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Analytical investigations of isonicotinic acid hydrazide (isoniazid). VI. Sensitive colorimetric determination of micro amounts of isoniazid using an indirect redox method

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Summary

An adequately sensitive colorimetric method for the determination of microquantities of isoniazid (INH) either in pure form or in pharmaceutical formulations is described. The method is based on the reduction of Fe(III) by INH, followed by the reaction of Fe(II) with 2,2′-dipyridyl (DPRL) to form, in an aqueous medium (pH 4.50 ± 0.05; t 50 ± 0.5 °C), the highly stable and intensely red-coloured [Fe(II)-(DPRL)$_3$]$^{2+}$ chelate complex which shows λ_{max} at 522 nm and obeys Beer's law from 0.25 to 12.0 ppm of the drug concentration. The regression line equation is $A = 1.48 \times 10^{-1}\, C + 5.05 \times 10^{-4}$ with a correlation coefficient of 0.9998 ($n = 7$). The molar absorptivity and Sandell's sensitivity, both referred to INH analysed, are 2.03×10^4 l mol^{-1} cm^{-1} and 6.76 ng cm^{-2}, respectively. The accuracy and precision of the method were checked; the results obtained from the determination of INH, using the proposed procedure and the official U.S.P. XXI method, were compared statistically by means of Student's t-test and by the variance ratio F-test and no significant difference was observed.

In recent analytical works, the determination of a drug, which is characterized as a reducing substance, may be carried out in two steps. Firstly, the drug reduces Fe(III) to Fe(II); the second step is the complexation of Fe(II) with a proper chelate ligand.

These ligands are structurally distinguished by the presence of the functional group -N = C-C = N- (ferroin group), such as 1,10-phenanthroline, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine), etc., reagents widely known for their high sensitivity to iron and especially to iron (II).

The chelate complexes formed are intensely coloured and so are suitable for colorimetry of the metal cation and consequently for the analytical estimation of the reducing drug.

This technique has been successfully applied for the purpose of colorimetric determination of isoniazid (INH) (Issopoulos and Economou, 1989;

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Issopoulos, 1990a), or other pharmaceutical substances such as levodopa, carbidopa and α-methyldopa (Issopoulos, 1990c), acetaminophen (Issopoulos, 1990b), cephalosporins of the first generation (Issopoulos, 1990d).

On the other hand a similar treatment was also employed in pharmaceutical analysis by others (Besada, 1987a,b; Sankar et al., 1988; Carmona et al., 1989; Koupparis et al., 1989).

In this work, based on the above-mentioned analytical process, a colorimetric procedure for the determination of microquantities of INH, either in pure form or in pharmaceutical formulations, has been developed under experimentally optimized conditions, using 2,2′-dipyridyl, as chelating agent.

A standard solution of 50 ppm of INH (Fluka, purum 99%, no. 58980), was prepared. Fe(III) reagent solution (50 μg Fe(III) per ml) was prepared by dissolving 435 mg of ammonium ferric sulphate dodecahydrate (Merck, p.a., no. 3776) in 10 ml 4 M H_2SO_4 and diluting to volume with water in a 1000 ml calibrated flask. The exact final concentration of Fe(III) was determined spectrophotometrically (Sandell, 1947). 2,2′-Dipyridyl (DPRL) reagent solution was prepared by dissolving 400 mg of DPRL (Merck, p.a., no. 3098) in a 1000 ml calibrated flask and diluting to volume with water. Sodium acetate (2 N)/acetic acid (2 N) buffer solutions (pH range 3.80–6.30) (Lurie, 1971) were used. Freshly prepared, de-ionized and thereafter doubly-distilled water was used throughout.

The recommended procedure was carried out as follows: into a 20 ml volumetric flask, the following components were pipetted in order: 5.0 ml Fe(III) reagent solution, 5.0 ml buffer solution, 0.1–4.8 ml standard solution of INH, containing 5–240 μg INH, respectively, and lastly 5.0 ml DPRL reagent solution.

The mixture, when necessary, was diluted to 23–24 ml with water, mixed by shaking and kept for 30 min in a water bath at 50 ± 0.5 °C, under light-protected conditions. Subsequently, the solution was cooled rapidly to room temperature (20 ± 2 °C), then diluted to volume with water and its absorbance was measured at λ = 522 nm, against a similarly treated INH blank solution. The examined concentration of the drug analysed was determined from a calibration graph constructed under the same experimental conditions, according to the regression line equation: $A = 1.48 \times 10^{-1}\ C + 5.05 \times 10^{-4}$ ($r = 0.9998$; $n = 7$).

The chemical part was integrated in two steps: the first includes the quantitative oxidation of INH by Fe(III), followed by the second step, in which the amount of Fe(II), resulting from the above-mentioned step, and which is equivalent to INH oxidized, was chelated, under experimentally optimized conditions with DPRL, in such a way that the performance became quantitative. The experimental determination, the choice and mainly the strict adherence to the conditions given, are considered as absolutely necessary, because of the probability of formation of Fe(II)-INH, 1 : 1 and 1 : 2 chelate complexes, being evident and obviously undesirable.

The absorption spectrum of the $[Fe(II)\text{-}(DPRL)_3]^{2+}$ formed was measured in the double-beam mode vs an INH blank solution, at a scan rate of 50 nm min^{-1} in the range 350–600 nm. Automatic baseline correction was used, while the same baseline was determined and checked with both sample and blank cells filled with INH blank solution. Maximum absorption occurred at λ = 522 nm. Beer's law was obeyed over the concentration range 0.25–12.0 ppm, with an optimum region of 0.50–10.0 ppm. The apparent molar absorptivity as well as Sandell's sensitivity (for log $I_0/I = 10^{-3}$), both referred to INH analysed, were calculated to be 2.03×10^4 l mol^{-1} cm^{-1} and 6.76 ng cm^{-2}, respectively (average of seven determinations).

The effect of pH on Fe(III) reduction by INH, as well as on formation of the $[Fe(II)\text{-}(DPRL)_3]^{2+}$ complex, was studied over the range pH 3.80–6.30, using a CH_3COONa (2 N)-CH_3COOH (2 N) buffer solution. The results of this study show that the absorbance remains practically constant within the above pH range and hence, all the absorbance measurements were carried out in solutions of pH 4.50 ± 0.05, since under these conditions the complex formed remains stable for at least 4 h.

On the other hand, the absorbance of the $[Fe(II)\text{-}(DPRL)_3]^{2+}$ complex was measured at three different temperatures (20, 35 and 50 °C) as

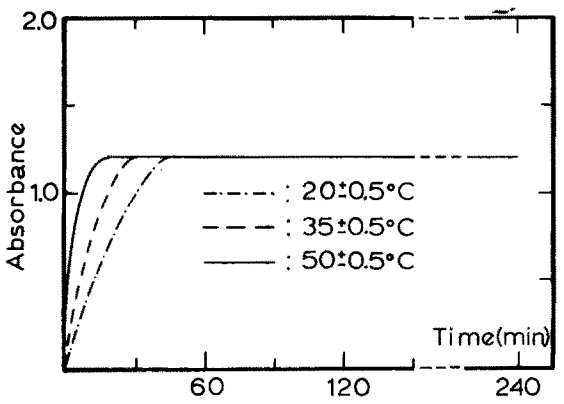

Fig. 1. Effect of reaction time on the absorbance in different temperatures. Concentration of INH: 8.25 ppm; pH = 4.50 ± 0.05.

a function of reaction time. At 20 ± 0.5 °C, formation of the complex had reached completion within 45 ± 2 min and the absorbance attained its maximum intensity; at 35 ± 0.5 °C, the rate of formation of the chelate complex was faster and was accomplished within 30 ± 2 min, while at 50 ± 0.5 °C the same chemical praxis was finished within 20 ± 2 min. Fig. 1 shows the effect of heating time on the formation of the complex.

In order to study the accuracy and precision of the new method, solutions of three different concentrations of INH (1.0, 5.0 and 10.0 ppm) were prepared and analysed in quintuplicate. The results of this analytical process are summarized in Table 1.

The experimentally determined concentrations, as well as the calculated analytical factors, such as SD, rsd%, SAE (standard analytical error) and confidence limits, were considered very satisfactory at least for the level of the concentrations examined.

Subsequently, this method was applied for the determination of INH in pure form or in pharmaceutical formulations (tablets, injections, capsules). Therefore, the same batches of pure form of INH and of Rimifon (Roche) tablets of 50 mg, Dianicotyl (Chropi) injections of 100 mg/2 ml and laboratory prepared capsules of 50 mg, were analysed five times, using the above method and also simultaneously by the official USP XXI (1985) method. The values of the recovery% and rsd% were:

(a) For INH in the pure form: 101.08 ± 0.8 and 0.97% (new method) and 99.89 ± 1.57 and 1.57% (USP method);

(b) For INH in Rimifon tablets: 102.66 ± 0.92 and 0.89% (new method) and 102.20 ± 1.15 and 1.13% (USP method);

(c) For INH in Dianicotyl injections: 103.10 ± 0.89 and 0.87% (new method) and 102.40 ± 0.64 and 0.62% (USP method);

(d) For INH in laboratory prepared capsules: 100.46 ± 1.05 and 1.05% (new method) and 99.70 ± 1.72 and 1.72% (USP method).

All the above sets of results were compared statistically by calculation of the *t*-test and *F*-test values (Saunders et al., 1957). The Student's *t*-test values at the 95% confidence level and d.f. = 8 (d.f., degrees of freedom) were (a) 1.412, (b) 0.689, (c) 1.526 and (d) 0.598, when the tabulated *t*-value was equal to 2.306. On the other hand, the variance ratio *F*-test values at the same confidence level and at $f_1 = f_2 = 4$, were (a) 2.38, (b) 1.33, (c)

TABLE 1

Accuracy and precision of the proposed method

No.	Isoniazid (in ppm)		rsd%	SAE	Confidence limits ($p = 0.05$; d.f. = 4; $t = 2.776$)
	Added	Found (± SD) [a]			
1– 5	1.0	0.999 ± 0.0120	1.21	0.0054	0.999 ± 0.015
6–10	5.0	4.984 ± 0.0376	0.75	0.0168	4.984 ± 0.047
11–15	10.0	10.008 ± 0.0842	0.84	0.0376	10.008 ± 0.104
Mean rsd%			0.935		
Mean SAE				0.0199	

[a] Average of five determinations.

2.50 and (d) 2.57, considerably inferior from the theoretical tabulated value of $F = 6.39$.

These results of the statistical analysis prove that there is no significant difference between the methods compared, as well as demonstrating that the precision of the new method described here does not differ significantly from the USP method.

The presence of the commonly used excipients for tablets, such as starch, talc, Mg stearate, acacia gum and phosphates did not interfere with the final estimation of INH, while the metabisulfite anion, which is added to some injectable formulations, can interfere with the determination of INH, however, it is present in such small amounts (usually added at the 0.1% level) that its influence can be taken to be negligible. Glucose and lactose did not interfere with the determination as reducing agents, but as is well known, they do interact significantly with INH on being allowed to stand over an extended period, forming the corresponding hydrazones (Wu, et al., 1970; Rao et al., 1971).

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