

Original Research Article

Development of Three Methods for Simultaneous Quantitative Determination of Chlorpheniramine Maleate and Dexamethasone in the Presence of Parabens in Oral Liquids

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Abstract

Purpose: To develop new selective, precise, and accurate methods for the simultaneous determination of chlorpheniramine maleate (CHP) and dexamethasone (DX) in the presence of methyl and propyl paraben in phenadone syrup.

Methods: In the first two methods, the predictive abilities of principal component regression (PCR) and partial least squares (PLS), respectively, were examined for the analysis of the quaternary mixture. The third method, high performance thin layer chromatography (HPTLC)-densitometric method, was based on the separation of the mixture on silica gel plates using chloroform: methanol (93:7, v/v) as a mobile phase.

Results: All the proposed methods were successfully applied to the analysis of raw materials and dosage form. For PCR method, recovery of chlorpheniramine maleate and dexamethasone in the dosage form was 98.89 ± 1.736 and 102.36 ± 1.86 %, respectively while for PLS method, recovery of chlorpheniramine maleate and dexamethasone was 98.94 ± 1.69 and 102.33 ± 1.84 , respectively. On the other hand, recovery of the two analytes by HPTLC method was 100.72 ± 1.05 and 102.29 ± 3.98 , respectively. The results obtained by applying the proposed methods were statistically analyzed and compared with those obtained by a reported HPLC method.

Conclusion: The proposed methods are fast, accurate and specific, and can be applied for the quantitative determination of the two analytes without interference from added excipient, thus obviating the need for preliminary extraction of analytes from the pharmaceutical formulation. Thus, they are suitable for use in quality control (QC) laboratories and pharmaceutical industry

Keywords: Chlorpheniramine maleate, Dexamethasone, Principal component regression, Partial least squares, High performance thin layer chromatography, Excipients

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INTRODUCTION

Chlorpheniramine maleate (CHP, Fig.1) [(3RS)-3-(4-chlorophenyl)-N, N-dimethyl-3-(pyridin-2-yl)propan-1-amine hydrogen (Z)-butenedioate] is an antihistaminic drug [1]. Dexamethasone (DX,

Fig.1) [9-fluoro-11 β , 17, 21-trihydroxy-16 α -methylpregna-1, 4-diene-3, 20-dione] is a corticosteroid anti-inflammatory agent [1]. CHP and DX were determined simultaneously in dosage form by HPLC methods[2,3], spectrofluorimetric method [4] chemometric

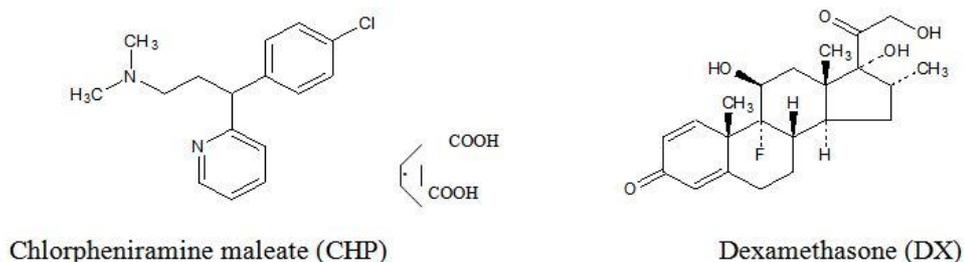


Figure 1: Chemical structures of Chlorpheniramine maleate and Dexamethasone

methods [5] and derivative spectrophotometric method [6]. Both drugs are formulated together in the local Egyptian market in the form of syrups for the relief of asthma [7] in addition to methyl paraben (MP) and propyl paraben (PP) as preservatives. To the best of our knowledge (upon extensive literature review), there is no reported method for the analysis of CHP and DX in presence of MP and PP. Therefore, the aim of this work was the application of multivariate calibration methods (PLS and PCR) and HPTLC methods for quantifying CHP and DX in their quaternary mixture with MP and PP. The results obtained are reproducible. The advantages of the proposed methods are their speed, simplicity and no need for preliminary separation step.

EXPERIMENTAL

Instruments

1. A double beam UV–visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm pathlength, connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7. The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min.
2. UV lamp with short wavelength 254 nm (USA).
3. TLC scanner 3 densitometer (Camag, Muttenz, Switzerland). The following requirements are taken into consideration:
 - Slit dimensions: 5mm × 0.2mm.
 - Scanning speed: 20 mm/S.
 - Spraying rate: 10 $\mu\text{L/s}$.
 - Data resolution: 100 $\mu\text{m/step}$.
4. TLC plates (20 cm × 10 cm) coated with silica gel 60F254 (Merck, Germany).

5. Sample applicator for TLC Linomat IV with 100 μL syringe (Camag, Muttenz, Switzerland).

Software

All multivariate calibration methods were implemented in Matlab® 7.1.0.246 (R14) using PLS toolbox software version 2.1. The t-test, F-test and ANOVA were performed using Microsoft® Excel 2013. All calculations were performed using intel® core™ i5-2400, 3.10 GHz, 4.00 GB of RAM under Microsoft Windows 7.

Reagents and chemicals

The reference compounds, chlorpheniramine maleate (CHP), dexamethasone (DX), methyl paraben (MP) and propyl paraben (PP), certified to contain 99.59, 99.73, 98.50 and 101.65 %, respectively, by the manufacturers, were kindly provided by The Arab Pharmaceuticals and Chemical Industries Company, Cairo, Egypt. Phenadone syrup was purchased from the Egyptian local market and had a labeled content of 0.4 mg mL⁻¹ CHP, 0.1 mg mL⁻¹ DX, 1 mg mL⁻¹ MP and 0.2 mg mL⁻¹ PP (Batch no.630351). Methanol, chloroform and 0.1 N HCl used were of spectroscopic grade.

Standard stock and working solutions

For PCR and PLS methods, stock standard solutions of CHP, DX, MP and PP were prepared separately by dissolving 100 mg of CHP, 50 mg DX, 50 mg MP and 100 mg PP in 100 mL methanolic HCl (1 mL methanol: 4 mL 0.1 N HCl). Corresponding working solutions were prepared by transferring accurately 25 mL from each stock standard solutions separately in 250 mL measuring flasks and volume was completed with methanolic HCl. Solutions (xc) and (xd) [0.125 mg mL⁻¹] were also prepared by methanolic HCl.

For HPTLC-densitometric method, stock standard solutions of CHP, DX, MP and PP were prepared separately by dissolving 100 mg of CHP, 100 mg DX, 200 mg MP and 100 mg PP in 100 mL methanol. Working solution of DX (0.2 mg mL⁻¹) was prepared by diluting 20 mL of its stock standard solutions into 100 mL measuring flasks with methanol.

PCR and PLS methods

Calibration

1. Construction of a training set

A training set composed of 17 mixtures was prepared by diluting different volumes of each of CHP, DX, MP and PP working solutions into a series of 25-mL measuring flasks, each flask was spiked with 250 µg of CHP and 125 µg of DX (from solutions Xc and Xd), in addition to mixture no. 18 that contains spiked concentrations of CHP and DX, 10 µg mL⁻¹ of MP and 2 µg mL⁻¹ of PP. All flasks were diluted to volume with methanolic HCl. The absorption spectra of all 18 mixtures were recorded between 200-300 nm. The data points of the spectra were collected at every 1 nm.

2. Pre-processing the data

Reject the regions from 200- 215 nm and above 290 nm. Mean centering of the data was performed.

3. Selection of the optimum numbers of latent variables

Root mean square error of cross validation (RMSECV) values were calculated using cross validation method, leaving out one sample at a time to select the optimum number of factors.

Validation

A validation set composed of 8 mixtures containing different concentrations of CHP, DX, MP and PP was prepared to check the performance of the developed models.

Application to pharmaceutical preparation (Phenadone syrup)

Five mLs of phenadone syrup equivalent to 2 mg of CHP, 0.5 mg of DX, 5 mg of MP and 1 mg of PP was diluted to 100 mL with methanolic HCl, further dilution was made by taking 5 mL of the above solution in 25-mL measuring flask, 250 µg of CHP and 125 µg of DX were spiked and volume was completed by methanolic HCl. The

general procedures described under calibration were followed and the concentration of each compound was calculated.

HPTLC-densitometric method

Different volumes (1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 µL) of CHP working solution (1.00 mg mL⁻¹) and (2.0, 4.0, 6.0, 8.0, 9.0 and 10.0 µL) of DX working solution (0.20 mg mL⁻¹) were spotted on two separate TLC plates using the Camag TLC sampler. Bands are spaced 1 cm apart from each other and from the bottom edge of the plate. TLC plates were developed at room temperature in a chromatographic tank previously saturated with the mobile phase chloroform: methanol (93:7 v/v), by ascending chromatography to a distance 8 cm from the bands at room temperature. The plates were left to dry then the bands were visualized under UV lamp (254 nm). The bands were scanned at 254 nm. The peak area was recorded and the calibration curve was constructed by plotting the integrated peak area versus the corresponding concentrations of CHP and DX and the regression equation was computed.

Assay of laboratory-prepared mixtures

In a series of 10-mL measuring flasks, aliquots of CHP, DX, MP and PP were transferred accurately from their corresponding stock and working solutions to prepare different mixtures and then complete to volume with methanol. Ten µL of the laboratory prepared mixtures was applied to a silica gel plate and continue under the above described conditions. The integrated peak were recorded and the concentrations of CHP and DX were calculated either by substituting in the corresponding regression equations or by comparing to a standard spotted, developed and scanned under the above mentioned conditions.

Application to commercial pharmaceutical preparation (phenadone syrup)

Five mLs of phenadone syrup equivalent to 2 mg of CHP, 0.5 mg of DX, 5 mg of MP and 1 mg of PP was diluted to 25 mL with methanol. On a TLC plate, four µL of the prepared solution was spotted and scanned under the above mentioned conditions.

The concentrations of CHP and DX were calculated either from the regression equation or by comparing to a standard spotted, developed and scanned under the same conditions.

RESULTS

Figure 2 shows the absorption spectra of CHP, DX, MP and PP. The main problem in the analysis of this mixture is that CHP and DX possess only a low absorbance in the UV-region comparing to MP and PP, moreover, the problem is further complicated because CHP and DX are present as minor components in the combination and MP is a major one. Thus, this work was devoted to the simultaneous determination of CHP and DX in the presence of MP and PP, which are available together in the form of syrups. This was achieved using multivariate calibration (PCR and PLS) and HPTLC-densitometric methods.

PCR and PLS

The multivariate calibration requires a careful experimental design of the standard composition of calibration set for providing the best predictions. Multilevel multifactor design [8] was used for the construction of the calibration set. PLS and PCR models were constructed using training set of 17 samples containing different ratios of CHP, DX, MP and PP in addition to mixture no. 18 that contains spiked concentrations of CHP and DX, $10 \mu\text{g mL}^{-1}$ of MP and $2 \mu\text{g mL}^{-1}$ of PP. The concentrations of CHP and DX were obtained in training and validation set by subtracting their concentrations in mix no. 18 in training set from their total concentrations in other mixtures. Table 1 shows the composition of the training set. PLS and PCR procedures were run on the calibration data of absorption (zero-order) UV spectra and concentrations in validation set were calculated at the optimum number of factors. Number of factors used for

constructing the models was 3 as shown in Figure 3. As the difference between the minimum RMSECV and other RMSECV values become smaller, the probability that each additional factor is significant becomes smaller [9]. The predicted concentrations of the calibration samples were plotted against the known concentrations to determine whether the model accounted for the concentration variation in the calibration set. Plots were expected to fall on a straight line with a slope of 1 and zero intercept. In order to validate proposed PLS and PCR methods, a validation set composed of 8 synthetic mixtures of CHP, DX, MP and PP were analyzed with the proposed PLS and PCR methods. Percent recovery of the validated samples are shown in Table 2.

The validation of the developed PCR and PLS models was assessed using several diagnostic tools. These tools were grouped into two categories in model diagnostic tools that are used to determine the quality of the model and sample diagnostic tools which are used to study the relationship between the samples and to identify unusual samples.

The predicted concentrations of the validation samples were plotted against the true concentration values. This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero (Table 3). The RMSEP was another diagnostic tool for examining the errors in the predicted concentrations, it indicates both the precision and accuracy [10]. The results in Table 3 indicating the high predictive abilities of the two models.

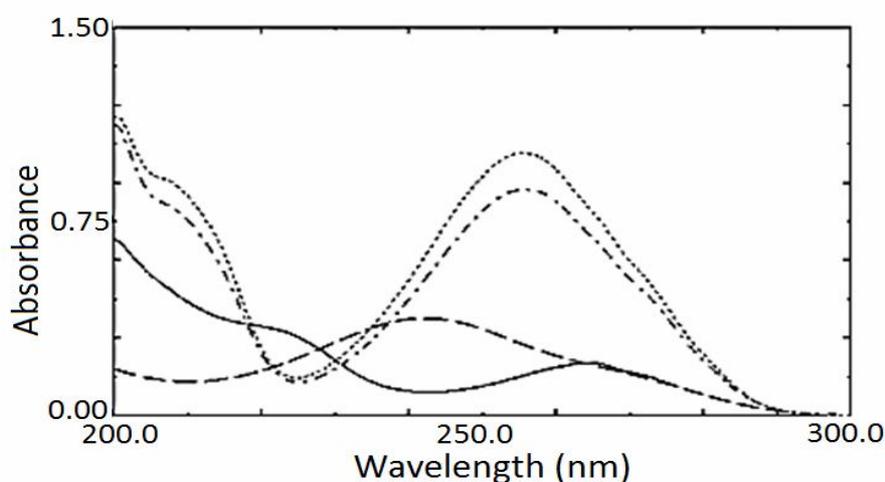
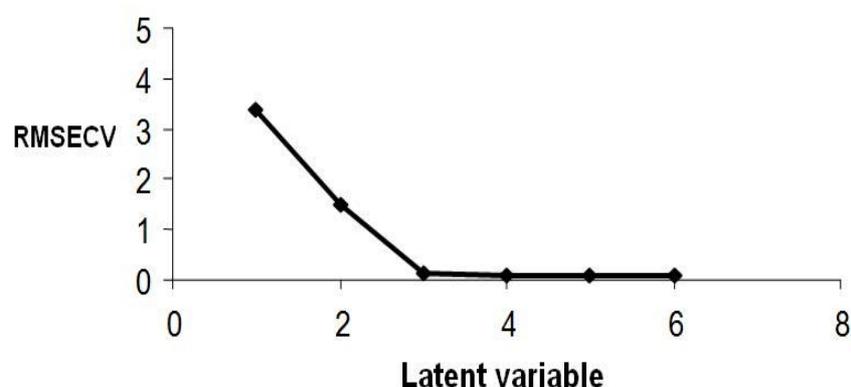


Figure 2: Absorption spectra for CHP (—), DX (-----), MP (.....) and PP (·-·) each $10 \mu\text{g mL}^{-1}$

Table 1: The concentration of different mixtures of CHP, DX, MP and PP used in the training set

Sample no.	CHP ($\mu\text{g mL}^{-1}$)	DX ($\mu\text{g mL}^{-1}$)	MP ($\mu\text{g mL}^{-1}$)	PP ($\mu\text{g mL}^{-1}$)
1	22	6	2	0.4
2	14	14	4	0.8
3	30	8	10	2
4	18	14	6	1.2
5	30	10	2	0.4
6	22	8	2	0.4
7	18	8	8	1.6
8	18	12	10	2
9	26	14	8	1.6
10	30	12	6	1.2
11	26	10	10	2
12	22	14	10	2
13	30	14	2	0.4
14	30	6	8	1.6
15	14	12	2	0.4
16	26	6	6	1.2
17	14	10	8	1.6
18	10	5	10	2

**Figure 3:** RMSECV plots for the cross validation results of the training set as a function of the number of factors used to construct the calibration models**Table 2:** Determination of CHP and DX in validation set by the proposed multivariate calibration methods

Mixture no.	Composition of mixture ($\mu\text{g.ml}^{-1}$)				Recovery %			
	CHP	DX	MP	PP	PLS method		PCR method	
					CHP	DX	CHP	DX
1	14	8	6	1.2	100.57	99.81	99.25	99.08
2	22	12	8	1.6	102.42	99.10	102.42	99.09
3	26	12	4	0.8	99.77	100.49	99.77	100.48
4	26	8	2	0.4	99.58	98.93	99.58	98.94
5	18	6	4	0.8	99.03	99.49	99.03	99.48
6	18	10	2	0.2	99.28	101.69	97.83	101.68
7	14	6	10	2	101.34	98.41	102.00	98.40
8	22	10	6	1.2	100.26	99.24	100.26	98.32
Mean					100.28	99.65	100.02	98.70
RSD* (%)					1.137	1.035	1.527	0.321

*Relative standard deviation

HPTLC- densitometric results

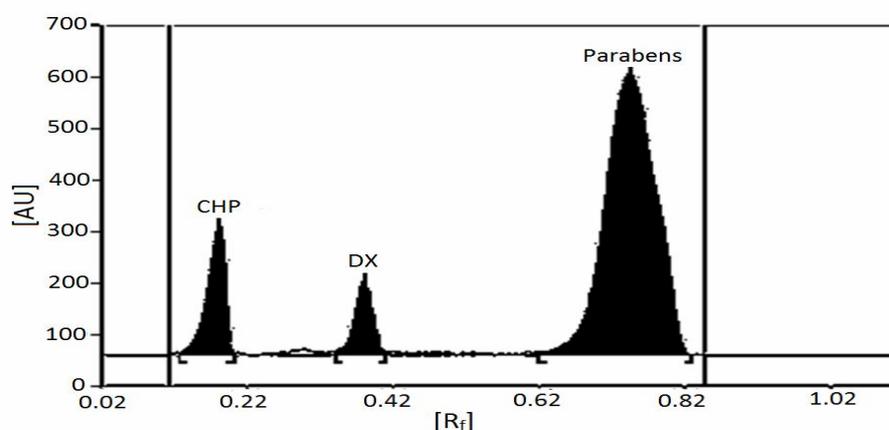
The proposed HPTLC method is applied as a simple and sensitive method for the determination of CHP and DX in presence of MP

and PP as preservatives. The proposed technique is based on the difference of RF values of CHP (RF = 0.12 ± 0.04), DX (RF = 0.37 ± 0.05) and Parabens (MP and PP) (RF = 0.67 ± 0.03) as shown in Fig.4.

Table 3: Summary of results obtained by applying the diagnostic tools for model validation of the multivariate calibration methods

Validation parameter	PCR		PLS	
	CHP	DX	CHP	DX
a) Predicted vs. known concentration plot				
1- Slope	0.9984	0.9976	0.9984	0.9976
2- Intercept	0.0371	0.0255	0.0366	0.0256
3- Correlation coefficient	0.9992	0.9984	0.9992	0.9988
b) Residual vs. actual concentration plot (\pm error in prediction)	0.2865	0.1071	0.2308	0.0948
c) RMSEP *	0.2579	0.1059	0.1924	0.0998

* Root mean square error of prediction

**Figure 4:** Thin layer chromatogram of CHP, DX and Parabens (MP and PP) using developing system, chloroform: methanol (93:7 v/v)

Different solvent systems were tried, and complete separation of the mixture was achieved using chloroform: methanol (93:7 v/v) as a mobile phase. This separation allows the determination of CHP and DX without any interference from Parabens. CHP and DX bands were scanned at 254 nm. To ensure good separation of bands, Parameters including resolution (R_s), peak symmetry, capacity factor (K') and selectivity factor (α) were calculated using parabens band. The resolution is always above 1.5 and an accepted value for symmetry and capacity factors was obtained, as shown in Table 4.

Table 4: Parameters of system suitability of the HPTLC method used

Parameter	CHP	DX	Paraben
Symmetry factor	0.91	1.05	0.98
Resolution (R_s) ^a	1.68	2.96	
Capacity factor (K')	0.82	0.45	

^aParameters were calculated using parabens as reference

The proposed HPTLC-densitometric method was applied for the determination of CHP and DX in laboratory prepared mixtures containing different ratios of CHP, DX and Parabens with mean accuracy of 101.46 ± 1.813 and 99.90 ± 3.271 for CHP and DX respectively. The results obtained are shown in Table 5. Assay validation sheet for HPTLC method was presented in Table 6. One-way ANOVA was applied for the purpose of comparison of developed chemometric and HPTLC methods. Table 7 shows that there was no significant difference between them for the determination of CHP and DX in presence of MP and PP.

Application to pharmaceutical preparation

The proposed methods were successfully applied for the determination of CHP and DX in phenadone syrup. The results are shown in Table 8. Each value indicated is the mean of 4 determination of the same commercial batch. The validity of the proposed methods was further assessed by applying the standard addition

technique. Results obtained are shown in Table 8. The results obtained for the analysis of CHP and DX in the pure powder form by the proposed method were statistically compared with those obtained by applying one of previously reported methods that used HPLC analysis (C18 column

and 0.005 M heptane sulphonic acid sodium salt in bi-distilled water: acetonitrile (70:30 v/v), pH 5) [2] but no significant difference was found between the results in terms of accuracy and precision (see Table 9).

Table 5: Determination of CHP and DX in laboratory prepared mixtures containing different percentages of MP and PP by the proposed HPTLC method

Concentration ($\mu\text{g band}^{-1}$)				Recovery %	
CHP	DX	MP	PP	CHP	DX
1	2	2	1	102.86	104.71
2	2	4	2	101.37	101.88
4	2	8	4	101.97	97.30
4	1	10	2	98.40	97.54
5	1	4	2	102.71	98.06
Mean				101.46	99.90
SD*				1.813	3.271

*Standard deviation

Table 6: Assay parameters and method validation obtained by applying the proposed HPTLC method for CHP and DX

Parameter	HPTLC method	
	CHP	DX
Range ($\mu\text{g band}^{-1}$)	1-6	0.4-2
Slope	1.7922	3.3431
Intercept	1.4857	1.6395
Mean	99.73	100.11
S.D.	2.096	1.914
Variance	4.393	3.663
Coff. of variation	0.021	0.019
Correl. Coef.(r)	0.9997	0.9996
*LOD ($\mu\text{g band}^{-1}$)	0.173	0.169
*LOQ($\mu\text{g band}^{-1}$)	0.524	0.512
** RSD% ^a	99.96 \pm 1.857	100.72 \pm 1.520
**RSD % ^b	100.08 \pm 2.882	98.88 \pm 2.913

*Limit of detection (LOD) and limit of quantification (LOQ) were determined by calculation; ** RSD%^a and RSD%^b are the intra-day and inter-day relative standard deviation, respectively (n = 4)

Table 7: One-way ANOVA parameters for the different proposed methods used for the determination of CHP and DX

Analyte	Source of variation	DF	Sum of squares	Mean square	F-value
CHP	Between exp.	2	6.842	3.421	1.594
	Within exp.	18	38.617	2.145	
DX	Between exp.	2	0.668	0.334	0.102
	Within exp.	18	59.155	3.286	

There was no significance difference between the methods using one-way ANOVA (F-test), where F tabulated = 3.555 at $p < 0.05$

DISCUSSION

Both PLS and PCR techniques are listed under the multivariate calibration methods. They were successfully applied for the simultaneous determination of multi-components thus solving the problem of overlapping spectra of the relatively small absorptivity substances. PLS and

PCR techniques are spectral analyzing methods where the data are fit to many data points. PLS and PCR procedures are designated to be full spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened by discarding particularly noisy wave-

Table 8A: Applying standard addition technique for determination of CHP and DX in phenadone syrup (Batch No. 630351) by multivariate calibration methods

Sample no.	Authentic added $\mu\text{g ml}^{-1}$		Multivariate calibration methods			
			PLS		PCR	
	CHP	DX	R% of CHP	R% of DX	R% of CHP	R% of DX
1	2	1	101.96	100.90	101.67	100.01
2	4	2	98.31	99.34	97.75	98.88
3	6	3	98.03	99.24	98.17	98.13
Mean \pm RSD			99.43 \pm 2.193	99.83 \pm 0.932	99.20 \pm 2.153	99.02 \pm 0.942
Found of CHP and DX in Phenadone syrup* (% \pm R.S.D.)			98.94 \pm 1.692	102.33 \pm 1.839	98.89 \pm 1.736	102.36 \pm 1.857

*Mean of 4 experiments

Table 8B: Applying standard addition technique for determination of CHP and DX in phenadone syrup (Batch No. 630351) by HPTLC-densitometric method

	Claimed taken ($\mu\text{g band}^{-1}$)	Found (% \pm S.D.) *	Pure added ($\mu\text{g band}^{-1}$)	Found (Recovery %)
CHP	1.6	100.72 \pm 1.053	1	99.75
			1.6	99.54
			2.4	97.74
Mean % \pm S.D.				99.01 \pm 1.105
DX	0.4	102.29 \pm 3.979	0.4	102.31
			0.8	98.23
			1.2	98.63
Mean % \pm SD				99.72 \pm 2.885

*Mean of 3 experiments

Table 9: Statistical comparison of the results obtained by the proposed methods and HPLC method [2] in respect of analysis of CHP and DX in pure powder form

Item	HPTLC method		PLS method		PCR method		Reported HPLC method [2]	
	CHP	DX	CHP	DX	CHP	DX	CHP	DX
Mean	99.73	100.11	100.06	99.88	100.06	99.88	99.93	99.96
R.S.D.	2.096	1.914	1.196	1.639	1.198	1.647	1.920	1.746
Variance	4.393	3.663	1.430	2.686	1.435	2.713	3.686	3.049
N	6	6	18	18	18	18	7	7
F test	1.192	1.201	2.578	1.135	2.569	1.124		
	(4.39)	(4.39)	(2.70)	(2.70)	(2.70)	(2.70)		
Student's t test	0.18	0.148	0.205	0.108	0.205	0.107		
	(2.201)	(2.201)	(2.069)	(2.069)	(2.069)	(2.069)		

lengths. The wavelengths used were in the range 215 – 290 nm in all cases. Wavelengths less than 215 nm were rejected due to the noisy content, while wavelengths > 290 nm were not used because corresponding components do not absorb in these regions. In this study, two calibration models were proposed, validated and then used for the prediction of unknown samples. Appropriate selection of the number of factors to be used to construct the model is key to achieving correct quantitation in PLS and PCR calibration. The most usual procedure for this purpose involves choosing the number of factors

that result in the minimum root mean square error of cross validation (RMSECV). However, this criterion is subjected to some constraints since, occasionally; the RMSECV does not reach a sharp minimum, but decreases gradually above a given number of factors. On the other hand, it is calculated from a finite number of samples, and so it is error-prone. For these reasons, the method developed by Haaland and Thomas [9] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that results in an insignificant difference between the

corresponding RMSECV and the minimum RMSECV.

Densitometry offers a simple way of quantifying directly on a TLC plate by measuring the optical density of the separated spots. The amounts of the compounds are determined by comparing them to a standard curve from reference materials chromatographed simultaneously under the same conditions [11]. In the presented work, samples are applied as bands using TLC Linomat IV sample applicator with 100 μ l syringe (Camag) where bands have many advantages over spots [12]. Trials were done to choose a developing system, which can affect separation of CHP, DX and Parabens. These systems include ethyl acetate: methanol (1:1 v/v) and methanol: chloroform: ammonia (2:2:1 v/v/v) which did not affect good separation of the three drugs. Complete separation of CHP, DX and Parabens was obtained using chloroform: methanol (93:7 v/v) as a mobile phase. A linear correlation was obtained between peak area of the separated bands and the corresponding concentration in the range of 1 – 6 μ g band⁻¹ for CHP and in the range of 0.4 - 2 μ g. band⁻¹ for DX.

The regression equations were computed and found to be

$$A_1 (\text{CHP}) = 1.79223 C_1 + 1.4857 \quad (r = 0.9997) \quad (1)$$

$$A_2 (\text{DX}) = 3.3431 C_2 + 1.6395 \quad (r = 0.9996) \quad (2)$$

where A_1 and A_2 are the integrated area under the peak $\times 10^{-3}$ for CHP and DX respectively. C_1 and C_2 are the concentrations in μ g band⁻¹ for CHP and DX, respectively, and r is the correlation coefficient.

CONCLUSION

PCR and PLS methods exhibit simplicity, greater convenience, lower analysis time and more economical than published HPLC methods. With HPTLC-densitometric method, several samples can be run simultaneously using a small quantity of mobile phase, unlike HPLC, thus lowering analysis time and cost per analysis, and it also provides high sensitivity and selectivity. The findings indicate high reproducibility of the proposed methods as well as high sensitivity, accuracy, reproducibility and specificity. Moreover, the methods are simple and inexpensive, thus favouring their application in quality control laboratories.

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