Rapid Validated Thin-Layer Chromatography–Densitometry for the Simultaneous Determination of Three Co-formulated Drugs Used for Common Cold Treatment

Preprint *in* JPC - Journal of Planar Chromatography - Modern TLC · March 2019

DOI: 10.1556/1006.2019.32.2.8

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Rapid Validated Thin-Layer Chromatography–Densitometry for the Simultaneous Determination of Three Co-formulated Drugs Used for Common Cold Treatment

Manal M. Fouad and **Christine M. El-Maraghy***

Key Words:

Paracetamol
Pseudoephedrine
Chlorpheniramine maleate
Thin-layer chromatography–densitometry
Common cold

Summary

A rapid validated thin-layer chromatography (TLC)–densitometric method has been developed for the simultaneous determination of 3 co-formulated drugs used for common cold and cough treatment. The studied drugs are paracetamol, pseudoephedrine, and chlorpheniramine maleate. The separation was achieved using silica gel 60 F_{254} plates and the developing system of methanol–toluene–acetic acid (44:16:1, *v/v*). Densitometry scanning was performed at 254 nm. The method was validated as per the International Conference on Harmonization (ICH) guidelines and was successfully applied for the analysis of pharmaceutical preparation containing the cited ternary mixture without interference from excipients. There is no previously published TLC–densitometric method for the determination of the previously mentioned ternary mixture. The suggested method is rapid and of low cost, so it can be used for quality control analysis.

1 Introduction

Paracetamol (PARA), pseudoephedrine (PSE), and chlorpheniramine maleate (CPM) have been co-formulated and widely used for symptomatic treatment of common cold [1, 2]. Paracetamol, *N*-(4-hydroxyphenyl)acetamide, is an analgesic and antipyretic agent. Pseudoephedrine, 2-methylamino-1-phenylpropan-1-ol, is used as a nasal mucosal vasoconstrictor and decongestant [3]. Chlorpheniramine maleate, 2-pyridinepropanamine-(4-chlorophenyl)-*N*,*N*-dimethyl-2-butenedioate, is a reversible competitive inhibitor of the interaction of histamine with H_1 receptors [3]. The structures of these compounds are shown in **Figure 1.** The literature review revealed their simultaneous quantification in pharmaceutical formulations and in plasma, including spectrophotometry for the determination of PSE, CPM, and dextromethorphan (DX) [4], chemometric methods for the determination of PARA, CPM, and guaiphenesin [5], high-performance liquid chromatography (HPLC) for the determination of PARA, CPM, and PSE in pharmaceuticals [6–9], and HPLC–mass spectrometry (MS) for their analysis in biological fluid [10–12]. To date, to the best of our knowledge, there was no reported thin-layer chromatography (TLC)–densitometric method for the simultaneous determination of PARA, PSE, and CPM. The TLC method has the advantages over the HPLC method of being more cost- and time-saving and not requiring pH adjustment of the mobile phase or tedious cleanup procedures. Thus, the aim of our present work was to conduct a comparative study between TLC–densitometry and the previously published HPLC method for the simultaneous determination of the 3 co-formulated drugs in bulk powder and pharmaceutical preparation.

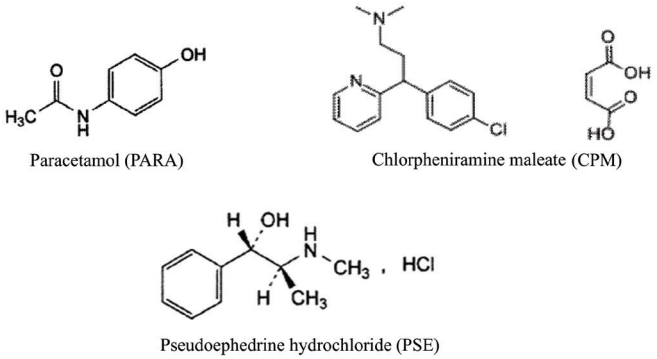

Figure 1

The chemical structures of paracetamol, pseudoephedrine hydrochloride, and chlorpheniramine maleate.

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2 Experimental

2.1 Instruments

A CAMAG TLC scanner (CAMAG, Muttenz, Switzerland) operated with winCATS software version 3.15, a Linomat IV autosampler (CAMAG), a 100-µL CAMAG microsyringe (Hamilton, Bonaduz, Switzerland), and pre-coated silica gel aluminum plates 60 F_{254} (20 cm × 20 cm; 250-µm thicknesses) (E. Merck, Darmstadt, Germany) were used.

2.2 Reagents and Chemicals

Paracetamol (El Nasr Company, Cairo, Egypt), chlorpheniramine maleate (ADWIC Pharmaceutical Company, Egypt), and pseudoephedrine hydrochloride (Delta Pharma Company, Cairo, Egypt) with purities of 99.98%, 99.73%, and 100.01%, respectively, as stated by the supplier were used.

Methanol (Riedel-de Haën, Sigma-Aldrich, Darmstadt, Germany), toluene (Euromedex, Souffelweyersheim, France), and glacial acetic acid (ADWIC) were used.

2.3 Pharmaceutical Preparation

Cetal Cold&Flu® tablets (batch No.: 1704157) were obtained from the local market. Each tablet was labeled to contain PARA 500 mg, CPM 2.0 mg, and PSE 30 mg.

2.4 Preparation of Standard Solutions

Stock standard solutions with concentrations of 2 mg mL^{-1} for PARA and 1 mg mL^{-1} for PSE and CPM were prepared using methanol as the solvent. Working standard solutions were freshly prepared by dilution with methanol to obtain solutions having concentrations of 1 mg mL^{-1} for PARA and 0.1 mg mL^{-1} for PSE and CPM.

2.5 Procedures

2.5.1 Chromatographic Conditions

The analysis was performed on pre-coated 20 cm × 20 cm silica gel 60 F_{254} aluminum sheets. The samples were applied to the plates using CAMAG Linomat IV applicator along with a 100-µL CAMAG micro syringe. Spots were applied 1.5 cm apart from each other and 2 cm from the bottom edge. A mixture of methanol, toluene, and acetic acid (44:16:1, *v/v*) was selected as the mobile phase. Densitometry scanning was performed at 254 nm. The chromatographic chamber was pre-saturated with the mobile phase for 20 min, and the developing distance on a TLC plate was 180 mm.

2.5.2 Construction of Calibration Curves

Accurately measured aliquots were transferred from the working standard solutions of each drug separately, equivalent to 50–600 µg per spot for PARA, 1–30 µg per spot for CPM, and 10–35 µg spot for PSE, and were applied in triplicate to TLC plates, and the previous conditions were applied. The relative peak area of each drug was plotted against the corresponding concentration, from which the regression equations were calculated.

2.5.3 Assay of Laboratory-Prepared Mixtures

Solutions containing different concentrations of PARA, CPM, and PSE were prepared from their respective working standard solutions and diluted with methanol. The average peak areas of each drug in the laboratory-prepared mixtures were calculated and processed as described. The concentration of the 3 drugs was calculated using the corresponding calculated regression equation.

2.5.4 Application to Pharmaceutical Preparation

Ten Cetal Cold&Flu® tablets were accurately weighted and finely powdered, and an amount equivalent to 500 mg PARA, 2 mg CPM, and 30 mg PSE was dissolved in 50-mL methanol in ultrasonic bath for 20 min. The solution was filtered and quantitatively transferred into 100-mL volumetric flask, and the volume was brought to the mark with methanol. An aliquot of 10 mL was transferred from the prepared stock solution into a 100-mL volumetric flask, completed to the volume with methanol to obtain solution concentrations of 500 µg mL^{-1} PARA, 2 µg mL^{-1} CPM, and 30 µg mL^{-1} PSE. The general procedure described above was followed, and the concentrations of PARA, CPM, and PSE were calculated using the corresponding regression equation. The validity of the results was assessed by applying the standard addition technique, by adding different concentrations of the 3 pure drugs to the same pharmaceutical preparation and proceeding as the previously mentioned procedure.

3 Results and Discussion

The aim of this work was to develop a TLC–densitometric method for the simultaneous determination of 3 co-formulated drugs, namely, PARA, CPM, and PSE, in bulk powder in pharmaceutical formulations. To the best of our knowledge, there was no reported TLC–densitometric method for the simultaneous determination of PARA, PSE, and CPM. TLC–densitometry has the advantages of being simple, cost-effective (for the instrument and the solvents used), and rapid, when compared to HPLC.

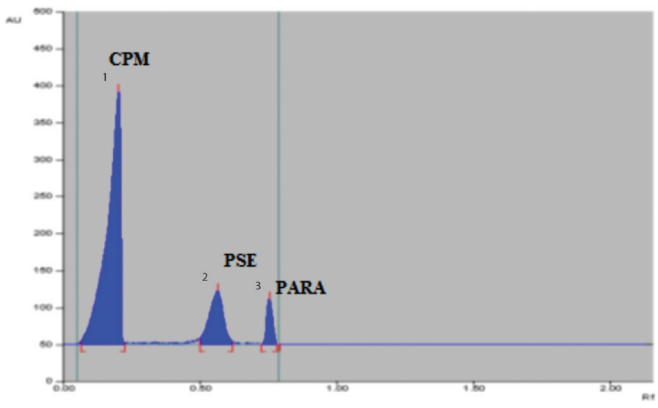

Figure 2

TLC densitogram of PARA (60 µg per spot), CPM (20 µg per spot), and PSE (20 µg per spot), using methanol–toluene–acetic acid (44:16:1, *v/v*) as the developing system measured at 254 nm.

3.1 Method Optimization

The chromatographic conditions were optimized by spotting the 3 drugs on TLC plates and developing different solvent systems in order to achieve the best separation. Initially, a system of methanol and toluene (10:2, *v/v*) was used, but PARA was very near to the solvent front. Increasing the polarity of the developing system by increasing the methanol ratio was done in order to move PARA away from the solvent front. Acetic acid was added dropwise to correct the streaking that may cause an error in the retardation factor (R_F) calculation. Complete separation of the 3 drugs was achieved by using methanol–toluene–acetic acid in a ratio of 44:16:1 (*v/v*). The average R_F values of PARA, CPM, and PSE were found to be 0.81 ± 0.02, 0.1 ± 0.01, and 0.71 ± 0.02, respectively, as shown in **Figures 2–5.**

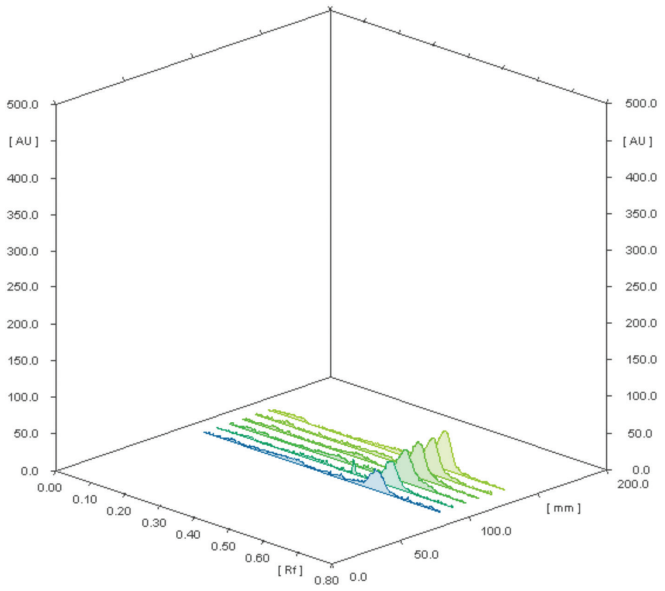

Figure 3

TLC densitogram of PSE (R_F = 0.71 ± 0.02) in the concentration range of 10–35 µg per spot.

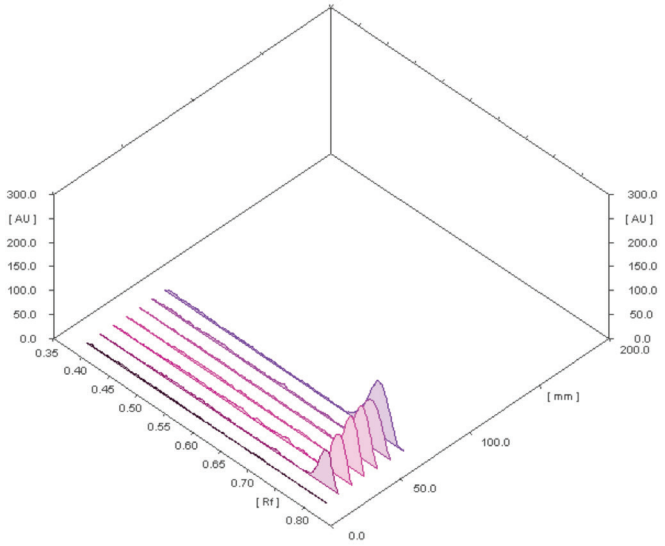

Figure 4

TLC densitogram of PARA (R_F = 0.81 ± 0.02) in the concentration range of 50–600 µg per spot.

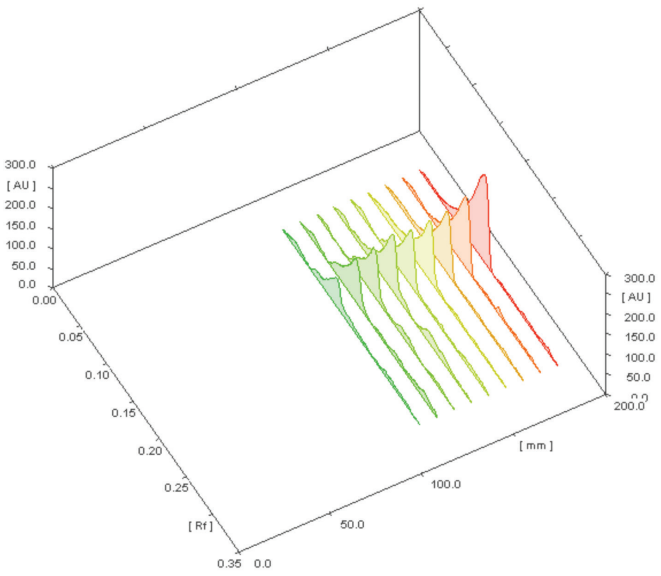

Figure 5

TLC densitogram of CPM (R_F = 0.1 ± 0.01) in the concentration range 1–30 µg per spot.

3.2 Method Validation

The International Conference on Harmonization (ICH) guidelines [13] for method validation were followed.

3.2.1 Linearity

Calibration graphs were found to be linear in the range of 50–600 µg per spot for PARA, 1–30 µg per spot for CPM, and 10–35 µg per spot for PSE.

3.2.2 Accuracy

The accuracy of the proposed chromatographic method was checked by the analysis of five different concentrations of each standard solution of PARA, CPM, and PSE in triplicate. Their concentrations were calculated using the corresponding regression equation, and then the mean percentage recovery and the standard deviation *(%RSD)* were calculated, as shown in **Table 1.**

3.2.3 Precision

Three replicates of 3 different concentrations for the studied drugs were analyzed on the same day to determine the intra-day precision. To confirm the inter-day precision, 3 replicates of each concentration were analyzed for 3 separate days by using the developed chromatographic method and calculating the relative standard deviation (Table 1).

3.2.4 Specificity

The specificity of the proposed method was tested by analysis of laboratory-prepared mixtures containing different ratios of PARA, CPM, and PSE. Satisfactory results were obtained as shown in **Table 2.**

3.2.5 Robustness

The robustness was investigated by analysis of samples under small changes in the mobile phase ratio to 42:18:1 (*v/v*), by changing the mobile phase volume and the time of the mobile

Table 1

Regression parameters for the determination of PARA, CPM, and PSE using TLC–densitometry.

Parameters		TLC–densitometry		
		PARA	CPM	PSE
Linearity range [μg per spot^{-1}]		50–600	1–30	10–35
Slope		212.75	1771.9	693.14
Intercept		1587.2	3515	810.55
SE of the slope		25.409	95.615	27.906
SE of the intercept		191.83	667.870	70.872
Standard deviation of residuals		160.70	76.67	67.20
Correlation coefficient (r)		0.9991	0.9976	0.9952
LOD [μg spot^{-1}]		19.7	0.07	0.31
LOQ [μg spot^{-1}]		48.6	0.43	0.96
Accuracy (%Recovery ± *%RSD*)		99.65 ± 1.41	101.65 ± 1.26	99.74 ± 1.74
Precision (*%RSD*)	Inter-day	1.54	1.23	1.67
	Intra-day	1.89	1.75	1.90

The inter-day and intra-day precision values of the samples: PARA (60, 200, and 400 μg per spot), CPM (3, 15, and 30 μg per spot), and PSE (15, 20, and 30 μg per spot)

Table 2

Results obtained for the analysis of laboratory-prepared mixtures, by the proposed methods, for the determination of PARA, CPM, and PSE.

Conc. (PAR–CPM–PSE)	%Recovery[a)]		
	PAR	CPM	PSE
200:5:20	99.67	98.17	100.20
250:2:10	101.4	102.52	100.75
400:2:10	101.56	101.2	101.83
450:5:20	99.00	101.68	101.00
500:2:30[b)]	98.54	99.77	98.77
Mean (% ± SD)	100.03 ± 1.38	100.66 ± 1.71	100.51 ± 1.13

[a)]Average of three determinations
[b)]The ratio of Cetal Cold&Flu® pharmaceutical preparation

phase saturation. These changes had no significant effect on the chromatographic resolution. The results indicated that the capacity of the proposed method remained unaffected by these small deliberate variations **(Table 3).**

Table 3

Robustness of the proposed TLC–densitometric method for the determination of PARA, CPM, and PSE.

Parameter	PARA	CPM	PSE
	R_F ± SD		
Mobile phase composition [methanol–toluene–acetic acid]			
(42:18:1, *v*/*v*)	0.81 ± 0.01	0.10 ± 0.01	0.71 ± 0.03
(43:15:0.5, *v*/*v*)	0.80 ± 0.02	0.12 ± 0.01	0.73 ± 0.02
Mobile phase volume (60, 100, and 120 mL)	0.81 ± 0.02	0.08 ± 0.01	0.71 ± 0.03
Duration of saturation (15, 30, and 40 min)	0.81 ± 0.01	0.11 ± 0.01	0.71 ± 0.02

Table 4

System suitability parameters of the developed TLC–densitometric method.

Parameter	TLC–densitometry		
	PARA	CPM	PSE
R_F	0.81 ± 0.02	0.10 ± 0.01	0.71 ± 0.02
Tailing factor (T)	0.92	0.71	0.89
Selectivity factor (α)	7.66	1.15	
Resolution (Rs)	2.2	2.45	–

Table 5

Quantitative determination of PARA, CPM, and PSE in the pharmaceutical preparation and application of standard addition technique.

Pharmaceutical Preparation	claimed	Added (μg per spot)	%Recovery[a)]
Cetal Cold&Flu® tablets (batch No.: 1704157) Each labeled to contain 500 mg PARA, 2 mg CPM, and 30 mg PSE	PARA	10	100.67
	500 μg per spot	20	101.53
	(99.43 ± 1.17)	30	99.72
	Mean ± S.D.		100.64 ± 0.90
	CPM	3	101.65
	2 μg per spot	4	101.33
	(100.79 ± 1.55)	5	100.86
	Mean ± S.D.		101.28 ± 0.39
	PSE	1	98.54
	30 μg per spot	2	99.31
	(99.54 ± 1.23)	3	99.75
	Mean ± S.D.		99.20 ± 0.61

[a)]Average of 3 determinations. The values between parentheses represent the %Recovery and SD of analysis of pharmaceutical preparation

Table 6

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

Parameter	TLC–densitometry			Reference method[b)]		
	PARA	CPM	PSE	PARA	CPM	PSE
Mean	99.43	100.79	99.54	99.21	98.99	99.21
SD	1.17	1.55	1.23	0.49	1.53	1.07
Variance	1.36	2.40	1.51	0.83	2.34	1.14
N	5	5	5	5	5	5
Student's *t*-test[a)]	0.426 (2.306)	0.186 (2.306)	0.621 (2.306)			
F-test[a)]	1.613 (6.400)	1.209 (6.400)	1.158 (6.400)			

[a)]The values between parenthesis are the theoretical values of *t*- and *F*-test at $P = 0.05$

[b)]The reference HPLC method using a C_{18} (150 mm × 4.6 mm, 5 μm) column and gradient mobile phase methanol–sodium perchlorate (0.043 M, 2 mL triethylamine, pH 5.0) at a flow rate of 1.0 mL, detection was at 204 nm for CPM and PSE and 300 nm for PARA

3.3 System Suitability

System suitability test parameters must be checked to ensure that the system was working correctly during the analysis. Method performance data including retardation factor (R_F), tailing factor (*T*), and resolution (*R*s) are listed in **Table 4.**

3.4 Analysis of Pharmaceutical Preparation

The proposed method was applied for the determination of PARA, CPM, and PSE in Cetal Cold&Flu® tablets. The results were satisfactory and in good agreement within the labeled amount. The interference of excipients in the pharmaceutical preparation was studied using a standard addition technique. According to the obtained results, good accuracy and precision were observed **(Table 5).** Consequently, the excipients in the pharmaceutical formulations did not interfere in the simultaneous analysis of the 3 studied drugs in pharmaceutical preparation.

3.5 Statistical Analysis

The results obtained by applying the proposed chromatographic method were statistically compared to the reference HPLC method [8]. The calculated *t*- and *F*-values were less than the theoretical values, which indicate that there was no significant difference between the proposed and the reference method with respect to accuracy and precision, as presented in **Table 6.**

4 Conclusion

The present work described the successful simultaneous quantitative analysis of PARA, PSE, and CPM in their laboratory-prepared mixtures and in pharmaceutical formulations using TLC–densitometry. The results showed that the developed TLC–densitometry had the advantages of being simpler than the HPLC, as it used simple mobile phase with no pH adjustment, sensitive, and economic, as it saves cost (inexpensive apparatus and solvents) and time (up to 20 samples could be applied onto a single plate per one development). The developed method can be used in routine quality control testing, allowing qualitative and quantitative determination with high accuracy and precision.

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Ms received: December 15, 2018
Accepted: January 29, 2019