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A sensitive colorimetric determination of microquantities of isonicotinic acid hydrazide (isoniazid)

Prodromos B. Issopoulos and Pantelis T. Economou

Laboratory of Analytical Chemistry, Department of Inorganic and Analytical Chemistry, University of Ioannina, Ioannina (Greece)

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Summary

The aim of this investigation was to develop a colorimetric method able to determine microquantities of isoniazid (INH), either in pure form or in pharmaceutical formulations. The method described is based on the reduction, under the optimum conditions of Fe(III) to Fe(II) by INH and then the Fe(II) resulted, is reacted with *o*-phenanthroline (*o*-Phen) to form the well-known highly stable orange-red coloured chelate complex $[Fe(II)\text{-}(o\text{-}Phen)_3]^{2+}$, the ferroin complex. The latter exhibits an absorption maximum at $\lambda = 510$ nm, with an apparent molar absorptivity of 2.28×10^4 $l \cdot mol^{-1} \cdot cm^{-1}$ (referred to drug analysed), while the Sandell's sensitivity was calculated as 6.0 $ng \cdot cm^{-2}$. Beer's law is obeyed over the range of 0.5–10.0 ppm of INH. The regression line equation is $A = 0.16C + 0.016$ with a correlation coefficient of 0.9995 ($n = 6$). The results obtained from the determination of INH, using the described procedure and the official U.S.P. XVIII method, were compared statistically by means of the Student's *t*-test and by the variance ratio *F*-test and no significant difference was found.

Introduction

Isoniazid (INH, pyridine-4-carboxyhydrazide) is the hydrazide of isonicotinic acid, and one of the principal drugs used in the treatment of pulmonary tuberculosis, but also appears to be effective in the treatment of extrapulmonary lesions, including meningitis and genitourinary diseases.

INH has also been successfully used in the treatment of lupus vulgaris, while in the treatment of leprosy, it has not been found as useful as dapsone and other sulphones although it has antileprotic activity.

Correspondence: P.B. Issopoulos, Laboratory of Analytical Chemistry, Department of Inorganic and Analytical Chemistry, University of Ioannina, Ioannina, Greece.

Several procedures, except those of the official Pharmacopoeias, have been reported in the scientific literature for the INH determination.

So, during the period 1983–1985 more than twenty interesting methods of several analytical techniques have been published. The most representative of these methods are classified in various categories as follows: titrimetric (Verma and Gulati, 1982), spectrophotometric (Fujita et al., 1983), chromatographic (Carr and Fish, 1983), potentiometric (Radhakrisnamurti and Rao, 1983), polarographic (Emel'yanenko et al., 1985). Another spectrophotometric procedure based on the reduction of Mo(VI) by INH in a warm sulphuric acid medium and the measurement of the absorbance of the isopoly-'molybdenum blue' formed are described by Issopoulos and have recently been accepted for publication (Issopoulos, 1989).

In the present paper, a simple, rapid, accurate and highly sensitive colorimetric procedure for the determination of microquantities of INH present in the pure form or in pharmaceutical formulations is described.

The method is based on the measurement of the Tris(*o*-phenanthroline-Fe(II)) chelate complex (ferroin complex) formed by reaction of INH with a mixture of *o*-phenanthroline and Fe(III), under optimum conditions.

Materials and Methods

Materials

INH (Fluka, purum 99%, Art. No. 58980) The standard solution was prepared fresh, as required, by dissolving the appropriate amount of INH in water to provide 20 ppm ($= 20\ \mu g \cdot ml^{-1}$) solution of INH (the concentration is expressed in terms of the anhydrous 100% pure substance). The resultant solution must be stored under light protected conditions at about 4 °C. The exact concentration of the standard solution was determined spectrophotometrically (Clarke, 1974).

o-Phen-iron(III) mixture 0.50 g of *o*-phenanthroline monohydrate (Fluka, puriss. p.a. No. 77500), 5.0 ml of 1 M HCl solution and 0.40 g of ammonium ferric sulphate dodecahydrate (Merck, p.a. No. 3776) were dissolved and then diluted with water to 250 ml. The solution is stable for at least four weeks, if it is stored in a dark cool place, e.g. in a refrigerator.

Freshly prepared, double distilled, de-ionized water was used throughout.

Apparatus

A Hitachi, Model 100-80, double-beam ratio recording spectrophotometric system, with matched 10.0-mm quartz cells, was used for all absorbance measurements during the development of this procedure.

A WTW Model 522 pH-meter, with a precision combined glass-calomel electrode E56, was utilized for all pH measurements.

A thermostated constant temperature bath, accurate to ± 0.5 °C was employed.

Recommended procedure

A volume V ml ($V = 1.25$–25.0 ml) of the INH standard solution corresponding to 25 μg–500 μg of INH, respectively, was transferred quantitatively into a 50-ml volumetric flask, and diluted to about 45 ml with water. The pH of this solution was adjusted to 5.0 ± 0.2, with the necessary volume of 1 N HCl or 1 N NaOH, and then 2.0 ml of the *o*-Phen-iron(III) mixture were added. The resultant solution was diluted to volume with water, was mixed by shaking, was transferred into a 50-ml testtube and finally was placed for 20–25 min into a water-bath thermostated at 50 ± 0.5 °C. After the heat treatment, the solution was immediately cooled to room temperature (about 20 °C) using a cold water-bath. The absorbance of the orange-red coloured solution was measured at $\lambda = 510$ nm, against a INH-blank solution prepared under the same conditions.

Results and Discussion

o-Phenanthroline is universally known as one of the principal reagents for the colorimetric determination of Fe(II). A strongly orange-red coloured complex of hexacovalent-type $(FeL_3)^{2+}$ can be formed quantitatively in the pH range 2.0–9.0, and which appears at an absorption maximum of $\lambda = 510$ nm. The solution of this coloured chelate complex shows no change in colour after many months and Lambert-Beer's law is closely followed (Onishi, 1986). The recommended procedure is based, as was reported previously, on the oxidation of INH by the Fe(III) of the *o*-Phen-Fe(III) mixture used, with simultaneous formation of an equivalent quantity of the above mentioned Tris(*o*-Phen-Fe(II)) orange-red chelate complex, also known as the ferroin complex. The optimum colour intensity was obtained at $pH = 5.0 \pm 1.0$, while the stoichiometric reaction was accomplished within 60 min at 25 °C or within 20 min at 50 °C.

Absorption spectrum

The absorption spectrum of the aforesaid chelate complex formed by the reducing action of INH, was scanned in the double-beam mode

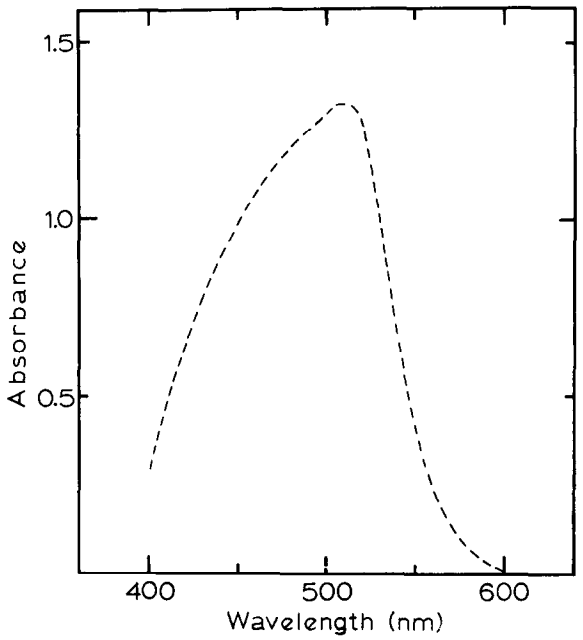

Fig. 1. Typical absorption spectrum of ferroin obtained by application of the proposed method. Concentration of INH used, 8.0 ppm; pH, 5.0 ± 0.2; scan rate, 50 nm · min^{-1}.

against a reagent blank, with a scan rate of 50 nm · min^{-1} in the range 390–600 nm. The automatic base-line corrector was employed, while the base-line was being determined and checked with both sample and blank cells filled with reagent blank. Fig. 1 shows this absorption spectrum.

The apparent molar absorptivity (i.e. referred to INH analysed) and other spectral characteristics, as well as the measured or calculated analytical factors, are presented in Table 1.

Calibration graph

The calibration graph for the determination of INH was traced according to the above described procedure. The linearity of this calibration graph (five replicates for six different concentrations) was checked by a linear least-squares treatment using a Macintosh SE computer.

Beer's law limits, the regression line equation, the correlation coefficient and the Sandell's sensitivity, regarding to this calibration graph, are summarized in Table 1.

TABLE 1

Spectral characteristics and other analytical factors

Characteristic	Numerical data
λ_{max} (nm)	510
Adherence to Beer's law (in ppm)	0.2–12.0
Optimum concentration range (in ppm)	0.5–10.0
ϵ(l · mol^{-1} · cm^{-1}) [a]	2.28×10^4
Sandell's sensitivity (ng · cm^{-2}) [a]	6.00
Regression line equation (r.l.e.): $A = mC \pm z$	
(A = Absorbance; C = Concentration (in ppm))	
Slope (m) (in ppm)	1.6×10^{-1}
Intercept (z)	1.6×10^{-2}
Correlation coefficient (r)	0.9995
Number of points on which the r.l.e. is based	6

[a] Average of six determinations (ϵ is referred to INH analysed).

Effect of pH on the absorbance

The effect of pH on the Fe(III) reduction by the INH analysed, as well as on the formation of the ferroin chelate complex, was studied over the range 1.0–8.0.

Fig. 2 shows that the absorbance is practically constant from pH 4.0 to 6.0. In more acidic or

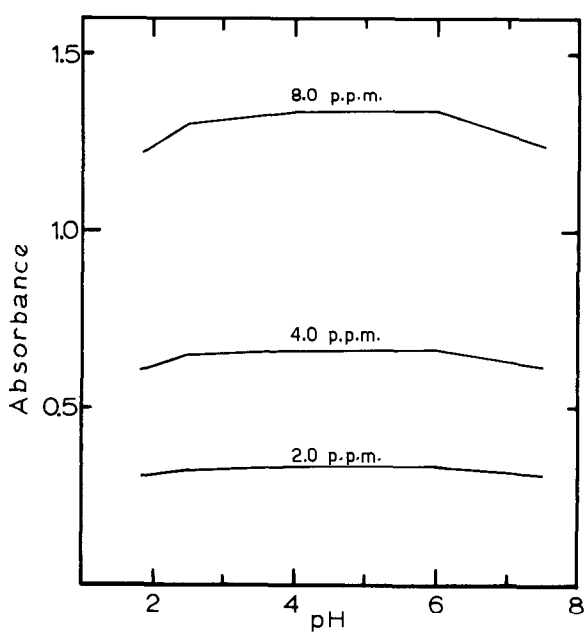

Fig. 2. Effect of pH on the absorbance at various concentrations.

more alkaline solutions, the absorbance decreased because of the incomplete complex formation or hydrolysis of the complex formed, respectively (Sandell, 1959; Skoog and West, 1970; Onishi, 1986).

To obtain the pH value required, the necessary volume of 1 N HCl or 1 N NaOH was added to the solution studied. The use of a buffer solution was avoided, because the presence of any foreign ion or molecule with undesirable properties (viz. reducing action, complexing ability, etc.) could interfere with formation of the complex examined and with its absorption spectrum.

According to the aforesaid conclusions, all the absorbance measurements were realized in solutions of three different concentrations of the same pH equal to 5.0 ± 0.2, since under this condition the complex measured is stable for at least 24 h.

Effect of reaction time in different temperatures, on the absorbance

The absorbance of the ferroin complex formed was measured as a function of time, following the mixing of the *o*-Phen-iron(III) mixture and the solution of INH examined, as well as of the temperature in which this formation take place.

At $25 \pm 0.5\,^{\circ}C$, the formation of the ferroin complex was completed within 60 min, and the absorbance measured reaches the maximum intensity, which from that time remains unchangeable for at least 24 h.

At $50 \pm 0.5\,^{\circ}C$, the formation rate of the discussed complex was faster and was accomplished within 20 min, with the same behaviour of its absorbance. In Fig. 3 the evolution of the above-mentioned phenomena is shown.

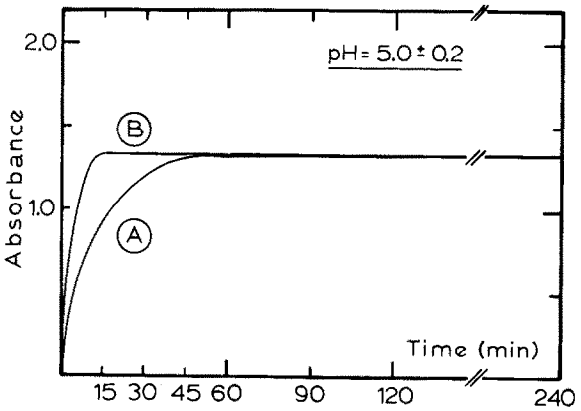

Fig. 3. Effect of reaction time on the absorbance in different temperatures. A, $25\,^{\circ}C \pm 0.5\,^{\circ}C$; B, $50\,^{\circ}C \pm 0.5\,^{\circ}C$; pH, 5.0 ± 0.2.

Accuracy and precision

In order to determine the precision and the accuracy of the proposed procedure, solutions containing four different concentrations of INH were prepared and were analysed in five replicates.

The analytical results obtained from this investigation are summarized in Table 2. The mean rsd% and SAE (standard analytical error) were so advantageous as to be considered very satisfactory for the level of assayed concentrations. All the

TABLE 2

Accuracy and precision of the described procedure

No.	Isoniazid in ppm		rsd%	SAE [a]	Confidence limits ($p = 0.05$; $(n-1) = 4$)
	Added	Found ± SD [b]			
1	0.5	0.491 ± 0.0167	3.40	0.0075	0.491 ± 0.021
2	2.0	2.010 ± 0.035	1.76	0.016	2.010 ± 0.044
3	6.0	6.002 ± 0.099	1.65	0.044	6.002 ± 0.122
4	10.0	9.972 ± 0.155	1.55	0.069	9.972 ± 0.191
Mean rsd%			2.09		
Mean SAE				0.034	

[a] SAE = standard analytical error ($SD/\sqrt{n}$).

[b] Average of five determinations ($n = 5$).

TABLE 3

Determination of INH by the proposed procedure compared with the official method

Samples	Proposed method		Official method [a]		t value [e]	F value [f]
	Recovery% ± SD [b]	rsd%	Recovery% ± SD [b]	rsd%		
Pure substance	100.04 ± 1.65	1.65	99.89 ± 1.57	1.57	0.146	1.10
Tablets, 50 mg (Roche) [c]	102.40 ± 1.14	1.11	102.20 ± 1.15	1.13	0.282	1.08
Injections, 100 mg/2 ml (Chropi) [c]	103.00 ± 0.79	0.77	102.40 ± 0.65	0.64	1.504	1.66
Capsules, 50 mg [d] (Laboratory-prepared)	100.20 ± 0.84	0.83	99.70 ± 1.72	1.72	0.589	0.24

[a] United States Pharmacopeia XVIII Revision, p. 349–351.
[b] Average of five determinations.
[c] Nominal content.
[d] The laboratory-prepared capsules contain Aerosil as inert excipient.
[e] Tabulated t value for $p = 0.05$ and d.f. = 8 is equal to 2.306.
[f] Tabulated F value for $p = 0.05$ and $f_1 = f_2 = 4$ is equal to 6.39.

above discussed points of view, are valid for the calculated confidence limits also.

Application of the proposed method in the field of the pharmaceutical analysis and statistical comparative study of the results

The results, obtained by application of the reported method in the assay of INH in pure form or in pharmaceutical formulations, were compared statistically by the Student's t-test and by the variance ratio F-test (Saunders and Fleming, 1979) with those obtained by application of the official U.S. Pharmacopeia's method (U.S.P. XVIII Revision, 1970) on the same samples.

As is shown in Table 3, the Student's t-test values at the 95% confidence level and d.f. = 8 (degrees of freedom) did not exceed the theoretical tabulated t value ($t = 2.306$), either every one alone or as the mean of all of them, indicating no significant difference between the methods compared.

On the other hand, the variance ratio F values at the same confidence level and for $f_1 = f_2 = 4$ also indicate that there is no significant difference between the precision of the method recommended and that officially applied.

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