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Simultaneous determination of some multicomponent dosage forms by quantitative thin layer chromatography densitometric method

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Abstract

In this study, simultaneous determination of naproxen with diflunisal (mixture I), paracetamol with chlorzoxazone (mixture II) and chlorphenoxamine hydrochloride with 8–chlorotheophylline and caffeine (mixture III) in multicomponent mixtures was conducted by a thin layer chromatography–densitometric method. The mobile phase ethyl acetate: methanol: ammonia 25% (85:15:5 v/v) was used for the separation of the components of mixtures (I) and (II) with R_f values of 0.16 for naproxen, 0.4 for diflunisal, 0.77 for paracetamol and 0.32 for chlorzoxazone. Efficient separation of the components of mixture (III) was attained using ethyl acetate as mobile phase with R_f values of 0.12, 0.62 and 0.42 for chlorphenoxamine hydrochloride, 8–chlorotheophylline and caffeine, respectively. Linearity ranges, mean recoveries and relative standard deviations in calibration graphs of the proposed method were calculated. The method has been successfully applied to pharmaceutical formulations, sugar-coated tablets, capsules and suppositories. The results obtained were statistically compared with those obtained by applying the reported alternate methods.

Keywords: Naproxen; Diflunisal; Paracetamol; Chlorzoxazone; Chlorphenoxamine hydrochloride; 8-Chlorotheophylline; Caffeine; Thin layer chromatography–UV densitometry

1. Introduction

Few methods have been reported for the quantitative determination of naproxen and diflunisal in their combination with other drugs including high-performance liquid chromatography (HPLC) [1–3]. Several methods describing the simultaneous determination of paracetamol and chlorzoxazone in dosage forms have been reported including UV spectrophotometry [4–7], derivative spectrophotometry [8,9] potentiometry [10], gas chromatography [11] and HPLC [12–14]. A literature survey reveals that there are only two methods reported for the determination of 8-chlorotheophylline in the presence of chlorphenoxamine hydrochloride and caffeine in the pharmaceu-

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tical formulations applying conductometry [15] and HPLC [16] techniques.

In this paper, a thin layer chromatography (TLC)–UV scanning densitometric technique is used for the simultaneous determination of the components of mixtures (I), (II) and (III) in their pharmaceutical dosage forms. A favorable advantage of TLC–UV densitometry over UV spectrophotometry [4–7] is its ability to separate the contents of the analyzed samples, thus eliminating the possibility of interference between active ingredients or due to additives, excepients or impurities. In addition, the method is amenable to the simultaneous analysis of six samples on the same TLC plate with precision and accuracy comparable with alternate UV and derivative spectrophotometry [4–9]. Other advantages of the TLC–UV method are its fast scanning speed, its low limit of detection and its broad linear ranges.

Although the HPLC technique was applied for the determination of components of mixtures (II) [12–14] and (III) [16], the TLC technique is less expensive without loss of accuracy and is therefore more suitable for application in developing countries.

2. Experimental

2.1. Materials

2.1.1. Mixture (I)

2.1.1.1. Working standards. Naproxen (99.75 ± 0.23%) and diflunisal (100.12 ± 0.51) were purchased from Rameda, Egypt.

2.1.1.2. Standard solutions. All solutions were freshly prepared.

1. Naproxen (1.0 mg ml^{-1}) in methanol.
2. Diflunisal (1.0 mg ml^{-1}) in methanol.
3. A standard solution of both analytes was prepared by dissolving 25.0 mg of each of naproxen and diflunisal in 15 ml methanol in a 25 ml volumetric flask and making up to volume with the same solvent. The final concentration ratio of naproxen to diflunisal is the same as that of the dosage form.

2.1.1.3. Pharmaceutical preparations analyzed.

- Maxipan tablet (batch No. 60549; Rameda, Egypt). Each tablet claimed to contain 200 mg naproxen and 200 mg diflunisal.
- Maxipan suppositories (batch No. 70646; Rameda, Egypt). Each suppository claimed to contain 250 mg naproxen and 250 mg diflunisal.

2.1.1.4. Sample preparation. Tablets. Twenty tablets were accurately weighed and powdered. An amount of the powdered tablets equivalent to 100.0 mg of each component was shaken with 60 ml methanol in a 100 ml volumetric flask. After 15 min of mechanical shaking, the volume was filled to the mark with the same solvent and filtered (1.0 mg ml^{-1}).

Suppositories. Ten suppositories were accurately weighed in a beaker, melted in a water bath with stirring and cooled. An amount equivalent to one suppository was transferred to a 100 ml conical flask, extracted with 4 × 50 ml hot methanol and cooled in a refrigerator each time. Filtration was carried out into 250 ml volumetric flask and the volume was filled to the mark with methanol (1.0 mg ml^{-1}).

2.1.2. Mixture (II)

2.1.2.1. Working standards. Paracetamol (99.85 ± 0.45%) and chlorzoxazone (99.65 ± 0.23%) were purchased from Amoun, Egypt.

2.1.2.2. Standard solutions. All solutions were freshly prepared.

1. Paracetamol (1.2 mg ml^{-1}) in methanol.
2. Chlorzoxazone (1.0 mg ml^{-1}) in methanol.
3. A standard solution of two actives was prepared by dissolving 30.0 mg of paracetamol and 25.0 mg of chlorzoxazone in 15 ml methanol in a 25 ml volumetric flask and filling up to the mark with the same solvent. The final concentration ratio of paracetamol to chlorzoxazone is the same as that of the capsules.

2.1.2.3. Pharmaceutical preparations analyzed. Myolgin capsule (batch No. 1640; Amoun, Egypt). Each capsule claimed to contain 300 mg paracetamol and 250 mg chlorzoxazone.

2.1.2.4. Sample preparation. Capsules. The contents of twenty capsules were transferred to a suitable weighed container and the average weight per capsule was determined. The combined contents were mixed and an accurately weighed portion equivalent to one capsule was shaken with 60 ml methanol in a 100 ml volumetric flask. After 15 min of mechanical shaking, the volume was made up using the same solvent and filter. Transfer of 10 ml of the solution to a 25 ml volumetric flask was followed by filling up to volume using methanol (1.2 and 1.0 mg ml^{-1} of paracetamol and chlorzoxazone, respectively).

2.1.3. Mixture (III)

2.1.3.1. Working standards. Chlorphenoxamine hydrochloride ($99.89 \pm 0.83\%$), 8-chlorotheophylline ($100.01 \pm 0.35\%$) and caffeine ($99.80 \pm 0.58\%$) were purchased from Eipico, Egypt.

2.1.3.2. Standard solutions. All solutions were freshly prepared.

1. Chlorphenoxamine hydrochloride (0.6 mg ml^{-1}) in methanol.
2. 8-Chlorotheophylline (0.4 mg ml^{-1}) in methanol.
3. Caffeine (1.0 mg ml^{-1}) in methanol.
4. A standard solution of the three actives was prepared by dissolving 30.0, 20.0 and 50.0 mg of chlorphenoxamine hydrochloride, 8-chlorotheophylline and caffeine, respectively, in 30 ml methanol in a 50 ml volumetric flask and filling up to the mark with the same solvent. The final concentration ratio of chlorphenoxamine hydrochloride to 8-chlorotheophylline and caffeine is the same as that of the dosage form.

2.1.3.3. Pharmaceutical preparations analyzed

- Emeral tablet (batch No. 980618; Eipico, Egypt). Each tablet claimed to contain 30 mg chlorphenoxamine hydrochloride, 20 mg 8-chlorotheophylline and 50 mg caffeine.
- Emeral suppository (batch No. 961774; Eipico, Egypt). Each suppository claimed to contain 60 mg chlorphenoxamine hydrochloride, 40 mg 8-chlorotheophylline and 100 mg caffeine.

2.1.3.4. Sample preparation. Tablets. The tablet coat was removed by washing with water and the tablets were dried. Twenty tablets were accurately weighed and powdered. An amount of the tablet mass equivalent to one tablet was shaken with 30 ml methanol in a 50 ml volumetric flask. After 30 min of mechanical shaking, the volume was made up using the same solvent and filtration was carried out (0.6, 0.4, 1.0 mg ml^{-1} for chlorphenoxamine hydrochloride, 8-chlorotheophylline and caffeine, respectively).

Suppositories. Ten suppositories were weighed accurately in a small beaker and melted on a water bath with stirring and cooled. An amount of the melted and cooled suppositories equivalent to one suppository was extracted with 3×30 ml hot methanol, cooled in a refrigerator each time, filtered into a 100 ml volumetric flask and filled to volume with methanol (0.6, 0.4, 1.0 mg ml^{-1} for chlorphenoxamine hydrochloride, 8-chlorotheophylline and caffeine, respectively).

2.2. TLC–UV densitometric method

2.2.1. Apparatus

A SHIMADZU (Kyoto, Japan) dual wavelength flying spot scanning densitometer CS 9301 PC. List of parameters: photomode, reflection; scan mode, zigzag; beam size, 12.

2.2.2. Solid phase

TLC precoated plates 20×20 cm^2 with 0.25 mm thickness silica gel G60 F_{254} (Merck, Darmstadt, Germany).

2.2.3. Mobile phases

1. Ethyl acetate: methanol: ammonia 25% (85:10:5 v/v) is used as mobile phase for mixtures (I) and (II).
2. Ethyl acetate (Merck, Germany) is used as mobile phase for mixture (III).

2.2.4. Procedure

2.2.4.1. Calibration. Different amounts ranging from 0.5 to 4.5 ml of each standard solution were transferred into eight 5 ml volumetric flasks and

the volume was made up using methanol. Aliquots of 20 μl of each solution were applied to a separate precoated thin layer chromatographic plate (20 × 20cm) using a 50 μl micropipette.

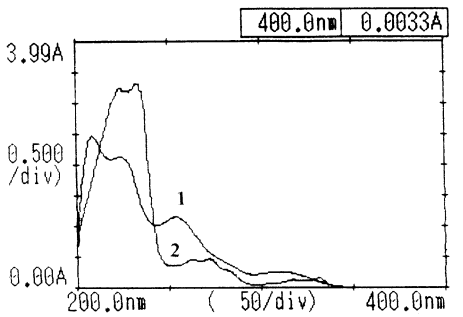

Fig. 1. Typical UV spectra: 1, diflunisal (1 mg%); 2, naproxen (1 mg%).

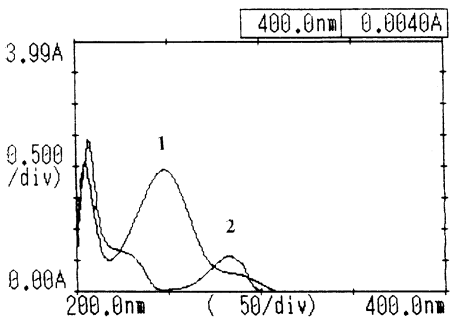

Fig. 2. Typical UV spectra: 1, paracetamol (1.2 mg%); 2, chlorzoxazone (1 mg%).

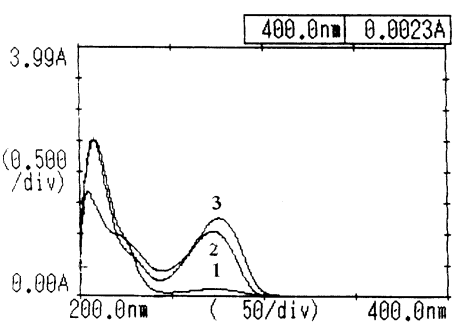

Fig. 3. Typical UV spectra: 1, chlorphenoxamine hydrochloride (1.2 mg%); 2, 8-chlorotheophylline (0.8 mg%); 3, caffeine (2 mg%).

2.2.4.2. Chromatographic conditions. The chromatographic chamber was equilibrated with ethyl acetate: methanol: ammonia 25% (85:10:5 v/v) for 1 h prior to use for mixtures (I) and (II), and ethyl acetate for 30 min for mixture (III). The chromatograms were developed at room temperature by ascending migration of the mobile phase over a distance of 16 cm. The plates were removed, dried and the spots were visualized under UV lamp at 254 nm. The chromatograms were scanned with the spectrodensitometer at the maximum of each drug as listed in Table 2.

The calibration curves were plotted representing the relationship between the recorded area under the peak and the corresponding concentrations.

2.2.5. Assay of pharmaceutical preparations

Performed as procedures in Sections 2.2.4.1 and 2.2.4.2, and calculate the concentrations of each drug from the corresponding regression equation. Results obtained are shown in Tables 3–5).

3. Results and discussion

Conventional spectrophotometric determination of the components of mixtures (I), (II) and (III) by using absorbance at λ_{max} is inapplicable due to the overlapping of their absorption spectra (Figs. 1–3). TLC densitometry overcomes this problem by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram.

The proposed TLC–UV densitometric method has the advantage of simultaneously determining the active ingredients in multicomponent dosage forms.

In this work, various developing systems were examined to separate diflunisal–naproxen (mixture I), paracetamol–chlorzoxazone (mixture II) and chlorphenoxamine hydrochloride–8-chlorotheophylline–caffeine (mixture III) by combining homogeneous design with solvent polarity optimization [17]. The separation results of those different polarity developers are shown in Table 1. It can be seen that components of mixtures (I) and (II) are well separated with ethyl

Table 1
Influence of the component and proportion of the developers on the separation[a]

Solvent system	P^*	$R_{f\times}100$						
		Naproxen	Diflunisal	Paracetamol	Chlorzoxazone	Chlorphenoxamine HCl	8-Chlorotheophylline	Caffeine
Ethyl acetate	4.3	94	94	0	0	12	62	42
Methanol/ ammonia (100:1.5)	6.75	96	96	92	94	84	96	93
Chloroform/methanol (9:1)	4.62	92	98	75	0	92	66	97
Ethyl acetate/ methanol/ammonia (85:10:5)	5.17	16	40	77	32	96	45	96

[a] The volume ratios of component (v/v) are included in parentheses; P^* is the polar parameter, $P^* = P_a \times A\% + P_b \times B\% + P_c \times C\% + \ldots$, where A, B, C, … are the percentage of volume of solvent a, b, c, …

Table 2
The analytical characteristics of mixtures(I), (II) and (III) with the TLC–UV method

Substances	Linear ranges[a] (μg)	Intercept	RSD of intercept	Slope	RSD of slope	r	λ_{max}
Mixture (I)							
Naproxen	2–16	0.36	2.01×10^{-3}	6.01	2.71×10^{-2}	0.9999	271
Diflunisal	2–18	1.95	1.52×10^{-2}	15.90	7.00×10^{-2}	0.9999	251
Mixture (II)							
Paracetamol	2.4–20	1.23	1.11×10^{-2}	17.09	7.20×10^{-2}	0.9999	249
Chlorzoxazone	2–18	3.5	2.13×10^{-2}	8.23	3.11×10^{-2}	0.9999	287
Mixture (III)							
Chlorphenoxamine hydrochloride	3–10.8	−1.0	5.70×10^{-3}	11.6	4.92×10^{-2}	0.9998	278
8-Chlorotheophylline	1–7	0.25	1.50×10^{-3}	13.68	6.10×10^{-2}	0.9958	271
Caffeine	2–18	4.0	2.61×10^{-2}	9.64	4.00×10^{-2}	1.00	273

[a] Eight concentrations were analyzed (average of threedeterminations).

Table 3
Comparison between the TLC–UV method and the reported method [18] for the determination of the naproxen–diflunisal mixture in pure form and in its pharmaceutical formulations

Sample	TLC UV method		Reported method[c]
	Found[a] (% ± CV)	Recovery[b] (% ± CV)	Found[a] (% ± CV)
Pure sample			
Naproxen	99.54 ± 0.41		99.75 ± 0.23
Diflunisal	100.67 ± 0.27		100.12 ± 0.51
Maxipan tablets B.N. 60549			
Naproxen	99.21 ± 0.51	99.45 ± 0.38	99.11 ± 0.15
Diflunisal	100.30 ± 0.83	99.81 ± 1.12	99.46 ± 1.13
Maxipan suppositories B.N. 70646			
Naproxen	99.89 ± 0.56	99.13 ± 0.37	98.59 ± 0.48
Diflunisal	100.13 ± 0.26	99.24 ± 0.35	99.74 ± 0.50

[a] Mean of six determinations.
[b] Recovery of standard solutions and the average of five experiments carried out by the TLC–UV densitometric method.
[c] Ref. [18] UV spectrophotometric method (Vireodet's method) in methanol at 251 and 262 nm.

acetate: methanol: concentrated ammonia solution 25% (85:10:5) as mobile phase. Ethyl acetate is the ideal mobile phase for the separation of the components of mixture (III); the R_f values of the cited drugs are presented in Table 1.

The equilibration time required before development is important to achieve homogeneity of the atmosphere thus minimizes the evaporation of the solvent from the TLC plate during the development, therefore, the saturation time of the tank has been optimized and found to be 1 h for ethyl acetate: methanol: ammonia (85:10:5 v/v) and 30 min for ethyl acetate. The TLC plates were activated at 100°C for 1 h to increase the efficiency of separation.

Application of 2–20 μg was carried out on the silica gel plates using a 50 μl micropipette. After the development, the chromatogram can be scanned densitometrically at the corresponding wavelengths (Table 2). By applying this technique, a linear correlation was obtained between the area under the peak and the concentration; the analytical data of the calibration curves including RSD for the intercept and slope are summarized in Table 2.

The reproducibility, repeatability and precision of the proposed method were tested by applying them for the determination of six different mixtures of pure samples and calculating their concentrations from the respective regression equations. The mean percentage recoveries range from 99.54 to 100.67% with CV values of 0.27–1.55% (Tables 3–5).

Three-day determinations were also performed, showing accurate results, and the mean percentage recoveries range from 98.87 to 100.51% with CV values of 1.0–1.4%.

For further study of the validity accuracy, reproducibility and precision of the proposed method, recovery experiments were carried out by analyzing samples of the pharmaceutical preparations applying the standard addition technique. The mean percentage recoveries lie in the ranges 99.45–99.81 and 99.12–100.63%. The precision of the proposed method was confirmed by the low

Table 4
Comparison between the TLC–UV method and the reported method [8] for the determination of paracetamol–chlorzoxazone mixture in pure form and in its pharmaceutical formulations

Sample	TLC–UV method		Reported method[c]
	Found[a] (% ± CV)	Recovery[b] (% ± CV)	Found[a] (% ± CV)
Pure sample			
Paracetamol	100.41 ± 0.45		99.85 ± 0.45
Chlorzoxazone	99.75 ± 0.55		99.65 ± 0.23
Mylogin capsules B. N. 1640			
Paracetamol	98.95 ± 0.56	99.22 ± 0.54	98.77 ± 0.68
Chlorzoxazone	101.16 ± 0.12	99.98 ± 0.45	99.72 ± 0.17

[a] Mean of six determinations.
[b] Recovery of standard solutions and the average of five experiments carried out by the TLC–UV densitometric method.
[c] Ref. [8] derivative spectrophotometric method at 270.8 and 278.9 nm in methanol for paracetamol and chlorzoxazone, respectively.

Table 5
Comparison between the TLC–UV method and the reported method [16] for the determination of chlorphenoxamine hydrochloride, 8-chlorotheophylline and caffeine mixture in pure form and in its pharmaceutical formulations

Sample	TLC–UV method		Reported method[c]
	Found[a] (% ± CV)	Recovery[b] (% ± CV)	Found[a] (% ± CV)
Pure sample			
Chlorphenoxamine hydrochloride	99.66 ± 0.97		99.89 ± 0.83
8-Chlorotheophylline	99.80 ± 0.56		100.01 ± 0.35
Caffeine	99.60 ± 0.37		99.80 ± 0.58
Emeral tablets B. N. 980618			
Chlorphenoxamine hydrochloride	99.57 ± 0.21	100.63 ± 0.38	99.98 ± 0.23
8-Chlorotheophylline	98.23 ± 0.85	100.34 ± 0.69	99.97 ± 0.63
Caffeine	101.22 ± 0.23	99.90 ± 0.44	100.49 ± 0.43
Emeral suppository B.N. 961774			
Chlorphenoxamine hydrochloride	98.56 ± 0.80	99.12 ± 0.92	98.25 ± 1.12
8-Chlorotheophylline	97.25 ± 0.41	99.51 ± 0.35	99.56 ± 0.55
Caffeine	99.67 ± 0.71	99.67 ± 0.51	99.71 ± 0.63

[a] Mean of six determinations.
[b] Recovery of standard solutions and the average of five experiments carried out by the TLC–UV densitometric method.
[c] Ref. [16] HPLC method with UV detection at 220 nm.

CV values which lie in the range 0.35–1.12% (Tables 3–5). The recoveries obtained are comparable with those of the reported methods as shown in Tables 3–5).

4. Conclusion

The method described is sensitive, precise, rapid and involves single-step sample preparation. A large number of samples can be analyzed within a short time as from six to eight samples can be applied to one plate, hence the method can be used for routine analysis in quality control and development laboratories for the simultaneous determination of multicomponent dosage forms.

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