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Original Scientific Paper

Simultaneous Kinetic Spectrophotometric Determination of Hydrazine and Isoniazid Using H-Point Standard Addition Method and Partial Least Squares Regression in Micellar Media

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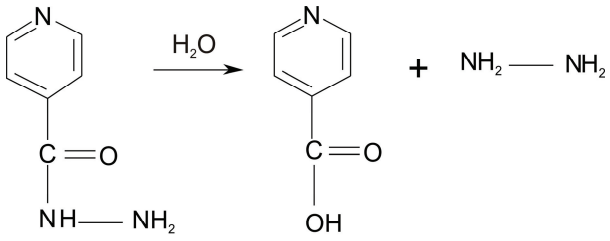
Abstract. The present study describes the application of simultaneous kinetic spectrophotometric determination of hydrazine (HZ) and isoniazid (INH), using H-point standard addition method (HPSAM) and partial least squares (PLS) calibration. The methods are based on the difference observed in the rate of iron (III) reduction with HZ and INH, in the presence of 2,2'-bipyridine (Bpy) and the subsequent complex formation between the resulted Fe^{2+} and Bpy in a solution containing sodium dodecyl sulfate (SDS) as a micellar medium. INH and HZ can simultaneously be determined between the range of 0.08−6.0 and 1.0−80.0 μg mL^{-1}, respectively. The results have shown that by the application of HPSAM, the simultaneous determination could be performed with the ratio of 1:1000 to 1:12.5 for INH-HZ. Through the HPSAM analysis, the relative standard deviations of HZ and INH were 2.5 and 1.2, respectively. The total relative standard error for applying the PLS method to 9 synthetic samples, in the concentration ranges of 0.0−20.0 μg mL^{-1} of HZ and 0.5−3.0 μg mL^{-1} of INH, was 3.19. Both proposed methods (PLS and HPSAM) were successfully applied to the simultaneous determination of HZ and INH in several commercially available isoniazid formulations and satisfactory results were obtained.

Keywords: Isoniazid, Hydrazine, Simultaneous determination, HPSAM, PLS

INTRODUCTION

Isonicotinic acid hydrazide, commercially known as isoniazid (INH), is an antitubercular drug and now is widely used together with other antituberculostatic drugs for the chemotherapy of tuberculosis. Determination of INH, as a bacteriostatic drug, is important in pharmaceutical preparations and biological fluids. INH has different metabolites that cause hepatoxicity and are readily absorbed by oral, dermal, or inhalation routes of exposure.[1] Hydrazine (HZ), one of isoniazid's principal degradation products, is a known carcinogen and considerably more toxic than isoniazid.[1,2] INH, itself, has been reported to be carcinogenic in mice[1] but the carcinogenic activity is probably due to the release of free hydrazine. This drug hydrolyzes by the passage of time and inappropriate storage conditions such as high temperature to HZ according to the following equation (Scheme 1):[2]



Scheme 1.

The determination of HZ, as an impurity or a synthetic intermediate, in pharmaceutical preparation of INH and biological fluids is important.

Several techniques, such as titrimetry,[3,4] electroanalytical techniques,[5,6] spectrophotometry,[7,8] fluorimetry[9,10] and chemiluminescence[11–13] methods, have been applied for the individual quantification of INH and HZ. A few reports on simultaneous determination of HZ and

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INH using chromatographic and electrochemical techniques have been published.[14–16] There is still a sustained interest in the development of simple and reliable methods for the simultaneous determination of INH with other active ingredients in pharmaceutical preparations.

Differential kinetic methods use different rates, at which two or more species react, with a common reagent, to resolve mixtures with no prior separation. Multivariate calibration methods are being successfully applied to the multicomponent kinetic determination to overcome some of the drawbacks of classical methods. Recently, soft algorithms such as principle component regression (PCR), partial least squares (PLS) and artificial neural network (ANN), which avoid the co-linearity problems, have been used for the simultaneous determination of the analytes having the same chemical properties that cannot be resolved with common methods.[17–19]

H-point standard addition method (HPSAM) is a modification of the standard addition method that transforms the incorrigible error, resulting from the presence of a direct interference during the determination of an analyte, into a constant systematic error.[20–23] This error can be evaluated and eliminated. By using this method, measurement of two or even three co-existing species is possible, within a mixture, but this simultaneous determination is not possible while using common standard addition methods. HPSAM can also be applied to the kinetic data for the simultaneous determination of binary mixtures or the calculation of analyte concentration, completely free from bias error.[23,24]

HPSAM was reported for the simultaneous spectrophotometric determination of binary mixtures of HZ and semicarbazide,[25] HZ and phenylhydrazine,[26] and HZ and acetylhydrazine.[27] Recently, we have reported a kinetic-spectrophotometric determination of ternary mixture of HZ and its derivatives using PCR and PLS methods.[28] We have also reported, for the first time ever, the application of PLS and PCR multivariate calibration methods and HPSAM analysis for the simultaneous kinetic-potentiometric determination of binary mixtures containing: HZ and phenylhydrazine,[29] HZ and thiosemicarbazide,[30] and levodopa and carbidopa.[31] Majidi *et al*. have reported voltammetric method for the simultaneous determination of HZ and INH using PLS and ANNs.[32] To the best of our knowledge, no spectrophotometric method, using chemometrics methods for simultaneous determination of HZ and INH, are reported.

Here we report the application of PLS and HPSAM for the simultaneous determination of HZ and INH. The difference observed in the rate of Fe^{3+} reduction to Fe^{2+},with HZ and INH, in the presence of 2,2'-bipyridine (Bpy) and subsequent complex formation between the resulted Fe^{2+} and Bpy in an anionic surfactant micellar medium (with λ_{max} = 520 nm) was the basic theme of both of the applied methods.

EXPERIMENTAL SECTION

Apparatus

A GBC UV-Visible Cintra 6 Spectrophotometer with 10-mm glass cells was used to record the kinetic spectrophotometric data. The data collection was done with an attached computer. Metrohm 781 pH-meter was used to adjust pH of the buffered solutions. PLS analysis was performed with the help of PLS toolbox in MATLAB 7.0 software.

Reagents

All of the chemicals were of analytical grade. Double distilled water was used for the preparation of the experimental solutions. The standard HZ solution (1000 μg mL^{-1}) was prepared in a 100-mL volumetric flask by dissolving 0.4061 g of hydrazinium sulfate in water and diluting it up to the mark. The standard INH solution (1000 μg mL^{-1}) was prepared in a 100-mL volumetric flask by dissolving 0.100 g of INH with water to the mark. The stock solution of 0.05 mol L^{-1} Fe^{3+} was prepared in a 100-mL volumetric flask by dissolving 2.43 g of ammonium ferric sulfate in water and diluting to the mark. 0.2 mol L^{-1} of sodium dodecyl sulfate (SDS) solution was prepared by dissolving 5.76 g of SDS in water in a 100-mL volumetric flask and diluting with water to the mark. The stock solution of 0.05 mol L^{-1} 2,2'-bipyridine (Bpy) was prepared in a 100-mL volumetric flask by dissolving 0.784 g of Bpy in ethanol and diluting it with water to the mark. Acetate buffer solution (1.0 mol L^{-1}) was prepared by using acetic acid and NaOH solutions and its pH was adjusted to 3.0. All of the chemicals were obtained from Merck.

Procedure

The iron(III) solution was prepared daily, in the presence of Bpy in micellar medium, by adding 4.0 mL Fe^{3+} solution (0.05 mol L^{-1}), 6.0 mL Bpy solution (0.05 mol L^{-1}), 10 mL buffer solution (pH 3.0) and 10 mL of SDS solution (0.2 mol L^{-1}). The resulting solution was diluted up to the mark of 100 mL with water. After thermostating the solution at 25 °C for 10 minutes, 2.4 mL of it was transferred into spectrophotometric glass cell and the absorbance was made auto-zero against the air before injecting analyte(s). Then, 100 μL of the solution, containing HZ or INH or a mixture of them in the range of the analyte determination, was injected with a 100-microliter syringe into the cell. The absorbance changes were recorded at 520 nm versus time (at the time intervals of every two seconds).

HPSAM was applied to the simultaneous determination of INH and HZ by measuring the absorbance of

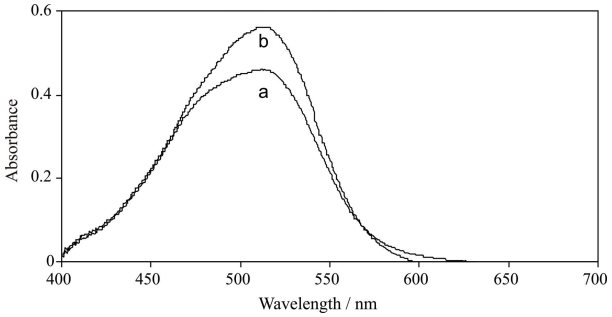

Figure 1. Absorption spectra of 15 μg mL^{-1} HZ (a) and 2.0 μgmL^{-1} INH and (b) Experimental conditions: 2.0×10^{-3} mol L^{-1} Fe^{3+} and 3.0×10^{-3} mol L^{-1} Bpy, 2.0×10^{-2} mol L^{-1} SDS, 1.0 mol L^{-1} acetate buffer (pH = 3.0, 25.0 °C), measured at 300 seconds.

solution at 120 and 300 seconds, for each sample. Synthetic samples, containing different concentration ratios of INH and HZ, were prepared and standard addition of HZ was made. Simultaneous determination of INH and HZ, with PLS method was performed by recording the absorbance spectra for each solution from 0.0 to 300 seconds.

RESULTS AND DISCUSSION

In the SDS micellar medium, the reaction of HZ and INH with Fe^{3+}, in the presence of Bpy, leads to a colored complex λ_{max} of which is 520 nm (Figure 1). The Fe^{3+} allows the spectrophotometric determination of a reducing agent, A_{red}, according to following reaction:[33]

$$n\,Fe^{3+} + 3n\,Bpy + A_{red} \longrightarrow n\left[Fe(Bpy)_3\right]^{2+} + A_{ox}$$

Scheme 2.

The above reaction is completed with the formation of an equivalent amount of $[Fe(Bpy)_3]^{2+}$ with respect to the *n*-electron-reducing agent, A_{red}. The Fe^{3+} reduction, in the presence of Bpy, and subsequent complex formation between the resulted Fe^{2+} and Bpy in a micellar medium (with λ_{max} = 520 nm) is completed in a few minutes in the presence of suitable reducing agents such as HZ and its derivatives. The reduction rate of Fe^{3+} is different in the presence of HZ and INH. This difference provides a possibility to resolve their mixtures using HPSAM and PLS method.

Effect of Variables

The effect of Fe^{3+} and Bpy concentrations on the reaction rate with HZ and INH and on the absorbance intensity was studied at a constant Fe^{3+} concentration (2.0×10^{-3} mol L^{-1}) and Bpy concentration in the range from 5.0×10^{-4} to 4.0×10^{-3} mol L^{-1}. For HZ, an increase at the Bpy concentration up to 1.5×10^{-3} mol L^{-1} causes an

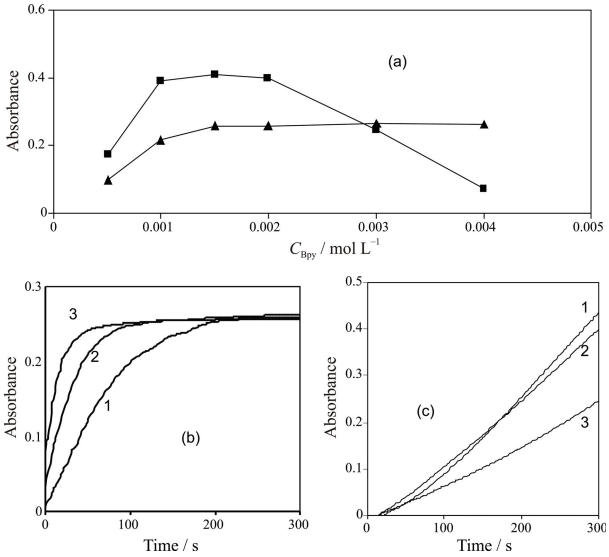

Figure 2. Effect of Bpy concentration on the absorbance of 10μg mL^{-1} of HZ (■) and 1.0 μg mL^{-1} INH (▲) (a) and effect of different concentrations 1.5×10^{-3} (1), 2.0×10^{-3} (2) and 3.0×10^{-3} mol L^{-1} (3) of Bpy on the reaction rate of 1.0 μg mL^{-1} INH (b) and 10 μg mL^{-1} HZ (c). Conditions: 2.0×10^{-3} mol L^{-1} Fe^{3+}, 2×10^{-2} mol L^{-1} SDS, 1.0 mol L^{-1} of acetate buffer of pH 3.0, 25 °C, measured at 300 seconds.

increase in the reaction rate and absorbance intensity, but at higher concentrations of Bpy, a decrease in both reaction rate and absorbance intensity was observed (Figure 2a, c). For INH, the reaction rate and absorbance intensity, increases by increasing the Bpy concentration up to 3.0×10^{-3} mol L^{-1} and remains nearly constant at higher concentrations (Figure 2a, b). In fact, increased Bpy/ Fe^{3+} ratio caused an increase in the reduction rate of Fe^{3+}/Bpy to Fe^{2+}/Bpy complex and consequently, an increase in the reaction rate and absorbance intensity.

The influence of Fe^{3+} concentration on the reaction rate and absorbance of the INH at constant Bpy concentration (3.0×10^{-3} mol L^{-1}) was also studied. The increase of Fe^{3+} concentration, up to 2.0×10^{-3} mol L^{-1}, caused an increase in the reaction rate and absorbance of HZ and INH. Beyond this amount, the increased Fe^{3+} concentration caused a decrease in the reaction rate of INH. Thus, for simultaneous determination of INH and HZ by HPSAM and PLS, 2.0×10^{-3} mol L^{-1} Fe^{3+} and 3.0×10^{-3} mol L^{-1} Bpy were the preferred and optimized concentrations during the present experiment. So, these concentrations were chosen for the further experiments.

Furthermore, the effect of micelles, on the reaction rate, was also investigated. In order to choose an appropriate micellar medium, different kinds of surfactants should be observed. To do this, the effect of surfactant type, on the reaction rates of INH and HZ with Fe^{3+} in the presence of Bpy, was investigated. According to our results, SDS as an anionic surfactant caused an in-

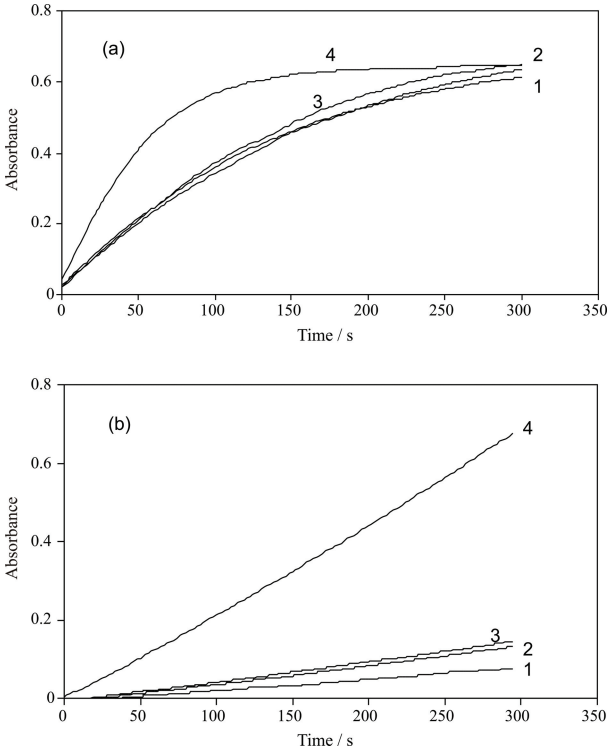

Figure 3. Reaction rate diagram of 2.5 μg mL^{-1} INH (a) 20.0 μg mL^{-1} HZ (b) with Fe^{3+} in the presence of different surfactants of 2×10^{-3} mol L^{-1} Triton X-100 (1), 2.0×10^{-2} mol L^{-1} CTAB (2), no surfactants (3), 2.0×10^{-2} mol L^{-1} SDS (4). Conditions: 3.0×10^{-3} mol L^{-1} Fe^{3+}, 2.0×10^{-3} mol L^{-1} Bpy, 25°C.

creased rate of HZ and INH reaction. While, cetyltrimethyl amonium bromide (CTAB) as a cationic surfactant and Triton X-100 (TX-100) as a non-ionic surfactant caused a bit decreased reaction rate (Figure 3). The decreasing effect of the reaction rate at concentrations lower than critical micelle concentration of SDS and two other surfactants, *viz*. CTAB and TX-100, could be attributed to the fact that micelles can change the effective microenvironment around the dissolved solutes as a consequence of their physicochemical properties, such as reaction mechanisms and/or the observed rate constant ratio of two or more species, interaction with a common reagent, control pathways, spectral profile and *etc*.[34] It was found that any decrease in the SDS concentration from 2.0×10^{-3} mol L^{-1} causes a decrease in the reaction rates. While, increased SDS concentration did not have any effect on the reaction rates. Therefore, 2.0×10^{-3} mol L^{-1} SDS was selected as the optimum concentration.

The effect of pH on the reaction rates of HZ and INH, over the pH range of 1.0−5.0, was examined using acetate buffer. An increase in pH up to 3.0 caused increased reaction rates for both analytes (Figure 4). So, pH 3.0 was chosen as the optimum pH value for the further studies.

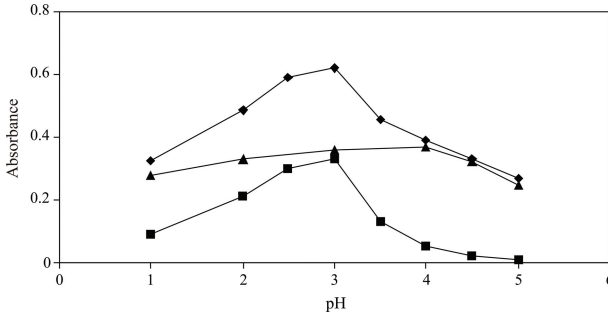

Figure 4. Effect of pH on the absorbance of 1.0 μg mL^{-1} of INH (▲), 15.0 μg mL^{-1} of HZ (■) and mixture of them (♦).Conditions: 2.0×10^{-2} mol L^{-1} SDS, 2.0×10^{-3} mol L^{-1} Fe^{3+} and 3.0×10^{-3} mol L^{-1} Bpy, 25 °C, measured at 300 s.

The effect of temperature on the reaction rates of HZ and INH was also studied over the range of 20−70 °C. An increase in the temperature lead to increased reaction rates for both analytes. However, for the sake of simplicity and better control over the temperature effects on the precision of experimental determinations, 25 °C was chosen as the optimized temperature.

Absorbance-Time Behavior

The absorbance-time behavior of the reaction of HZ, INH and a mixture of them with Fe^{3+} in the presence of Bpy, at the optimized conditions for both methods, is shown in Figure 5. As shown in Figure 5, the reaction rate for the reduction of Fe^{3+} with 1.0 μg mL^{-1} INH is faster than that of 10 μg mL^{-1} HZ. This difference provides the possibility for studying their mixtures using HPSAM and PLS method.[35] Under optimized conditions, the characteristics of calibration graphs, for the determination of HZ and INH, are given in Table 1.

H-point standard addition method (HPSAM)

To select the appropriate times for HPSAM, the following principles were followed: At two selected times (t_1 and t_2), the analyte signals must be linear with concentration, and the interfering signal must remain constant, even if the analyte concentration is changed. The analyt-

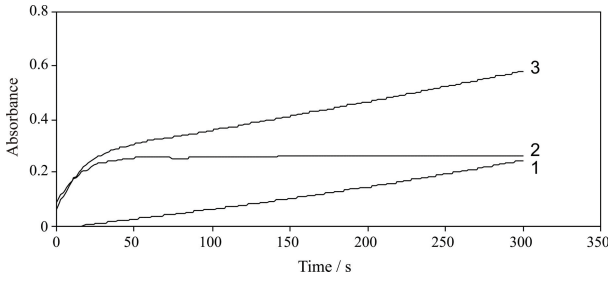

Figure 5. Absorbance-time profiles of 10 μg mL^{-1} HZ (1), 1.0 μg mL^{-1} INH (2) and mixture of them (3) recorded at 520 nm. Conditions: 2×10^{-3} mol L^{-1} Fe^{3+}, 3×10^{-3} mol L^{-1} Bpy, 2×10^{-2} mol L^{-1} SDS, 1.0 mol L^{-1} of acetate buffer (pH 3.0), 25.0 °C.

Table 1. Calibration graph characteristics for the determination of HZ and INH.

Compound	Slope	Intercept	Correlation	Linear range / μg mL^{-1}	Detection limit / μg mL^{-1}
HZ	0.0124	0.0315	0.9991	1.0−80.0	0.80
INH	0.2731	0.0022	0.9992	0.08−6.0	0.06

Table 2. Application of signal increment version of HPSAM to a synthetic mixture of 5.0 μg mL^{-1} HZ (as actual HZ concentration) and 1.0 μg mL^{-1} of INH

Time interval / s	$\gamma(\mathrm{HZ})_{\mathrm{found}}$ / μg mL^{-1}
100−300	5.30
120−300	5.10
150−400	5.09
100−250	5.41
100−400	5.12

ical signals of the mixture of analyte and interfering species should be equal to the sum of individual signals of two compounds. In addition, the slope difference of two straight lines, obtained at t_1 and t_2, must be as large as possible, so that we can achieve accurate results. To select the appropriate times for the application of HPSAM, time pairs of 100-250, 120-250, 100-300, 120-300, 150-400 and 200-400 seconds, which present the same absorbance for INH, were examined and the corresponding H-point plots were drawn. The higher the value for the slope increment, the lower the error for the analyte concentration[35], while greater time increments cause higher sensitivity and greater slope for two time axes. Also, accuracy of the determinations was affected by slope increments of H-point plots. However, the time pair, which gives the greatest slope increment, lower error and shortest analysis time, was selected. For this reason, the time pair of 120-300 seconds was used (Table 2).

According to the HPSAM theory, for binary mixture of INH-HZ, the resulting absorbance of their reaction with Fe^{3+}, in the presence of Bpy and SDS in acidic medium, was measured at 520 nm at the 120 and 300 seconds. The following equations show the relation between them:

$$A_{120} = b_0 + b + M_{120}C_i \quad (1)$$

$$A_{300} = A_0 + A' + M_{300}C_i \quad (2)$$

Where A_{120} and A_{300} was the absorbance measured at 120 and 300 seconds, respectively, b_0 and A_0 was the original absorbance of INH at 120 and 300 seconds, respectively, b and A' are the absorbences of HZ at 120 and 300 seconds, respectively. M_{120} and M_{300} are the slopes of the standard addition calibration lines at 120 and 300 seconds, respectively, and C_i is the added analytical concentration. Since, A_{120} is the same as A_{300} at point H (Figure 6) and INH is not to evolve over time (then $A' = b$), the coordinates of H will be:

$$b_0 + b + M_{120}(-C_{\mathrm{H}}) = A_0 + A' + M_{300}(-C_{\mathrm{H}}) \quad (3)$$

Hence,

$$-C_{\mathrm{H}} = \left[(A' - b) + (A_0 - b_0)\right] / (M_{120} - M_{300}) \quad (4)$$

$$A' = b$$

$$-C_{\mathrm{H}} = (A_0 - b_0) / (M_{120} - M_{300}) \quad (5)$$

Which is equivalent to the existing C_{HZ} ($= b_0/M_{120} = A_0/M_{300}$).

Substitution of C_{INH} into Eqs. (1) and (2) yields $A_{\mathrm{H}} = b$ (Figure 6) and the overall equation for the absorbance at H-point simplifies to:

$$A' = b = A_{\mathrm{H}} = A_{\mathrm{INH}} \quad (6)$$

In this special system, HZ can be considered as the analyte and INH as the interfering species. By plotting the analytical signal versus the added HZ concentration at the selected time pairs, two straight lines are obtained that have a common point with coordinates H ($-C_{\mathrm{H}}$, A_{H}), where C_{H} is the unknown HZ concentration and A_{H} is the analytical signal due to the INH species, as shown in Fig. 6. The INH concentration was calculated in each test solution by the calibration method with a single standard and ordinate value of the A_{H}. The results of several experiments, for the analysis of HZ and INH mixtures in different concentration ratios, are shown in Table 3. To check the reproducibility of the method,

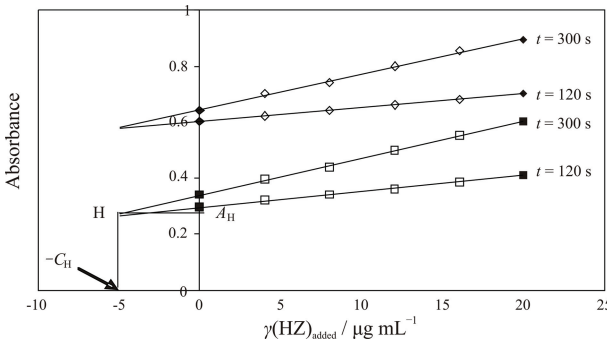

Figure 6. Plot of H-point standard addition method for fixed HZ (5.0 μg mL^{-1}) and 1.0 (■) and 2.5 μg mL^{-1} INH (♦).

Table 3. Results of several experiments for the analysis of HZ and INH mixtures at different concentration ratios using HPSAM (θ = 25 °C)

		Mass concentration / μg mL^{-1}			
		Spiked		Found	
A-*C* equation	*r*	INH	HZ	INH	HZ
$A_{300} = 0.0158C_i + 0.3004$	0.9991	0.60	9.00	0.62	8.75
$A_{120} = 0.0046C_i + 0.2006$	0.9972				
$A_{300} = 0.0159C_i + 0.4062$	0.9988	1.25	6.25	1.14	6.60
$A_{120} = 0.0064C_i + 0.3433$	0.9908				
$A_{300} = 0.0132C_i + 0.2408$	0.9999	0.50	7.00	0.55	7.20
$A_{120} = 0.0032C_i + 0.1688$	0.9991				
$A_{300} = 0.0134C_i + 0.3376$	0.9978	1.00	5.00	0.98	5.11
$A_{120} = 0.0055C_i + 0.2972$	0.9976				
$A_{300} = 0.0185C_i + 0.6432$	0.9984	1.00	10.00	1.03	10.40
$A_{120} = 0.0092C_i + 0.5436$	0.9977				
$A_{300} = 0.0116C_i + 0.7707$	0.9984	2.00	5.00	1.98	5.19
$A_{120} = 0.0054C_i + 0.7385$	0.9983				

four replicates were performed with 5.0 μg mL^{-1} HZ and 1.0 μg mL^{-1} INH and the relative standard deviation (RSD) was obtained for binary mixtures. The results have shown that RSDs were recorded to be 2.5 (for the mean of 5.05 μg mL^{-1} HZ) and 1.2 (for the mean of 0.975 μg mL^{-1} INH), respectively. Thus, the precision of the method was satisfactory.

Table 4. Values of the HZ and INH concentrations (μg mL^{-1}), used in calibration and prediction solutions

Sample number	Calibration set		Prediction set	
	HZ	INH	HZ	INH
1	5	0.5	5	1.2
2	5	2	9	3
3	5	2.5	9	0.9
4	5	3	0.8	2
5	9	1	13	2
6	9	1.2	17	1.2
7	9	2	17	2.5
8	9	2.5	20	2
9	12	1	20	3
10	12	1.5		
11	13	0.5		
12	13	1.2		
13	13	2.5		
14	13	3		
15	17	0.5		
16	17	2		
17	17	3		
18	20	0.5		
19	20	1		
20	20	1.2		
21	20	2.5		

Partial least squares (PLS) method

The theories and applications of chemometrics methods such as PLS, to the analysis of multi-component mixtures have been discussed by several workers. PLS modeling is a powerful multivariate statistical tool that has been successfully applied to the quantitative analysis of spectrophotometry,[36–41] fluorimetry[42] and electrochemical[29–32] data. PLS has been used in multicomponent analysis to simultaneously resolve the mixtures of analytes either by equilibrium or by kinetic-based methods. In the PLS regression, a relationship is sought between a single response variable (Y) and a data matrix (X). The first step, in the simultaneous determination of species by PLS-1 methodology, involves the construction of calibration matrix for the binary mixture of HZ and INH. For constructing the calibration set, a factorial design was applied at five levels to extract a great deal of quantitative information using only a few experimental trials. In this research, a synthetic set of 30 solutions, including different concentrations of HZ and INH, was prepared. A collection of 21 solutions was selected as the calibration set and the other 9 were used as the prediction set (Tables 4 and 5). Their composition was randomly designed to obtain more information from the calibration procedure. Changes in the solution absorbance were recorded during a time period of 300 seconds.

To select the number of factors in the PLS algo-

Table 5. Composition of prediction set, their predictions by PLS and statistical parameters for the system

	Mass concentration / μg mL^{-1}				Recovery / %	
	Synthetic		Prediction			
Sample	HZ	INH	HZ	INH	HZ	INH
1	5.0	1.2	4.70	1.23	94.0	102.5
2	9.0	3.0	9.17	2.88	101.8	96.0
3	9.0	0.5	8.62	0.49	95.7	98.0
4	0.8	2.0	0.80	1.95	100.0	97.5
5	13.0	2.0	13.15	1.97	101.1	98.5
6	17.0	1.2	17.31	1.28	101.8	106.6
7	17.0	2.5	17.81	2.38	104.7	95.2
8	20.0	2.0	20.74	1.92	103.7	96.0
9	20.0	3.0	19.60	2.90	98.0	96.6
			Mean recovery / %		100.1	98.4
			RSE single / %		3.1	3.7
			RSE total / %		3.1	

rithm, a cross-validation method, leaving out one sample methods was employed.[28] The prediction error was calculated for each species for the prediction set. This error was expressed as the prediction residual error sum of squares (PRESS):

$$\mathrm{PRESS} = \sum_{i=1}^{m} \left(\hat{C}_i - C_i \right)^2 \tag{7}$$

Where m is the total number of calibration sample, $\hat{C}_i$ represents the estimated concentration, and C_i is the reference concentration for the i-th sample left out of the calibration during cross validation. The results of the PRESS plot, against the number of factors for each individual component, have shown that the optimum number of factors yielding the smallest error (PRESS) was obtained to be 3 for HZ and INH. To find out minimum factors, the F-statistics was also used to carry out the significant determination.[28]

The validation step of PLS methodology was carried out by running PLS on the prediction set. The results are shown in Table 5. The obtained results are quite acceptable for both analytes. The prediction error of a single component, in the mixture, is calculated as the relative standard error (RSE) of predicted concentration:

$$\mathrm{RSE}(\%) = 100 \times \left(\frac{\sum_{j=1}^{N} \left(\hat{C}_j - C_j \right)^2}{\sum_{j=1}^{N} \left(C_j \right)^2} \right)^{1/2} \tag{8}$$

Where N is the number of samples, C_i is the concentration of the component in the j-th mixture and $\hat{C}_i$ is the estimated concentration. The total prediction error of N samples is calculated as follows:

$$\mathrm{RSE_t}(\%) = 100 \times \left(\frac{\sum_{i=1}^{M} \sum_{j=1}^{N} \left(\hat{C}_{ij} - C_{ij} \right)^2}{\sum_{i=1}^{M} \sum_{j=1}^{N} \left(C_{ij} \right)^2} \right)^{1/2} \tag{9}$$

Where C_{ij} is the concentration of the i-th component in the j-th sample and $\hat{C}_{ij}$ is the estimated concentration.

Selectivity

In order to assess the possible analytical applications of the proposed method, the effect of common excipients, used in pharmaceutical preparations and various coexisting compounds at different concentrations, on the absorbance of synthetic sample solutions containing a mixture of 1.0 and 10 μg mL^{-1} INH and HZ, respectively, were studied. The undissolved material was filtered before the measurement. The recovery results are given in Table 6. As shown by the results, no interference was observed from any of the tested excipients and only coexisting compounds of ascorbic acid, riboflavin and pyridoxine hydrochloride because of their reducing properties appeared to cause interference in this procedure. The interference of ascorbic acid was eliminated when the synthetic sample solution was measured after the time equal to or greater than one hour.

Application

The proposed methods were applied to the determination of HZ and INH in several commercially available INH pharmaceutical samples. Twenty tablets of each sample were accurately weighted and their solutions

Table 6. Recovery of 1.0 μg mL^{-1} INH and 10 μg mL^{-1} HZ from the solutions with various additives, used as excipients, and various co-existing compounds (n = 3)

Additive	Mass concentration of additive / μg mL^{-1}	Recovery / %			
		HPSAM		PLS	
		HZ	INH	HZ	INH
Galactose	250	102.0 ± 2.5	99.0 ± 1.0	98.1 ± 2.2	103.7 ± 0.3
Glucose	250	104.1 ± 1.2	100.6 ± 2.1	103.4 ± 1.5	102.5 ± 1.0
Lactose	250	103.5 ± 1.1	98.5 ± 0.9	102.6 ± 1.6	99.2 ± 1.7
Fructose	250	103.1 ± 2.2	102.0 ± 1.1	102.7 ± 1.2	103.2 ± 1.3
Calcium sulfate	250	104.0 ± 1.8	100.6 ± 1.2	103.7 ± 0.8	103.4 ± 2.1
Surbitol	100	102.0 ± 2.2	97.1 ± 1.8	96.0 ± 1.1	106.0 ± 2.2
Sodium citrate	50	105.0 ± 4.5	96.0 ± 1.1	96.5 ± 2.0	107.0 ± 1.5
Ascorbic acid	1	140.0 ± 3.2	105.0 ± 0.8	115.4 ± 3.1	122.0 ± 1.1
Thiamin hydrochloride	100	104.5 ± 1.5	98.0 ± 2.1	104.7 ± 0.7	98.3 ± 2.0
Riboflavin	1	107.0 ± 1.3	103.0 ± 1.5	110.0 ± 0.9	114.0 ± 1.9
Pyridoxine hydrochloride	1	105.0 ± 0.8	105.0 ± 1.0	104.0 ± 1.2	106.0 ± 1.4
Calcium panthotenate	100	105.0 ± 1.3	102.0 ± 0.4	99.5 ± 0.6	102.8 ± 1.4
Nicotinic acid	100	110.0 ± 2.5	104.0 ± 0.8	120.5 ± 1.5	97.3 ± 1.6
Streptomycin sulfate	100	96.0 ± 0.8	98.0 ± 0.9	103.3 ± 0.7	96.6 ± 1.8

Table 7. Results of the determination of HZ and INH quantification in the pharmaceutical samples of INH

Sample[a]	Mass concentration / μg mL^{-1}							
	Nominal		Spiked		Found[b]			
					HPSAM		PLS	
	INH	HZ	INH	HZ	INH	HZ	INH	HZ
1[c]	1.0	*	-	-	1.02 ± 0.03	-	1.03 ± 0.04	-
1[c]	1.0	*	0.5	1.0	1.55 ± 0.08	1.05 ± 0.10	1.57 ± 0.12	1.04 ± 0.13
1[c]	1.0	*	1.0	0.5	2.10 ± 0.16	5.22 ± 0.30	2.08 ± 0.10	5.18 ± 0.24
2[d]	3.0	*	-	-	3.12 ± 0.20	-	3.20 ± 0.24	-
2[d]	3.0	*	1.0	1.0	4.12 ± 0.22	1.02 ± 0.08	4.10 ± 0.15	1.00 ± 0.12
2[d]	3.0	*	2.0	10.0	5.28 ± 0.34	9.72 ± 0.40	5.15 ± 0.27	9.84 ± 0.36
3[e]	5.0	*	-	-	4.12 ± 0.24	0.85 ± 0.06	4.20 ± 0.16	0.78 ± 0.08
3[e]	5.0	*	0.5	2.0	4.58 ± 0.32	2.92± 0.14	4.60 ± 0.26	2.90 ± 0.10
3[e]	5.0	*	0.5	5.0	4.62 ± 0.36	5.95 ± 0.42	4.55 ± 0.20	5.88 ± 0.33
4[f]	4.0	*	-	-	2.35 ± 0.14	1.57 ± 0.12	2.40 ± 0.10	1.64 ± 0.13
4[f]	4.0	*	1.0	1.0	3.30 ± 0.20	2.76 ± 0.15	3.36 ± 0.25	2.80 ± 0.18
4[f]	4.0	*	2.0	5.0	4.38 ± 0.27	6.78 ± 0.32	4.42 ± 0.24	6.70 ± 0.03

[a] Samples 1, 2, 3 and 4 were the pharmaceutical samples of INH. Samples 1 and 2 were not degraded and stored under appropriate conditions of temperature and moisture; samples 3 and 4 were degraded under inappropriate conditions of temperature and moisture after 45 days and 1 year, respectively. [b] Average ± standard deviation (n = 6). [c] and [e] INH (100 mg per tablet); Daru Pakhsh, Tehran, Iran. [d] and [f] INH (300 mg per tablet); Daru Pakhsh, Tehran, Iran. * Unknown amount.

were prepared by dissolving them in water and then filtering the solutions. The prepared solutions, containing aliquot amounts of INH and HZ, were analyzed (n = 3). The quantitative results of this analysis are summarized in Table 7. The good agreement between these results and the nominal labeled values indicates the successful applicability of HPSAM and PLS for the simultaneous determination of HZ and INH in pharmaceutical samples.

CONCLUSION

During the present work, it was shown that the application of HPSAM and PLS could be well adopted for the simultaneous determination of INH and HZ. The HPSAM and PLS models are suitable for the simultaneous kinetic determination of INH and HZ, but the PLS method was faster than HPSAM. Both of the methods are inexpensive than chromatographic methods. Furthermore, while working with these methods, one does not need to use toxic organic solvents. In other words, they belong to green chemistry. As the new, inexpensive and sensitive techniques, the proposed methods offer good selectivity, accuracy and precision that can be applied for a wide range of INH and HZ concentrations.

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SAŽETAK

Simultano kinetičko spektrofotometrijsko određivanje hidrazina i izonijanida u micelarnom mediju metodom dodatka standarda HPSAM i regresijom parcijalnih najmanjih kvadrata

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Opisano je simultano kinetičko spektrofotometrijsko određivanje hidrazina (HZ) i izonijazida (INH) upotrebom metode standardnog dodatka HPSAM (engl. *H-Point Standard Addition Method*) i regresijom parcijalnih najmanjih kvadrata (PLS, engl. *Partial Least Squares*). Metode se temelje na razlici u brzini redukcije željeza (III) s HZ odnosno INH u prisutnosti 2,2'-bipiridina (Bpy) i nastajanja kompleksa rezultirajućeg Fe^{2+} s Bpy u otopini natrijevog dodecil sulfata kao micelarnom mediju. Spojevi se mogu odrediti istovremeno u rasponu masenih koncentracija od 0,08 μg mL^{-1} do 6,0 μg mL^{-1} za HZ i od 1,0 μg mL^{-1} do 80,0 μg mL^{-1} za INH. Rezultati su pokazali da se primjenom metode HPSAM istovremeno mogu odrediti oba spoja zastupljena u omjeru INH:HZ od 1:1000 do 1:12,5. Relativna standardna devijacija određivanja HZ metodom HPSAM bila je 2,5; a INH 1,2. Ukupna relativna standardna pogreška pri određivanju HZ (raspon koncentracija od 0,0 μg mL^{-1} do 20,0 μg mL^{-1}) i INH (raspon koncentracija od 0,5 μg mL^{-1} do 3,0 μg mL^{-1}) u 9 sintetičkih uzoraka metodom PLS bila je 3,19. Obje predložene metode (PLS i HPSAM) uspješno su primijenjene za simultano određivanje HZ i INH u nekoliko komercijalno dostupnih formulacija izonijazida.