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Simultaneous determination of phenytoin, barbital and caffeine in pharmaceuticals by absorption (zero-order) UV spectra and first-order derivative spectra—multivariate calibration methods

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Abstract

The quantitative predictive abilities of partial least squares (PLS-1) and principle component regression (PCR) on absorption (zero-order) UV spectra are compared with the results obtained by the use of these multivariate calibration methods on first-order derivative spectra. Both approaches were satisfactorily applied to the simultaneous determination of these drugs in synthetic and pharmaceutical mixtures. Significant advantages were found in the simultaneous determination of phenytoin, barbital and caffeine in binary and ternary mixtures, by application of different multivariate calibration methods when the calibration matrix was performed using the first-order derivative spectra. The proposed method was validated by applying it to the analysis of binary and ternary mixtures of phenytoin, barbital and caffeine. Determinations were made over the concentration ranges of 0.24–22.0, 0.01–27.0 and 0.049–27.0 $\mu g\,ml^{-1}$ for phenytoin, barbital and caffeine, respectively, in the binary and 0.45–22.0, 0.05–26.0 and 0.05–20.0 $\mu g\,ml^{-1}$ for phenytoin, barbital and caffeine, respectively, in the ternary mixtures. The relative standard errors in the determinations were less than 3% in most cases.
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Keywords: Phenytoin; Caffeine; Barbital; Simultaneous determination; PLS; PCR; First derivative spectra

1. Introduction

Phenytoin, 5,5-diphenylhydantoin, is one of the cyclic uredies which is related in structure to the barbiturates [1]. It was reported to be the least hypnotic, most strongly anticonvulsant and most effective against grand mal [2]. It has been widely used in the management of patients with epilepsy, generalized convulsion and partial seizure [3,4]. Different methods for the determination of this drug have been reviewed [5–7]. Anticonvulsants are currently quantified by techniques including solid phase micro-extraction gas chromatography [8], molecular imprinted in the chromatographic mode [9], fluorescence [10], radio-immunoassay [11], pholarography [12] and liquid chromatography–tandem mass spectroscopy [13].

Up to now, some immunoassay and high-performance liquid chromatography (HPLC) methods with UV detection have also been developed for determination of phenytoin [14–18]. Other reported methods involve gravimetry, titrimetry, stripping voltametry and chromatography. With the exception of HPLC methods, most procedures suffer from some drawbacks. Titrimetric and gravimetric methods are time-consuming; they lack simplicity, sensitivity and selectivity [19–22].

However, few simple and inexpensive direct spectrophotometric methods with good sensitivity and selectivity have been reported for the determination of phenytoin [23–27].

There are many drugs such as phenobarbital, levodoppa, caffeine and barbital, which may increase or decrease phenytoin effect in the body [28]. In addition, some of them interfere in determination of the others, such as interference of caffeine and barbital in determination of phenytoin, so here is a need for selective and sensitive spectrophotomet-

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ric method which could be utilized in detecting and distinguishing between phenytoin, barbital and caffeine, without the need for any pretreatment or prior separation. Simultaneous determination of several compounds in mixture can be a difficult task, especially when their analytical characteristics are not very different. In order to resolve complex spectra and to avoid time-consuming, clean-up and separation procedure attempted to simultaneous determination using derivative techniques and chemometrics methods. PLS and PCR are simple and powerful factor analysis multivariate tools that have been successfully applied to multicomponent analysis of complex mixtures [29]. One of the clearest explanations of these methods was given by Haaland and Thomas [30]. PLS is related to PCR in that a spectral decomposition is performed. PCR decomposition is significantly influenced by variations, which have no relevance to the analyte concentrations, whereas in PLS, the spectral decomposition is weighted to the concentration [31]. In this work, we use multivariate methods on first-order derivative and absorption UV spectra for simultaneous determination of phenytoin, barbital and caffeine in mixture. There is no any previous report for simultaneous spectrophotometric determination of these compounds in synthetic sample or pharmaceutical compounds.

2. Experimental

2.1. Chemicals and reagents

All experiments were performed with analytical-reagent grade and were used directly without further purification (all from Merck). Triply distilled water was used to prepare buffer and reagent solutions. Methanol, sodium hydroxide and Na_2HPO_4 used were of analytical grade. Pharmaceuticals grade were obtained from Sigma. Capsules of phenytoin and barbital and tablet of Cafergot was kindly donated by Darou Pakhsh., Iran. Capsules were labeled as containing 30 mg phenytoin and 50 mg barbital. Tablet of Cafergot was used and labeled as containing 100 mg caffeine and 1 mg ergotamine. Serum samples were prepared by injecting blank serum of rat with appropriate amounts of the stock solutions of phenytoin, barbital and caffeine.

2.2. Apparatus and software

A photodiode array UV–vis spectrophotometer model (multispec-1501 Shimadzu) equipped with 10-mm quartz cells was used to make absorbance measurements. The UV spectra of mixtures were recorded over the wavelength 190–300 nm and digitized absorbance was sampled at 0.5 nm intervals. Spectral bandwidth was 1 nm, scan speed was 2800 nm min^{-1} and $\Delta\lambda$ was 5 nm. Measurements of pH were made with a metrohm 654 pH meter (Metrohm Ltd., CH-9100-Hesau Switzerland) using a combined glass electrode. To calibrate the pH meter in various binaries, methanol–water mixtures were used; the 0.01 M solutions of oxalate and succinate buffers were employed. PLS and PCR programs were performed using chemometrics toolbox of MATLAB and first derivative spectra was obtained in MATLAB. (Version 6, Math Work, Inc.). All programs were run on a Pentium (III), 633 MHz, personal computer, with windows 98 operating system.

2.3. Stock and standard solutions

Stock solutions of phenytoin (500 $\mu g\,ml^{-1}$), barbital (500 $\mu g\,ml^{-1}$) and caffeine (500 $\mu g\,ml^{-1}$) were prepared by dissolving appropriate amount of these compounds in water–methanol 1:1 (v/v) mixture. A 0.05 M Na_2HPO_4/NaH_2PO_4 buffer, pH 12 was prepared by weighing the appropriate amount of Na_2HPO_4 and diluting with water–methanol 1:1 (v/v). The desired pH was obtained by adding the required amount of NaOH solution. Working solutions were prepared daily by adequate dilution of stock solutions in optimum conditions. The solutions used to prepare the binary and ternary mixtures were prepared in duplicate by placing them in 10-ml volumetric flasks. Two sets of standard solutions were prepared, the calibration set contained 25 standard solutions and the prediction set contained 15 standard solutions, so that the concentration of each drug in resulting solutions was in its own linear dynamic range. To a series of 10-ml volumetric flasks, 5 ml of buffer solution, NaH_2PO_4/Na_2HPO_4 (0.05 M) at pH 12, was added. Aliquots of phenytoin, barbital or caffeine solutions, containing appropriate amount of these drugs in the range of calibrations, were also added. Then, solutions were diluted to 10 ml with water–methanol 1:1 (v/v). UV spectra of the mixtures were recorded in the wavelength range 190–300 nm versus a solvent blank, and digitized absorbance was sampled at 0.5 nm intervals.

2.4. Procedure to determine phenytoin, barbital and caffeine in pharmaceutical formulations and serum

The contents of 20 tablets or capsules of each compound were individually weighed and powdered or evacuated. Then, an accurately weighed portion of the powder (100–200 mg) was transferred into 100-ml calibrated flasks and diluted to volume with solvent (as in standard solution, water–methanol 1:1), shaken well for 15 min and the solutions were filtered through 0.45 μm membrane filter. Then, 1 ml aliquots were transferred from each flask to 25-ml volumetric flasks and completed to volume with water–methanol 1:1 (v/v). Ternary and binary synthetic mixtures of phenytoin, caffeine and barbital were prepared by diluting known amounts of their stock solutions in water–methanol 1:1 (v/v), to obtain final concentrations in the range of calibration graph. Only a 1:3 dilution for serum must be made,and then mixtures assayed as described under optimized proposed procedure.

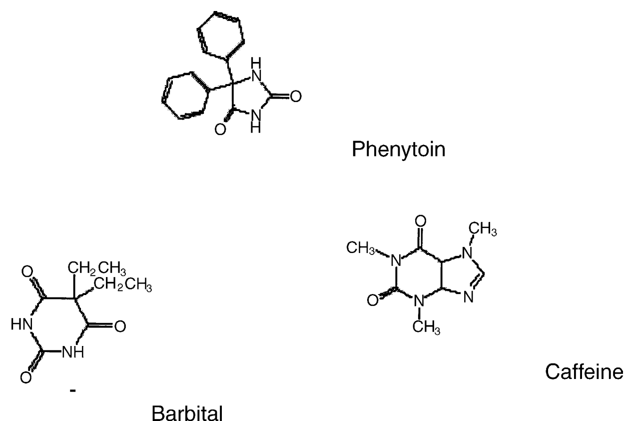

Fig. 1. Chemical structure of the analyzed drugs (phenytoin, barbital and caffeine).

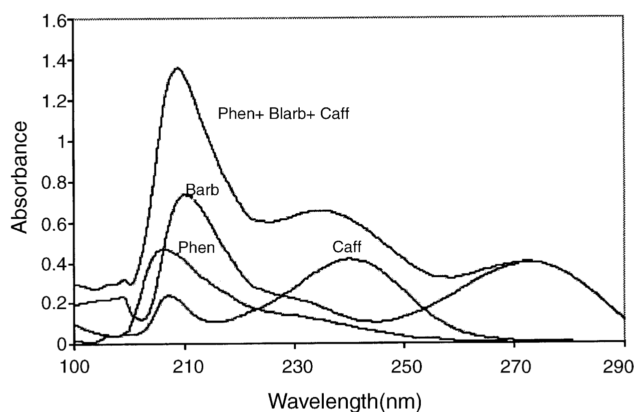

Fig. 2. Absorption (zero-order) UV spectra of 8 μg ml^{-1} of phenytoin, 10 μg ml^{-1} of barbital and 8 μg ml^{-1} of caffeine in water–methanol 1:1 (v/v) solution.

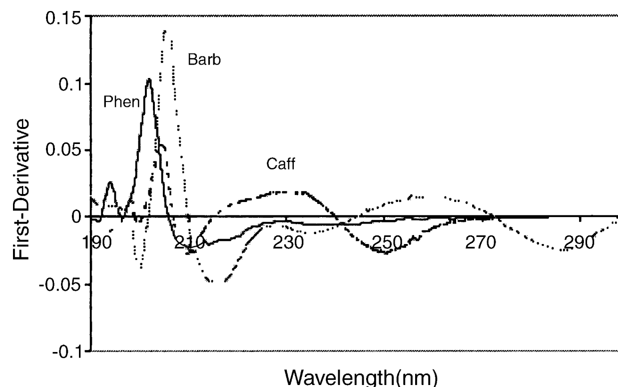

Fig. 3. First-order derivative spectra of 8 μg ml^{-1} phenytoin, 10 μg ml^{-1} barbital and 8 μg ml^{-1} of caffeine in water–methanol 1:1 (v/v) solution.

3. Results and discussion

3.1. Optimization of conditions

The chemical structures of phenytoin, barbital and caffeine are shown in Fig. 1. Fig. 2 shows the absorption UV spectra of these drugs and the mixture of them. First-order derivatives spectra for the drugs are shown in Fig. 3. As these figures show there is a clear overlapping between them; the spectral overlapping of the drugs prevents resolution of the mixtures by direct spectrophotometric measurements. Thus, the univariate analysis can not be applied to resolve their mixtures. The optimum conditions for quantitative estimation of considered compound were established via a number of preliminary experiments. In an attempt to reduce the degree of spectral overlapping of the considered drugs, the spectrum of each component was recorded in different organic solvents as well as in different buffer solutions. The absorption spectra of each drug in different organic solvent–water binary mixtures was investigated (ethanol, methanol, acetone). Among these, methanol–water 1:1 (v/v) gave better sensitivity and selectivity; thus, this mixture was chosen as solvent for further studies. The influence of pH values on the spectrum of each drug at a constant concentration, 6 μg ml^{-1}, was investigated separately. This study was made over the pH range of 2.5–13.5. Although no significant change was observed in spectrum of caffeine in all ranges of pH, the spectra of phenytoin and barbital show maximum absorbance at all wavelengths at high pH value (pH > 11.5). For achieving higher sensitivity and selectivity, pH 12 was selected as an optimum pH value for simultaneous determination of binary and ternary mixtures of these compounds. The wavelength interval ($\Delta\lambda$) used for calculation of derivative spectra was optimized for each drug separately and, accordingly, $\Delta\lambda$ were obtained. $\Delta\lambda = 5$ nm was considered to be optimum which gives the best signal-to-noise ratio for all drugs.

3.2. One-component calibration

To find the linear dynamic range of each component, calibration graph were obtained. The absorption spectra were recorded over 190–300 nm against a solvent blank. Linear range for each drug was determined by plotting the absorbances at its λ_{max} (phenytoin, 207 nm; barbital, 210 nm; and caffeine, 230 nm) versus sample concentration. Calibration curves were linear between 0.24 and 25.0 μg ml^{-1} of phenytoin, 0.01 and 27.0 μg ml^{-1} of barbital and 0.05 and 28.0 μg ml^{-1} of caffeine. Characteristic parameters for the regression equations of individual calibration by absorption UV spectra are shown in Table 1.

3.3. Multivariate methods

3.3.1. Simultaneous resolution of binary and ternary mixtures by application of PLS and PCR on absorption (zero-order) UV spectra and first-order derivative spectra

The PLS and PCR techniques are typical full-spectrum methods, more powerful than the ones based on measurement at only one wavelength, such as direct spectrophotometry because the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of

Table 1
Characteristic parameters for the regression equations of individual calibration by absorption UV spectra

Compound	Equation	R^{2a}	LOD[b] ($\mu g\,ml^{-1}$)	LOQ[b] ($\mu g\,ml^{-1}$)	S_b	S_a	R.S.E. (%)
Phenytoin	$A = 0.0799C_i + 0.0181$	0.9998	0.06	0.08	3.10×10^{-5}	2.5×10^{-4}	0.20
Barbital	$A = 0.0721C_i - 0.0354$	0.9985	0.06	0.07	1.51×10^{-4}	6.0×10^{-3}	0.65
Caffeine	$A = 0.0561C_i + 0.0052$	0.9985	0.04	0.06	5.10×10^{-4}	7.3×10^{-4}	0.50

C_i: concentration in $\mu g\,ml^{-1}$; S_b: S.D. of the slope [35]; S_a: S.D. of the intercept [35].
[a] The square of correlation coefficient.
[b] Limit of detections and limit of quantifications calculated by Miller & Miller method [35].

quantitative spectral analysis of mixtures. The first step in simultaneous determination of phenytoin, barbital and caffeine in mixtures by multivariate methods involved constructing the calibration matrix. In this work, we performed the calibration with the absorption spectra and the first-order derivative spectra. The wavelength range used was 190–300 nm in all cases. The multivariate calibration requires a careful experimental design of the standard composition of calibration set for providing the best predictions. In order to select the mixtures that provide more information from calibration set, their compositions were randomly designed. Four sets of standard solutions were prepared; the calibration sets contained 25 standard solutions for both binary and ternary determinations, so that the concentration of each drug in resulting solutions was in its own linear dynamic range The calibrations curves were constructed over the concentrations ranges between 0.24 and 22.0 $\mu g\,ml^{-1}$ for phenytion, 0.01 and 27.0 $\mu g\,ml^{-1}$ for barbital and 0.049 and 27.0 $\mu g\,ml^{-1}$ for caffeine in binary mixtures and 0.45 and 22.0 $\mu g\,ml^{-1}$ for phenytoin, 0.05 and 26.0 $\mu g\,ml^{-1}$ for barbital and 0.05 and 20.0 $\mu g\,ml^{-1}$ for caffeine in ternary mixtures.

Table 3
Composition of synthetic samples in ternary mixtures of prediction set

Mixture	Caffeine ($\mu g\,ml^{-1}$)	Barbital ($\mu g\,ml^{-1}$)	Phenytoin ($\mu g\,ml^{-1}$)
1	1.92	1.29	0.45
2	5.75	3.70	3.56
3	13.93	6.09	6.40
4	0.13	0.20	8.30
5	19.20	5.00	1.50
6	2.30	2.50	11.75
7	13.73	9.70	6.15
8	5.00	12.55	6.00
9	2.03	2.80	14.75
10	9.50	18.35	7.00
11	7.36	16.99	2.94
12	1.20	2.60	17.66
13	17.88	4.00	5.00
14	2.25	25.33	2.66
15	1.50	3.00	21.88

3.3.2. Data processing and model building

The digitized absorbance of calibration mixtures was gathered in a 25 × 221 data matrix (**Y**) and absorbances of prediction matrixes were collected in a 15 × 221 data matrix ($\mathbf{Y}_{un}$). The composition of the prediction sets of binary and ternary mixtures is given in Tables 2 and 3, respectively. The derivative of each spectrum was calculated with MATLAB. Also, a vector of concentration of each drug in calibration matrix was made (*c*). PLS and PCR methods were run on the calibration data of absorption (zero-order) UV spectra and first-order derivative spectra and concentrations in prediction sets were calculated at the optimum number of factors. The selection of the number of factors used in the calibration with these methods is very important for achieving the best prediction. As a first approach the number of factors were estimated by cross-validation method, leaving out one sample at a time

Table 2
Composition of synthetic samples in binary mixtures of prediction set

Mixture	Caffeine	Barbital ($\mu g\,ml^{-1}$)	Caffeine	Phenytoin ($\mu g\,ml^{-1}$)	Barbital	Phenytoin ($\mu g\,ml^{-1}$)
1	0.20	0.00	1.45	0.25	2.55	2.90
2	3.30	0.30	2.63	0.60	4.17	7.66
3	3.50	1.80	4.30	1.10	7.66	10.50
4	4.60	9.80	5.50	2.50	12.50	11.76
5	11.76	12.90	7.20	6.60	14.28	14.00
6	14.36	14.50	1.63	14.50	16.90	13.80
7	13.47	25.55	2.80	17.80	19.80	13.00
8	15.95	24.88	8.70	20.77	8.00	17.80
9	6.30	5.60	14.50	16.50	0.35	18.70
10	7.82	6.00	18.20	2.50	0.80	2.15
11	10.52	10.30	11.25	7.65	0.60	1.50
12	21.5	8.70	4.15	8.00	3.40	20.00
13	13.96	18.90	16.55	22.00	24.32	12.50
14	26.20	2.55	22.00	2.70	27.00	11.55
15	5.50	22.85	15.50	1.70	1.00	21.88

Table 4
Predicted concentrations obtained by application of PLS method on first-order derivative spectra and absorption UV spectra. for simultaneous determination of phenytoin, barbital and caffeine in ternary mixtures

Sample	PLS on absorption UV spectra			PLS on first-order derivative spectra		
	Phenytoin ($\mu g\,ml^{-1}$)	Barbital ($\mu g\,ml^{-1}$)	Caffeine ($\mu g\,ml^{-1}$)	Phenytoin ($\mu g\,ml^{-1}$)	Barbital ($\mu g\,ml^{-1}$)	Caffeine ($\mu g\,ml^{-1}$)
1	0.50	1.57	1.97	0.3	1.30	1.95
2	3.20	3.72	5.72	3.56	3.72	5.70
3	6.60	6.66	12.5	6.38	6.64	13.99
4	8.35	0.25	0.01	8.28	0.16	0.12
5	1.72	5.05	19.20	1.70	5.80	19.22
6	11.8	2.66	2.53	11.79	2.66	2.57
7	5.70	98.5	13.95	5.9	9.70	13.95
8	6.35	12.64	4.82	6.30	12.64	4.80
9	14.3	2.48	20.3	14.78	2.46	20.3
10	6.50	17.90	9.46	6.77	18.19	9.40
11	2.50	17.09	9.34	2.90	17.00	9.34
12	17.10	2.50	1.74	17.59	2.50	1.73
13	5.50	4.32	17.95	5.30	3.91	17.95
14	2.88	25.44	2.25	2.67	25.40	2.24
15	21.95	3.00	1.47	21.95	3.1	1.37

and plotting the prediction residual sum of squares (PRESS) versus the number of factors for each individual component. The (PRESS) for each number of factors was calculated by comparing the predicted concentration of compounds in each sample with known concentration of compounds in standard solutions. The PRESS can defined as:

$$\mathrm{PRESS} = \left(\sum_{j=1}^{N}(\hat{C}_j - C_j)\right)^2 \tag{1}$$

where N is the total number of calibration samples; C_j, the reference concentration for jth sample and $\hat{C}_j$ represents the estimated concentration of C_j. The PRESS values provide a measure of how well the training set is predicting the concentration for each number of factors. The F ratio probability is used to determine the significance of PRESS values greater than minimum. As the difference between the minimum PRESS and other PRESS values becomes smaller, the probability that each additional factor is significant becomes smaller [32]. In order to validate proposed methods we prepared four sets of synthetic mixtures which involved binary or ternary mixture of phenytoin, barbital or caffeine; 15 synthetic test samples in each set were analyzed with the proposed methods. Predicted concentrations obtained by application of PLS method on first-order derivative spectra and absorption UV spectra for simultaneous determination of phenytoin, barbital and caffeine in ternary mixtures are given in Table 4.

The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E.) of the prediction concentration [33],

$$\mathrm{R.S.E.}\ (\%) = 100 \times \left(\frac{\sum_{j=1}^{N}(\hat{C}_j - C_j)^2}{\sum_{j=1}^{N}(C_j)^2}\right)^{1/2} \tag{2}$$

where N is the number of samples; C_j, the concentration of the component in the jth mixture and $\hat{C}_j$ is the estimated concentration. The method was evaluated using statistical comparison between different applied methods on ternary mixtures of phenytoin, barbital and caffeine by models optimised; the results are in Tables 5 and 6.

3.3.3. Percent recovery study

This study was performed on the sets of prediction containing 15 binary or ternary mixtures of phenytoin, barbital and caffeine. The mean recoveries obtained for analytes in each set of binary samples by applying each method are summarized in Table 5; as can be seen the agreement between experimental and predicted values by all of the methods is good, but however, the better results for simultaneous determination of phenytoin, caffeine and barbital in binary mixtures can be obtained by application of PLS on first derivative spectra, and in the case of caffeine and barbital, better determination could be performed by applying PCR on first derivative spectra. In these three models, known concentrations of all tested samples in prediction sets were compared with the predicted concentrations by constructed models and equations and R^2 obtained when plots of predicted versus actual concentrations were constructed; very good agreement between actual and predicted values for all component are represented. The percentage of recoveries was also calculated for each component by application of PLS and PCR on UV absorption spectra and first-order derivative spectra in ternary mixtures; then mean recovery was calculated and the mean recovery values were obtained, The results obtained by application of constructed models by each method for simultaneous determination of drugs in ternary mixtures are presented in Table 6. The limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected [35].

Table 5
Correlation statistics of predicted vs. actual values for phenytoin and caffeine and barbital in prediction set of binary samples

Method	Compound	Linearity (μg ml^{-1})	R^{2}[a]	PRESS	Factor	Mean recovery (%)	LOD[b] (μg ml^{-1})	R.S.E. (%)
1	Phenytoin	0.030–28.0	0.9968	0.7	3	103.03	0.025	1.01
	Barbital	0.010–27.0	0.9983	1.24	5	102.19	0.010	0.80
2	Phenytoin	0.200–23.0	0.9976	0.07	4	99.03	0.015	0.40
	Barbital	0.010–27.0	0.9994	0.08	4	103.59	0.010	0.30
2	Phenytoin	0.240–22.0	0.9994	0.05	3	99.81	0.24	0.30
	Caffeine	0.049–22.0	0.9970	0.08	3	100.2	0.042	1.03

(1) PLS on absorption UV spectra, (2) PLS on first-order derivative spectra.
[a] The square of correlation coefficients.
[b] Limit of detections and limit of quantifications calculated by Miller and Miller method [35].

Table 6
Statistical comparison between different applied methods on ternary mixtures of phenytoin, barbital and caffeine by models optimized

Drug	Method	PRESS	Factors	Mean recovery (%)	LOD[a] (μg ml^{-1})	R.S.E. (%)
Phenytoin	1	1.25	6	100.57	0.33	1.16
	2	0.05	5	98.5	0.34	0.41
	3	3.3	4	94.89	0.43	2.01
	4	1.78	4	96.28	0.44	1.36
Barbital	1	1.2	4	103.82	0.05	0.83
	2	0.09	3	99.0	0.05	0.67
	3	1.1	4	106.87	0.05	0.78
	4	0.9	4	102.84	0.05	0.74
Caffeine	1	0.09	5	103.01	0.04	2.44
	2	0.09	4	1.042	0.05	2.02
	3	1.25	3	110.60	0.05	2.39
	4	1.12	3	1.08	0.04	2.12

(1) PLS on absorption UV spectra, (2) PLS on first-order derivative spectra, (3) PCR on absorption UV spectra, (4) PCR on first-order derivative spectra.
[a] Limit of detections and limit of quantifications calculated by Miller and Miller method [35].

The statistical comparisons between the two multivariate calibrations methods employed for ternary mixtures are also included in Table 6. By considering these results and also the values of relative standard error, it can be seen that application of PLS method on first-order derivative spectra represents better results for simultaneous determination of phenytoin, barbital and caffeine in ternary mixtures.

It is obvious that the predictive ability of PCR for phenytoin is less than that for caffeine and barbital. The observed trend is possibly due to significant overlapping of phenytoin

Table 7
Simultaneous determination of phenytoin, barbital and caffeine in pharmaceutical mixtures and spiked serum by application of PLS on first-order derivative spectra

	Amount taken (μg)			Found (μg)		
	Phenytoin	Barbital	Caffeine	Phenytoin	Barbital	Caffeine
Mixture						
1	3.0	20.0	6.0	3.2 ± 0.1	2.3 ± 0.1	5.8 ± 0.6
2	5.0	8.0	15.0	5.2 ± 0.3	8.3 ± 0.5	14.9 ± 0.2
3	15.0	12.0	8.0	14.8 ± 0.4	11.9 ± 0.2	7.9 ± 0.3
4	20.0	16.0	3.0	19.8 ± 0.4	16.3 ± 0.1	2.8 ± 0.1
5	12.0	0.3	10.0	12.0 ± 0.1	0.3 ± 0.2	10.0 ± 0.2
6	8.0	24.0	1.0	7.5 ± 0.3	24.1 ± 0.4	1.1 ± 0.8
7	0.5	0.5	20.0	0.47 ± 0.2	0.48 ± 0.3	20.1 ± 0.5
Spiked serum						
1	20.0	10.0	5.0	22.0 ± 0.1	10.5 ± 0.6	4.0 ± 0.8
2	10.0	25.0	10.0	9.0 ± 0.3	26.0 ± 0.2	10.8 ± 0.6
3	5.0	15.0	20.0	5.3 ± 0.3	14.5 ± 0.5	20.9 ± 0.3
4	2.0	2.0	15.0	1.8 ± 0.3	2.0 ± 0.3	15.9 ± 0.6

spectra with two other drugs; it is noteworthy that in most cases the application of PLS and PCR to first-order derivative spectra improved the performance of PLS modeling for phenytoin. On the other hand, it can be observed that the use of first-order derivative spectra has no significant effect on the predicting ability of the calibration models obtained by both methods for barbital and caffeine, and similar results were obtained by application of PLS and PCR methods with the use of the absorbance data or first derivative data, respectively. This paper testifies the high potential of PLS and PCR methods for multicomponent determinations even in the presence of strong spectral overlap between the analysts; in fact, both allow the effective resolution of binary and ternary mixtures of phenytoin, barbital and caffeine mixtures. However, PLS calibration can implicitly model some interference, whenever the calibration solutions and samples have similar compositions and interferences are included in variable concentrations in the calibration set. This allows the multicomponent determination without previous separation and makes the method more robust [34].

4. Application

In order to assess the applicability of the proposed method to the analysis of real samples, it was applied to the determination of these drugs in different mixtures of pharmaceutical formulation and serum samples. Thus, different mixtures of the commercially available phenytoin, barbital and caffeine tablet or capsule were prepared and analyzed by application of PLS to derivative spectra. Each measurement was repeated three times and relative standard deviation of prediction was also calculated. The results are shown in Table 7. As it is seen, in all mixtures the calculated values are in satisfactory agreement with the declared values. Serum samples were prepared by spiking blank serum with appropriate amounts of the stock solutions of phenytoin, barbital and caffeine.

5. Conclusion

Based on the results obtained in this work, application of UV spectrophotometric method for simultaneous determination of phenytoin, barbital and caffeine in binary and ternary mixtures by multivariate calibration of synthetic and pharmaceutical samples is an effective and accurate way. PLS and PCR using a calibration matrix constructed with the first-order derivative absorption spectra have been successfully applied to simultaneous analysis of these drugs in synthetic and pharmaceutical mixtures. It can be concluded that derivative techniques in combination with multivariate calibration methods are a good approach for obtaining reliable results for binary and ternary mixtures analysis in pharmaceutical mixtures. Their application is simpler and less expensive than the application of alternative techniques like chromatographic or immunoassay analysis. This technique is simple, fast, precise and affordable. Also, it requires no complex pretreatment or chromatographic separations of the samples containing analytes.

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