Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, Nos 6–8, pp. 1023–1027, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Fluorimetric determination of hydrazine in isoniazid formulations with 2-hydroxy-1-naphthaldehyde*

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Keywords: *Hydrazine determination; isoniazid; iproniazid; 2-hydroxy-1-naphthaldehyde.*

Introduction

Hydrazine is a toxic agent that may induce mutagenesis, carcinogenesis and hepatic injury. Isoniazid and other hydrazide drugs are unstable substances that can yield different degradation products including hydrazine. Thus, the presence of hydrazine in isoniazid and its formulations must be controlled and currently is limited to no more than 0.0128% in isoniazid, according to WHO [1].

Hydrazine can be determined in isoniazid and isoniazid preparations using 4-dimethylaminobenzaldehyde in hydrochloric acid solution as reagent, to form the yellow 4-dimethylaminobenzaldazine, by direct [2–4] or difference spectrophotometry [5]. Other methods have also been reported, such as thin-layer chromatography using 4-dimethylaminocinnamaldehyde [6] as the visualising agent, high-performance liquid chromatography with ultraviolet detection [7] and gas chromatography with nitrogen-phosphorus detection [8]. The last two methods use benzaldehyde as derivatising agent.

This paper describes the application of 2-hydroxy-1-naphthaldehyde as a sensitive fluorimetric reagent for hydrazine in isoniazid and its formulations based on the formation of 2,2′-dihydroxy-1-naphthaldazine (DHND). The use of this reaction for the colorimetric determination of hydrazine in steam boiler waters [9] has recently been proposed. Isoniazid also reacts quickly with the reagent to form the non-fluorescent isonicotinoylhydrazone of 2-hydroxy-1-naphthaldehyde (IHHN) which is a very stable substance that does not undergo hydrolysis at 100°C.

*Presented at the "International Symposium on Pharmaceutical and Biomedical Analysis", September 1987, Barcelona, Spain.

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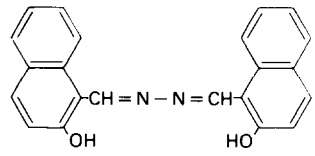
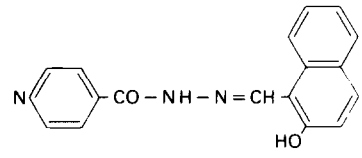


DHND



IHHN

Experimental

Apparatus

Fluorescence measurements were made with a 'Shimadzu RF-510' double-monochomator spectrofluorimeter, equipped with a xenon light source and 1-cm quartz cells. The spectral band widths for both monochromators were set at 5 nm.

Reagents

Hydrazine stock solution (0.01 M) was prepared from hydrazine sulphate and standardised by bromate titration. Hydrazine standard solution (1 ppm) was prepared by appropriate dilution of the stock solution immediately before use. Isoniazid stock solution (1000 ppm). 2-Hydroxy-1-naphthaldehyde [Ega-Chemie] (0.01 M) in ethanol. Buffer solution, 1 M sodium acetate–0.1 M acetic acid. Ethylenediaminetetraacetic acid disodium salt (0.05 M). Chloroform. Anhydrous sodium sulphate.

Procedure

To 5 ml of sample containing 0.1–0.8 μg of hydrazine and 500 ppm of isoniazid in a glass-stoppered tube, 1 ml of buffer solution, 1 ml of EDTA solution and 1 ml of reagent solution were added and warmed in a boiling water-bath for 15 min. After the solution had cooled 5 ml of chloroform were added and shaken vigorously for 1 min. The mixture was transferred to a 25-ml separating funnel, the organic layer was passed through anhydrous sodium sulphate and its fluorescence intensity was measured at 512 nm with excitation at 416 nm, against a reagent blank as reference.

Determination of hydrazine in isoniazid formulations

Twenty isoniazid tablets were weighed and powdered. A weight of powder equivalent to about 125 mg of isoniazid was transferred to a 250-ml calibrated flask. The powder was extracted with about 25 ml of 0.01 M hydrochloric acid for 10 min and the mixture was diluted to volume with distilled water and filtered.

Liquid formulations were directly diluted in distilled water to obtain a 500 ppm solution of isoniazid. The solutions were treated as described under Procedure.

Results and Discussion

The azine was obtained by reaction of one part of hydrazine with two parts of 2-hydroxy-1-naphthaldehyde for 30 min at 100°C and then chromatography with chloroform–ethanol (95:5, v/v) on a silica gel thin layer plate. The eluate yields a pure solid product with the following properties: m.p., 308.5°C; I.R., bands at 1650 and 1630 cm^{-1} corresponding to the C = N groups; MS, m/z at 340(40.9%), 323(19.4%), 170(100%), 115(35.0%).

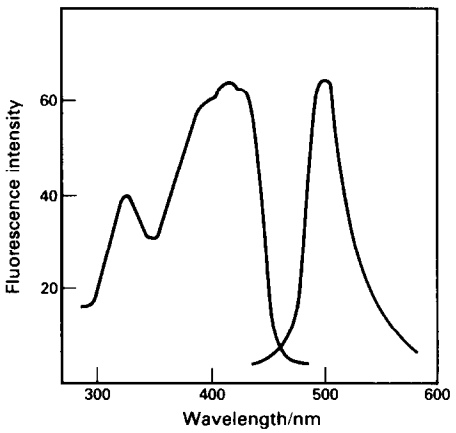


Figure 1
Excitation and emission fluorescence spectra of DHDN 2.94 × 10^{-6}M in chloroform.

Figure 1 shows the fluorescence excitation and emission spectra of 2,2′-dihydroxy-1-naphthaldazine in chloroform. The excitation spectrum shows two maximum at 416 and 445 nm with emission at 512 nm. The emission shows a maximum at 512 nm with excitation at 416 or 445 nm.

Different water-immiscible liquids were tested as solvents for the extraction of 2,2′-dihydroxy-1-naphthaldazine. The fluorescence intensities, corrected for the corresponding blank intensity, for 100 ppb of hydrazine were in the following order: chloroform = benzene = toluene > 1,1,1-trichloroethane > 1,2-dichloroethane > carbon tetrachloride > hexane > dichloromethane > cyclohexane > methyl isobutyl ketone > ethyl acetate > ethyl ether.

The maximum constant fluorescence intensity was observed when the pH of reagent solution was maintained between 4.8 and 6.2 by the addition of approximately 1 M acetate buffer.

The extraction of the azine into chloroform was found to be very rapid and no change was observed in the efficiency of extraction when the shaking time was varied from 30 s to 30 min.

The fluorescence intensity reached a maximum value immediately after extraction and remained unaltered for more than 3 days.

Analytical parameters

The fluorescence intensities gave a linear relationship in the range 10–160 ppb of hydrazine in the presence of 500 ppm of isoniazid. This range represents free hydrazine levels from 0.002 to 0.032% in isoniazid. The regression equation for the calibration graph was $F = 0.347c + 1.97$, where F is the measured relative fluorescence and c the concentration of hydrazine solution in ppb ($n = 5$). The regression coefficient was 0.999.

The detection limit, defined as the sample concentration giving a fluorescence intensity that is three times the standard deviation of the fluorescence intensities of the blank solution, is 3.2 ppb.

The precision and accuracy of the proposed method were studied by determining hydrazine ten times in a standard solution (80 ppb). The standard deviation, relative standard deviation and relative error were found to be 1.9 ppb, 2.2% and 1.6% respectively (95% confidence level).

Recovery studies were carried out by adding hydrazine sulphate solutions to the pharmaceutical preparations and determining the concentration of hydrazine. The recovery of hydrazine was found to be in the range 98.7–102.5%.

Interference studies

A systematic study of the effect of foreign substances on the assay of 100 ppb of hydrazine was undertaken under the optimum conditions. The tolerance limit of a substance was fixed as the maximum amount causing an error of not greater than 2% in the fluorescence. The most serious inorganic interference was due to Ti^{4+} and Be^{2+} [9], which caused a disturbance even at 1:1 m/m ratio. No interferences were observed with commonly used excipients such as talc, magnesium stearate, starch, lactose and glucose. The results obtained for some organic chemicals showed satisfactory selectivity (Table 1).

Applications of the method

Samples were prepared containing isoniazid and vitamin B_6 and analysed by the proposed method after heating, because the content of hydrazine in the drug was below the detection limit. The results obtained for isoniazid solution are summarised in Fig. 2. In the case of tablets, the generation of hydrazine was more slow, but the curves obtained showed a similar shape.

This method has also been applied to the determination of hydrazine in other hydrazine drugs, such as iproniazid, nialamide, phenelzine and hydralazine. With

Table 1
Selectivity data

Substance	Tolerance ratio† (Substance)/(N_2H_4) m/m
Oxalate, citrate, urea	10,000*
Phenol, glycine, methionine	5000
Vitamin B_6	2000*
Vitamin B_1	1000
Phenylephrine, paracetamol, sulphanilamide	800
Bencydamine	600

*Maximum amount studied.
†See text for definition; concentration of hydrazine = 100 ppb.

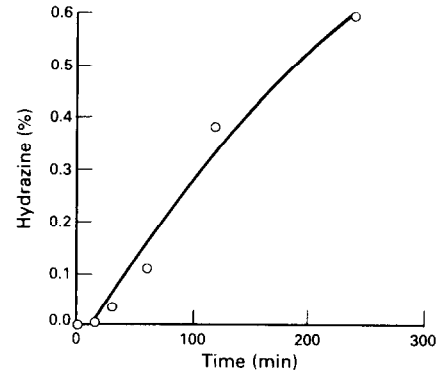


Figure 2
Generation of hydrazine in injectables of isoniazid and vitamin B_6 at 100°C.

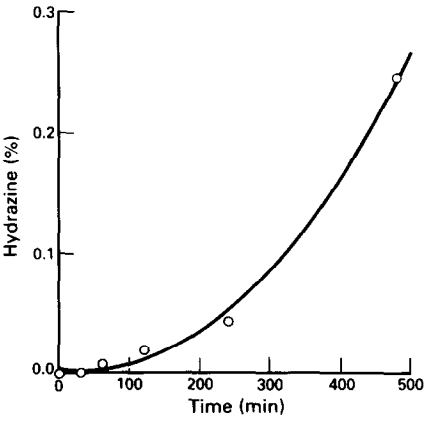


Figure 3
Generation of hydrazine in injectables of iproniazid at 100°C.

iproniazid the analytical parameters obtained (calibration graph, recovery, detection limit, etc.) were essentially the same as those obtained with isoniazid. The generation of hydrazine in iproniazid solution after heating is shown in Fig. 3. In the case of hydralazine, a strong yellow reaction product masked the detection of hydrazine. With nialamide and phenelzine the procedure is not applicable for quantitative determination owing to interference from other reaction products that are fluorescent. However, when the drug concentration falls to 250 ppm the method can be used for the detection of at least 20 ppb of hydrazine, and this represents the detection of free hydrazine at a concentration of 0.008% in nialamide or phenelzine.

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[*Received for review 23 September 1987*]