

SCIENCE PAPERS AND DISCUSSIONS

THE DETECTION AND DETERMINATION OF ISONICOTINYL HYDRAZIDE

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*iso*NICOTINYL HYDRAZIDE has recently been introduced as an anti-tuberculosis drug, but very little has been published concerning its qualitative reactions and quantitative determination. Although the tests and methods described in this preliminary communication have not yet received exhaustive study it is hoped they might provide a basis on which to gain further experience. Since one of the main routes for the administration of *isonicotinyl* hydrazide is oral, its determination in the presence of tablet excipients is of particular importance and hence this aspect has been given special consideration.

1. QUALITATIVE TESTS

*iso*Nicotinyl hydrazide was expected to show reducing properties associated with the hydrazide side chain, and also to give reactions characteristic of the pyridine nucleus. Accordingly the qualitative tests proposed fall into 2 groups as shown in Table I.

TABLE I
QUALITATIVE TESTS FOR *ISONICOTINYL* HYDRAZIDE DEPENDING ON
(a) THE HYDRAZIDE SIDE CHAIN, AND (b) THE PYRIDINE NUCLEUS

	Test*	Result
(a)	1. Add 1 ml. of solution of silver nitrate.	White precipitate, which is slightly soluble in water, and soluble in dilute nitric acid; on warming a silver mirror forms.
	2. Add 1 ml. of a 5 per cent. aqueous solution of selenium dioxide.	Red precipitate.
	3. Add 1 ml. of a saturated solution of 1:2-naphthaquinone-4-sulphonic acid in ethanol (50 per cent.), followed by a few drops of sodium hydroxide solution.	A bright red colour.
	4. Add 1 ml. of a 1 per cent. aqueous solution of sodium pentacyano-amine ferroate and stand for about 10 minutes.	A red colour.
	5. Reflux 0.1 g. of <i>isonicotinyl</i> hydrazide with 20 ml. of 5N sodium hydroxide for 15 minutes. Dilute 1000 times with water. To 20 ml. add 5 ml. of a 2 per cent. solution of <i>p</i> -dimethylamino-benzaldehyde in a 10:1 mixture of ethanol and hydrochloric acid; stand for a few minutes.	A yellow colour.
(b)	6. Add 1 ml. of a 1 per cent. solution of mercuric chloride.	White precipitate.
	7. To 0.1 g. of borax in a test-tube, add 5 ml. of ethanol containing about 0.15 mg. of <i>isonicotinyl</i> hydrazide and 5 ml. of 5 per cent. solution of 1-chloro-2:4-dinitrobenzene in ethanol. Heat to dryness on a boiling water bath and then for a further 10 minutes. Add 10 ml. of methanol and stir.	Reddish-purple solution.

* Unless otherwise stated all the reactions were carried out by adding the reagent to 5 ml. of a 5 per cent. aqueous solution of *isonicotinyl* hydrazide.

Specificity of the Tests

- Tests 1 and 2. These tests are of low specificity and serve simply to characterise the reducing properties of *isonicotinyl* hydrazide.
- Test 3. 1:2-Naphthaquinone-4-sulphonic acid reacts in alkaline solution with compounds which contain 2 removable hydrogen atoms attached to 1 nitrogen atom or 1 carbon atom.
- Test 4. This reaction is given by aliphatic and aromatic hydrazines,² red to violet colours being formed by many hydrazine derivatives and hydrazides, e.g., phenylhydrazine, semicarbazide, benzoyl hydrazide.
- Test 5. In this reaction *isonicotinyl* hydrazide is hydrolysed to give hydrazine which then reacts with the *p*-dimethylaminobenzaldehyde to give an azine,³ $(\text{CH}_3)_2\text{N}\cdot\text{C}_6\text{H}_4\cdot\text{CH}:\text{N}:\text{N}:\text{CH}\cdot\text{C}_6\text{H}_4\cdot\text{N}(\text{CH}_3)$.
- Test 6. Mercuric chloride forms a double salt with pyridine,⁴ and pyridine carboxylic acids,⁵ and, in a similar manner, with *isonicotinyl* hydrazide.
- Test 7. This reaction is fairly specific, being given by only γ -picoline and γ -ethylpyridine out of about 30 pyridine derivatives tried.⁸

2. QUANTITATIVE DETERMINATION

(a) Chlorodinitrobenzene Method

This method was evolved from the qualitative test and optimum conditions for it are incorporated in the procedure given below. This method is applicable to the determination of *isonicotinyl* hydrazide and to its determination in the presence of the usual tablet excipients.

Reagent. 1-Chloro-2:4-dinitrobenzene solution, 5 per cent. w/v in dehydrated ethanol.

Apparatus. Water bath fitted with a 2½ inch wide brim to prevent ethanol vapour escaping over the side of the bath and igniting.

Procedure. Weigh accurately an amount of finely ground tablet containing about 0.06 g. of *isonicotinyl* hydrazide into a 100-ml. flask, add 80 ml. of dehydrated ethanol, shake for 5 minutes and make up to 100 ml. with ethanol. Dilute 5.0 ml. to 100 ml. with ethanol and transfer 5.0 ml. of this dilution to a dry test-tube containing 0.1 g. of borax. Add 5 ml. of the chlorodinitrobenzene solution, mix and place the tube in a boiling water bath for 15 minutes. Cool in ice for 1 minute, add 25.0 ml. of methanol, shake and filter. Read the extinction of 1 cm. of the filtrate at about 530 m μ or, using a suitable filter, against a blank prepared as above omitting the sample, and obtain the amount of *isonicotinyl* hydrazide present from a calibration curve (Fig. 1).

Notes on method. 1. The water bath is preferably heated over a flame which is adjusted so that the water is kept just at the boil initially, to prevent the ethanol from boiling out of the tubes. Under these conditions

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the ethanol evaporates off in about 5 minutes, leaving a dry, purple coloured mass in the tube.

2. The colour of the final solution decreases by about 3 per cent. in 1 hour on standing in daylight.

3. The reproducibility is about ± 3 per cent., and recoveries of 102.5 per cent. and 101.3 per cent. were obtained in 2 determinations on known mixtures of isonicotinyl hydrazide with tablet excipients.

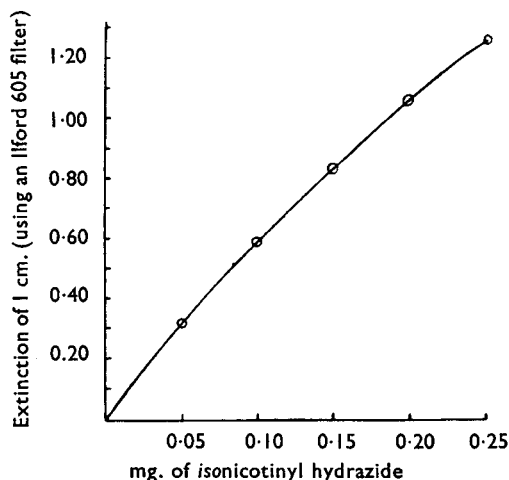


Fig. 1. Calibration curve for the determination of isonicotinyl hydrazide by the chlorodinitrobenzene method. (Each point is the mean of three determinations.)

(b) Azine Method

Pesez and Petit³ have described a method for the determination of hydrazine by coupling with *p*-dimethylaminobenzaldehyde to give an azine (*vide supra*) and since isonicotinyl hydrazide can be quantitatively hydrolysed to give hydrazine with either acid or alkali, this method is applicable to the determination of isonicotinyl hydrazide. If sugars or other aldehydes or ketones are present, reaction occurs during hydrolysis with the hydrazine, which precludes the use of this method for the determination of isonicotinyl hydrazide in tablets containing sugars.

Reagents. 1. Sulphuric acid 20N.

2. Sodium hydroxide 5N.

3. *p*-Dimethylaminobenzaldehyde reagent, freshly prepared each day. Dissolve 0.4 g. of *p*-dimethylaminobenzaldehyde in a mixture of 20 ml. of absolute ethanol and 2 ml. of hydrochloric acid.

Procedure. Weigh accurately about 0.1 g. of isonicotinyl hydrazide into a 100-ml. quickfit conical flask, add either 20 ml. of 5N sodium hydroxide or 10 ml. of 20N sulphuric acid, attach an air condenser and boil gently for 15 minutes. Cool, add 40 ml. of water down the condenser, mix well and transfer to a 100-ml. flask, washing in with water and making up to 100 ml. Dilute 10.0 ml. to 100 ml. with water and dilute 5.0 ml. of this to 100 ml. with water. Transfer 20.0-ml. to a 50-ml. flask,

add 5 ml. of *p*-dimethylaminobenzaldehyde reagent, stand for 10 minutes and dilute to 50 ml. Read the extinction of 1 cm. using a suitable filter against a blank prepared by diluting 5 ml. of the reagent to 50 ml. with water. Obtain the amount of *isonicotinyl* hydrazide present from a calibration curve (Fig. 2).

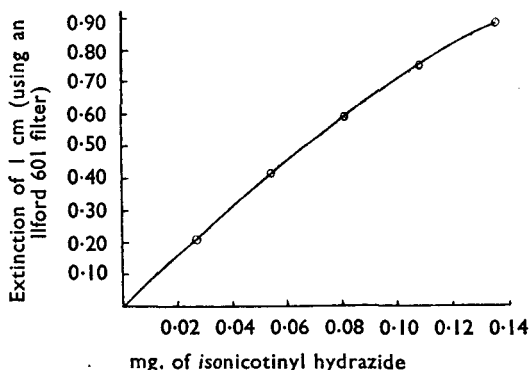


FIG. 2. Calibration curve for the determination of *isonicotinyl* hydrazide by the azine method. (Each point is the mean of three determinations.)

Notes on method. 1. The colour of the final solution remains constant for at least 1 hour on standing.

2. The calibration curve can be prepared using hydrazine sulphate, since complete hydrolysis of the *isonicotinyl* hydrazide to hydrazine occurs.

3. The reproducibility of the determination is about ± 1 per cent.

(c) Spectrophotometric Determination

The ultra-violet absorption spectrum of *isonicotinyl* hydrazide in 0.01N hydrochloric acid has $\lambda_{\max.} = 266 \text{ m}\mu$ (Fig. 3) and since in neutral and alkaline solution a slightly lower intensity of absorption is found, acid solution using 0.01N hydrochloric acid was utilised for its determination. A plot of optical density against concentration of *isonicotinyl* hydrazide is linear over the concentration range of 0.0007 per cent. to 0.0012 per cent. and the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 266 $\text{m}\mu$ calculated from the optical density of an 0.001 per cent. solution of recrystallised *isonicotinyl* hydrazide is 429.2. The method was applied successfully to the determination of *isonicotinyl* hydrazide in known mixtures with tablet excipients and 3 determinations gave recoveries of 99.95, 99.9 and 99.1 per cent.

Procedure. Weigh accurately an amount of finely ground tablet containing about 0.1 g. of *isonicotinyl* hydrazide into a 100-ml. flask. Add about 80 ml. of water, shake for 5 minutes, and dilute to 100 ml. Dilute 10.0 ml. to 100 ml. with water and transfer 10.0 ml. of this dilution to a 100 ml. flask. Add 10.0 ml. of 0.1N hydrochloric acid and dilute to 100 ml. with water. Prepare a blank from the tablet excipients in an exactly similar manner. Read the optical density of 1 cm. at 266 $\text{m}\mu$ against the blank.

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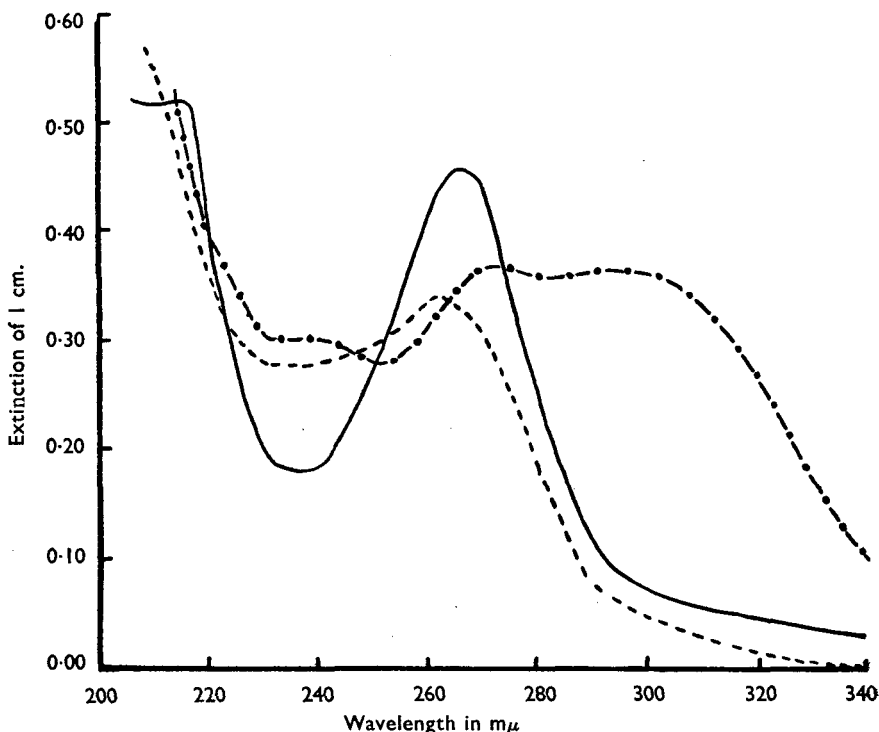


Fig. 3. Ultra-violet absorption curves of *isonicotinyl hydrazide*.
 ——— 0.001 per cent. solution in 0.01N hydrochloric acid.
 - - - 0.001 per cent. solution in water.
 -●-●- 0.001 per cent. solution in 0.01N sodium hydroxide.

(d) Polarographic Determination

isoNicotinyl hydrazide in solutions of *pH* from 1 to 8 is reducible at the dropping mercury electrode giving 2 waves about 0.2 volt apart; in solutions of *pH* greater than 8 the 2 waves tended to coalesce. With the cathode ray polarograph⁹ the peak potentials for reduction in 0.01N hydrochloric acid/*N* potassium chloride using a silver chloride anode, were 0.55 volt and 0.75 volt. The second wave was chosen for quantitative purposes because of a small subsidiary wave on the first wave at low concentrations. In the supporting electrolyte mentioned the plot of current against concentration was linear over the concentration range 0.0001 per cent. to 0.02 per cent. The *isonicotinyl hydrazide* content of tablet granules was determined with a precision of ± 1 per cent. the excipients having no interfering effect.

(e) Titration Methods

Cänback⁶ has described a method of determination for *isonicotinyl-hydrazide* by titration with iodine in slightly alkaline solution and his results indicate a reproducibility of about 0.5 per cent. He has applied this method to the determination of *isonicotinyl hydrazide* in tablets.

A preliminary study has been made of two alternative methods which might be of value for the determination of *isonicotinyl* hydrazide in the presence of substances which would interfere with the iodine titration. These are based on (a) acidimetric titration in a non-aqueous solvent with perchloric acid and (b) titration with nitrite. In the first method, excess of perchloric acid in glacial acetic acid is added to the sample dissolved in a mixture of equal volumes of monochlorobenzene and glacial acetic acid, followed by the potentiometric back titration with a standard solution of pyridine in monochlorobenzene. Glass and silver/silver chloride electrodes are used and the end-point is obtained graphically from a plot of potential against volume of pyridine solution added. 4 titrations of pure recrystallised *isonicotinyl* hydrazide gave results with a maximum spread of 1.5 per cent. and provided an empirical factor of 1 ml. of 0.1N \equiv 0.007114 g. of $C_6H_7ON_3$, compared with the theoretical value of 0.006857 calculated from an equivalent weight equal to half the molecular weight of *isonicotinyl* hydrazide.

In the second method *isonicotinyl* hydrazide in dilute hydrochloric acid solution is titrated with 0.05M sodium nitrite solution, using starch-iodide as external indicator. 6 titrations gave a maximum spread of about 1 per cent. and 3 titrations using recrystallised *isonicotinyl* hydrazide provided an empirical factor of 1 ml. of 0.05M \equiv 0.006553 g. of $C_6H_7ON_3$, compared with a theoretical factor of 0.006857 calculated on the assumption that 1 mol. of sodium nitrite reacts with 1 mol. of *isonicotinyl* hydrazide.

SUMMARY AND CONCLUSIONS

1. Qualitative reactions for *isonicotinyl* hydrazide are given.
2. 2 colorimetric methods of determination, based on reaction with (a) 1-chloro-2:4-dinitrobenzene and (b) *p*-dimethylaminobenzaldehyde, and ultra-violet spectrophotometric and polarographic methods are proposed.
3. The spectrophotometric, polarographic and chlorodinitrobenzene methods have been applied to the determination of *isonicotinyl* hydrazide in tablets.
4. 2 titration procedures for *isonicotinyl* hydrazide, (1) with perchloric acid in a non-aqueous solvent, and (2) with nitrous acid, are given.

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