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Simultaneous Determination of Isoniazid and Hydrazine by Spectrophotometric H-Point Standard Addition Method

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A new, simple, inexpensive and sensitive method for the simultaneous microdetermination of hydrazine and isoniazid has been developed. The H-point standard addition method was applied to the simultaneous determination of hydrazine and isoniazid. The method is based on the difference between the rates of their hydrazone formation with 4-dimethylaminobenzaldehyde. The experimental parameters, such as temperature and concentrations of 4-dimethylaminobenzaldehyde, sodium dodecyl sulfate and acidity were optimized to minimize the errors. The linear ranges of hydrazine and isoniazid were 0.01-1.0 and 1.0-80.0 μg mL^{-1}, respectively. The detection limits for hydrazine and isoniazid were 0.005 and 0.8 μg mL^{-1}, respectively. The proposed method was successfully applied to the simultaneous determination of hydrazine and isoniazid in several commercially available isoniazid formulations and satisfactory results were obtained.

Key Words: Isoniazid, Hydrazine, 4-Dimethylaminobenzaldehyde, Simultaneous determination, H-point standard addition method.

INTRODUCTION

Isoniazid (INH, Isonicotinic acid hydrazide) is an antitubercular drug and now is widely used together with other antituberculostatic agents for the chemotherapy of tuberculosis. The determination of INH is important in pharmaceutical preparation and biological fluids as a bacteriostatic drug. 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 principal degradation products, is a known carcinogen and considerably more toxic than INH[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[2]. This drug hydrolyzed in during the time and inappropriate storage conditions such as high temperature to hydrazine according to the following equation[2]:

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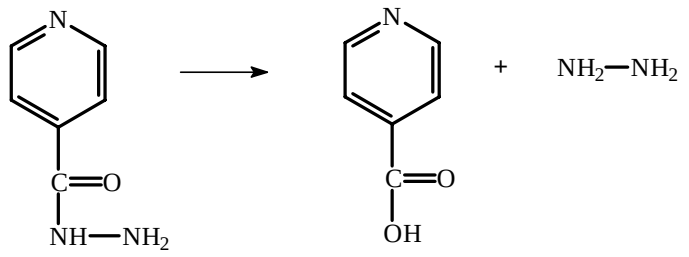


Isoniazid Isonicotinic acid Hydrazine

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

Numerous methods that have been used for the quantification of INH and HZ are titrimetry[3,4], electroanalytical techniques[5,6], spectrophotometry[7,8], fluorometry[9,10] and chemiluminescence[11-13] methods.

A few reports on simultaneous determination of HZ and 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. Recently, Majidi *et al.*[17] reported voltammetric method for the simultaneous determination of HZ and INH using partial least squares and artificial neural networks. To our knowledge, it has not reported any spectrophotometric method using H-point standard addition method (HPSAM) and/or chemometrics methods for simultaneous determination of HZ and INH.

HPSAM is a modification of the standard addition method that transforms the incorrigible error resulting from the presence of a direct interference in the determination of an analyte into a constant systematic error[18-21]. This error can be evaluated and eliminated. By using this method, it is possible to measure two and even three species that exist together within the mixture that cannot be measured simultaneously with common standard addition methods. This method can also be applied to kinetic data for the simultaneous determination of binary mixtures or the calculation of analyte concentration completely free from bias error[21,22].

In this paper, it is shown that H-point standard addition method can be employed for the simultaneous determination of HZ and INH. The difference in the rates of reactions in 460 nm with DAB in the presence of SDS in acidic media is the basis of this method. Based on this work, a new, simple, sensitive, inexpensive, precise and accurate method is proposed for the simultaneous determination of HZ and its derivative.

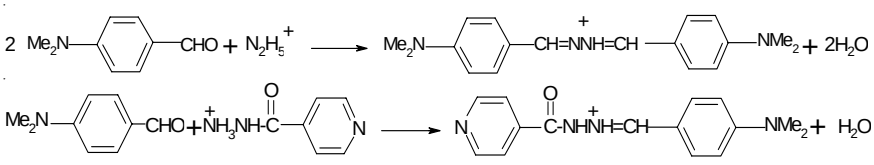
EXPERIMENTAL

A GBC UV-Visible Cintra 6 Spectrophotometer model, attached to a Pentium (IV) computer, with 1 cm glass cells was used to record the kinetic spectrophotometric data. Analytical grade of the materials and double distilled water were used. A standard solution of hydrazine (100 μg mL^{-1}) was prepared by dissolving 0.0406 g of hydrazinium sulfate (Merck) in water and diluting to 100 mL with water. The standard solution of isoniazid (1000 μg mL^{-1}) was prepared by dissolving 0.1000 g of isoniazid (Merck) in water and diluting to 100 mL with water. The 0.15 M solution of sodium dodecyl sulphate (SDS) was prepared by dissolving 4.33 g of SDS (Merck) in water and diluting to 100 mL with water. HCl solution (0.4 M) was prepared by diluting concentrated hydrochloric acid with water. The 0.04 M solution of 4-dimethylaminobenzaldehyde (DAB) was prepared by dissolving 0.298 g of DAB in 0.4 M HCl and diluting to 100 mL with 0.4 M HCl. The minimum number of dilution steps possible was used for preparation of more dilute solutions. All other common laboratory chemicals were of the best grade available and used without further purification.

Recommended procedure: Appropriate amounts of HZ and INH (from the stock solutions or from real samples) were added to a series of 10 mL volumetric flasks and different amounts of standard solution of INH were added to the flasks and 1 mL of 0.15 M SDS were also added to the flasks. Then, 0.04 mmol of DAB solution was added to initiate the reaction and the solution was diluted to mark with water and placed in 70°C water bath for each sample solutions. A stopwatch was started just after the addition of the DAB solution. A portion of the solution was transferred into a 1 cm glass cell to measure the absorbance change with time at 460 nm. The simultaneous determination of HZ and INH standard solutions with HPSAM was performed by measuring the absorbances at 5 and 20 min after initiation of the reaction for each sample solution. HZ and INH standard solutions can be determined simultaneously with the concentration ranges of 0.01-1.0 and 1.0-80.0 μg mL^{-1}, respectively. The procedure was repeated for some mixtures to show the applicability of the method.

RESULTS AND DISCUSSION

In the SDS micellar medium, reaction of HZ and INH with DAB lead to form a colour complex whose λ_{max} is 460 nm (Fig. 1). The reaction follows the stoichiometric equation:



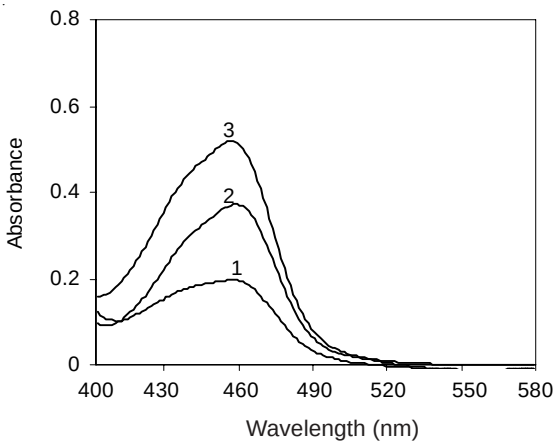


Fig. 1. Absorption spectra of 20 µg mL^{-1} of INH (1), 0.2 µg mL^{-1} of HZ (2) and their mixture (3) in the presence of 0.004 M DAB, 0.015 M SDS, 0.04 M HCl and 70°C

Preliminary studies showed that the reactions of HZ and INH with DAB in the presence of SDS in acidic media are not instantaneous and take some time to completed. SDS micellar media strongly enhance the rate and equilibrium constants of the above reactions[23]. It was also observed that the reaction rate of HZ is higher than that of INH and is completed within a few minutes. The reaction of HZ with DAB in presence of SDS in an acidic media was completed at 5 min after mixing, while the reaction of INH with DAB in the same conditions was relatively slow (Fig.2). Difference in kinetic behaviour of two analytes accompanied with the treatment of data using HPSAM permits simultaneous analysis of HZ and INH. The effects of different temperatures and concentrations of DAB, SDS and acid on the reaction rates were investigated to find the optimized conditions for application of HPSAM.

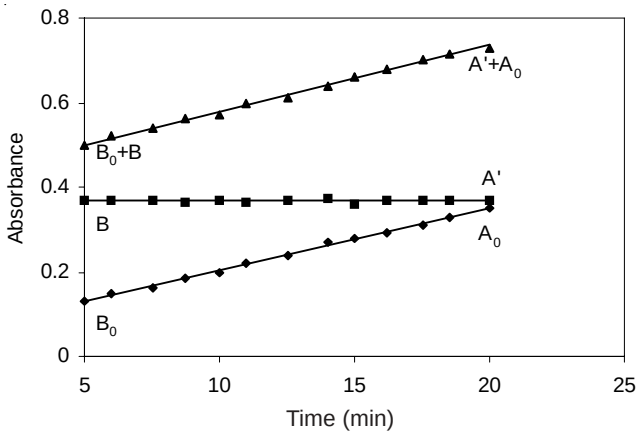


Fig. 2. Absorbance-time curves for a 0.2 µg mL^{-1} of HZ (■). 20.0 µg mL^{-1} of INH (◆) and their mixture ( ) in the presence of 0.004 M DAB, 0.015 M SDS, 0.04 M HCl and 70°C

Effect of DAB concentration: The effect of DAB concentration on the reaction rate of HZ and INH mixture was studied over the range of 0.0004 - 0.008 M. As Fig.3 shows, the absorbance for a mixture of HZ and INH increases by increasing the DAB concentration up to 0.004 M and remaines nearly constant at higher concentrations. Thus, the optimum concentration 0.004 M DAB was selected for further work.

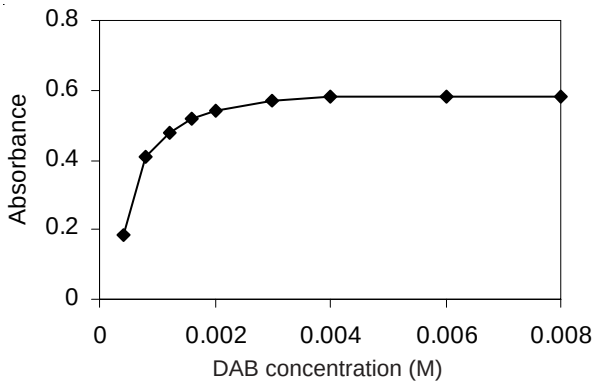


Fig. 3. Effect of DAB concentration on the reaction of a mixture of 0.2 μg mL^{-1} HZ-20.0 μg mL^{-1} INH in the presence of 0.015 M SDS, 0.04 M HCl and 70°C

Effect of SDS concentration: The effect of SDS concentration on the rate of reaction was studied in the range of 0.00-0.04 M. As Fig. 4 shows, the absorbance for a mixture of HZ and INH increases by increasing the SDS concentration up to 0.015 M and remaines nearly constant at higher concentrations. Thus, 0.015 M concentration was chosen as the optimum SDS concentration.

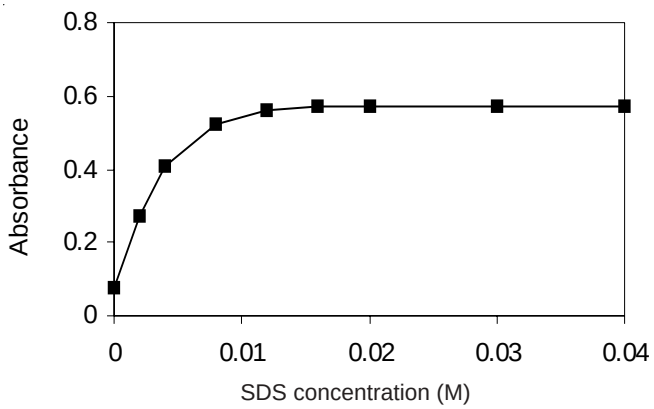


Fig. 4. Effect of the SDS concentration on the reaction of a mixture of 0.2 μg mL^{-1} HZ-20.0 μg mL^{-1} INH in the presence of 0.004 M DAB, 0.04 M HCl and 70°C

Effect of HCl concentration: The effect of hydrochloric acid concentration on the rate of the reaction was studied in the range of 0.008-0.1

M. The absorbance for a mixture of HZ and INH increases by increasing the HCl concentration up to 0.04 M and decreases at higher concentrations. Thus, a 0.04 M HCl concentration was chosen as the optimum concentration for further studies.

Effect of temperature: The effect of temperature on the rate of reaction was studied in range of 10 to 80°C. The reaction of HZ with DAB was completed at 5 min after mixing the reactants at 25°C and increasing temperature has no considerable effect, but the absorbance decrease at higher temperature than 70°C. The reaction of INH was relatively slow and an increase in the temperature caused an increase in the reaction rates. Thus, a 70°C temperature was selected as the optimum.

H-Point standard addition method: For the selection of appropriate times for applying HPSAM, consider an unknown sample containing an analyte X and an interference Y. In the present work, either HZ and INH can be considered as the analyte or the interferent. If reactions X and Y have different kinetic mechanisms, for concentration determination of X by HPSAM needs to select two times (t_1 and t_2), in which Y has the same absorbance[24]. In addition, the slope difference of the two straight lines obtained at t_1 and t_2 must be as large as possible in order to get good accuracy[25]. As shown previously by Compains-Falco *et al.*[18], the higher the value of the slope increment, the lower is the error for the analyte concentration. For this reason, the time pairs of 5 and 20 min that gave the best accuracy, the lowest error and the shortest analysis time were used. The reaction of HZ was completed in 5 min, while the reaction of INH was relatively slow. According to the HPSAM theory, for binary mixture of HZ and INH, the resulting absorbance of the reaction of HZ and INH with DAB in the presence of SDS in acidic media are measured at 460 nm at times of 5 and 20 min. The following equations show the reaction between them,

$$A_5 = B_0 + B + M_5Ci \quad (1)$$

$$A_{20} = A_0 + A' + M_{20}Ci \quad (2)$$

where A_5 and A_{20} are the analytical signals measured at 5 and 20 min, respectively, B_0 and A_0 are the original analytical signals of INH at 5 and 20 min, respectively, B and A' are the analytical signals of HZ at 5 and 20 min, respectively (Fig.2). M_5 and M_{20} are the slopes of the standard addition calibration lines at 5 and 20 min, respectively, and Ci is the added analytical (X) concentration. Fig.5 shows the two straight lines obtained intersect at the point H (C_H, A_H) ≡ ($-C_{INH}$, A_{HZ}). At the intersect,

$$B_0 + B + M_5(-C_H) = A_0 + A' + M_{20}(-C_H) \quad (3)$$

Hence

$$-C_H = [(A' - B) + (A_0 - B_0)] / (M_4 - M_{20}) \quad (4)$$

because of the fast reaction between HZ and DAB, A' equals to B, hence

$$-C_H = (A_0 - B_0) / (M_0 - M_{20}) \quad (5)$$

which is equivalent to the existing C_{INH} (= $B_0/M_5 = A_0/M_{20}$).

Substituating of C_{HZ} into Eqs. (1) and (2) yields $A_H = B$ and the overall equation for the absorbance at H-point simplifies to

$$A' = B = A_H = A_{HZ} \quad (6)$$

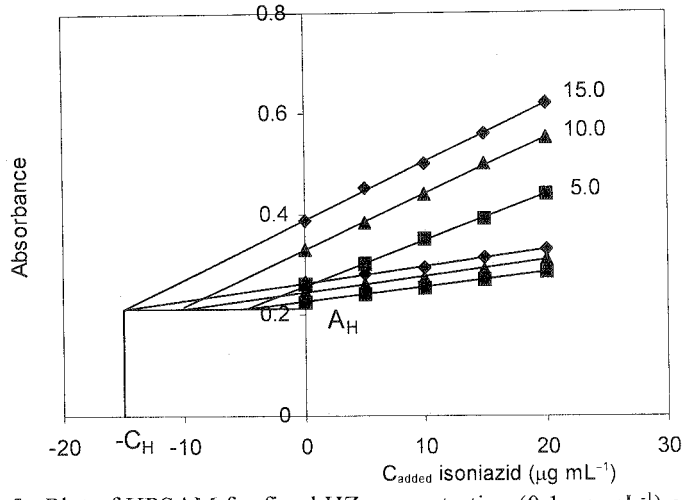


Fig. 5. Plot of HPSAM for fixed HZ concentration (0.1 μg mL^{-1}) and 5.0 (■) 10.0 (▲) and 15.0 (◆) μg mL^{-1} of INH

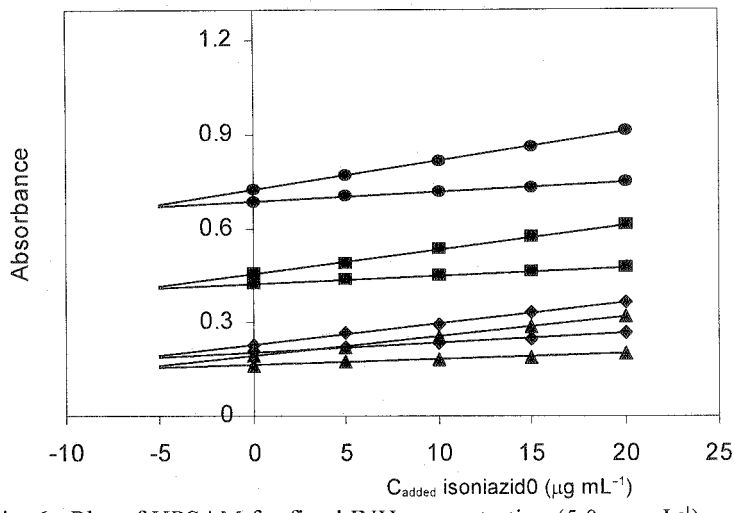


Fig. 6. Plot of HPSAM for fixed INH concentration (5.0 μg mL^{-1}) and 0.08 (▲) 0.1 (◆) 0.2 (■) and 0.3 (●) μg mL^{-1} of HZ

The intercept of the straight lines represented by eqns. 1 and 2 would thus directly yield the unknown INH concentration (C_{INH}) and the analytical signal of species HZ (A_{HZ}) in the original samples. The concentration of HZ was calculated from this analytical signal from a calibration graph (Table-1).

TABLE-1
CHARATERISTICS OF CALIBRATION GRAPHS FOR
THE DETERMINATION OF HZ AND INH

Analyte	Slope (μg mL $^{-1}$)	Intercept	Correlation coefficient (n = 12)	Range (μg mL^{-1})	Limits of detection (LD)[a] (μg mL^{-1})
HZ	1.8931	0.0073	0.9997	0.01-01.0	0.005
INH	0.0095	0.0037	0.9995	1.00-80.0	0.800

[a]Defined as LD = $3S_b$/ m, where LD, S_b and m are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively.

According to the theory of the proposed method, the absorbance values for the different mixtures of HZ and INH solutions were measured at 5 and 20 min.

A summary of the obtained results for various analyte concentrations is given in Table-2. The concentration was calculated directly by solving a system of equations of two straight lines. The HZ concentrations were calculated from the absorbance values (A_H) obtained on the basis above equations.

TABLE-2
RESULTS OF SEVERAL EXPERIMENTS FOR THE
ANALYSIS OF HZ AND INH MIXTURES IN DIFFERENT
CONCENTRATION RATIOS (TEMP. = 70ºC)

A-C equation	r	Taken (μg mL^{-1})		Found (μg mL^{-1})	
		HZ	INH	HZ	INH
A_{20} = 0.0173 Ci + 0.3543	0.9994	0.01	20.00	0.010	19.00
A_5 = 0.0053 Ci + 0.1284	0.9996				
A_{20} = 0.0184 Ci + 0.8383	0.9990	0.05	40.00	0.049	39.45
A_5 = 0.0045 Ci + 0.2909	0.9989				
A_{20} = 0.0195 Ci + 1.1617	0.9992	0.06	50.00	0.060	52.30
A_5 = 0.0038 Ci + 0.3404	0.9992				
A_{20} = 0.0181 Ci + 0.4162	0.9995	0.10	10.00	0.101	10.10
A_5 = 0.0025 Ci + 0.2586	0.9990				
A_{20} = 0.0116 Ci + 0.3876	0.9994	0.10	15.00	0.110	14.90
A_5 = 0.0033 Ci + 0.2640	0.9989				
A_{20} = 0.0343 Ci + 0.6115	0.9996	0.20	5.00	0.189	5.02
A_5 = 0.0081 Ci + 0.4788	0.9992				
A_{20} = 0.0240 Ci + 1.2503	0.9992	0.60	1.00	0.620	1.05
A_5 = 0.0208 Ci + 1.2469	0.9993				
A_{20} = 0.0216 Ci + 0.6860	0.9989	0.02	30.00	0.019	29.46
A_5 = 0.0066 Ci + 0.2440	0.9950				
A_{20} = 0.0098 Ci + 0.7182	0.9986	0.30	5.00	0.296	5.01
A_5 = 0.0031 Ci + 0.6846	0.9981				

Accuracy and Precision of the method: Under the optimum conditions, the simultaneous determination of several synthetic mixed samples

with different concentrations of HZ and INH were analyzed by HPSAM. Table-2 shows the accuracy of the results is satisfactory when the concentrations ratio of HZ and INH varied from 2000:1 to 1:0.6.

To check the reproducibility of the method, five replicates were performed and the relative standard deviation (RSD) was obtained for binary mixtures. Table-3 shows, the precision of the results is satisfactory.

TABLE-3
RESULTS OF FIVE REPLICATE EXPERIMENTS FOR THE ANALYSIS HZ AND INH MIXTURES (TEMP. = 70ºC)

A-C equation	r	Taken (μg mL^{-1})		Found (μg mL^{-1})	
		HZ	INH	HZ	INH
A_{20} = 0.0170 Ci + 0.3843	0.9995	0.10	10.00	0.097	10.07
A_5 = 0.0036 Ci + 0.2493	0.9992				
A_{20} = 0.0191 Ci + 0.3710	0.9996	0.10	10.00	0.094	9.96
A_5 = 0.0049 Ci + 0.2305	0.9992				
A_{20} = 0.0181 Ci + 0.4162	0.9998	0.10	10.00	0.100	10.10
A_5 = 0.0025 Ci + 0.2586	0.9990				
A_{20} = 0.0177 Ci + 0.4374	0.9986	0.10	10.00	0.096	10.20
A_5 = 0.0029 Ci + 0.2862	0.9985				
A_{20} = 0.0132 Ci + 0.3990	0.9990	0.10	10.00	0.102	9.96
A_5 = 0.0021 Ci + 0.2886	0.9989				
Mean				0.098	10.05
Standard deviation				0.003	0.10
RSD%				3.060	0.99

Selectivity: In order to assess the possible analytical applications of the proposed method, the effect of common excipients used in pharmaceutical preparations were studied by analyzing synthetic sample solutions containing 5.0 μg mL^{-1} of INH and 10-fold concentration of each excipient. The undissolved material was filtered before measurement. The recovery results are given in Table-4. No interference was observed from any of the excipients tested, which showed recoveries in the range of 97.5-104.0 %. The effect of some common co-existing compounds on the recovery of 5.0 μg mL^{-1} of INH was studied by analyzing synthetic samples, as for the excipient study, but with 100-fold concentration of each co-existing compound (Table-5). No interference was observed from any of co-existing compounds studied, which showed recoveries in the range of 98.5-105.0 %.

Application of the method: The proposed method was applied to determine of HZ and INH in several commercially available INH formulations. Ten tablets of each sample were accurately weighed and their solutions were prepared by dissolving them in water and filtering the solutions. In these tablets, HZ was not found. Therefore, HZ and INH were spiked to prepared solutions from tablets and HZ and INH were simultaneously

determined (n=3). The quantitative results of this analysis are summarized in Table-6. The good agreement among these results and the nominal values labeled indicate the successful applicability of HPSAM for the simultaneous determination of HZ and INH in pharmaceutical samples. Moreover, to evaluate the applicability of the proposed method for real samples it was applied to determine simultaneously HZ and INH in blood plasma, serum and drinking water (Table-7).

TABLE-4
RECOVERY OF 5.0 μg mL^{-1} OF INH FROM SOLUTIONS WITH A 10-FOLD CONCENTRATION OF VARIOUS ADDITIVES USED AS EXIPIENTS

Additive	Recovery (%) (n = 3)	Additive	Recovery (%) (n = 3)
Galactose	103.0 (±1.3)	Starch	97.5 (±1.9)
Glucose	104.0 (±0.8)	Talc	99.0 (±1.5)
Lactose	98.0 (±1.3)	Sodium citrate	102.0 (±0.7)
Sugar	102.0 (±1.1)	Calcium sulfate	102.0 (±1.2)
Sorbitol	102.0 (±1.8)	–	–

TABLE-5
RECOVERY OF 5.0 μg mL^{-1} OF INH FROM SOLUTIONS WITH A 100-FOLD CONCENTRATION OF VARIOUS CO-EXISTING COMPOUNDS

Additive	Recovery (%) (n = 3)	Additive	Recovery (%) (n = 3)
Ascorbic acid	104.0 (±0.8)	Riboflavin	102.4 (±1.2)
Nicotinic acid	100.2 (±1.0)	Pyridoxine hydrochloride	98.5 (±1.4)
Nicotinamide	106.1 (±1.1)	Calcium panthothenate	101.9 (±2.1)
Thiamine hydrochloride	100.2 (±1.3)	Streptomycin sulfate	105.0 (±1.1)

TABLE-6
RESULTS OF DETERMINATION OF HZ AND INH QUANTITATION IN PHARMACEUTICAL SAMPLES USING HPSAM (THREE REPLICATE)

Sample	Nominial (μg mL^{-1})		Added (μg mL^{-1})		Found* (μg mL^{-1})		Recovery (%) of added compound	
	HZ	INH	HZ	INH	HZ	INH	HZ	INH
1[a]	–	10.00	–	–	–	9.89	–	–
	–	10.00	0.10	2.00	0.098	11.88	98.0	94.0
	–	10.00	0.20	10.00	0.189	19.55	94.5	95.5
	–	10.00	0.08	15.00	0.080	25.20	100.0	101.3
2[b]	–	10.00	–	–	–	9.86	–	–
	–	10.00	0.10	5.00	0.100	14.98	100.0	99.6
	–	10.00	0.09	14.00	0.092	25.00	102.2	107.1
	–	10.00	0.30	20.00	0.310	31.10	103.3	105.5

[a]Isoniazid (100 mg per tablet); Daru Pakhsh, Tehran, Iran, [b]Isoniazid (300 mg per tablet); Daru Pakhsh, Tehran, Iran, *Mean value (n = 3).

TABLE-7
RESULTS OF SIMULTANEOUS DETERMINATION OF HZ AND INH IN DRINKING WATER, BLOOD PLASMA AND SERUM SAMPLES USING HPSAM (THREE REPLICATE)

Sample	A-C equation	Spiked found ($\mu g\ mL^{-1}$)		Found ($\mu g\ mL^{-1}$)[a]	
		HZ	INH	HZ	INH
Drinking water	A_{20} = 0.0167 Ci + 0.3534	0.20	5.00	0.196	5.07
	A_5 = 0.0041 Ci + 0.2537			(98)	(98)
Plasma 1	A_{20} = 0.0178 Ci + 0.4479	0.05	5.00	0.048	4.90
	A_5 = 0.0038 Ci + 0.2792			(96)	(98)
Plasma 2	A_{20} = 0.0194 Ci + 0.4584	0.10	30.00	0.100	30.96
	A_5 = 0.0036 Ci + 0.2338			(100)	(103)
Plasma 3	A_{20} = 0.0185 Ci + 0.4396	0.30	10.00	0.292	10.18
	A_5 = 0.0039 Ci + 0.2862			(97)	(102)
Serum 1	A_{20} = 0.0188 Ci + 0.4126	0.15	10.00	0.144	10.12
	A_{20} = 0.0175 Ci + 0.4074			(96)	(101)
Serum 2	A_5 = 0.0040 Ci + 0.2791	0.20	20.00	0.21	2076
	A_{20} = 0.0183 Ci + 0.4233			(105)	(104)
Serum 3	A_5 = 0.0044 Ci + 0.2706	0.08	4.00	0.076	4.23
				(95)	(106)

[a]Values of recovery are given in parenthesis.

Conclusion

In this work it is shown that the application of HPSAM can be well adopted for simultaneous determination of HZ and INH. This method is cheaper than chromatographic methods, furthermore, this method does not require any organic solvents. In other words it belongs to green chemistry. The proposed method is inexpensive and sensitive method offers good selectivity, accuracy and precision that can be applied for a wide range of HZ and INH concentrations. The main advantages of the proposed method compared to many routine analytical methods for simultaneous determination of HZ and INH, are its simplicity, its ability to determine the lower concentrations of hydrazine in the presence of high amounts of isoniazid.

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