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Individual and simultaneous determinations of phenothiazine drugs using PCR, PLS and (OSC)–PLS multivariate calibration methods

MOHAMMAD ALI KARIMI[1,2,3]*, MOHAMMAD MAZLOUM ARDAKANI[4], REZA BEHJATMANESH-ARDAKANI[2], MOHAMMAD REZA HORMOZI NEZHAD[5] and HAMZEH AMIRYAN[2]

[1]*Department of Chemistry, Payame Noor University of Sirjan, Sirjan,* [2]*Department of Chemistry, Payame Noor University of Ardakan, Ardakan,* [3]*Department of Chemistry, Shahid Bahonar University of Kerman, Kerman,* [4]*Department of Chemistry, Yazd University, Yazd and* [5]*Department of Chemistry, Persian Gulf University, Bushehr, Iran*

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Abstract: Individual and simultaneous determinations of some phenothiazine drugs are described. The individual determination method is based on the reaction of chlorpromazine hydrochloride (CPH), promethazine hydrochloride (PH), trifluoperazine hydrochloride (TFPH), trimipramine maleate (TPM) and thioridazine hydrochloride (TRDH) with complex of $[Fe(Bpy)_3]^{3+}$. In the presence of phenothiazine derivatives, $[Fe(Bpy)_3]^{3+}$ is reduced easily to the coloured complex $[Fe(Bpy)_3]^{2+}$, which shows an absorption maximum at 525 nm. The individual method is highly sensitive and suitable for 0.3–190 μg ml^{-1} concentrations, with detection limits in the range 0.18–2.46 μg ml^{-1}. Simultaneous kinetic–spectrophotometric determination of ternary mixture of CPH, PH and TPM using principal component regression (PCR), partial least squares (PLS) and orthogonal signal correction (OSC)–PLS multivariate calibration methods is also described. The simultaneous methods are based on the difference observed in the reduction rate of the $[Fe(Bpy)_3]^{3+}$ complex with CPH, PH and TPM in acidic media. The results showed that the simultaneous determination of CPH, PH and TPM can be performed in the concentration ranges of 0.5–120.0, 0.3–80.0 and 5.0–100.0 μg ml^{-1}, respectively, for three methods (PCR, PLS and OSC–PLS). The root mean square errors of prediction (RMSEP) of CPH, PH and TPM were 0.346, 0.663 and 0.820 (for PCR) 0.317, 0.659 and 0.830 (for PLS) and 0.087, 0.124 and 0.085 (for OSC–PLS), respectively. The proposed methods were successfully applied to the individual and simultaneous determination of phenothiazine derivatives in pharmaceutical preparations, the results of which compared well with those obtained by the official method, and several synthetic (spiked) samples, whereby satisfactory results were obtained.

Keywords: individual and simultaneous determinations; phenothiazine drugs; PLS; PCR; OSC–PLS.

* Coresponding author. E-mail: m_karimi@pnu.ac.ir or ma_karimi43@yahoo.com
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INTRODUCTION

Phenothiazine derivatives, an important group of neuroleptics, are used as antihistamines, tranquilizers, anti-emetics and antiparkinson.[1] The therapeutic importance of these drugs has prompted many workers to develop methods for their individual and simultaneous determinations in body fluids, as well as in pharmaceutical.[2–4] There are various analytical procedures for the individual assay of phenothiazines, the most important of which being titrimetry,[5] conductometry,[6] voltammetry,[7] spectrofluorometry,[8] chemiluminescence,[9] HPLC[10] and GLC[11] methods. Many spectrophotometric methods for their determination have already been proposed based on the oxidation of the drugs to a coloured radical cation and the subsequent measurement of absorbance.[12–20] Unfortunately, some of these methods have some disadvantages, such as the use of non-aqueous media,[16] low sensitivity,[17] a heating step,[18] a very strong acid,[19] a low linear range and critical working conditions,[12,15] a narrow linear range of application[20] and a very narrow limit of detection.[13] In addition to this, the simultaneous determination of phenothiazine derivatives in binary and ternary mixtures was also reported.[21–26] Gutierrez *et al*. proposed a stopped-flow method for the simultaneous determination of perphenazine (PP) and chlorpromazine hydrochloride (CPH).[21] Chen *et al*. reported the simultaneous flow-injection determination of CPH and promethazine hydrochloride (PH) by a photochemical reaction.[22] The simultaneous kinetic determination of phenothiazine drugs was also reported.[23] Fasanmade reported a multivariate calibration method based on principal component regression (PCR) for the simultaneous ultraviolet (UV) determination of an oxidation product of CPH sulphoxide.[24] Shamsipur *et al*. tested partial least-squares (PLS) regression, singular value decomposition-based PLS, and an artificial neural network (ANN) as calibration procedures for the simultaneous determination of PH, CPH, and PP by both conventional and derivative spectrophotometry.[25] Recently, Hemmateenejad *et al*. reported the simultaneous determination of a ternary mixture of PH, CPH and PP based on the net analyte signal (NAS)–ANN model using conventional and derivative absorbance spectra.[26] To the best of our knowledge, no chemometrics methods for the simultaneous determination of these drugs using kinetic–spectrophotometric methods have been reported.

The theories and applications of chemometric methods, such as PCR and PLS, to the analysis of multi-component mixtures have been discussed by several workers.[27–32] Multivariate calibration methods have been successfully applied to multi-component kinetic determination in order to overcome some of the drawbacks of classical methods. Soft algorithms, such as PCR, PLS and ANN, which avoid co-linearity problems, have been used for the simultaneous determination of analytes having the same chemical properties, which cannot be resolved with common methods.

The formation of the complex between Fe(III) and 2,2'-bipyridine (bpy) is the basis of existing spectrophotometric methods for the determination of trace amounts of reducing agents.[33] Reducing agents can be determined through reducing the $[Fe(bpy)_3]^{3+}$ complex, followed by treating the coloured complex of $[Fe(bpy)_3]^{2+}$. Recently, a kinetic–spectrophotometric determination of a ternary mixture of hydrazine and its derivatives by PCR and PLS methods based on the difference observe in the rate of reduction of $[Fe(bpy)_3]^{3+}$ with hydrazine, thiosemicarbazide and phenylhydrazine in a micellar media of sodium dodecyl sulphate (SDS) and buffer of pH 3.0 was reported.[34] In this paper, a new, simple, rapid and sensitive indirect spectrophotometric method for the individual microdetermination of five phenothiazine drugs, containing CPH, PH, trifluoperazine hydrochloride (TFPH), trimipramine maleate (TPM) and thioridazine hydrochloride (TRDH). Also, in this study, principal component regression (PCR), partial least squares (PLS) and orthogonal signal correction (OSC)–PLS multivariate calibration methods for the analysis of ternary mixtures of CPH, PH and TPM, using the observed difference in the reduction rate of $[Fe(bpy)_3]^{3+}$ with these drugs in acidic media, were used.

EXPERIMENTAL

Apparatus and software

A GBC UV–Visible Cintra 6 Spectrophotometer with 1-cm glass cells, attached to a Pentium IV computer was used for recording the absorbance spectra and the kinetic spectrophotometric data. A Metrohm 780 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 1.00 and 7.00. Measurements of the pH were made with a Metrohm 691 pH-meter using a combined electrode. The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using Matlab software. PLS and PCR analysis were performed using PLS and PCR toolboxes in the Matlab program version 7.0.

Reagents and standard solutions

All reagents were of analytical reagent grade. Triply distilled water was used throughout. Stock solutions (1000 μg ml^{-1}) of chlorpromazine hydrochloride, promethazine hydrochloride, trifluoperazine hydrochloride, trimipramine maleate and thioridazine hydrochloride (all from Biochemicals Inc., USA) were prepared by dissolving 100 mg each of the phenothiazine salts in distilled water and diluting to the mark in 100 ml volumetric flasks. These solutions were spectrophotometrically stable for at least 48 h. Standard solutions were prepared by appropriate dilution of the above solutions. A stock solution of 5.0×10^{-2} M 2,2'-bipyridine was prepared by dissolving 0.784 g of 2,2'-bipyridine (Merck) in water and diluting to the mark in a 100 ml volumetric flask. A stock solution of 5×10^{-2} M of Fe(III) was prepared by dissolving 2.43 g of $Fe(NH_4)(SO_4)_2\cdot12H_2O$ (Merck) in water and diluting to the mark in a 100 ml volumetric flask. A buffer solution of pH 4 was prepared using sodium acetate and hydrochloric acid at appropriate concentrations.

General procedure

The $[Fe(bpy)_3]^{3+}$ complex as the oxidizing agent for both the individual and simultaneous method of determination was prepared daily in a 25 ml volumetric flask by the addition

of 2.5 ml of buffer solution (pH 4.0), 0.2 ml of Fe(III) solution (0.05 M) and 1.0 ml of bpy solution (0.05 M) and then diluting with water to the mark. For each measurement in the individual determination, 2.0 ml of the above solution was transferred to a spectrophotometer cell, then an appropriate volume of CPH, PH, TFPH, TPM or TRDH in the range of 0.5–120, 0.3–100, 7–190, 5–100 and 3–120 μg ml^{-1}, respectively, was injected into the cell using a micro-syringe and the absorbance was recorded at 525 nm after 200 s. In the simultaneous determination method, the temperature was thermostated at 25 °C for 10 min, 2.4 ml of solution was transferred into the glass cell of the spectrophotometer and the absorbance of this solution was zeroed before injecting the analyte(s). Then, an appropriate volume of CPH, PH, TPM or a mixture of them in their concentration ranges was injected into the cell using a micro-syringe and absorbance was recorded at 525 nm every 2.0 s.

Sample preparation procedure for tablets and injections

Twenty tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg of phenothiazine salt was transferred into a 100 ml volumetric flask and diluted to the mark with water. The powder was completely dispersed using a mechanical stirrer and the solution was filtered. A suitable aliquot of this solution within the working range of the individual phenothiazine was treated as described in the recommended procedure.

An accurately measured volume from the injections was appropriately diluted to obtain 500 μg ml^{-1} of phenothiazine salt solution. A suitable aliquot of this solution was taken and the recommended procedure followed for the analysis of the drug content.

RESULTS AND DISCUSSION

The Fe(III)–bpy system allows the spectrophotometric determination of a reducing agent, A_{red}, as follows:[33,34]

$$n[Fe(bpy)_3]^{3+} + A_{red} \rightarrow n[Fe(bpy)_3]^{2+} + A_{ox}$$

The above reaction is completed with the formation of an equivalent amount of $[Fe(bpy)_3]^{2+}$ with respect to the *n*-electron reductant, A_{red}. The reduction of $[Fe(bpy)_3]^{3+}$ to the complex $[Fe(bpy)_3]^{2+}$ (with λ_{max} = 525 nm) is completed in the presence of a suitable reducing agent, such as phenothiazine derivatives, in a few minutes. A linear correlation was found between the absorbance at λ_{max} for each drug and the concentration in the range given in Table I. The intercepts, slopes and correlation coefficients for the calibration data of the phenothiazine drugs are also presented in Table I.

The reduction rate of $[Fe(bpy)_3]^{3+}$ with CPH, PH and TPM is different. This difference provides the possibility for resolving their mixtures using PCR, PLS and OSC–PLS multivariate calibration methods. The characteristics of the calibration curves for the determination of CPH, PH and TPM for PCR, PLS and OSC–PLS are given in Table I.

A series of experiments was conducted to establish the optimum analytical parameters to achieve maximum sensitivity in the individual determination of CPH, PH, TFPH, TPM and TRDH and the simultaneous determination of CPH, PH and TPM. The experimental parameters, such as the concentrations of the reagents, temperature and pH of the solution were optimized. The optimization process gave similar results for both methods (individual and simultaneous determinations).

TABLE I. Analytical parameters for the determination of phenothiazine drugs

Parameter	CPH	PH	TFPH	TPM	TRDH
Colour	Pink	Pink	Pink	Pink	Pink
λ_{max} / nm	525	525	525	525	525
Stability, h	48	48	48	48	48
The Beer law limits, μg ml^{-1}	0.5–120	0.3–80	7–190	5–100	3–120
Detection limit[a], μg ml^{-1}	0.33	0.18	1.60	1.20	1.20
Molar absorptivity, cm^2 mol^{-1}	0.263	0.227	0.631	0.356	0.350
The Sandell sensitivity μg cm^{-2} per A unit	13.5	14.1	7.6	11.5	11.6
Regression equation[b]					
Regression coefficient (r)	0.9982	0.9984	0.9988	0.9989	0.9987
Slope (b / cm^3 mg^{-1})	13.5	14.1	6.5	11.5	11.6
Intercept (a)	0.1688	0.0490	0.1238	0.0941	0.1149
RSD[c] / %	1.2	1.6	0.9	1.4	1.5

[a]Theoritical detection limit ($3S_b$ or three times the standard deviation of the blank);[23] [b]$A = a + bC$, where A is the absorbance for concentration c in mg cm^{-3}; [c]relative standard deviation (calculated from five determinations)

Absorption spectra

The reagent blank does not absorb in visible range of the spectrum but when CPH, PH, TFPH, TPM or TRDH has reacted with $[Fe(bpy)_3]^{3+}$, a pink-coloured cationic product, $[Fe(bpy)_3]^{2+}$, is formed, with a peak of absorbance at 525 nm. The absorption spectra of the products and reagent blank are shown in Fig. 1.

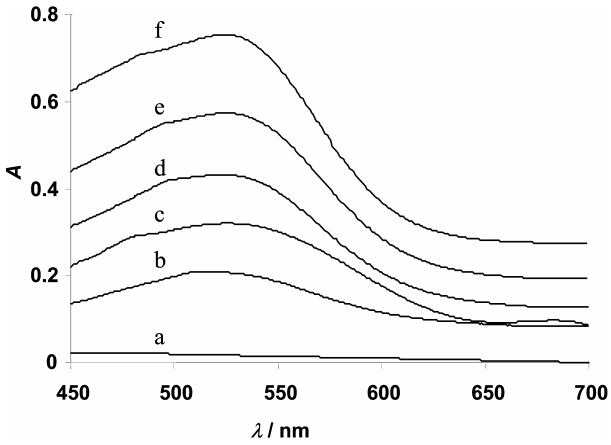

Fig. 1. Absorption spectra of the Fe(III)–bpy reagent blank (a), Fe(III)–bpy complex in the presence of 40 μg ml^{-1} of TFPH (b), TRDH (c), TPM (d), CPH (e) and PH (f).

Effect of Fe(III) and bpy concentrations

The effect of the Fe(III) and bpy concentrations, in the ranges 5.0×10^{-4}–-1.0×10^{-2} and 5.0×10^{-4}–5.0×10^{-3} M, respectively, were studied. At a constant concentration of Fe(III) of 4.0×10^{-4} M, the bpy concentration was varied in the above-mentioned range. For each CPH, PH, TFPH, TPM and TRDH in their individual determination and in the ternary mixture of CPH, PH, and TPM in their simultaneous (for the three PCR, PLS and OSC–PLS methods) determi-

nation, increasing the bpy concentration up to 2.0×10^{-3} M resulted in an increase in the reaction rate and the absorbance. However, at higher concentrations of bpy, a decrease in the reaction rate and absorbance was observed. This might be due to the fact that high concentrations of bpy would result in a positive interference from Fe(III), which could have arisen from incomplete conversion of Fe(II) into the $[Fe(bpy)_3]^{2+}$ complex *via* mixed ligand complex formation. Therefore, the bpy concentration of 2.0×10^{-3} M was selected as the optimum concentration.

The effect of the Fe(III) concentration on the reaction rate and absorbance of CPH, PH, TFPH, TPM and TRDH at constant concentration of bpy (2.0×10^{-3} M) was studied. Increasing the Fe(III) concentration up to 4.0×10^{-4} M resulted in an increase in the reaction rate and absorbance in the individual determinations of CPH, PH, TFPH, TPM and TRDH and the simultaneous determination of CPH, PH and TPM. However, at higher concentrations of Fe(III), a decrease in the reaction rate and absorbance was observed. Thus, for both the individual and simultaneous (for each three PCR, PLS and OSC–PLS methods) determinations of these drugs, a Fe(III) concentration of 4.0×10^{-4} M was chosen as the optimum concentration for further studies.

Effect of pH

The effects of pH on the absorbance and reduction of $[Fe(bpy)_3]^{3+}$ by CPH, PH, TFPH, TPM and TRDH and the formation of the $[Fe(bpy)_3]^{2+}$ complex, as well as on the reaction rates were studied over the pH range 1.0–6.0. The effect of pH on the absorbance of the solution mixture is shown in Fig. 2. The absorbance and reaction rate increased with increasing pH up to 4.0, but thereafter decreased. Therefore, for both the individual and simultaneous (for each three PCR, PLS and OSC–PLS methods) determinations of these drugs, a pH value of 4.0 (acetate buffer) was chosen as the optimal pH for further studies.

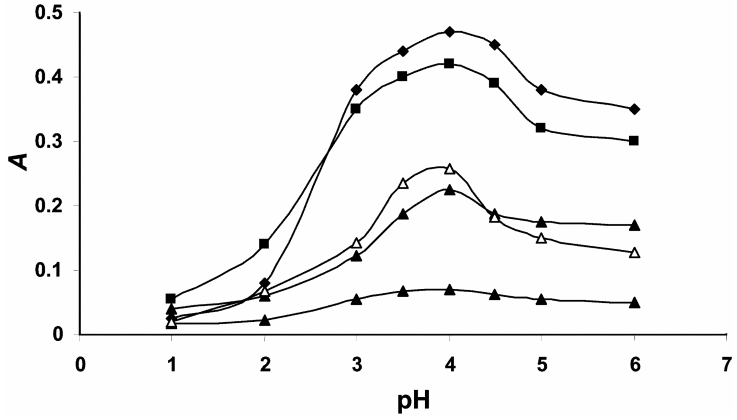

Fig. 2. Effect of pH on the absorbance of 20 μg ml^{-1} of TPM (●), TFPH (▲), TRDH (Δ), CPH (♦) and PH (■). Conditions: 4×10^{-4} M Fe(III); 2×10^{-3} M bpy; 25 °C temperature.

Effect of temperature, reaction time and stability of the colour

The effect of temperature on the absorbance and reaction rates was studied in the range of 25–70 °C. From the results, it can be concluded that increasing the temperature led to an increase in the reaction rates for each five analytes but temperature had no effect on the absorbance. However, for the sake of simplicity and for a better control of temperature effects on the precision of determinations, 25 °C was chosen as the optimal temperature. In the individual determinations, at room temperature (25 °C), it was necessary to wait for at least 2 min after drug addition before the absorbance was measured to allow its reaction with the $[Fe(bpy)_3]^{3+}$ complex to go to completion. Therefore, all absorbance values were measured after 200 s from the initiation of the reaction. The coloured products were stable for at least 48 h.

Absorbance–time behaviour

Under the optimized conditions, the reactions of CPH, PH and TPM with $[Fe(bpy)_3]^{3+}$ complex showed different kinetic behaviours (Fig. 3). These differences in the reaction rates allowed multivariate calibration methods to be designed as techniques for the simultaneous determination of CPH, PH and TPM.

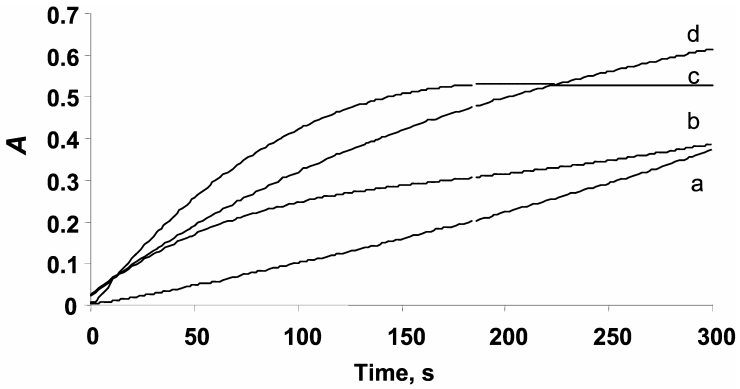

Fig. 3. Absorbance changes of the Fe(III)/bpy complex *vs*. time in the reaction with: 10 μg ml^{-1} of TPM (a), CPH (b), 1 PH (c) and a mixture of them (d).

Accuracy and precision

In the individual determination method, the accuracy of the method was established by analyzing the pure drugs at three concentration levels and the precision by determining the relative standard deviation (*RSD*) for seven replicate analyses on the same solution containing three different concentration levels for each drug (Table II).

Multivariate calibration and statistical parameters

Multivariate calibration methods, such as PCR, PLS and OSC–PLS, require a suitable experimental design of a standard belonging to the calibration set in

order to provide for a good prediction. The first step in the simultaneous determination of the drugs by the PCR, PLS and OSC–PLS methodologies involved constructing a calibration mixture for the mixtures of CPH, PH and TPM. A synthetic set of 40 solutions of mixtures of CPH, PH and TPM were prepared according to the experimental design of three factors at three levels. The concentration ranges used were 0.5–120.0, 0.3–80.0 and 0.50–100.0 μg ml^{-1} for CPH, PH and TPM, respectively. From these series, 27 solutions were chosen for the calibration set (Table III) and the other 13 were used as the prediction set (Table IV). Changes in the absorbance of the solutions were recorded during a period of 300 s.

TABLE II. Accuracy and precision data

Pheothiazine derivative	Amount, μg ml^{-1}		*RSD* / % (n = 7)
	Taken	Found	
CPH	2	2.0	1.9
	50	50.6	2.4
	110	111.7	2.9
TFPH	8	7.9	3.1
	60	61.1	2.0
	180	177.2	2.5
PH	0.5	0.5	2.8
	10	10.3	1.6
	60	59.2	2.5
	5	4.8	2.7
TPM	30	30.4	2.6
	70	68.9	2.1
	20	19.6	2.5
TRDH	75	76.4	1.9
	100	98.7	2.8

TABLE III. Calibration set for constructing the PCR and PLS models in the determination of CPH, PH and TPM

Sample	Concentration, μg ml^{-1}			Sample	Concentration, μg ml^{-1}		
	CPH	PH	TPM		CPH	PH	TPM
1	5.0	1.0	9.0	15	7.0	3.0	15.0
2	5.0	1.0	12.0	16	7.0	6.0	9.0
3	5.0	1.0	15.0	17	7.0	6.0	12.0
4	5.0	3.0	9.0	18	7.0	6.0	15.0
5	5.0	3.0	12.0	19	10.0	1.0	9.0
6	5.0	3.0	15.0	20	10.0	1.0	12.0
7	5.0	6.0	9.0	21	10.0	1.0	15.0
8	5.0	6.0	12.0	22	10.0	3.0	9.0
9	5.0	6.0	15.0	23	10.0	3.0	12.0
10	7.0	1.0	9.0	24	10.0	3.0	15.0
11	7.0	1.0	12.0	25	10.0	6.0	9.0
12	7.0	1.0	15.0	26	10.0	6.0	12.0
13	7.0	3.0	9.0	27	10.0	6.0	15.0
14	7.0	3.0	12.0				

TABLE IV. Prediction set for constructing the PCR, PLS and O–PLS models in the determination of CPH, PH and TPM

Sample	Amount added μg ml^{-1}			Amount predicted, μg ml^{-1}								
				PCR			PLS			O–PLS		
	CPH	PH	TPM	CPH	PH	TPM	CPH	PH	TPM	CPH	PH	TPM
1	5.75	2.25	9.00	5.79	2.04	9.55	5.78	2.04	9.52	5.70	2.22	8.82
2	6.00	2.00	9.00	5.85	2.08	8.21	5.84	2.08	8.21	5.95	1.90	8.92
3	6.00	3.10	9.75	5.94	3.17	9.67	5.94	3.16	9.61	5.95	3.14	9.75
4	4.50	3.50	8.25	4.87	2.92	10.20	4.86	2.91	10.19	4.60	3.43	8.21
5	9.00	1.75	12.75	8.95	1.74	13.01	8.94	1.75	13.04	8.92	1.81	12.69
6	9.50	1.25	13.00	9.41	1.36	12.55	9.41	1.36	12.55	9.5	1.22	13.05
7	7.75	3.25	12.25	7.91	3.08	13.25	7.90	3.10	13.33	7.78	3.35	12.25
8	8.50	3.25	13.25	8.62	3.21	13.06	8.61	3.23	13.13	8.65	3.18	13.25
9	9.25	5.00	15.00	9.36	4.47	16.63	9.33	4.50	16.73	9.12	4.91	15.02
10	8.50	6.00	15.25	9.00	5.43	18.74	9.03	5.45	18.80	8.57	6.35	15.26
11	8.75	4.50	14.25	9.42	3.21	19.97	9.39	3.23	20.00	8.67	4.69	14.28
12	7.50	5.50	13.00	8.24	3.75	20.06	8.21	3.75	20.02	7.33	5.53	13.11
13	15.50	3.20	21.50	15.23	3.08	21.90	15.2	3.12	22.06	15.20	3.16	21.68

To select the number of factors in the PCR, PLS and OSC–PLS algorithm, a cross-validation leaving out one sample method was employed.[35] The prediction error was calculated for each compound for the prediction set. This error was expressed as the prediction residual error sum of squares (*PRESS*):

$$PRESS = \sum_{i=1}^{m} (c_i^{\mathrm{E}} - c_i)^2 \tag{1}$$

where m is the total number of calibration samples, c_i^{E} represents the estimated concentration and c_i is the reference concentration for the i-th sample left out of the calibration during cross validation. A plot of *PRESS* against the number of factors for a mixture of the components is shown in Fig. 4. To find the smallest number of factors, F-statistics was employed to perform the significant determination.[35] The optimal number of factors yielding the smallest error (*PRESS*) for the three compounds was found to be 3 for PCR, PLS and OSC–PLS. The validation step of the methodologies was performed by running PCR, PLS and OSC–PLS on the prediction set.

For the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction (*RMSEP*) and the relative standard error of prediction (*RSE*) can be employed:[28]

$$RMSEP = \sqrt{\sum_{i=1}^{N} \frac{(c_i^{\mathrm{E}} - c_i)^2}{n}} \tag{2}$$

$$RSE = \sqrt{\frac{\sum_{i=1}^{N}(c_i^{\mathrm{E}} - c_i)^2}{\sum_{i=1}^{N} c_i^2}} \times 100 \quad (3)$$

where c_i^{E} represents the estimated concentration, c_i and n are the actual analyte concentration and the number of samples, respectively.

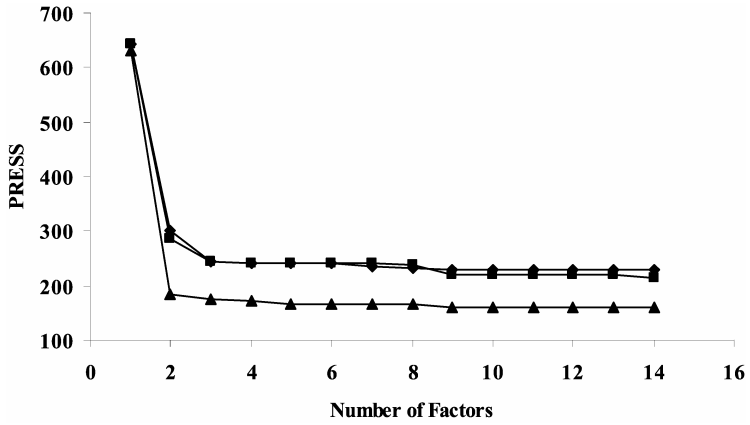

Fig. 4. Plot of *PRESS* against the number of factors for a mixture of CPH, PH, TPM for the PCR (♦), PLS (■) and O–PLS (▲) methods.

The square of the correlation coefficient (R^2), which is an indication of the quality fit of all the data to a straight line, is given by:

$$R^2 = \frac{\sum_{i=1}^{N}(c_i^{\mathrm{E}} - c_{\mathrm{m}})^2}{\sum_{j=1}^{N}(c_i - c_{\mathrm{m}})^2} \quad (4)$$

where c_{m} represents the mean of the actual concentration in the prediction set.[36]

The values of *RSE*, *RMSEP* and R^2 for each component using PLS, PCR and OSC–PLS are given in Table V, from which it can be seen that the obtained values for the statistical parameters were almost the same for the PLS and PCR methods, while the best results were obtained using the OSC–PLS method.

TABLE V. Statistical parameters calculated for the prediction set using the PCR, PLS and O–PLS models

Component	*RSE* / %			*RMSEP*			R^2		
	PCR	PLS	O–PLS	PCR	PLS	O–PLS	PCR	PLS	O–PLS
CPH	3.97	3.78	1.03	0.346	0.317	0.087	0.9869	0.9879	0.9988
PH	4.50	4.36	3.30	0.663	0.659	0.124	0.8836	0.8812	0.9949
TPM	5.08	4.80	0.65	0.820	0.830	0.085	0.7273	0.7335	0.9998

Interference studies

Interference by commonly associated excipients in pharmaceutical preparation, such as talc, glucose, starch, lactose, dextrose, sodium alginate and magnesium stearate, was investigated by preparing synthetic mixtures containing 20 μg ml^{-1} of each drug and 10-fold excess amounts of the excipients. The tolerance limit was defined as the concentration which gave an error of 3 % or less in the determination of 20 μg ml^{-1} of drug. The results are presented in Table VI, from which it is clear that the method is free from interferences of excipient species. Only ascorbic acid appeared to interfere with drugs in this method. The interference of ascorbic acid was eliminated when the synthetic sample solution was measured after times greater or equal to one hour.

TABLE VI. Recovery of 20 μg ml^{-1} of phenothiazine drugs from solutions with a 10-fold concentration of various additives used as excipients

Additive	Recovery of phenothiazine drug ± *RSD*[a], %				
	CPH	TPM	TRDH	PH	TFPH
Talc	100.6±1.1	101.0±0.8	100.8±0.9	101.8±1.4	101.0±0.6
Glucose	101.9±1.2	100.8±1.3	102.8±1.5	99.6±1.1	100.5±0.8
Starch	98.7±1.3	100.2±0.7	100.4±0.9	99.2±1.4	103.0±1.2
Lactose	102.5±1.0	100.6±1.7	101.9±1.3	100.8±0.8	98.0±1.5
Dextrose	98.5±1.3	101.3±0.8	101.6±1.3	102.6±1.4	101.4±1.1
Sodium alginate	101.6±1.0	100.9±1.5	01.6±1.0	100.2±0.7	101.4±1.4
Magnesium stearate	100.6±1.4	100.4±0.6	102.8±1.6	100.2±0.7	101.7±1.4

[a]Average of four determinations

Application

The proposed individual method was successfully applied to the determination of CPH, PH, TFPH, TPM and TRDH in pharmaceutical preparations. The same samples were also analyzed by the British Pharmaceutical (BP) official method,[37] and recovery percent, standard deviation (*SD*), T-test and F-test values were calculated (Table VII). The results reveal that similar degrees of accuracy and precision are afforded by both methods.

TABLE VII. Results of the determination of the studied drugs in pharmaceutical formulations

Drug and formulation[a]	Label claim mg/tablet or mg/ml	Found[b] (recovery ± *SD* / %)		Student's T-value[c]	F-value[d]
		Proposed method	Official BP method		
		CPH			
Tablet (1)	25	99.42±0.86	99.23±1.14	1.64	3.85
Tablet (1)	100	98.89±0.96	100.76±0.92	1.44	1.90
Injection (1)	25	99.85±0.54	101.24±0.82	1.96	2.65
		TFPH			
Tablet (2)	1	99.34±1.12	99.64±0.89	2.24	2.84
Tablet (2)	5	100.8±0.70	99.36±0.58	1.85	2.75

TABLE VII. Continued

Drug and formulation[a]	Label claim mg/tablet or mg/ml	Found[b] (recovery ± *SD* / %)		Student's T-value[c]	F-value[d]
		Proposed method	Official BP method		
		TFPH			
Tablet (2)	10	99.04±1.06	100.60±0.81	1.45	2.90
Injection (2)	1	98.96±0.70	100.34±0.58	1.76	3.85
		PH			
Tablet (1)	25	99.10±0.82	99.56±0.76	2.34	2.65
Injection (1)	25	98.28±0.56	98.56±0.64	2.50	3.75
		TPM			
Tablet (1)	25	101.60±0.48	99.42±0.72	1.90	1.82
Injection (1)	100	101.84±0.64	100.30±0.43	1.58	2.95
		TRDH			
Tablet (3)	10	100.80±0.94	99.92±0.82	2.15	2.88
Tablet (3)	25	100.40±0.76	100.74±0.54	2.60	2.05
Tablet (3)	100	101.24±0.98	100.16±0.42	2.05	3.25

[a]Marketed by: 1 – Tehran Chimi; 2 – Iran Daru Pakhsh; 3 – Pars Minoo; [b]average of five determinations ± standard deviation; [c]tabulated Student's T-value at the 95 % confidence level is 2.78; [d]tabulated F-value at the 95 % confidence level is 6.39

In order to assess the applicability of the proposed simultaneous determination methods (PCR, PLS and OSC–PLS) to the analysis of real samples, it was applied to the determination of the three phenothiazine derivatives in different synthetic mixtures. Thus, six different mixtures used in commercially available CPH, PH and TPM tablets were prepared and analyzed. Each measurement was repeated 3 times. The deviation results (Table VIII) show that the calculated values for all mixtures were in satisfactory agreement with the declared values.

TABLE VIII. Simultaneous determination of phenothiazines in tablet mixtures by application of PLS, PCR and OSC–PLS

Sample	Taken, mg			Recovery, %								
				PCR			PLS			O–PLS		
	CPH	PH	TPM	CPH	PH	TPM	CPH	PH	TPM	CPH	PH	TPM
1	6.00	3.00	11.00	95.66 ±0.98	103.53 ±1.26	95.63 ±1.04	102.42 ±1.22	101.24 ±0.96	101.25 ±1.13	100.64 ±0.62	101.20 ±1.05	97.36 ±1.40
2	7.00	3.50	12.00	97.85 ±1.06	99.42 ±0.86	99.00 ±0.92	99.20 ±0.82	105.20 ±1.15	104.34 ±0.74	98.82 ±1.30	97.23 ±0.65	104.17 ±0.81
3	10.00	5.00	17.00	98.50 ±0.75	104.00 ±1.47	95.52 ±0.58	98.50 ±0.56	102.10 ±1.18	96.47 ±0.83	103.23 ±1.17	105.32 ±1.22	96.25 ±0.85
4	6.50	3.20	10.00	97.38 ±0.68	106.20 ±1.80	97.80 ±0.66	104.52 ±0.67	97.20 ±1.24	100.52 ±0.89	99.05 ±0.57	102.06 ±1.25	99.08 ±0.98
5	9.50	5.00	16.50	96.31 ±0.94	103.20 ±0.72	96.54 ±1.33	100.66 ±0.78	102.76 ±1.14	101.35 ±1.69	94.78 ±1.85	98.32 ±0.94	103.45 ±1.63
6	10.00	4.50	13.00	98.30 ±0.63	105.05 ±1.08	100.92 ±0.90	96.24 ±0.66	103.02 ±0.88	99.48 ±0.96	103.88 ±0.86	102.76 ±1.56	102.29 ±1.39

CONCLUSIONS

This individual determination method is simple, rapid, quite selective and highly sensitive in comparison to other reported methods. The other advantages of the present method over the previous methods include rate of development and stability of the colour of the product, wide range of determination without the necessity for heating or extracting, low detection limit with high accuracy and precision. The high λ_{max} (in the visible region) of the proposed method is a decisive advantage, since interference from associated excipients was not observed. Furthermore, in this method, toxic organic solvents are not required. In other words, it belongs to green chemistry. Thus, it is hoped that this method can be used as an alternative for the rapid and routine microdetermination of bulk samples and various pharmaceutical formulations. In short, the proposed method is a step towards this direction.

In this study, it was shown that the application of multivariate calibration methods, such as PCR, PLS and OSC–PLS, could be well applied for the simultaneous determination of CPH, PH and TPM. The three proposed methods are cheaper than chromatographic methods. Furthermore, in these methods, the use of toxic organic solvents is not required. In other words, they belong to green chemistry. The proposed methods as new, inexpensive and sensitive methods offers good selectivity, accuracy and precision and can be applied for a wide range of CPH, PH and TPM concentrations.

И З В О Д

ПОЈЕДИНАЧАНА И УПОРЕДНА ОДРЕЂИВАЊА ФЕНОТИАЗИНСКИХ ЛЕКОВА ПОМОЋУ CR, PLS И (OSC)–PLS КАЛИБРАЦИОНИХ МЕТОДА СА ВИШЕ ПРОМЕНЉИВИХ

MOHAMMAD ALI KARIMI[1,2,3], MOHAMMAD MAZLOUM ARDAKANI[4], REZA BEHJATMANESH-ARDAKANI[2], MOHAMMAD REZA HORMOZI NEZHAD[5] и HAMZEH AMIRYAN[2]

[1]Department of Chemistry, Payame Noor University of Sirjan, Sirjan, [2]Department of Chemistry, Payame Noor University of Ardakan, Ardakan, [3]Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, [4]Department of Chemistry, Yazd University, Yazd and [5]Department of Chemistry, Persian Gulf University, Bushehr, Iran

У раду је описано појединачно и упоредно одређивање неких фенотиазинских лекова. Метода појединачног одређивања заснована је на реакцији хлорпромазин-хидрохлорида (CPH), прометазин-хидрохлорида (PH), трифлуороперазин-хидрохлорида (TFPH), тримипрамин-малеата (TPM) и тиоридазин-хидрохлорида (TRDH) са комплексним јоном $[Fe(bpy)_3]^{3+}$. У присуству фенитазинских деривата, $[Fe(bpy)_3]^{3+}$ се лако редукује формирајући обојен комплексни јон $[Fe(bpy)_3]^{3+}$, који показује абсорбциони максимум при таласној дужини од 525 nm. Метода је веома осетљива и погодна за одређивањас у опсегу концентрација 0,3–190 μg ml^{-1}, са границом детекције од 0,18–2,46 μg ml^{-1}. У раду је такође описано спектрофотометријско одређивање тројних смеша CPH, PH и TPM применом мултиваријантних метода калибрације (PCR, PLS и OSC–PLS). Методе симултаног одређивања заснивају се на различитој брзини редукције комплексног јона $[Fe(бру)_3]^{3+}$ са CPH, PH и TPM у киселој средини. Резултати указују на то да CPH, PH и TPM могу бити упоредно одређени методама PCR, PLS и OSC–PLS у

областима концетрације 0,5–120,0; 0,3–80,0 и 5,0–100,0 μg ml^{-1}, респективно. Средња квадратна грешка предвиђања концентрације CPH, PH и TPM износи 0,346; 0,663 и 0,820 за методу PCR, 0,317; 0,659 и 0.830 за методу PLS и 0,087; 0,124 and 0,085 за методу OSC–PLS, респективно. Предложене методе су успешно примењене за појединачно и истовремено одређивање фенотиазинских деривата у фармацеутским препаратима, чији се резултати добро слажу са званичном методом, и на неколоко синтетичких узорака, при чему су добијени задовољавајући резултати.

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