DRUG FORMULATIONS AND CLINICAL METHODS

TLC-Densitometric and RP-HPLC Methods for Simultaneous Determination of Dexamethasone and Chlorpheniramine Maleate in the Presence of Methylparaben and Propylparaben

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Validated simple, sensitive, and highly selective methods are applied for the quantitative determination of dexamethasone and chlorpheniramine maleate in the presence of their reported preservatives (methylparaben and propylparaben), whether in pure forms or in pharmaceutical formulation. TLC is the first method, in which dexamethasone, chlorpheniramine maleate, methylparaben, and propylparaben are separated on silica gel TLC F_{254} plates using hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v) as the developing phase. Separated bands are scanned at 254 nm over a concentration range of 0.1–1.7 and 0.4–2.8 µg/band, with mean ± SD recoveries of 99.12 ± 0.964 and 100.14 ± 0.962%, for dexamethasone and chlorpheniramine maleate, respectively. Reversed-phase HPLC is the second method, in which a mixture of dexamethasone and chlorpheniramine maleate, methylparaben, and propylparaben is separated on a reversed-phase silica C_{18} (5 µm particle size, 250 mm, 4.6 mm id) column using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40, v/v, pH 3) as the mobile phase. The drugs were detected at 220 nm over a concentration range of 5–50 µg/mL, 2–90 µg/mL, 4–100 µg/mL, and 7–50 µg/mL, with mean ± SD recoveries of 100.85 ± 0.905, 99.67 ± 1.281, 100.20 ± 0.906, and 99.81 ± 0.954%, for dexamethasone, chlorpheniramine maleate, methylparaben paraben, and propylparaben, respectively. The advantages of the suggested methods over previously reported methods are the ability to detect lower concentrations of the main drugs and to show better resolution of interfering preservatives; hence, these methods could be more reliable for routine QC analyses.

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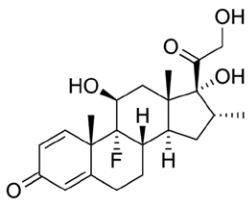
Dexamethasone (DEX), 9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1, 4-diene-3,20-dione, is a type of steroid medication. It has immunosuppressant and anti-inflammatory effects. It is more potent than cortisol in its glucocorticoid effect, while having minimal mineralocorticoid effect (1). DEX is an official drug in the British Pharmacopoeia (BP; 2; Figure 1). Chlorpheniramine maleate (CHL), 3-(4-chlorophenyl)-*N,N*-dimethyl-3-(pyridin-2-yl)propan-1-amine hydrogen (Z)-butenedioate, is used as a first-generation alkylamine antihistamine for prevention of the symptoms of allergic conditions such as rhinitis and urticaria. It is an official drug in the BP (2; Figure 1).

The combination of the two drugs is used to treat hay fever, urticaria, bronchial asthma, and rheumatic arthritis, and the drugs are formulated together, in addition to methylparaben paraben (MTP) and propylparaben (PRP) as preservatives, in the form of syrups for relief of asthma (1). The chemical structures, MWs, and molecular formulae are shown in Figure 1. DEX and CHL are determined by pharmacopeial and nonpharmacopeial methods. DEX is assayed in the BP via spectrophotometry, and CHL is assayed in the BP via nonaqueous titration (2).

DEX and CHL have been determined simultaneously in dosage form by HPLC methods (3–5), spectrofluorometry (6), chemometric methods (7), derivative spectrophotometry (8), and densitometric and chemometric methods in the presence of MTP and PRP as preservatives (9). Reviewing the literature in hand, there were methods found for the determination of CHL and DEX in the presence of MTP and PRP but without separation of MTP and PRP, and other methods that separated MTP and PRP but did not calibrate them. The advantage of our present work over other studies is that MTP and PRP can be separated by both the TLC and the reversed-phase (RP) HPLC methods and can be calibrated and quantified by the RP-HPLC method.

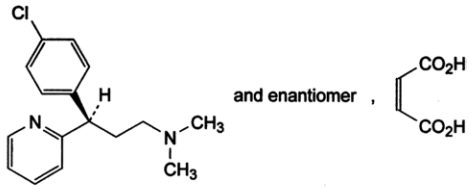
The aim of this study was to develop accurate, reproducible, very selective, and more-sensitive TLC and RP-HPLC methods for the determination of DEX and CHL in the presence of MTP and PRP, which are included as preservatives in the dosage form, and to determine MTP and PRP using the RP-HPLC method.



DEX

$C_{22}H_{29}FO_5$

Molecular weight392.5



CHL

$C_{16}H_{19}ClN_2$, C4H4O4

Molecular weight 390.9

Figure 1. Chemical structure, MW, and molecular formula of DEX and CHL.

Experimental

Apparatus

(a) *TLC method*.—TLC aluminum plates (20 × 20 cm) coated with 0.25 mm silica gel 60 F_{254} (Merck, Germany); TLC Scanner 3 Densitometer (CAMAG, Muttenz, Switzerland); CAMAG Linomat IV with 100 µL syringe; Sonix TV ss-series ultrasonicator (Newtown, CT); and UV lamp with short wavelength (254 nm; Vilber Lourmat, Marne LaVallée, France).

(b) *RP-HPLC method*.—Agilent Technologies 1200 series HPLC system (USA). The Eclipse XDB-C_{18} (5 µm particle size, 250 mm, 4.6 mm id) column was equilibrated and saturated for 30 min at a flow rate of 1 mL/min before the injection of the samples. All determinations were performed at 30°C. The detector was set at 220 nm.

Materials and Reagents

(a) *Pure standard*.—DEX (Batch No. 366R112) and CHL (Batch No. 372R112) were kindly supplied by EVA Pharma Co. for Pharmaceutical Industries (Cairo, Egypt). Purity was reported to be 99.30% and 99.5% for DEX and CHL, respectively, according to the company's analysis certificate.

(b) *Pharmaceutical formulation*.—Apidone® syrup (Batch No. 130304, labeled to contain 0.5 mg DEX, 2 mg CHL, 5 mg MTP, and 1 mg PRP per 5 mL) and Phenadone® syrup (Batch No. 230149, labeled to contain 0.5 mg DEX, 2 mg CHL, 5 mg MTP, and 1 mg PRP per 5 mL) were purchased from the local market.

All chemicals and solvents used were of analytical grade.

(c) *Acetonitrile*.—HPLC grade (E. Merck, Germany).

(d) *Deionized water*.—Sedico Pharmaceutical Co. (6th October City, Egypt).

(e) *Acetone, hexane, ammonia, phosphoric acid, ammonium acetate, chloroform, acetic acid, and methanol*.—Elnasr Pharmaceutical Chemicals Co. (Cairo, Egypt).

(f) *TLC plates*.—20 × 20 cm Plates coated with silica gel 60 F_{254} (1.05554.0001; Merck KGaA, Darmstadt, Germany).

(g) *Preparation of standard solutions*.—Stock solutions (1 mg/mL) of DEX, CHL, MTP, and PRP were prepared in methanol and in ammonium acetate buffer (pH 3)–acetonitrile (60 + 40, v/v) for the TLC-densitometric and RP-HPLC methods, respectively. From stock solutions, 100 µg/mL working solutions were prepared in methanol and in ammonium acetate buffer (pH3)–acetonitrile (60 + 40, v/v) for the TLC-densitometric and RP-HPLC methods, respectively.

(h) *Laboratory-prepared mixtures*.—Mixtures containing different ratios of DEX, CHL, PRP, and MTP were prepared using their respective stock standard solutions in methanol and in 0.1 M ammonium acetate buffer–acetonitrile (60 + 40, v/v, pH 3) for the TLC-densitometric and RP-HPLC methods, respectively.

(i) *Sample preparation*.—Three and 6 mL aliquots of Phenadone and Apidone syrups were transferred separately to two 10 mL and two 100 mL measuring volumetric flasks and diluted with methanol and with ammonium acetate buffer (pH 3)–acetonitrile (60 + 40, v/v) for the TLC-densitometric and RP-HPLC methods, respectively. For TLC, 10 µL was spotted equivalent to 0.3 µg DEX and 1.2 µg CHL. The general procedures described under calibration were followed, and the concentration of each compound was calculated.

Method Development

Most previously reported methods were for the determination of DEX and CHL alone, without taking into consideration their preservatives. Hence, it was necessary to develop and validate simple, more-sensitive, and more-selective TLC-densitometric and RP-HPLC methods for the simultaneous determination of DEX and CHL in the presence of their preservatives in bulk material and in pharmaceutical formulation.

Construction of Calibration Curves

(a) *TLC method*.—Into a set of 10 mL volumetric flasks, different aliquots equivalent to 0.1–1.7 mg DEX and 0.4–2.8 mg CHL were accurately transferred from their standard stock solution (1 mg/mL) and diluted to volume with methanol. To TLC plates (20×20 cm), a 10 µL aliquot of each solution was applied as a 6 mm wide band using the CAMAG Linomat IV applicator. The bands were spaced 5 mm from each other and 10 mm apart from the bottom edge of the plate. Linear ascending development was performed in a chromatographic chamber previously saturated with hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v) as a developing system for 15 min at room temperature to a distance of 8 cm. The integrated peak areas were recorded using a scanning wavelength of 254 nm under the specified instrumental conditions. The calibration curves were constructed by plotting the integrated peak area ÷ 10^3 versus the corresponding concentrations of each component, and regression equations were computed.

(b) *RP-HPLC method*.—Accurate aliquots equivalent to 50–500, 20–900, 40–1000, and 70–500 µg of DEX, CHL, MTP, and PRP, respectively, were separately transferred from their respective working standard solutions (100 µg/mL) into four separate series of 10 mL volumetric flasks and diluted to volume with the mobile phase. Triplicate injections were made for each concentration, and the peak area was used to construct the calibration curve for each component from which its regression equation was computed. Chromatographic separation was carried out by isocratic elution using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40 v/v; pH 3) as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 µL, scanning was carried out at 220 nm at 30°C, and the run time was 15 min.

Application to Pharmaceutical Formulation

The procedure mentioned under the *Linearity* and *Construction of Calibration Curves* sections was followed for the previously prepared pharmaceutical formulation working solution. The concentrations of DEX and CHL were calculated from their respective regression equations, and the percentage recoveries were then calculated.

Results and Discussion

TLC-densitometric and RP-HPLC methods are useful techniques for the determination and resolution of drug mixtures. TLC-densitometric and RP-HPLC methods offer a very simple way to quantify studied drugs in the presence of other components. MTP and PRP are used as preservatives for DEX and CHL when they are used in syrup, and most of the methods reported in the literature review determined DEX and CHL in their binary mixtures, without taking into consideration the determination of their preservatives. CHL shows low absorbance in the UV region, and DEX and CHL have very low concentrations compared to MTP and PRP. Hence, the presented methods aim to develop and validate a highly selective and sensitive analytical method for the simultaneous determination of DEX and CHL in the presence of MTP and PRP. Both MTP and PRP were calibrated using the RP-HPLC method, and their LOD and LOQ values were calculated (Table 1) and quantified in drug products.

TLC Method

This method offers high sensitivity and selectivity for the analysis of DEX and CHL in the presence of the reported preservatives used, in which the good separation is shown by the difference in the R_f values.

In the present work, samples were applied as bands using the TLC Linomat IV sampler with 100 µL syringe (CAMAG), because bands have several advantages over spots, as proved in the literature (10).

A linear correlation was obtained between the peak area ÷ 10^3 and the corresponding concentration. The regression equation for DEX (r = 0.9998) was calculated as follows:

$$y = 3.9361x + 1.2430$$

The regression equation for CHL (r = 0.9999) was calculated as follows:

$$y = 2.1051x + 0.3207$$

where y is the peak area ratio, x is the concentration (µg/mL), and r is the correlation coefficient (Table 1).

RP-HPLC Method

An accurate, sensitive, and highly selective isocratic RP-HPLC method was used in this work for the analysis of DEX and CHL, in combination and in the presence of the reported preservatives, using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40 v/v; pH 3) as the mobile phase, with a retention time (t_R) of 4.195, 6.020, 7.827, and 13.531 min for CHL, MTP, DEX, and PRP respectively.

A linear correlation was obtained between the peak area (y) and the corresponding concentration x (µg/mL), as shown in Table 1. The regression equations were calculated as follows: for DEX, $y = 19.5566x + 7.8646$ (r = 0.9995); for CHL, $y = 43.6541x + 223.3064$ (r = 0.9997); for MTP, $y = 69.6423x - 147.7657$ (r = 0.9997); and for PRP, $y = 109.6862x + 652.0822$ (r = 0.9999).

Results obtained by applying the proposed TLC and RP-HPLC methods showed that the concentrations of DEX, CHL, MTP, and PRP can be simultaneously determined in prepared mixtures, with mean ± SD recoveries for the TLC-densitometric

Table 1. Regression and analytical parameters of the proposed TLC and RP-HPLC methods for the determination of DEX and CHL in the presence of MTP and PRP

	TLC method		RP-HPLC method			
Parameters	DEX	CHL	DEX	CHL	MTP	PRP
Calibration range	0.1–1.7 µg/band	0.4–2.8 µg/band	5–50 µg/mL	2–90 µg/mL	4–100 µg/mL	7–50 µg/mL
Slope	3.9361	2.1051	19.5566	43.6541	69.6423	109.6862
Intercept	1.2430	0.3207	7.8646	223.3064	147.7657	652.0822
Correlation coefficient (r)	0.9997	0.9999	0.9995	0.9997	0.9997	0.9999
Accuracy (mean ± SD), %	99.12 ± 0.964	100.14 ± 0.962	100.85 ± 0.905	99.67 ± 1.281	100.20 ± 0.906	99.81 ± 0.954
Precision RSD, %[a]						
Repeatability	0.985	1.232	0.988	1.354	0.897	0.988
Intermediate precision	1.513	1.576	0.898	0.957	1.098	1.101
LOD	0.03 µg/band	0.12 µg/band	1.03 µg/mL	0.29 µg/mL	0.09 µg/mL	0.85 µg/mL
LOQ	0.09 µg/band	0.37 µg/band	3.14 µg/mL	0.88 µg/mL	0.28 µg/mL	2.57 µg/mL

[a] Repeatability results of the intraday RSDs and intermediate precision results of the interday RSDs of 0.3, 0.5, and 0.7 µg/band DEX and 0.6, 0.8, and 1 µg/band CHL (as determined by the TLC method) and 10, 15, and 20 µg/mL DEX, CHL, MTP, and PRP (as determined by RP-HPLC method).

method of 99.12 ± 0.964 and 100.14 ± 0.962% for DEX and CHL, respectively (Table 2), and mean recoveries for the RP-HPLC method of 100.85 ± 0.905, 99.67 ± 1.281, 100.20 ± 0.906, and 99.81 ± 0.954% for DEX, CHL, MTP, and PRP, respectively (Table 3).

Method Optimization

TLC-densitometric method.—To optimize chromatographic resolution of the developed TLC-densitometric method, it was necessary to study the effects of different factors.

(a) *Mobile phase*.—Different developing systems of different composition and ratios were tested, i.e., chloroform–methanol (9 + 1, v/v), chloroform–methanol–glacial acetic acid (8 + 2 + 0.2, v/v/v), chloroform–methanol–ammonia (8 + 2 + 0.2, v/v/v), and toluene–acetone (5 + 5, v/v), to obtain maximum separation among DEX, CHL, MTP, and PRP. The best mobile phase was hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v). This selected mobile phase allows good separation of the quaternary mixtures with good R_f values (0.11, 0.28, 0.35, 0.46) for DEX, MTP, PRP, and CHL, respectively, without tailing of the separated bands, as shown in Figures 2 and 3.

(b) *Scanning wavelength*.—Different scanning wavelengths (230 and 254 nm) were evaluated for good sensitivity of DEX and CHL in the presence of MTP and PRP and minimum noise. The wavelength 254 nm was found to be the best wavelength regarding the sensitivity of all components. Peaks were symmetrical and sharp, with minimum noise.

Table 2. Determination of DEX and CHL in Phenadone and Apidone syrups by the proposed TLC method and results of the standard addition technique

				Standard addition technique		
Pharmaceutical formulation	Drug	Taken, µg/band[a]	Found, %[b]	Pure added, µg/band	Found, µg/band	Recovery, %
Phenadone syrup	DEX	0.3	99.05 ± 1.265	0.2	0.201	100.73
				0.3	0.302	100.52
				0.4	0.403	100.73
						100.68 ± 0.138[b]
	CHL	1.2	101.98 ± 0.982	0.8	0.788	98.50
				1.2	1.215	101.25
				1.6	1.589	99.31
						99.69 ± 1.417[b]
Apidone syrup	DEX	0.3	101.95 ± 0.589	0.2	0.203	101.50
				0.3	0.301	100.33
				0.4	0.404	101.00
						100.94 ± 0.585[b]
	CHL	1.2	100.95 ± 1.298	0.8	0.812	101.50
				1.2	1.197	99.75
				1.6	1.589	99.31
						100.19 ± 1.155[b]

[a] Average of three determinations.

[b] Data reported as the mean ± SD.

Table 3. Determination of DEX and CHL in Phenadone and Apidone syrups by the proposed RP-HPLC method and results of the standard addition technique

				Standard addition technique		
Pharmaceutical Formulation	Drug	Taken, µg/mL[a]	Found, %[b]	Pure added, µg/mL	Found, µg/mL	Recovery, %
Phenadone syrup	DEX	6	101.84 ± 0.564	5	4.929	98.58
				6	5.989	99.82
				7	7.022	100.31
						99.57 ± 0.897[b]
	CHL	24	101.50 ± 0.457	20	20.199	101.00
				24	23.897	99.57
				28	28.587	101.03
						100.53 ± 0.827[b]
Apidone syrup	DEX	6	102.01 ± 1.324	5	4.953	99.06
				6	5.091	101.82
				7	5.079	101.57
						100.82 ± 1.515[b]
	CHL	24	101.97 ± 0.978	20	20.058	100.29
				24	23.967	99.86
				28	28.168	100.60
						100.25 ± 0.369[b]

[a] Average of three determinations.

[b] Data reported as the mean ± SD.

(c) *Band dimensions*.—The slight spread of the developed bands due to ordinary diffusion should be considered, and bandwidth and interspaces between bands should be chosen carefully to avoid both the spread of bands outside the scanning tracks and interference between adjacent bands. Different band dimensions were tested for symmetrical and sharp peaks. The best bandwidth chosen was 6 mm, and the best interspace between bands was 5 mm.

(d) *Slit dimensions of scanning light beam*.—The slit dimensions of the scanning light beam must ensure complete coverage of the band dimensions on the scanned track without any interference of adjacent bands. Different slit dimensions were tested, and 6 × 0.3 mm proved to be the slit dimension of choice that provided the highest sensitivity.

RP-HPLC method.—It was necessary to study the effects of different parameters that affect the sensitivity, selectivity, and efficiency of the chromatographic separation in order to optimize the proposed RP-HPLC method.

(a) *Mobile phase*.—Different mobile phases with different compositions and polarities were tested to achieve the chromatographic separation, i.e., water–acetonitrile, ammonium acetate buffer–methanol, and ammonium acetate buffer–acetonitrile. Complete separation among the studied components was obtained by isocratic elution using 0.1 M ammonium acetate buffer–acetonitrile, adjusted to pH 3. In addition, the effect of the ammonium acetate buffer–acetonitrile ratio was studied in order to improve resolution. Maximum resolution was obtained using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40, v/v).

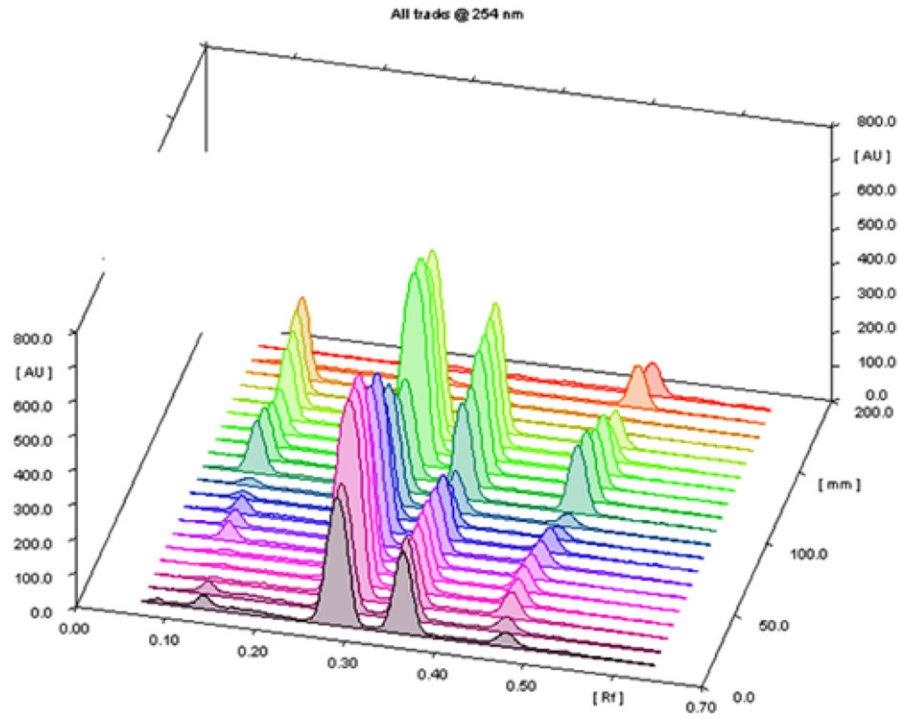


Figure 2. TLC densitogram of a mixture of DEX (R_f = 0.11) in the concentration range 0.1–1.7 µg/band, and of MTP (R_f = 0.28), PRP (R_f = 0.35), and CHL (R_f = 0.46) in the concentration range 0.4–2.8 µg/band, using hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v) as the mobile phase and with scanning of the separated bands at 254 nm. AU, absorbance unit.

The separation was obtained at t_R = 4.195, 6.020, 7.827, and 13.531 min for CHL, MTP, DEX, and PRP respectively, as shown in Figure 4.

(b) *Effect of pH and temperature*.—Different pH values (2.5–7) were tested, with pH 3 giving the best chromatographic resolution among DEX, CHL, MTP, and PRP. In addition, different temperature values (25–45°C) were tested, with 30°C being the optimum temperature for the best chromatographic peak symmetry.

(c) *Scanning wavelength*.—Different scanning wavelengths (205, 210, 220, 254, and 280 nm) were evaluated for their ability to enhance the sensitivity of the method. Scanning at 220 nm gave less noise and good sensitivity for all the studied components, as shown in Figure 4.

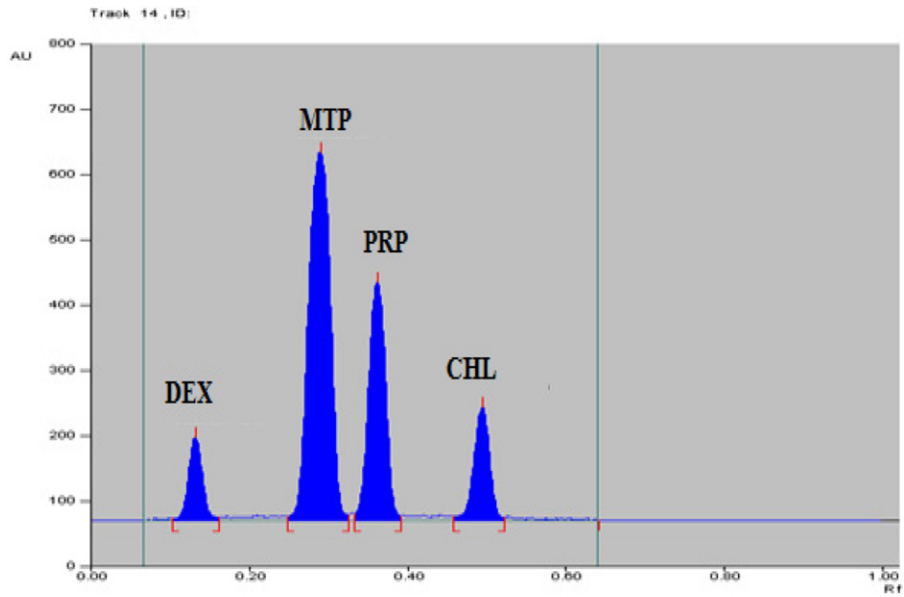


Figure 3. TLC chromatogram of resolved mixture of DEX (R_f = 0.11) in the concentration range 0.1–1.7 µg/band, and of MTP (R_f = 0.28), PRP (R_f = 0.35), and CHL (R_f = 0.46) in the concentration range 0.4–2.8 µg/band, using hexane–acetone–ammonia (5.5 + 4.5 + 0.5, v/v/v) as the mobile phase and with scanning of the separated bands at 254 nm.

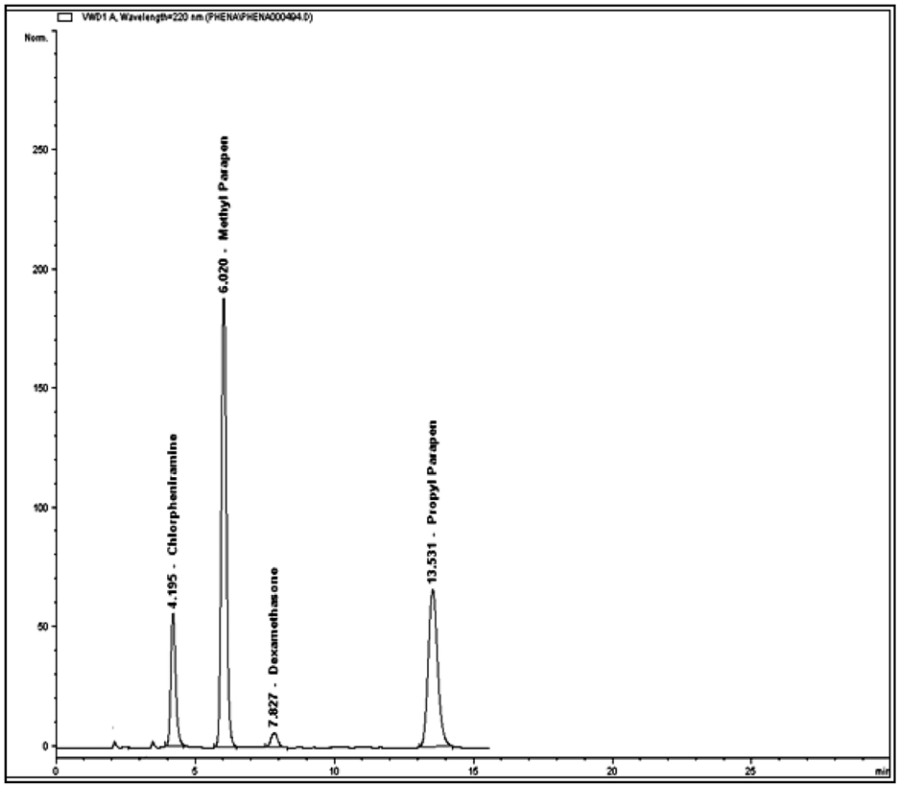


Figure 4. RP-HPLC chromatogram of separated peaks of CHL (t_R = 4.195 min), MTP (t_R = 6.020 min), DEX (t_R = 7.827 min), and PRP (t_R = 13.531 min) using 0.1 M ammonium acetate buffer–acetonitrile (60 + 40, v/v pH 3) as the mobile phase delivered at a flow rate of 1 mL/min and with scanning at 220 nm.

(d) *Flow rate*.—Different flow rates were tested for their ability to provide the best separation within acceptable run times. The best flow rate was obtained at 1 mL/min.

Method validation.—The method was validated according to International Conference on Harmonization (ICH) guidelines (11), as follows:

(a) *Linearity*.—Under optimum chromatographic conditions, linear relationships were obtained between the integrated peak area ÷ 10^3 and the corresponding concentrations for the TLC-densitometric method, and between the integrated peak area and the corresponding concentrations for the RP-HPLC method. Results were calculated and are presented in Table 1.

(b) *Accuracy*.—Accuracy was assessed using the standard addition technique and by analysis of market pharmaceutical preparations by the proposed methods (Tables 2 and 3). The resulting synthetic mixtures were assayed, and the results obtained were compared to the expected results. The good recoveries of pure drug samples suggest the good accuracy of the proposed methods (Tables 4 and 5).

(c) *Precision*.—Repeatability and intermediate precision were studied for the presented methods. Repeatability was calculated by the analysis of three different concentrations of pure components in triplicate on the same day. The experiment was repeated on the same concentrations three times on 3 consecutive days to determine the intermediate precision. Good results and acceptable RSDs were obtained (Table 1).

(d) *Specificity*.—The specificity of the method was ensured by how accurately and specifically the analytes of interest are determined in the presence of other components (other drugs and preservatives; 11). Specificity was confirmed, as shown in the TLC and RP-HPLC chromatograms in Figures 2–4. Good results were also obtained by applying the method on Phenadone and Apidone syrups (Tables 4 and 5), which proves that tablet additives do not interfere with any of the separated components.

(e) *LOD and LOQ*.—LOD and LOQ were calculated using the following equations (12):

$$LOD = (3.3 \times SD)/S$$

$$LOQ = (10 \times SD)/S$$

where S = slope. The low values of LOD and LOQ indicate the high sensitivity of proposed methods (Table 1).

(f) *Robustness*.—Small changes in TLC-densitometric method parameters (e.g., changing the hexane in the mobile

Table 4. Specificity of the proposed TLC method for the determination of DEX and CHL and their preservatives in laboratory-prepared mixtures[a]

	DEX			CHL		
Mixture No.	Claimed taken, µg/band	Found, µg/band	Recovery, %	Claimed taken, µg/band	Found, µg/band	Recovery, %
1	0.3	0.298	99.33	1.2	1.194	99.50
2	0.4	0.405	101.24	1.6	1.595	99.69
3	0.6	0.605	100.50	0.6	0.604	100.67
Mean ±SD			100.36 ± 0.961			99.95 ± 0.627

[a] Average of three determinations per mixture.

Table 5. Specificity of the proposed RP-HPLC method for the determination of DEX, CHL, MTP, and PRP in laboratory-prepared mixtures[a]

	DEX			CHL			MTP			PRP		
Mixture No.	Claimed taken, µg/mL	Found, µg/mL	Recovery, %	Claimed taken, µg/mL	Found, µg/mL	Recovery, %	Claimed taken, µg/mL	Found, µg/mL	Recovery, %	Claimed taken, µg/mL	Found, µg/mL	Recovery, %
1	5	5.083	101.66	20	19.712	98.56	35	35.321	100.92	20	20.352	101.76
2	45	45.720	101.60	50	50.205	100.41	10	9.952	99.52	10	9.837	98.37
3	45	44.279	98.42	45	44.685	99.30	10	9.934	99.34	10	9.952	99.52
4	10	9.927	99.27	15	14.989	99.93	35	35.449	101.28	20	20.021	100.11
Mean ± SD			100.24 ± 1.645			99.55 ± 0.801			100.27 ± 0.978			99.94 ± 1.412

[a] Average of three determinations per mixture.

phase by ±1% and the ammonia solution by ±0.05 mL) did not make significant changes in R_f or area under the peaks, e.g., the RSDs were 0.019 and 0.018 for DEX and CHL, respectively, for changing the amount of ammonia. Variations of the RP-HPLC method parameters (e.g., changing the acetonitrile in the mobile phase by ±1%, the flow rate by ±0.05 mL/min, the pH by ±0.01, the temperature by ±1°C, and the scanning wavelength by ±1 nm) did not make significant changes in t_R or symmetry of the peaks. For example, RSDs were 0.103, 0.089, 0.091, and 0.101% for CHL, MTP, PRP, and DEX, respectively, when the acetonitrile in the mobile phase was varied by ±1%.

(g) *System suitability*.—ICH guidelines (11) state that system suitability tests are an integral part of many analytical methods, especially LC methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. Parameters including capacity factor (13), symmetry factor, resolution, and selectivity factor were calculated according to the ICH (11) and the U.S. Pharmacopeia (14), as shown in Tables 6 and 7.

Results obtained by the suggested TLC and RP-HPLC methods for the determination of DEX and CHL in their pharmaceutical formulation were statistically compared to those obtained by applying the pharmacopeial method (2). The obtained values were found to be less than the theoretical ones, confirming accuracy and precision at the 95% confidence level, as shown in Table 8.

Table 6. Parameters of the system suitability test of the proposed TLC method

	Obtained value				
Parameter	DEX (R_f = 0.11)	CHL (R_f = 0.25)	MTP (R_f = 0.33)	PRP (R_f = 0.46)	Reference value
Capacity factor, K′	8.09	5	2.03	1.17	1–10 Acceptable
Symmetry factor	1	0.99	1.08	1	~1
Resolution, Rs	2.67		1.50	2.00	>1.5
Selectivity, α	2.26		1.28	1.35	α > 1

Table 7. Parameters of the system suitability test of the proposed RP-HPLC method

	Obtained value				
Parameter	CHL (t_R =4.195)	MTP (t_R =6.020)	DEX (t_R =7.827)	PRP (t_R =13.531)	Reference value
Column capacity, K′	1.97	3.27	4.55	8.59	1–10 Acceptable
Resolution, Rs	5.63		4.70	10.75	>1.5
Tailing factor	1.03	0.93	0.94	0.86	1, For a typical symmetric peak
Column efficiency, N	2653	5605	4942	7758	Increases with the efficiency of the separation
Height equivalent to theoretical plate, cm/plate	0.009	0.004	0.005	0.003	The smaller the value, the higher the column efficiency

Conclusions

The present work is concerned with the development and validation of TLC and RP-HPLC methods for the simultaneous determination of DEX, CHL, MTP, and PRP without any sample pretreatment and without interference from pharmaceutical formulation excipients. The advantages of the presented methods over any reported method are being able to determine the studied drugs along with their preservatives in a short analysis time and with more sensitivity and selectivity using one simple mobile phase for all components. Moreover, the presented methods were successfully applied for the determination of DEX and CHL in Phenadone and Apidone syrups without interference from pharmaceutical formulation excipients. The TLC densitometric method was more sensitive to determine DEX and CHL and to separate MTP and PRP while a previously published method could not separate them (9). The RP-HPLC method could easily determine MTP and PRP in the presence of DEX and CHL, and it was also more sensitive for the determination of CHL. The presented methods can be easily used for QC of the studied drugs.

Table 8. Statistical comparison of the results obtained by applying the proposed TLC and RP-HPLC methods and the official pharmacopeial methods for the determination of DEX and CHL in pure form

	TLC method		RP-HPLC method		Official method[a]	
Parameters	DEX	CHL	DEX	CHL	DEX	CHL
Mean recovery, %	99.12	100.14	100.85	99.67	100.12	100.31
SD	0.964	0.962	0.905	1.281	0.705	1.040
n	6	6	6	6	6	6
Student's *t*-test (2.228)[b]	2.047	0.291	1.562	0.943	—	—
F-test (5.050)[b]	1.866	1.169	1.646	1.517	—	—

[a] Official reference methods are the BP spectrophotometric method and nonaqueous titration for DEX and CHL, respectively (**2**).

[b] Figures within parentheses represent the corresponding tabulated values of *t* and *F* at $P = 0.05$.

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