Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 6, pp. 421–426, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Colorimetric and titrimetric assay of isoniazid

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Abstract: Two methods are proposed for the determination of isoniazid in pure form or in tablets. In the first method chlorpromazine hydrochloride, when treated with 2-iodoxybenzoic acid as an oxidant in 50% w/v *o*-phosphoric acid solution, is oxidized to chlorpromazine free radical which absorbs at 530 nm. The red free radical is readily reduced quantitatively by isoniazid to the colourless chloropromazine. The addition of isoniazid to a red solution of chlorpromazine free radical results in a decrease in absorbance in direct proportion to the quantity of isoniazid. This forms the basis for the quantitative determination of micro-quantities of isoniazid (3–18 μg ml^{-1}). The second method involves the titrimetric determination of isoniazid using *N*-bromophthalimide as a titrant. The end-point is determined either directly using methyl red or amaranth as indicator, or by a back titration method in which a known excess of *N*-bromophthalimide solution is added to isoniazid solution and then the residual unreacted reagent is determined iodometrically. The results by the proposed procedures were in good agreement with those obtained by the official methods.

Keywords: *Isoniazid; colorimetry; titrimetry; chlorpromazine hydrochloride; 2-iodoxybenzoate;* N-*bromophthalimide.*

Introduction

Isoniazid is an effective drug widely used in the treatment of tuberculosis. Existing analytical procedures for the assay of isoniazid include titrimetry [1–7], spectrophotometry [8], colorimetry [9–13], fluorimetry [14], atomic absorption spectrometry [15], polarography [16], coulometry [17], and high-performance liquid chromatography [18].

In this work, two methods are proposed for the determination of isoniazid in pure form or in tablets. The former involves the production of stable chlorpromazine free radical (CPZ°) using 2-iodoxybenzoate as an oxidant in *o*-phosphoric acid. The free radical, which is a red compound with an absorption maximum at 530 nm, is reduced by isoniazid to chlorpromazine (CPZ) resulting in a colourless solution.

The applications of chlorpromazine hydrochloride have been studied by Lee [19] as an analytical reagent for the detection of certain metallic ions and oxidizing agents. The sulphur atom in the molecule is very susceptible to oxidation. When an oxidant reacts with a large excess of CPZ hydrochloride in an acid medium, the product of oxidation is a red free radical compound which absorbs at 530 nm [20].

A wide variety of oxidants [20] such as ceric, ferric permanganate, bromate, iodate, nitrite and *o*-iodobenzoate ions have been shown to oxidize CPZ to CPZ° in aqueous solution. Ascorbic acid, ferrous ammonium sulphate, sodium thiosulphate and the reduced form of the coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, are reducing substances which can reduce CPZ° free radical [20].

In this study, a new oxidant, 2-iodoxybenzoate [6] is used for the production of CPZ° free radical which, when reduced by isoniazid, provides a new sensitive method for isoniazid.

The reported titrimetric methods for isoniazid include bromimetry [1, 4], nitrimetry [2, 4] and oxidimetry, the latter using hexacyanoferrate [5], iodine monochloride [3], 2-iodoxybenzoate [6] or permanganate [7] as titrants.

The second method proposed in this work involves the oxidimetric determination of isoniazid by using *N*-bromophthalimide (NBP) as a titrant. The molar ratio was calculated and the equivalent point was determined either by direct method using methyl red or amaranth as indicator or by back titration method.

This reagent has been used successfully in this laboratory as an organic brominating and oxidizing agent for many pharmaceutical compounds [21–24].

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Experimental

Apparatus and reagents

Absorption spectra were recorded on a Pye-Unicam SP 1800 UV–vis spectrophotometer with 1-cm cells. Chlorpromazine hydrochloride was obtained from May and Baker Ltd (Dagenham, UK). A 3% aqueous solution was prepared and protected from light in a dark bottle. This solution was stable for at least 3 months at room temperature. *o*-Iodoxybenzoic acid was prepared as described by Banerjee *et al.* [25]. A 5×10^{-4} M solution was prepared by dissolving 0.14 g of free acid in a slight excess (about 6 ml) of 1 M potassium hydroxide and diluting it to 1 l with deionized water. This solution was stable for at least 2 months at room temperature. *N*-Bromophthalimide was prepared according to Jolles [26]. A 0.01 M solution was prepared by dissolving 2.26 g in sufficient glacial acetic acid then diluting to 1 l with the same solvent. This solution was standardized iodometrically. It was stable for over 2 months, if protected from light. A 0.02 M solution of sodium thiosulphate was prepared by dissolving 4.98 g in deionized water and diluting to 1 l. A 1% aqueous solution of starch mucillage and a 50% (w/v) aqueous solution of *o*-phosphoric acid (Merck) were also prepared.

A solution containing 1 mg ml^{-1} isoniazid (Analar grade) (BDH, Poole, Dorset, UK) was prepared in deionized water. Other concentrations were prepared by dilution of this solution. A 0.01 M aqueous solution was also prepared.

Procedures

1. *Colorimetric method.* The CPZ° free radical solution was prepared by mixing 1 volume of 5×10^{-4} M *o*-iodoxybenzoate solution, and 2 volumes of 50% (w/v) *o*-phosphoric acid in a conical flask. The solution was allowed to stand for 10 min and then 1 volume of 3% CPZ hydrochloride solution was added and mixed. This solution is stable for at least 3 h at room temperature when protected from light. Deionized water must be used for the preparation of all reagents. An aliquot of the standard or sample of isoniazid solution in water (containing 30–180 μg) was pipetted into a 10 ml volumetric flask and 4 ml of the CPZ° reagent were added. The thoroughly mixed solution was allowed to stand for 10 min at room temperature and diluted to volume with deionized water. The absorbance was measured at 530 nm using water in the reference cell (A_1). A reagent blank was prepared and its absorbance was measured at 530 nm against water (A_2).

A calibration graph was made by plotting ΔA versus the concentration of isoniazid, where ΔA is the difference in absorbance between the blank (A_2) and test (A_1) solutions. The quantity of isoniazid in the sample was determined from the calibration graph or by using a regression equation.

2. *Titrimetric method.*

(a) Direct method. A volume of solution containing 1–15 mg of isoniazid was pipetted into a conical flask and 5 ml of dil. hydrochloric acid were added. The solution was titrated with 0.01 M NBP using amaranth or methyl red as indicator: at the end-point, decolourization occurs. A blank titration was carried out in the same manner.

(b) Back titration method. A measured volume of 0.01 M NBP was added to a solution containing 1–15 mg of isoniazid in a stoppered conical flask. 10 ml of 10% potassium iodide solution was added and the liberated iodine was titrated against 0.02 M sodium thiosulphate solution (V_1) using starch as indicator. A blank titration was carried out in the same manner (V_2). The amount of isoniazid was calculated from the following equations.

Isoniazid (mg) = $[E.P. - a] \times 0.6855$ (direct method), where $E.P.$ is the sample end-point and a is the blank end-point. Or isoniazid (mg) = $(V_2 - V_1) \times 0.6855$ (back titration method) where V_1 and V_2 are volumes of thiosulphate solution consumed by the test and blank solutions, respectively.

The molar ratio between isoniazid and NBP was calculated by titrating different aliquots of 0.01 M isoniazid solution with 0.01 M NBP by both methods.

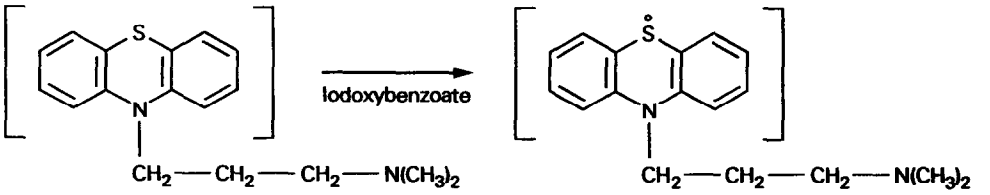
Procedures for isoniazid tablets

Twenty tablets were pulverized and an accurately weighed amount of the powder equivalent to 100 mg of isoniazid was dissolved in water and filtered into a 100 ml volumetric flask. The residue was washed several times with water and the combined filtrates were diluted to volume with water. The concentration of isoniazid was determined by using

the 'direct' or 'back' titration procedure described above.

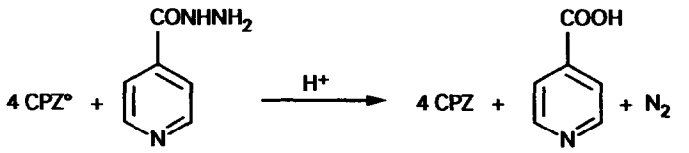
Stoichiometry of the reactions

In the colorimetric procedure chlorpromazine is oxidized by iodoxybenzoate to chlorpromazine free radical:



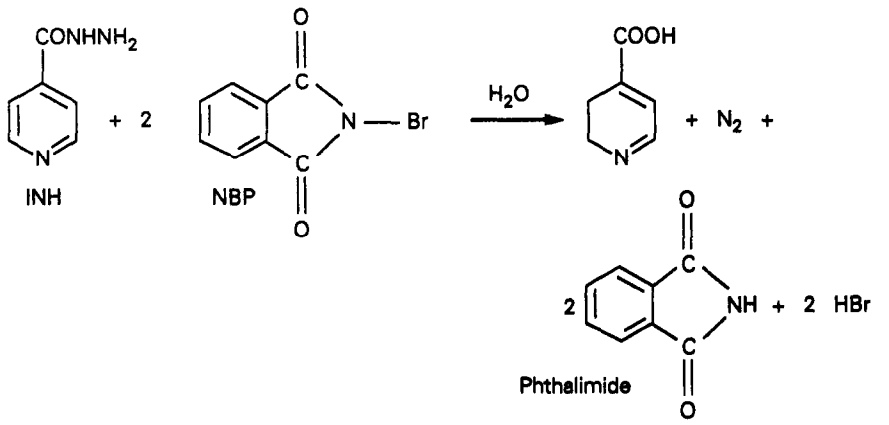
CPZ (colourless) CPZ° (coloured)

The isoniazid reduces the coloured CPZ° free radical to the colourless CPZ. The reaction involves 4 moles of free radical per mole of isomazid as shown:



Isoniazid Isonicotinic acid

In the titrimetric methods, isoniazid requires 2 moles of NBP (Table 1) even after a 1 h reaction time. The reaction is:



INH NBP Phthalimide

Although the generation of CPZ° must be carried out in an acidic medium, the free radical is not stable in all types of acids. It is stable in 50% (w/v) sulphuric acid, but in this medium reduction of the free radical by isoniazid is slow and non-stoichiometric. However, in the presence of *o*-phosphoric acid at a final concentration of at least 10% (w/v), the free radical generated is stable for at least 3 h. This concentration was selected for the assay procedure.

When CPZ hydrochloride solution was added to increasing quantities of *o*-iodoxybenzoate in the presence of 10% (w/v) *o*-phosphoric acid, CPZ° was produced in direct proportion to the quantity of *o*-iodoxybenzoate.

When increasing quantities of isoniazid were added to a solution containing CPZ°, there was

Table 1
Molar ratio of isoniazid and *N*-bromophthalimide

Isoniazid (mmol)	*N*-Bromophthalimide (mmol) Direct (methyl red indicator)	Back	Molar ratio
0.02	0.041	0.038	
0.05	0.108	0.101	
0.08	0.163	0.160	1:2
0.10	0.198	0.203	

Table 2
Determination of isoniazid by the colorimetric and titrimetric methods

Proposed colorimetric method		Proposed titrimetric method			USP method [2]
			Recovery (%)*		
Amount taken ($\mu g\ ml^{-1}$)	Recovery (%)*	Amount taken (mg)	Direct (methyl red indicator)	Back	Recovery (%)*
3	101.1	1	99.4	101.4	100.8
6	100.8	2	100.8	100.7	99.8
9	100.0	4	99.4	100.0	101.1
12	99.9	5	100.0	101.4	
15	100.6	8	101.0	99.5	
18	100.9	10	101.4	100.6	
		12	100.3	99.9	
		15	100.0	101.2	
Mean ± RSD†	100.54 ± 0.49		100.29 ± 0.74	100.60 ± 0.72	100.58 ± 0.69
Student's *t*-test	0.101 (2.365)‡		0.585 (2.262)‡	0.041 (2.262)‡	
Variance ratio	1.984 (5.790)‡		1.143 (19.350)‡	1.089 (19.350)‡	

Regression equation ($A = a + bc$) $A = -0.003933 + 0.02744c$ ($r = 0.9985$).§
*Each recovery % is the average of three determinations.
†Mean ± RSD : mean recovery % ± relative standard deviation.
‡The tabulated values at $P = 0.05$ [27].
§A: Absorbance difference at λ 530 nm, a: intercept, b: slope, c: concentration in $\mu g\ ml^{-1}$, r: correlation coefficient.

an immediate decrease in the colour of the solution which was complete after 10 min standing. The degree of reduction in absorbance of the solution is directly proportional to the quantity of isoniazid in the range 3–18 $\mu g\ ml^{-1}$ (Table 2).

Results and Discussion

Assay results

The percentage recoveries of isoniazid are summarized in Table 2. The results were compared with those obtained by using the USP [2] method. Statistical analysis [27] (Student's *t*-test, *F*-ratio) showed that there was no significant differences between these results. Moreover, the proposed procedures have advantages of simplicity, accuracy, sensitivity to small amount (3–18 $\mu g\ ml^{-1}$ and 1–15 mg can be determined by the colorimetric and titrimetric methods, respectively) and speed.

Table 3 shows that the results of the assay of isoniazid in tablet formulations by the two methods are in good agreement with those obtained by the USP method [2].

Table 3
Determination of isoniazid in tablet formulations

	Proposed colorimetric method		Proposed titrimetric method			
				Recovery (%)*		Official method [2] Recovery (%)*
Tablet	Amount taken ($\mu g\ ml^{-1}$)	Recovery (%)*	Amount taken (mg)	Direct (methyl red indicator)	Back	
Isocid†	5	99.3	5	99.4	100.7	101.0
(50 mg isoniazid per tablet)	8	99.0	8	99.0	101.0	98.9
	10	99.1	10	100.0	99.9	99.0
Mean ± RSD		99.14 ± 0.18		99.44 ± 0.51	100.54 ± 0.55	99.65 ± 1.15
Isocid fort†	5		5			
(200 mg isoniazid per tablet)	8	100.0	8	100.8	99.5	100.7
	10	101.0	10	101.0	100.5	99.9
Mean ± RSD		99.1		100.3	101.4	99.4
		100.04 ± 0.99		100.71 ± 0.33	100.50 ± 0.95	100.00 ± 0.64

*Average of three determinations.
†CID Company, Giza, Egypt.

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