, sensitive and selective High Performance Thin Layer Chromatography (HPTLC) method has been developed and validated for determination of carbinoxamine maleate and pseudoephedrine HCl in their binary mixture without previous separation. Chromatographic separation was performed on aluminum plates precoated with silica gel $60F_{254}$ using acetone: methanol:ethylacetate:triethylamine (5:4:2:0.2, *v:v:v:v*) as a developing system. The bands were scanned at 215 nm. The proposed method has been validated as per ICH guidelines and its linearity was evident in the ranges of 0.4-8.0 and 4-24 µg/ band for carbinoxamine maleate and pseudoephedrine HCl, respectively. The developed method has been applied for determination of the above mentioned drugs in their pharmaceutical formulation where no interference from methyl paraben present as excipient has been detected. Statistical comparison of the results obtained by the developed HPTLC method and those obtained by the reported HPLC method showed no significant difference between them. The developed method is sensitive, accurate and precise and can be easily used for quality control analysis of the studied drugs.

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1. Introduction

Chemically, carbinoxamine maleate (CRM) is designated as 2-[(4-chlorophenyl)(2-pyridinyl)methoxy]-*N*,*N*-dimethylethan amine (2Z)-2-butenedioate (Figure 1) [1]. It is a monoethanol amine derivative, a sedating antihistamine with antimuscarinic, significant sedative and serotonin antagonist effects. It is used for the relief of allergic conditions such as rhinitis, and is a common ingredient of compound preparations for symptomatic treatment of coughs and the common cold (Figure 1) [1].

Chemically, pseudoephedrine HCl (PSH) is designated as (1S,2S)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride [2]. It is a stereoisomer of ephedrine that has the same action. PSH and its salts are given by mouth for the symptomatic relief of nasal congestion and are commonly combined with other ingredients in preparations intended for the relief of cough and cold symptoms (Figure 1) [1].

A review in the literature revealed that carbinoxamine maleate has been determined by spectrophotometric methods and first-order UV-spectroscopic method [3-6], HPLC method [6,7], LC-MS/MS method for the quantification of carbinoxamine in human plasma [8], and gas liquid chromatographic method [9], while for PSH, it has been determined with other components in different pharmaceutical formulations such as, spectrophotometric methods [10,11] and second derivative photodiode array spectroscopy [12], HPLC methods [13-17], stability indicating HPLC method [18].

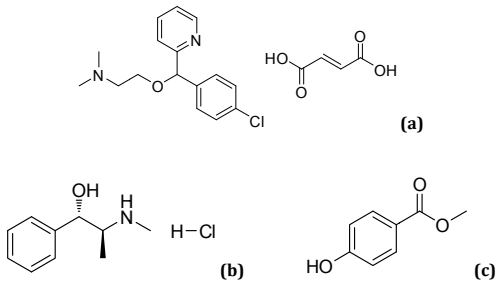

Figure 1. Chemical structure of CRM (a), PSH (b) and MP (c).

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The only reported method for determination of both drugs simultaneously was HPLC method [19] without taking in consideration presence of methyl paraben as formulation excipient. Methyl paraben (MP) is a hydroxybenzoates preservative; alkyl esters of *p*-hydroxy benzoic acid, with antibacterial and antifungal properties (Figure 1). Hydroxy benzoates are used as preservatives in pharmaceutical preparations in usual concentrations of up to 0.25% [1].

The advantage of HPTLC is that several samples can be run simultaneously using a small quantity of developing system, unlike HPLC, thus lower in analysis time and cost per analysis. So, the focus of the present study was to develop an accurate, specific and selective method for simultaneous determination of carbinoxamine maleate and pseudoephedrine HCl without interference from oral drops excipient (methyl paraben).

2. Experimental

2.1. Instrumentation

A sample applicator for TLC method was linomat V applicator with 100 µL syringe, (Camage, Muttenz, Switzerland). TLC scanner, densitometer, (Camag, Switzerland) was used controlled by WINCATS software, (V 3.15, Camage). HPTLC aluminum plates (20×20 cm) coated with 0.25 mm silica gel $60F_{254}$, (Merck, Germany), were used. UV lamp with short wavelength 254 nm, (VL-6.LC, MARNE LA VALLEE cedex 1, FRANCE), was used. Also, sonix TV ss-series ultrasonicator (USA) was used. There are some parameters that had been adjusted during TLC scanning; radiation source was a deuterium lamp, Slit dimension was 6×0.45 mm, scanning mode was "absorbance" and scanning speed was 20 mm/s.

2.2. Materials and reagents

2.2.1. Pure standards

Carbinoxamine maleate and pseudoephedrine HCl pure standards were kindly supplied by Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt with purity of 99.69 and 98.83%, respectively, according to the manufacturer certificate. Methyl paraben was kindly supplied by the Pharaonia Pharmaceuticals, Alexandria, Egypt, with purity of 99.02% according to the manufacturer certificate.

2.2.2. Pharmaceutical formulation

Rhinostop® oral drops (batch No. 134089) was Labeled to contain 2 mg CRM and 25 mg PSH in each mL, was manufactured by Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt.

2.2.3. Chemicals and solvents

All chemicals used throughout this work were of analytical grade and were used without further purification: Methanol, acetone, ethylacetate, triethylamine, (El-Nasr Pharmaceutical Chemicals Co., Abu Zabaal, Cairo, Egypt).

2.2.4. Solutions

Stock standard solutions of CRM and PSH (1 and 4 mg/mL, respectively): 100 and 400 mg of CRM and PSH, respectively, were accurately and separately weighed into two 100 mL volumetric flasks and dissolved in methanol.

Working standard solutions of CRM and PSH (0.1 and 0.2 mg/mL): They were prepared by diluting 10 and 5 mL each of CRM and PSH, respectively, from their respective stock solutions into two separate 100 mL volumetric flasks and the volume was completed to the mark with methanol.

Preparation of pharmaceutical formulation: 4 mL of Rhinostop® oral drops equivalent to 100 mg PSH and 8 mg CRM were accurately transferred into a 100 mL volumetric flask and then the volume was completed to the mark with methanol to prepare 1 mg/mL PSH (and the corresponding amount of 80 µg/band CRM) stock solution from which a working solution of 0.1 mg/mL PSH (corresponding to 8 µg/band of CRM) was prepared in methanol.

2.3. Procedure

2.3.1. Chromatographic conditions

It was performed using pre-coated silica gel HPTLC aluminum plates (20×10 cm). The plates were pre-washed with methanol and activated at 100 °C for 15 minutes prior to samples application. Samples were applied in the form of bands (6 mm length, 5 mm spacing and 10 mm from the bottom edge of the plate). Linear ascending development was performed in a chromatographic tank previously saturated with acetone: methanol:ethylacetate:triethylamine (5:4:2:0.2, *v*:*v*:*v*:*v*) for half an hour at room temperature to a distance of about 80 mm. The developed plates were air dried and then scanned at 215 nm.

2.3.2. Linearity and construction of calibration curves

Aliquots equivalent to 0.4-8.0 and 4-24 mg were accurately and separately transferred from CRM and PSH stock standard solutions (1 and 4 mg/mL), respectively, into a series of 10 mL volumetric flasks and the volume was completed to the mark with methanol, 10 µL of each solution were applied in triplicates on the pre-washed HPTLC plates to obtain the concentration range of 0.4-8.0 and 4-24 µg/band for CRM and PSH, respectively. The procedure under chromatographic conditions was then followed. The integrated peak area was then recorded and a calibration curve for each drug was constructed by plotting the integrated peak area versus the corresponding concentration of each drug and the regression equations were then computed.

2.3.3. Application to pharmaceutical formulation

The procedure under linearity was followed on Rhinostop® oral drops and the concentrations of CRM and PSH were determined using the computed regression equations. To assess the accuracy of the proposed method, standard addition technique was applied.

3. Results and discussion

The main task of this work was to develop sensitive, selective and accurate HPTLC-densitometric method for the determination of CRM and PSH in their binary mixture and in Rhinostop® oral drops without interference of Rhinostop® excipient; methylparaben with satisfactory precision for good analytical practice (GAP).

Thin layer chromatography has become a well-established technique for the assay of drugs either in binary or in multi-component mixtures [20-27]. The proposed method is based on the difference in the retardation factor (R_F) between the CRM, PSH and MP.

3.1. Method optimization

The experimental conditions had been optimized to get the desired chromatographic resolution and efficiency with sharp symmetric peaks.

3.1.1. Developing system

Table 1. Regression parameters of the proposed HPTLC-Densitometric methods for determination of carbinoxamine maleate and pseudoephedrine HCl.

Parameters	CRM	PSH
Linearity		
Range (µg/band)	0.4-8.0	4-24
Slope	1880.40	213.46
Intercept	943.54	290.64
r (correlation coefficient)	0.9999	0.9998
Accuracy	99.51	99.90
Precision (%RSD)		
Repeatability [a]	0.981	1.180
Intermediate precision [b]	1.608	1.432
Roubustness (% RSD)		
Saturation time ±5 min	0.147	0.551
Triethylamine ratio ±0.02 mL	0.603	0.788
Scanning wavelength ±1 nm	0.073	0.864
LOD* (µg/band)	0.12	1.16
LOQ* (µg/band)	0.36	3.50

[a] The intraday (n = 3), average of three different concentrations repeated three times within day.
[b] The interday (n = 3), average of three different concentrations repeated three times in three successive days.
* LOD and LOQ values were calculated using a visual non-instrumental method [24].

Table 2. Determination of carbinoxamine maleate and pseudoephedrine HCl in pharmaceutical formulation by the proposed HPTLC-Densitometric method and application of standard addition technique.

Pharmaceutical formulations		Taken (µg/band)	% Recovery±SD	Standard addition technique	
				Pure added (µg/band)	% Recovery
Rhinostop® oral drops (B.N. 134089)	CRM	1	95.74±1.489	0.8	99.89
Labeled to contain 2 mg CRM and 25 mg PSH/mL				1.0	98.12
				1.2	100.84
				Mean±SD	99.62±1.380
	PSH	12.5	106.64±1.442	4.0	100.74
				6.0	98.79
				8.0	101.75
				Mean±SD	100.43±1.505

Initially, trial experiments were performed in a view to select a suitable developing system for the suitable separation of the three studied components. Several solvent systems were tried to permit good separation without tailing, e.g., chloroform: methanol (4:6, *v*:*v*), acetone:methanol (6:4, *v*:*v*), acetone:methanol:ethyl acetate (5:4:2, *v*:*v*:*v*) and acetone: methanol:ethyl acetate:triethyl amine (5:4:2:0.2, *v*:*v*:*v*:*v*). Using the first system, tailed peaks were obtained with bad separation and very low R_f value of PSH peak. Replacement of chloroform by acetone in the second system increased the R_f value of PSH but gave tailed peaks with bad separation. Addition of ethyl acetate in the third system, improved CRM and MP peaks shape and symmetry but gave broad peak of PSH. Addition of TEA (0.2 mL) to the third system, improved PSH peak shape and symmetry with the best separation between the three components.

3.1.2. Saturation time

The saturation time required before development is important to achieve homogeneity of the atmosphere, thus, minimizing the evaporation of the solvent from HPTLC plate during the development. The saturation time of the developing system has been optimized and found to be 30 min.

3.1.3. Scanning wavelength

Different scanning wavelengths (210, 215, 220 and 254 nm) were tried in order to enhance the sensitivity of the method; scanning at 254 nm gave bad sensitivity for PSH and considerable sensitivity for CRM and high sensitivity for MP. While scanning at 215 nm gave the best sensitivity for PSH and CRM which is very important for quality control analysis of them. R_f values of the separated components were 0.33, 0.46 and 0.77 for PSH, CRM and MP, respectively, obtained by using a developing system containing acetone:methanol:ethyl acetate:triethyl amine (5:4:2:0.2, *v*:*v*:*v*:*v*). As shown in the densitogram Figure 2, the peaks are symmetric and well separated.

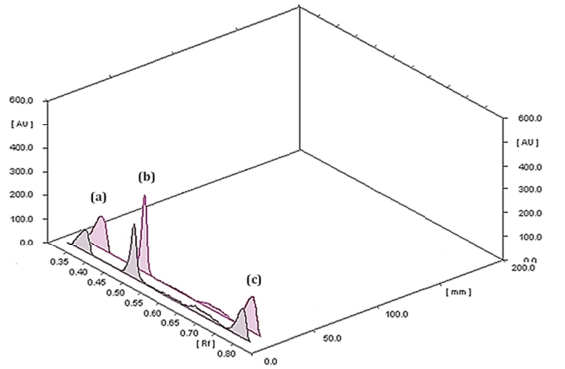

Figure 2. HPTLC densitogram of (a) pseudoephedrine HCl (R_f = 0.33), (b) carbinoxamine maleate (R_f = 0.46), (c) methylparaben (R_f = 0.77) using acetone:methanol:ethylacetate:triethylamine (5:4:2:0.2, *v*:*v*:*v*:*v*) as a developing system.

A linear relationship between the concentrations of CRM and PSH and the integrated peaks area in the range of 0.4-8.0 µg/band and 4-24 µg/band for CRM and PSH, respectively, and the regression equations were computed and found to be:

$$A_1 = 1880.40 \times C_1 + 943.54 \quad r_1 = 0.9999 \text{ for CRM} \qquad (1)$$

$$A_2 = 213.46 \times C_2 + 290.64 \quad r_2 = 0.9998 \text{ for PSH} \qquad (2)$$

where A_1, A_2 are the integrated peak area, C_1, C_2 are the concentrations in µg/band and r_1, r_2 are the correlation coefficients of CRM and PSH, respectively. Regression equation parameters are given in Table 1.

The validity of the proposed method for analysis of CRM and PSH was studied by applying the proposed method on Rhinostop® oral drops, Table 2. It was further assessed by applying standard addition technique, which showed that there was no interference from MP, Table 2.

Table 3. Statistical comparison of the proposed method and the reported one for determination of CRM and PSH in their pharmaceutical formulation.

Items	HPTLC		Reported method HPLC [19] [b]	
	CRM	PSH	CRM	PSH
Mean	99.51	99.90	99.97	101.06
SD	1.584	1.004	1.2895	0.854
RSD%	1.592	1.005	1.2899	0.845
n	8	8	6	6
Student's t-test	0.576 (2.178) [a]	1.748 (2.178) [a]		
F-value (3.972)	1.508 (4.875) [a]	1.384 (4.875) [a]		

[a] Figures between parenthesis represent the corresponding tabulated values of t and F at p= 0.05.

[b] An isocratic reversed phase LC analysis conducted on a CN column, with a mobile phase consisting of acetonitrile-methanol-phosphate buffer (pH = 5.3)-water (140:170:40:100) and at a detection wavelength of 262 nm and a flow rate of 1 mL/min.

Statistical analysis of the results obtained by applying the developed HPTLC-densitometric method with those obtained by the reported HPLC method [19], showed no significant difference with 95% confidence regarding both accuracy and precision, Table 3.

3.2. Method validation

Method validation was performed according to ICH guidelines [28].

3.2.1. Linearity and range

Linearity of the proposed method was evaluated and it was evident in the range of 0.4-8.0 and 4-24 µg/band for CRM and PSH, respectively. Good linearity was evident from the high value of correlation coefficient as shown in Table 1.

3.2.2. Accuracy

Accuracy of the method was checked by applying the proposed method for determination of different blind samples of pure CRM and PSH. The concentrations were calculated from the corresponding regression equations and the results were presented in Table 1. Accuracy of the method was further assured by applying the standard addition technique on pharmaceutical formulation where good recoveries were obtained revealing no interference from excipients, as given in Table 2.

3.2.3. Precision

Repeatability: Three concentrations (0.8, 1.0 and 2.0 µg/band) of CRM and (8, 10 and 14 µg/band) of PSH were analyzed three times intra-day using the proposed method. Good %RSD values were obtained confirming the repeatability of the method as given in Table 1.

Intermediate precision: The previous procedure was repeated inter-day on three different days for the analysis of the three chosen concentrations. Acceptable % RSD values were obtained as given in Table 1.

3.2.4. Specificity

The specificity of the proposed method was demonstrated by the HPTLC chromatogram as shown in Figure 2 where complete separation of CRM, PSH and MP was achieved. Also, the absence of any peaks at the retention time of the studied drug and the good results obtained on applying the method to Rhinostop® oral drops, presented in Table 2, prove that there was no interference from excipients.

3.2.5. Limit of detection and limit of quantitation

ICH recommendations [24] were followed using a visual non-instrumental method to calculate the values of LOD and LOQ of the two studied components where LOD is the concentration at which the signal to noise ratio is equal to 3:1 while LOQ is the concentration at which the signal to noise ratio is equal to 10:1. Low values of both LOD and LOQ indicated the high sensitivity of the developed method, Table 1.

3.2.6. Robustness

Robustness is the capacity of the method to remain unchanged with small deliberate variations in method parameters e.g. changing triethyl amine volume in the developing system 2±0.1 mL, changing saturation time 30±5 min and changing the scanning wavelength ±3 nm. The low value of %RSD shows that the method is robust and that the deliberate small change in the studied factors did not lead to significant changes in R_f values, area or symmetry of the peaks. The % RSD was calculated, and the results are given in Table 1.

3.2.7. System suitability

An overall system suitability testing was done to determine if the operating system were performed properly. Parameters including resolution (R_s), selectivity factor (α) and peak asymmetry (tailing factor) were calculated where good results were obtained as given in Table 4.

Table 4. System suitability testing parameters of HPTLC densitometric method.

Parameters	CRM	PSH	Reference values [29]
Resolution (R_s)	2.55		> 1.5
Selectivity factor (α)	1.39		>1
Tailing factor (T)	1.01	0.9	-

The results obtained by applying the proposed method were statistically compared with those obtained by applying the reported HPLC method [19] and the calculated F and t-values showed no significant differences between them, as shown in Table 3.

4. Conclusion

The proposed HPTLC densitometric method is precise, accurate, sensitive and cost effective method and can be used for the routine analysis of CRM and PSH in their pharmaceutical formulation without interference of MP (excipient). The advantage of the developed HPTLC-densitometric method is that several samples can be run simultaneously using a small quantity of developing system.

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