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Development and validation of chemometrics-assisted spectrophotometric and liquid chromatographic methods for the simultaneous determination of two multicomponent mixtures containing bronchodilator drugs

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Abstract

Three methods are developed for the determination of two multicomponent mixtures containing guaiphenesine (GU) with salbutamol sulfate (SL), methylparaben (MP) and propylparaben (PP) [mixture 1]; and acephylline piperazine (AC) with bromhexine hydrochloride (BX), methylparaben (MP) and propylparaben (PP) [mixture 2]. The resolution of the two multicomponent mixtures has been accomplished by using numerical spectrophotometric methods such as partial least squares (PLS-1) and principal component regression (PCR) applied to UV absorption spectra of the two mixtures. In addition HPLC method was developed using a RP 18 column at ambient temperature with mobile phase consisting of acetonitrile–0.05 M potassium dihydrogen phosphate, pH 4.3 (60:40, v/v), with UV detection at 243 nm for mixture 1, and mobile phase consisting of acetonitrile–0.05 M potassium dihydrogen phosphate, pH 3 (50:50, v/v), with UV detection at 245 nm for mixture 2.The methods were validated in terms of accuracy, specificity, precision and linearity in the range of 20–60 $\mu g\,ml^{-1}$ for GU, 1–3 $\mu g\,ml^{-1}$ for SL, 20–80 $\mu g\,ml^{-1}$ for AC, 0.2–1.8 $\mu g\,ml^{-1}$ for PP and 1–5 $\mu g\,ml^{-1}$ for BX and MP. The proposed methods were successfully applied for the determination of the two multicomponent combinations in laboratory prepared mixtures and commercial syrups.
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1. Introduction

Guaiphenesin (GP) is an expectorant used for treatment of productive cough which is associated with salbutamol sulfate (SL) as a bronchodilator used in the management of reversible airways obstruction and asthma in addition to methylparaben (MP) and propylparaben (PP) as preservatives [mixture 1]; acephylline piperazine (AC) as bronchodilator is associated with bromhexine hydrochloride (BX) as a mucolytic used for the treatment of respiratory disorders associated with productive cough in addition to methylparaben (MP) and propylparaben (PP) as preservatives [mixture 2]. Both mixtures are used as cough sedatives. The UV absorption spectra of both mixtures display considerable overlap, where the application of the conventional spectrophotometry and its direct derivative and derivative ratio technique failed to resolve it. No analytical method has been reported for the simultaneous determination of the studied compounds in their multicomponent mixture.

Five components in cough mixture containing guaiphenesine (GU) with acetaminophen, *p*-amino phenyl, caffeine, chlorpheniramine maleate were determined using partial least squares (PLS) [1] and principal component regression (PCR) [2]. HPLC was used for assay of mixtures containing GU with terbutaline and ambroxol [3], dextromethorphan, pseudoephedrine hydrochloride and acetaminophen [4], phenylpropanolamine hydrochloride and diphenylpyraline hydrochloride [5], terbutaline and bromhexine [6], dextromethorphan and sodium benzoate [7], pholcodine and ephedrine hydrochloride [8], paracetamol, caffeine, phenylpropanolamine hydrochloride and chlorpheniramine maleate [9] and paracetamol, caffeine, DL methylephedrine hydrochloride and chlorpheniramine maleate [10]. HPLC was used for the assay of SL with troventol [11], ipratropium bromide, fenoterol and terbutaline [12]. Ternary mixture of AC with phenobarbitone and papaverine hydrochloride was determined using second derivative of the ratio spectrum-zero-crossing and HPLC methods

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[13]. BX was determined using absorption spectrophotometry and PLS multivariate calibration [14,15]. HPLC was used for determination of multicomponent mixtures containing BX with ambroxol hydrochloride and clenbuterol [16], sulfamethoxypyridazine, sulfamethoxazole, sulfadimethoxine and trimethoprim [17], promethazine hydrochloride, noscapine hydrochloride, ephedrine hydrochloride [18], methylparaben and propylparaben [19], clorprenaline and decloxizine [20,21].

This work concerns PLS-1, PCR and HPLC methods for determination of GU, SL, MP and PP (mixture 1); and AC, BX, MP and PP (mixture 2) with highly overlapping UV absorption spectra. The simultaneous determination of such compounds in their studied mixtures by conventional, derivative and derivative ratio spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range. So HPLC and PLS-1 or PCR calibration methods can be used to overcome this problem. The proposed methods reduced the duration of the analysis. They are simple, sensitive, and suitable for routine determination of the components in the studied mixtures.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–Visible spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells and connected to an IBM compatible computer. HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. PLS and PCR analysis were carried out using PLS-Toolbox software version 2.1 PC [22] for use with MATLAB5.

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–Vis detector, separation and quantitation were made on a 250 mm × 4.6 mm (i.d.) Shim-pack RP18 column (4.6 μm particle size). The detector was set at λ 243 and 245 nm for mixtures 1 and 2, respectively. Data acquisition was performed on class-VP software.

2.2. Materials and reagents

Pharmaceutical grade of GU (Sterllar Chemical Lab., India), SL (Hermes Chemicals Co., India), AC (NEHTA Enterprise, India), BX (Ven Petrochem & Pharma, India), MP (Clariant, UK) and PP (Clariant) were used and certified to contain 99.7, 99.9, 99.7, 99.8, 99.9 and 99.8%, respectively. Acetonitrile and methanol used were HPLC grade (BDH, Poole, UK). Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric and phosphoric acids used were analytical grade.

The pharmaceutical combination of GU with SL, MP and PP {Bronchovent syrup} (Batch No. 427091), was manufactured by MISR company for pharmaceutical industries (Mataria, Cairo, Egypt). Each 5 ml was labeled to contain 50 mg of GU, 2 mg of SL, 3 mg of MP and 1.5 mg of PP.

The pharmaceutical combination of AC with BX, MP and PP {Mucophylline syrup} (Batch No. 401093), was manufactured by MISR company for pharmaceutical industries (Mataria, Cairo, Egypt). Each 5 ml was labeled to contain 100 mg of AC, 4 mg of BX, 4.5 mg of MP and 0.5 mg of PP.

2.3. Procedure

2.3.1. HPLC conditions

The mobile phase for mixture 1 was prepared by mixing acetonitrile and 0.05 M potassium dihydrogen phosphate (pH was adjusted to 4.3 using potassium hydroxide) in a ratio of 60:40 (v/v). The mobile phase for mixture 2 was prepared by mixing acetonitrile and 0.05 M potassium dihydrogen phosphate (pH was adjusted to 3 using phosphoric acid) in a ratio of 50:50 (v/v), the flow rate was 1.5 ml min^{-1}. All determinations were performed at ambient temperature.

2.3.2. Standard solutions and calibration

2.3.2.1. Stock solutions for mixture 1. Stock standard solution of GU was prepared by dissolving 50 mg of GU in 100 ml of methanol. Stock standard solutions of SL, MP, PP were prepared by separately dissolving 50 mg of each compound in 100 ml of methanol. Further dilution of 10 ml of each of SL, MP solutions and 3 ml of PP solution to 100 ml with methanol was carried out.

2.3.2.2. Stock solutions for mixture 2. Stock standard solution of AC was prepared by dissolving 100 mg of AC in 100 ml of 70% methanol. Stock solutions of BX, MP and PP were prepared by separately dissolving 50 mg of each compound in 100 ml of 70% methanol. Further dilution of 10 ml of each of BX, MP solutions and 1 ml of PP solution to 100 ml with 70% methanol was carried out.

2.3.2.3. Multivariate calibration. A training set of 25 laboratory prepared mixtures with different concentrations of each compound were prepared by dilution of the stock standard solutions with 0.1 M sodium hydroxide for mixture 1 in the concentration range of 20–60 μg ml^{-1} for GU, 1–3 μg ml^{-1} for SL, 1–5 μg ml^{-1} for MP and 0.6–1.8 μg ml^{-1} for PP; and dilution of the stock standard solutions with 0.1 M hydrochloric acid for mixture 2 in the concentration range of 20–80 μg ml^{-1} for AC, 1–5 μg ml^{-1} for BX, 1–5 μg ml^{-1} for MP and 0.2–1.8 μg ml^{-1} for PP (Tables 1 and 2).

The UV absorption spectra were recorded over the wavelength range of 232–300 nm with 0.8 nm intervals for mixture 1 and 235–275 nm with 0.4 nm intervals for mixture 2. The computations were made using PLS-Toolbox software version 2.1.

PLS-1 and PCR models were applied to the UV absorption spectra using four latent variables (or principal components) for determination of the studied components of mixture 1. For mixture 2 PLS-1 was applied using four latent variables for determination of AC, BX and five latent variables for determination

Table 1
Concentration data for the different mixtures used in the calibration set and internal validation for the determination of GU, SL, MP and PP (mixture 1)

No.	Mixture composition ($\mu g\,ml^{-1}$)				Internal validation (%recovery)							
	GU	SL	MP	PP	PLS-1				PCR			
					GU	SL	MP	PP	GU	SL	MP	PP
1	40	2	3	1.2	100.4	100.2	100.4	99.7	100.4	100.2	100.4	99.7
2	40	1	1	1.8	99.8	99.9	99.7	100.3	99.8	99.9	99.7	100.3
3	20	1	5	0.9	99.9	100.1	100.2	99.7	99.9	100.1	100.2	99.7
4	20	3	2	1.8	99.5	100.1	100.0	99.8	99.5	100.1	100.0	99.8
5	60	1.5	5	1.2	100.3	100.0	99.9	100.2	100.3	100.0	99.9	100.2
6	30	3	3	0.9	100.1	100.2	100.0	100.6	100.1	100.2	100.0	100.6
7	60	2	2	0.9	100.0	99.9	100.3	99.5	100.0	99.9	100.3	99.5
8	40	1.5	2	1.5	99.8	99.9	100.1	101.0	99.8	99.9	100.1	101.0
9	30	1.5	4	1.8	99.8	99.9	100.0	99.8	99.8	99.9	100.0	99.8
10	30	2.5	5	1.5	100.4	99.9	100.1	100.0	100.4	99.9	100.1	100.0
11	50	3	4	1.2	99.9	100.1	100.0	99.9	99.9	100.1	100.0	99.9
12	60	2.5	3	1.8	100.1	99.9	99.9	100.1	100.1	99.9	99.9	100.1
13	50	2	5	1.8	100.1	100.2	100.0	100.1	100.1	100.2	100.0	100.1
14	40	3	5	0.6	99.8	99.9	99.9	100.0	99.8	99.9	99.9	100.0
15	60	3	1	1.5	100.1	100.1	100.4	99.9	100.1	100.1	100.4	99.9
16	60	1	4	0.6	99.8	100.2	100.1	99.0	99.8	100.2	100.1	99.0
17	20	2.5	1	1.2	99.4	99.8	99.9	100.0	99.4	99.8	99.9	100.0
18	50	1	3	1.5	100.1	100.0	99.8	100.4	100.1	100.0	99.8	100.4
19	20	2	4	1.5	99.6	99.9	99.9	99.9	99.6	99.9	99.9	99.9
20	40	2.5	4	0.9	100.0	99.9	99.9	100.3	100.0	99.9	99.9	100.3
21	50	2.5	2	0.6	100.0	99.8	99.7	101.2	100.0	99.8	99.7	101.2
22	50	1.5	1	0.9	99.9	99.9	100.3	99.8	99.9	99.9	100.3	99.8
23	30	1	2	1.2	100.3	100.0	99.8	100.3	100.3	100.0	99.8	100.3
24	20	1.5	3	0.6	100.5	99.9	99.8	100.7	100.5	99.9	99.8	100.7
25	30	2	1	0.6	100.4	100.4	100.3	99.4	100.4	100.4	100.3	99.4
Mean[a]					100.0	100.0	100.0	100.1	100.0	100.0	100.0	100.1
S.D.[a]					0.29	0.15	0.20	0.48	0.29	0.15	0.20	0.48

[a] Mean and S.D., percentage recovery from the label claim amount.

of MP and PP. In PCR five principal components were used for determination of AC, BX, MP and PP.

2.3.2.4. HPLC calibration. Further dilutions of the stock solutions for each compound were made using the specified mobile phase to reach the concentration range of 20–60 $\mu g\,ml^{-1}$ for GU, 1–3 $\mu g\,ml^{-1}$ for SL, 20–80 $\mu g\,ml^{-1}$ for AC, 1–5 $\mu g\,ml^{-1}$ for BX, 1–5 $\mu g\,ml^{-1}$ for MP and 0.2–1.8 $\mu g\,ml^{-1}$ for PP. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified conditions described previously. The peak area values were plotted against corresponding concentrations. Linear relationship was obtained.

2.3.3. Pharmaceutical sample preparation

2.3.3.1. For mixture 1. A volume of the syrup equivalent to 50 mg of GU, 2 mg of SL, 3 mg of MP and 1.5 mg of PP was diluted to 100 ml with methanol. Further dilution was carried out with 0.1 M sodium hydroxide (for PLS-1 and PCR methods) or mobile phase (for HPLC method) to reach the calibration range of each compound.

2.3.3.2. For mixture 2. A volume of the syrup equivalent to 100 mg of AC, 4 mg of BX, 4.5 mg of MP and 0.5 mg of PP was diluted with 70% methanol. Further dilution was carried out with 0.1 M hydrochloric acid (for PLS-1 and PCR methods) or mobile phase (for HPLC method) to reach the calibration range of each compound.

The general procedures for PCR, PLS-1 and HPLC methods described under calibration were followed and the concentration of each compound was calculated.

2.4. Validation of the methods

2.4.1. Linearity

The linearity of the HPLC method for determination of GU, SL, MP and PP in mixture 1; AC, BX, MP and PP in mixture 2 was evaluated by analyzing a series of different concentrations of each compound. In this study seven concentrations were chosen, ranging between 20 and 60 $\mu g\,ml^{-1}$ for GU, 1 and 3 $\mu g\,ml^{-1}$ for SL, 1 and 5 $\mu g\,ml^{-1}$ for MP, and 0.2 and 1.8 $\mu g\,ml^{-1}$ for PP in mixture 1; and between 20 and 80 $\mu g\,ml^{-1}$ for AC, 1 and 5 $\mu g\,ml^{-1}$ for BX, 1 and 5 $\mu g\,ml^{-1}$ for MP, and 0.2 and 1.8 $\mu g\,ml^{-1}$ for PP in mixture 2. Each concentration was repeated three times; this approach will provide information on the variation in peak area between samples of same concentration. The high value of the correlation coefficient and the intercept value that was not statistically ($P < 0.05$) different from zero (Table 3) validated the linearity of the calibration graphs.

Table 2
Concentration data for the different mixtures used in the calibration set and internal validation for the determination of AC, BX, MP and PP (mixture 2)

No.	Mixture composition ($\mu g\,ml^{-1}$)				Internal validation (%recovery)							
	AC	BX	MP	PP	PLS-1				PCR			
					AC	BX	MP	PP	AC	BX	MP	PP
1	50	3	3	1	100.1	100.3	99.9	100.0	100.1	100.3	99.7	101.7
2	50	1	1	1.8	100.1	99.7	100.2	99.8	100.1	100.2	99.9	99.6
3	20	1	5	0.6	99.9	100.0	100.0	100.2	99.9	99.7	100.2	98.8
4	20	5	2	1.8	100.1	100.2	100.0	100.3	100.1	100.1	98.9	101.1
5	80	2	5	1	100.3	100.2	100.0	100.1	100.3	99.9	99.7	101.6
6	35	5	3	0.6	99.9	99.7	100.0	100.2	99.9	99.9	99.6	103.7
7	80	3	2	0.6	99.9	100.1	99.7	101.6	99.9	100.1	99.6	102.0
8	50	2	2	1.4	100.1	99.9	100.0	101.0	100.1	99.9	100.5	101.0
9	35	2	4	1.8	99.9	99.7	100.1	100.1	99.9	99.9	99.9	100.6
10	35	4	5	1.4	100.0	100.1	100.0	100.1	100.0	100.0	100.4	99.2
11	65	5	4	1	100.0	100.1	100.1	99.5	100.0	100.1	99.7	101.8
12	80	4	3	1.8	100.0	99.9	99.9	99.8	100.0	99.9	100.1	99.7
13	65	3	5	1.8	100.0	100.0	99.9	100.1	100.0	100.1	100.5	98.7
14	50	5	5	0.2	100.0	99.9	99.9	99.1	100.0	99.9	99.5	94.3
15	80	5	1	1.4	100.0	99.8	100.1	99.8	100.0	99.8	101.1	98.7
16	80	1	4	0.2	100.0	99.8	100.2	99.3	100.0	100.2	100.1	100.2
17	20	4	1	1	99.8	99.9	100.0	100.0	99.8	99.9	101.0	98.7
18	65	1	3	1.4	99.9	100.3	99.9	100.1	99.9	100.0	99.5	100.8
19	20	3	4	1.4	99.9	100.1	100.1	99.9	99.9	100.2	100.2	99.9
20	50	4	4	0.6	100.0	100.0	100.1	99.9	100.0	100.0	100.5	98.2
21	65	4	2	0.2	100.0	100.3	100.3	101.1	100.0	100.3	100.3	101.0
22	65	2	1	0.6	100.0	99.6	99.5	100.2	100.0	99.8	100.2	99.3
23	35	1	2	1	100.3	100.1	99.9	99.8	100.3	99.7	98.9	101.7
24	20	2	3	0.2	100.2	99.7	99.6	100.3	100.2	99.7	99.8	95.5
25	35	3	1	0.2	100.0	100.4	100.7	98.0	99.9	100.2	102.4	97.1
Mean[a]					100.0	100.0	100.0	100.0	100.0	100.0	100.1	99.8
S.D.[a]					0.13	0.21	0.23	0.68	0.13	0.19	0.72	2.10

[a] Mean and S.D., percentage recovery from the label claim amount.

2.4.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each compound. The data for each concentration level were evaluated by one-way ANOVA. A 8 days × 2 replicates design was performed. Statistical comparison of the results was performed using the *P*-value of the *F*-test. Three univariate analyses of variance for each concentration level were made. Since the *P*-value of the *F*-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

2.4.3. Range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in pharmaceutical product, to give accurate, precise and linear results. The calibration range of the proposed HPLC method was given in Table 3.

2.4.4. Detection and quantitation limits

According to ICH recommendations [28] the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Table 3.

2.4.5. Selectivity

Methods selectivity was achieved by preparing six mixtures of the studied compounds at various concentrations within the linearity range for HPLC. The external validation of the PLS-1 and PCR models was achieved by evaluation of the prediction ability of the two models to a prediction set of six new laboratory prepared mixtures, different from those of the calibration set. The concentrations of each compound were falling within the ranges of calibration matrix. The laboratory prepared mixtures were analyzed according to the previous procedures described under the proposed methods. Satisfactory results were obtained (Tables 4 and 5), indicating the high selectivity of the proposed methods for determination of the studied components in their mixture.

2.4.6. Accuracy

This study was performed by addition of known amounts of the studied compounds to a known concentration of the commercial pharmaceutical syrup (standard addition method). The resulting mixtures were analyzed and the results obtained were compared with the expected results. The excellent recoveries of

Table 3
Characteristic parameters of the calibration equations for the proposed HPLC method for simultaneous determination of GU, SL, MP and PP (mixture 1) and AC, BX, MP and PP (mixture 2)

Parameters	Mixture 1				Mixture 2			
	GU	SL	MP	PP	AC	BX	MP	PP
Calibration range ($\mu g\,ml^{-1}$)	20–60	1–3	1–5	0.2–1.8	20–80	1–5	1–5	0.2–1.8
Detection limit ($\mu g\,ml^{-1}$)	1.71×10^{-2}	1.70×10^{-2}	1.76×10^{-2}	1.60×10^{-2}	1.72×10^{-2}	1.79×10^{-2}	1.79×10^{-2}	1.52×10^{-2}
Quantitation limit ($\mu g\,ml^{-1}$)	5.68×10^{-2}	5.68×10^{-2}	5.87×10^{-2}	5.33×10^{-2}	5.72×10^{-2}	5.98×10^{-2}	5.97×10^{-2}	5.08×10^{-2}
Regression equation (Y)[a]								
Slope (b)	3.11×10^{3}	4.95×10^{4}	5.73×10^{4}	4.83×10^{4}	7.88×10^{3}	2.99×10^{4}	5.45×10^{4}	6.53×10^{4}
Standard deviation of the slope (S_b)	0.27×10^{2}	4.24×10^{2}	5.08×10^{2}	3.89×10^{2}	0.68×10^{2}	2.70×10^{2}	4.91×10^{2}	5.01×10^{2}
Relative standard deviation of the slope (%)	0.86	0.86	0.89	0.80	0.86	0.90	0.90	0.77
Confidence limit of the slope[b]	3.08×10^{3}–3.14×10^{3}	4.91×10^{4}–4.99×10^{4}	5.69×10^{4}–5.78×10^{4}	4.80×10^{4}–4.87×10^{4}	7.81×10^{3}–7.95×10^{3}	2.96×10^{4}–3.02×10^{4}	5.41×10^{4}–5.50×10^{4}	6.49×10^{4}–6.58×10^{4}
Intercept (a)	0.85×10^{2}	-3.32×10^{2}	-1.47×10^{2}	0.39×10^{2}	1.98×10^{3}	-0.89×10^{2}	-4.70×10^{2}	0.27×10^{2}
Standard deviation of the intercept (S_a)	0.27×10^{2}	6.63×10^{2}	1.53×10^{3}	4.53×10^{2}	3.66×10^{3}	5.81×10^{2}	1.48×10^{3}	5.83×10^{2}
Confidence limit of the intercept[b]	(-1.07×10^{3})–(1.24×10^{3})	(-1.23×10^{3})–(5.70×10^{2})	(-1.63×10^{3})–(1.34×10^{3})	(-3.79×10^{2})–(4.58×10^{2})	(-1.57×10^{3})–(5.54×10^{3})	(-6.53×10^{2})–(4.75×10^{2})	(-1.91×10^{3}) – (9.67×10^{2})	(-5.12×10^{2}) – (5.66×10^{2})
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	3.48×10^{2}	2.83×10^{2}	6.63×10^{2}	2.30×10^{2}	1.36×10^{3}	2.16×10^{2}	6.41×10^{2}	2.96×10^{2}

[a] $Y = a + bC$, where C is the concentration of compound in $\mu g\,ml^{-1}$ and Y is the peak area.
[b] 95% confidence limit.

standard addition method (Tables 4 and 5) suggested that good accuracy of the proposed methods.

2.4.7. Robustness

Variation of pH of 0.05 M potassium dihydrogen phosphate of the mobile phase by ±0.2 and the organic strength of the mobile phase by ±2% did not have significant effect on chromatographic resolution in HPLC method. Variation of strength of sodium hydroxide and hydrochloric acid by ±0.02 M did not has significant effect on PLS-1 and PCR methods.

2.4.8. Analytical solution stability

Analytical solution stability of the studied compounds in the mobile phase, 0.1 M sodium hydroxide and 0.1 M hydrochloric acid exhibited no chromatographic or absorbance changes for 1 h when kept at room temperature, and for 5 h when stored refrigerated at 5 °C.

2.5. Analysis of pharmaceutical syrup

The proposed PLS-1, PCR and HPLC methods were applied to the simultaneous determination of GU, SL, MP and PP; AC, BX, MP and PP in commercial syrup. Five replicate determinations for mixtures 1 and 2 were made. Satisfactory results were obtained for each compound in a good agreement with label claims (Tables 4 and 5). No published method has been reported for simultaneous determination of these components in their mixtures. Therefore, the results of the proposed PLS-1, PCR methods were compared with those of the proposed HPLC method. Statistical comparison between the results was performed with regards to accuracy and precision using Student's *t*-test and the *F*-ratio at 95% confidence level (Tables 4 and 5). There is no significant difference between the results.

Table 4
Determination of GU, SL, MP and PP (mixture 1) in laboratory prepared mixtures and commercial syrup using the proposed methods

	Mean ± S.D.[a]		
	PLS-1	PCR	HPLC
Synthetic mixtures			
For GU	100.1 ± 0.22	100.1 ± 0.22	99.9 ± 0.32
For SL	100.0 ± 0.14	100.0 ± 0.14	100.0 ± 0.26
For MP	100.0 ± 0.22	100.0 ± 0.22	99.7 ± 0.49
For PP	99.8 ± 0.77	99.8 ± 0.77	99.5 ± 0.58
Commercial syrup			
For GU	99.3 ± 0.72	99.3 ± 0.72	99.5 ± 0.63
t	0.55	0.55	(2.18)[b]
F	1.31	1.31	(4.28)[b]
For SL	100.9 ± 0.24	100.9 ± 0.24	100.4 ± 0.40
t	2.27	2.27	(2.18)[b]
F	0.36	0.36	(4.28)[b]
For MP	99.3 ± 0.61	99.3 ± 0.61	99.2 ± 0.98
t	0.37	0.37	(2.18)[b]
F	2.58	2.58	(4.28)[b]
For PP	97.9 ± 0.82	97.9 ± 0.82	99.8 ± 0.81
t	2.07	2.07	(2.18)[b]
F	1.02	1.02	(4.28)[b]
Recovery[c]			
For GU	99.8 ± 0.50	99.8 ± 0.50	99.7 ± 0.50
For SL	99.7 ± 0.48	99.7 ± 0.48	99.8 ± 0.36
For MP	100.1 ± 0.59	100.1 ± 0.59	99.9 ± 0.45
For PP	100.1 ± 0.78	100.1 ± 0.78	99.7 ± 0.60

[a] Mean and S.D., percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of 50% of the nominal content.

Table 5
Determination of AC, BX, MP and PP (mixture 2) in laboratory prepared mixtures and commercial syrup using the proposed methods

	Mean ± S.D.[a]		
	PLS-1	PCR	HPLC
Synthetic mixtures			
For AC	100.0 ± 0.19	100.0 ± 0.18	99.7 ± 0.52
For BX	100.1 ± 0.34	100.2 ± 0.31	99.9 ± 0.48
For MP	100.0 ± 0.40	99.9 ± 0.43	99.9 ± 0.61
For PP	99.7 ± 0.46	100.0 ± 0.41	99.9 ± 0.73
Commercial syrup			
For AC	99.7 ± 0.74	99.7 ± 0.74	99.6 ± 0.55
t	0.29	0.29	(2.18)[b]
F	1.81	1.81	(4.28)[b]
For BX	100.7 ± 0.38	100.7 ± 0.38	100.5 ± 0.30
t	1.09	1.09	(2.18)[b]
F	1.60	1.60	(4.28)[b]
For MP	95.4 ± 0.66	95.3 ± 0.63	95.9 ± 0.50
t	1.60	1.97	(2.18)[b]
F	1.74	1.59	(4.28)[b]
For PP	95.5 ± 1.14	95.9 ± 2.29	96.7 ± 0.93
t	2.16	0.86	(2.18)[b]
F	1.50	6.06	(4.28)[b]
Recovery[c]			
For AC	99.4 ± 0.20	99.4 ± 0.20	99.8 ± 0.47
For BX	99.6 ± 0.31	99.6 ± 0.31	100.0 ± 0.53
For MP	100.4 ± 0.51	100.9 ± 0.45	99.9 ± 0.72
For PP	98.0 ± 0.84	97.7 ± 1.00	99.8 ± 0.65

[a] Mean and S.D., percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of 50% of the nominal content.

3. Results and discussion

3.1. Spectral features

Figs. 1 and 2 show the UV absorption spectra of GU with SL, MP and PP; and AC with BX, MP and PP, respectively, at their nominal concentrations. As can be seen, the UV absorption spectra of GU, SL, MP and PP are overlapped; and the UV absorption spectra of AC is highly overlapped with BX, MP and PP spectra. The simultaneous determination of such drugs in their studied mixtures by conventional, derivative and derivative ratio spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range. HPLC and PLS-1 or PCR calibration methods can be used to overcome this problem.

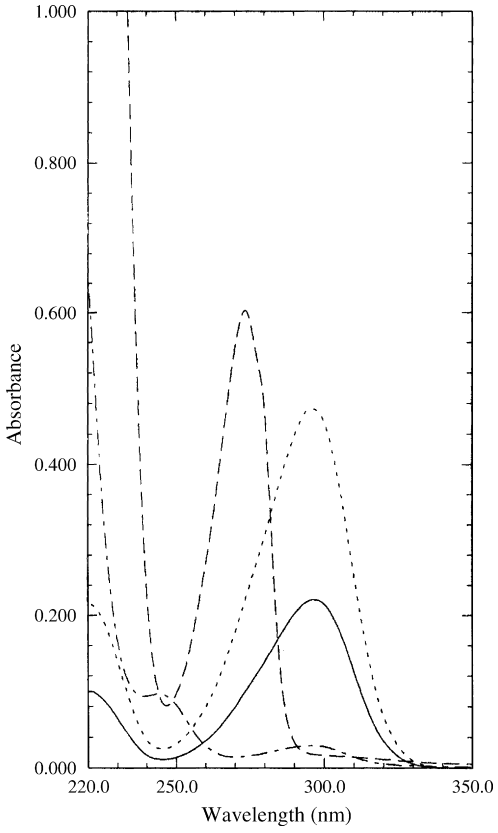

Fig. 1. UV absorption spectra of 50 μg ml^{-1} of guaiphensin (– – –), 2 μg ml^{-1} of salbutamol sulfate (– – –), 3 μg ml^{-1} of methylparaben (- - -) and 1.5 μg ml^{-1} of propylparaben (—) in 0.1 M sodium hydroxide.

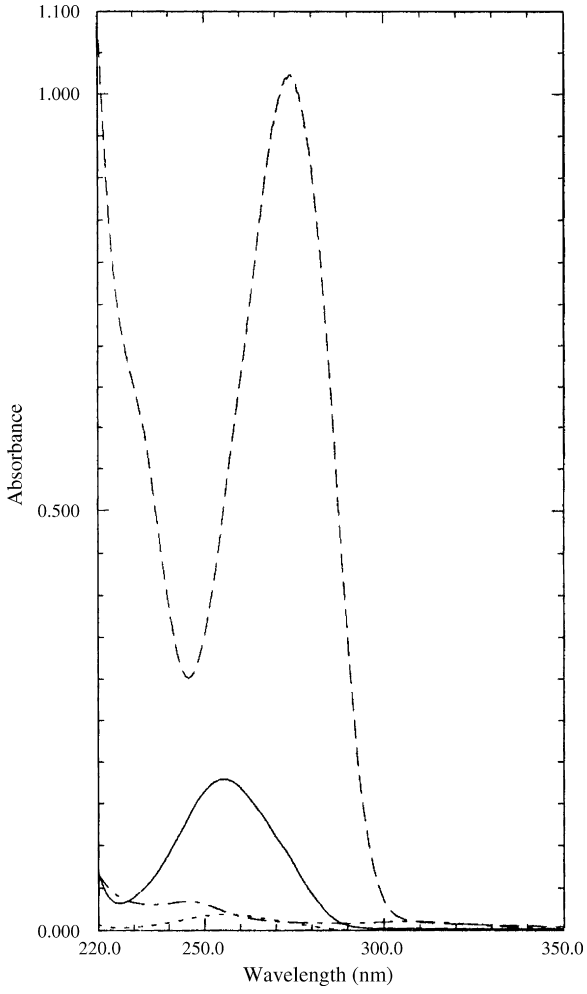

Fig. 2. UV absorption spectra of 40 μg ml^{-1} of acephylline piperazine (– – –), 1.6 μg ml^{-1} of bromhexine hydrochloride (– – –), 1.8 μg ml^{-1} of methylparaben (—) and 0.2 μg ml^{-1} of propylparaben (- - -) in 0.1 M hydrochloric acid.

3.2. Multivariate calibration

To improve the analysis for these compounds, two chemometric approaches based on PLS-1 and PCR calibration were evaluated.

3.2.1. Calibration matrix and selection of spectral zones for analysis by PLS-1 and PCR

The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used [23]. PLS procedures are designated to be full spectrum computational procedures, thus wavelength selection is seemingly unnecessary, and so all available wavelengths are often used. However, measurements from spectral wavelengths that are non-informative in a model degrade performance. Original and reconstructed spectra of the calibration matrix were compared in order to select the range of wavelengths. The range was obtained by all regions in which the difference between each component of the mixture and the others was maximum. Besides, the regions in which each component of the mixture was best reconstructed were also considered. The wavelength range of 232–300 nm with 0.8 nm intervals was selected for mixture 1 and wavelength range of 235–275 nm with 0.4 nm intervals was selected for mixture 2. Wavelengths less than 232 or 235 nm for mixtures 1 and 2, respectively, were rejected due to the noise appeared and the differences between the laboratory prepared mixtures and pharmaceutical syrup spectra, and wavelengths more than 300 or 275 nm for mixtures 1 and 2, respectively, were not used because GU and SL in mixture 1 and BX, PP in mixture 2 have neglected absorption at the concentrations used in this region. So any absorbance values obtained at these wavelengths would have introduced a significant amount of noise in the calibration matrix, thereby decreasing the precision.

Multilevel multifactor design [24] was used for the construction of the calibration set. A calibration set of 25 samples was prepared for calibration. A five-level, four-factor calibration design was used in concentrations ranging between 20 and 60 μg ml^{-1} for GU, 1 and 3 μg ml^{-1} for SL, 1 and 5 μg ml^{-1} for MP, and 0.6 and 1.8 μg ml^{-1} for PP in mixture 1; and between 20 and 80 μg ml^{-1} for AC, 1 and 5 μg ml^{-1} for BX, 1 and 5 μg ml^{-1} for MP, and 0.2 and 1.8 μg ml^{-1} for PP in mixture 2. The concentrations details are given in Tables 1 and 2.

3.2.2. Selection of the optimum number of factors

An appropriate choice of the number of principal components or factors is necessary for PCR and PLS-1 calibrations. The number of factors should account as much as possible for the experimental data without resulting in over fitting. Various

criteria have been developed to select the optimum number [25]. Cross-validation methods leaving out one sample at a time was employed [26]. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root mean squares error of cross-validation (RMSECV) was calculated for each method as follows:

$$\text{RMSECV} = \sqrt{\frac{\text{PRESS}}{n}}$$

where n is the number of training samples,

$$\text{PRESS} = \sum (Y_{\text{pred}} - Y_{\text{true}})^2$$

where Y_{pred} and Y_{true} are predicted and true concentrations in $\mu g\,ml^{-1}$, respectively.

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both precision and accuracy of predictions. It was recalculated upon addition of each new factor to the PLS-1 and PCR models.

The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations. Satisfactory correlation coefficient (r) values between actual and predicted concentrations are obtained for the studied components in the training set by PLS-1 and PCR optimized models (Table 3) indicating good predictive abilities of the models. The RMSECV obtained by optimizing the calibration matrix of the absorption spectra for the PLS-1 and PCR methods are shown in Table 6 indicating good accuracy and precision.

The optimum number of factors was selected by following the criterion of Haaland and Thomas [27]. The selected model is that with the smallest number of factors such that RMSECV for that model is not significantly greater than RMSECV from the model with additional factor. A number of factors of 4 was found to be optimum for mixture 1 using PLS-1 and PCR, while for mixture 2 PLS-1 was applied using four latent variables for determination of AC, BX and using five latent variables for determination of MP and PP, while PCR was applied for determination of AC, BX, MP and PP using five principal components.

PLS-1 may have the largest advantage when analyzing systems that have constituent concentrations and absorbance that are widely varied. This is clear in mixture 2, where PP concentration and absorbance is low relatively to the other components of mixture 2, so PLS-1 prediction was better than PCR pediction for PP in mixture 2.

3.3. HPLC method

The developed HPLC method has been applied for simultaneous determination of GU, SL, MP and PP [mixture 1]; AC, BX, MP and PP [mixture 2]. The mobile phase composition and pH were studied and optimized. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–0.05 M potassium dihydrogen phosphate in a ratio of (60:40, v/v) for mixture 1 and (50:50, v/v) for mixture 2. Increasing acetonitrile concentration to more than 75% led to inadequate separation of the four peaks in each mixture. At lower acetonitrile concentration (<35%) separation occurred but with excessive tailing and increased retention time for PP peak in mixture 1, PP and BX peaks in mixture 2. Variation of apparent pH of 0.05 potassium dihydrogen phosphate of the mobile phase for mixture 1 resulted in maximum capacity factor (K') value at apparent pH 6.5, at apparent pH 3–5 improved resolution for the four compounds was observed. However at apparent pH 4.3 optimum resolution with reasonable retention time was observed. Variation of apparent pH of 0.05 potassium dihydrogen phosphate of the mobile phase of mixture 2 resulted in maximum capacity factor (K') value at apparent pH 6.0, at apparent pH 2.5–4 improved resolution for the four peaks was observed. However, at apparent pH 3 optimum resolution with reasonable retention time was observed. Quantitation based on peak area was achieved with UV detection at 243 and 245 nm for mixtures 1 and 2, respectively. The specificity of the HPLC method is illustrated in Figs. 3 and 4 where complete separation of the four compounds of each mixture was noticed.

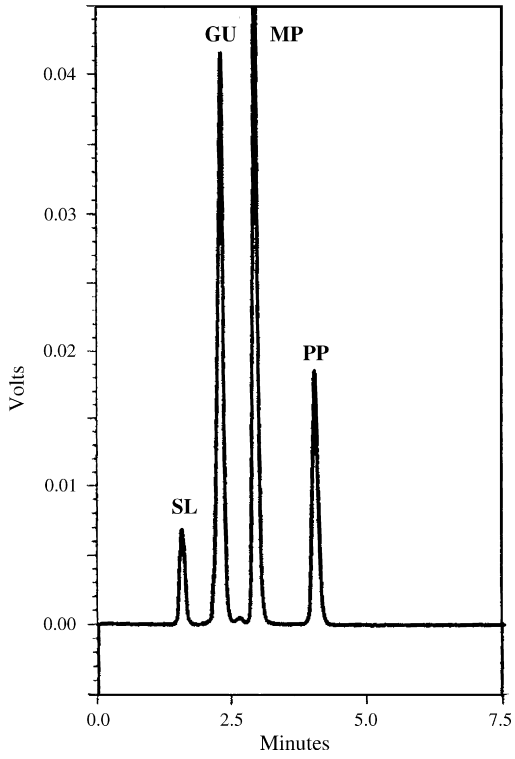

Fig. 3. HPLC chromatogram of 20 μl injection of syrup sample containing 50 $\mu g\,ml^{-1}$ of guaiphensin (GU), 2 $\mu g\,ml^{-1}$ of salbutamol (SL), 3 $\mu g\,ml^{-1}$ of methylparaben (MP) and 1.5 $\mu g\,ml^{-1}$ of propylparaben (PP).

The average retention time ± standard deviation were found to be 1.7 ± 0.02, 2.3 ± 0.04, 3.0 ± 0.04 and 4.1 ± 0.03 for SL, GU, MP and PP, respectively, in mixture 1; and 1.6 ± 0.02, 3.2 ± 0.03, 5.8 ± 0.04 and 6.5 ± 0.02 min for AC, MP, PP and BX, respectively, in mixture 2 for 10 replicates.

To determine the linearity of HPLC detector response, calibration standard solutions for each compound were prepared as described in text. Linear correlation was obtained between peak area versus concentration of each compound. Characteristic parameters for regression equations of the HPLC method were given in Table 3.

Table 6
RMSECV and statistical parameter values for simultaneous determination of GU, SL, MP and PP (mixture 1); AC, BX, MP and PP (mixture 2) using PLS-1 and PCR methods

	Method	RMSECV	Intercept	Slope	r	S.E. of intercept	S.E. of slope
Mixture 1							
GU	PLS-1	1.06×10^{-1}	1.40×10^{-3}	0.99996	0.9999	5.26×10^{-2}	5.26×10^{-2}
	PCR	1.06×10^{-1}	1.43×10^{-3}	0.99996	0.9999	5.26×10^{-2}	5.26×10^{-2}
SL	PLS-1	3.90×10^{-3}	1.60×10^{-5}	0.99999	0.9999	1.95×10^{-3}	9.18×10^{-4}
	PCR	3.90×10^{-3}	1.60×10^{-5}	0.99999	0.9999	1.95×10^{-3}	9.18×10^{-4}
MP	PLS-1	5.80×10^{-3}	1.00×10^{-5}	0.99999	0.9999	2.31×10^{-3}	6.95×10^{-4}
	PCR	5.80×10^{-3}	-6.00×10^{-6}	1.00000	0.9999	2.21×10^{-3}	6.95×10^{-4}
PP	PLS-1	4.50×10^{-3}	9.20×10^{-5}	0.99993	0.9999	2.25×10^{-3}	1.77×10^{-3}
	PCR	4.50×10^{-3}	9.20×10^{-5}	0.99993	0.9999	2.25×10^{-3}	1.77×10^{-3}
Mixture 2							
AC	PLS-1	3.88×10^{-2}	1.24×10^{-4}	1.00000	0.9999	1.66×10^{-2}	3.06×10^{-4}
	PCR	3.90×10^{-2}	9.60×10^{-5}	1.00000	0.9999	1.65×10^{-2}	3.04×10^{-4}
BX	PLS-1	7.90×10^{-3}	4.60×10^{-5}	0.99998	0.9999	3.07×10^{-3}	9.27×10^{-4}
	PCR	7.80×10^{-3}	3.80×10^{-5}	0.99999	0.9999	2.73×10^{-3}	8.22×10^{-4}
MP	PLS-1	1.63×10^{-2}	4.20×10^{-5}	0.99999	0.9999	2.21×10^{-3}	6.67×10^{-4}
	PCR	1.88×10^{-2}	3.08×10^{-4}	0.99990	0.9999	7.02×10^{-3}	2.12×10^{-3}
PP	PLS-1	1.47×10^{-2}	1.40×10^{-5}	0.99999	0.9999	1.25×10^{-3}	1.09×10^{-3}
	PCR	1.75×10^{-2}	5.55×10^{-4}	0.99945	0.9999	5.65×10^{-3}	4.92×10^{-3}

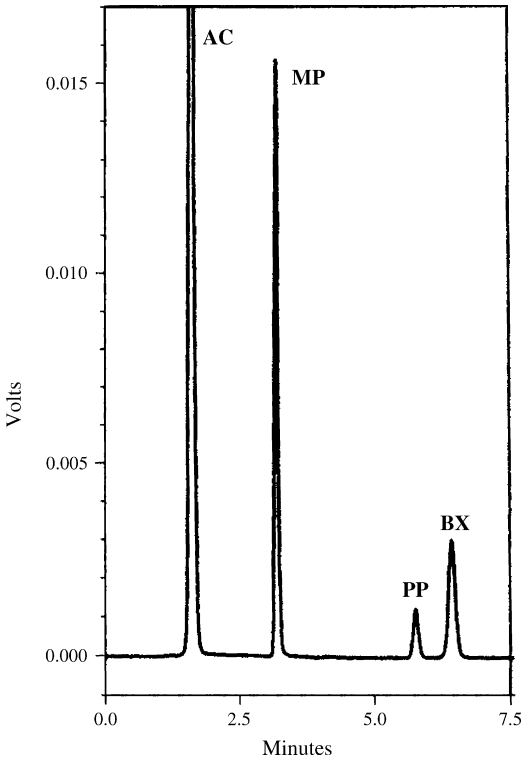

Fig. 4. HPLC chromatogram of 20 μl injection of syrup sample containing 40 μg ml^{-1} of acephylline piperazine (AC), 1.6 μg ml^{-1} of bromhexine hydrochloride (BX), 1.8 μg ml^{-1} of methylparaben (MP) and 0.2 μg ml^{-1} of propylparaben (PP).

4. Conclusion

For analytical purposes it is always of interest to establish methods capable of analysing a large number of samples in a short time period with acceptable accuracy and precision. Spectroscopic techniques can generate large amounts of data within a short period of analysis; however, when coupled with chemometrics tools, the quality of the spectral information can be markedly increased, converting this combined technique into a powerful and highly convenient analytical tool.

A comparative study of the use of HPLC and multivariate calibration (PLS-1 and PCR) methods for the resolution of GU, SL, MP and PP (mixture 1); AC, BX, MP and PP (mixture 2) has been accomplished, showing that multivariate calibration methods provide, with adequate software support, a clear example of the high resolving power of this technique. Although the HPLC method is more specific than the multivariate calibration methods, it needs expensive equipment and materials. Multivariate calibration methods are less expensive by comparison and they do not require sophisticated instrumentation and any prior separation step. The proposed HPLC, PLS-1 and PCR methods were found to be suitable for the determination of GU, SL, MP and PP; AC, BX, MP and PP in their commercial syrup.

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