Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1473–1477, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Determination of isoniazid in pharmaceutical preparations by reaction with radiochloramine-B*

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Abstract: A method has been developed for the determination of isoniazid (INH) at microgram level in pharmaceutical preparations based on the oxidation of isoniazid by radiochloramine-B. Interference of vitamin C has been overcome by precipitation as lead ascorbate and that of rifampicin and *p*-aminosalicylic acid by selective extraction. Amounts as low as 25 μg of INH can be determined.

Keywords: *Radiochloramine-B; activity measurement; isoniazid; drug analysis.*

Introduction

Drugs used for tuberculosis may be divided into two major categories [1]. Those of first choice combine the greatest level of efficacy with an acceptable degree of toxicity: these agents include isoniazid (INH), rifampicin, ethambutol and streptomycin. Because of microbial resistance or patient-related factors, it may be necessary to resort to a "second-line" drug: this category includes pyrazinamide, ethionamide, *p*-aminosalicylic acid (PAS), viomycin and cycloserine. Among them INH is still considered to be the primary drug for the chemotherapy of tuberculosis.

Except for a fluorimetric method which determines INH (isonicotinic acid hydrazide) at microgram level [2], all other methods reported are applicable at milligram level by titrimetry employing iodine monochloride [3], 2-iodoxybenzoate [4], chloramine-T [5] and *N*-bromosuccinimide (NBS) method [6]. Titrimetric procedures, in general, cannot be used to determine substances at low concentrations. The titrimetric method using NBS is, however, reported to be applicable at microgram level. The main disadvantage of this method is that the reagent is not stable and no interference studies have been carried out. Chloramine-B (CAB) is a stable oxidant and hence it has an edge over NBS. CAB has been used for the determination of hydrazines [7] at milligram level. It is stated that the end-point with CAB is indistinct. However, in the presence of excess of potassium bromide the end-point is sharp, and accurate results can be obtained. Its application can be extended to trace levels if radiochloramine-B (RACAB) is used instead of CAB. With this in view, RACAB has been prepared and applied to the determination of INH. Interference by vitamin C, rifampicin and PAS has been studied.

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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Experimental

Reagents

CAB (Fluka AG) (0.01 M) was prepared and standardized iodometrically [8]. INH (Riedel) 1000 ppm was prepared by dissolving 0.1 g in 100 ml distilled water. This solution was prepared fresh before use and standardized by the NBS method [6]. A working solution of 100 ppm was prepared from the above by suitable dilution with water. ^{36}Cl in the form of $H^{36}Cl$ (sp. act., 400 μCi/g) supplied by "BARC" (India) was used to prepare RACAB as described in an earlier communication [9]. The radioactivity of samples was measured using a Geiger-Muller (G.M.) counter. All other reagents used were of analytical grade.

Calibration graph

Aliquots containing INH between 25–250 μg were taken in a separating funnel to which 1 ml of 0.5 N H_2SO_4 was added followed by 2.5 ml of a solution of 0.01 M RACAB. The mixture was diluted to 10 ml, and after 5 min (for completion of the reaction), 5 ml carbon tetrachloride was added and the unreacted RACAB was extracted for 10 min. The organic layer was separated and RACAB was re-extracted into aqueous solution by shaking with 5 ml 0.1 N NaOH for 5 min. Two millilitres of the alkali layer were pipetted into an empty planchet, dried under an IR lamp and its activity was measured using a G.M. counter. A blank was performed under similar conditions and the activity lost due to INH (equivalent to $^{36}Cl^-$ released by INH in the reaction) was calculated in each case. A calibration graph was constructed by plotting the activity released against the concentration of INH in standard solutions. The correlation coefficient obtained for seven determinations in the range 25–250 μg INH was 0.9961.

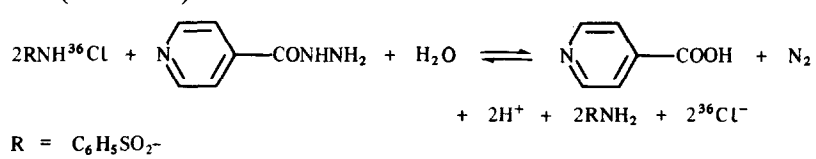
Procedure

Determination of INH in aqueous solutions. Five millilitres of aqueous samples containing not more than 250 μg INH were treated with excess RACAB in acidic solution as described above, and the concentration of INH was determined from the calibration graph.

Determination of INH in pharmaceutical preparations. A finely ground tablet was stirred with about 20 ml distilled water. The residual solid was filtered off on Whatman No. 42 paper and washed with water. The filtrate was diluted to 100 ml. This was again diluted 10-fold, and aliquots containing not more than 250 μg INH were taken and the INH content was determined as described above. The results are given in Table 1.

Results and Discussion

RACAB oxidizes INH to isonicotinic acid in a reaction in which 4 equivalents of oxidant are consumed (Scheme 1).



Scheme 1
Oxidation of INH by RACAB.

Table 1
Determination of INH in pharmaceutical preparations

Sample tablet	Recovery of INH in the tablet by the present method, $n = 3$ (%)	NBS method, $n = 2$ (mg)
1 Docina 300 (Ashok Pharm)	98.1	310
2 Isokin Forte (Pfeizer)	100.0	308
3 Isonex Forte 300 (Warner Hindustan)	98.0	304
4 Isokin 300 (Warner Hindustan)	99.3	302
5 Isocaldin Syrup (Retort Lab)	97.4	308
6 Tibirim INH (Ranbaxy)	102.7*	—
7 Inapas granules (Neo Pharma)	97.0*	—

Sample tablets 1 and 3 contain INH (300 mg); 2 contains thiacetazone, INH (300 mg); 4 contains vitamin B_6, INH (300 mg); 5 contains vitamin B_6, sorbital, INH (300 mg); 6 contains rifampicin, INH (300 mg); 7 contains PAS (sodium salt), INH (100 mg).
n, Number of determinations.
%Recoveries are against independent method.
* % Recovery against label strength.

Various parameters were optimized for the quantitative conversion of INH to isonicotinic acid and as a result, the following optimum experimental conditions were used. (1) Overall acidity of the reaction mixture, 0.05 N sulphuric acid; (2) reaction time 5 min; (3) extraction of unused RACAB with 5 ml carbon tetrachloride for 10 min; and (4) re-extraction of RACAB from carbon tetrachloride with 5 ml 0.1 N NaOH for 5 min.

Substances that do not interfere when present in concentrations at up to 10-fold molar excess of INH include glucose, maltose, sucrose, glycine, thiacetazone, vitamins B_1, B_2, B_6, etc. Some INH drugs contain vitamin C which interferes considerably. This interference can be overcome by precipitating vitamin C as lead ascorbate.

Assay of INH in presence of vitamin C

Interference of vitamin C in quantities up to 1000 μg was overcome in the following way.

To 1 ml of a solution of INH containing 100 μg and 1 ml of a solution of vitamin C containing 300–1000 μg, 1 ml of 1% lead acetate was added. The solution was made alkaline (pH 8) by adding 0.1 N NaOH. The precipitate (lead ascorbate) was removed by centrifugation. To the supernatant solution 2 ml 5% sodium sulphate was added to precipitate the excess lead as lead sulphate and the solution was centrifuged. To the supernatant solution, sulphuric acid was added to give a concentration of 0.05 N and

then 2.5 ml a solution of RACAB was added. The reaction was allowed to proceed for 5 min. Then 5 ml carbon tetrachloride was added and the unreacted RACAB was extracted as described above. INH recovery was found to be satisfactory as seen from Table 2.

Assay of INH in presence of rifampicin

Rifampicin is a macrocyclic antibiotic used for tuberculosis. But it should never be used alone because of the rapidity with which resistance may develop. It finds its greatest usefulness in combination with INH in the treatment of pulmonary tuberculosis. The combination of INH and rifampicin is probably as effective for sensitive micro-organisms, as are regimens that utilize three or more agents. Rifampicin has been found to interfere in the determination of INH by RACAB.

To remove the interference, rifampicin (John Baker Inc.) at different concentrations in combination with INH has been subjected to selective extraction to remove rifampicin and then INH has been assayed as described below.

One millilitre of rifampicin solution (containing 250–1000 μg) and 1 ml INH solution (containing 100 μg) diluted to 5 ml was extracted twice with 5 ml of benzene. The INH remained in the aqueous layer and rifampicin was extracted completely into the organic layer. To the aqueous layer containing INH, 1 ml of 0.5 N sulphuric acid and 2.5 ml of RACAB solution were added and the determination of INH was carried out as described earlier. The recovery of INH was found to be satisfactory as seen from Table 3. (For routine work it is recommended that benzene be replaced by toluene on the grounds of safety.)

Assay of INH in presence of PAS

p-Aminosalicylic acid (PAS) is a second-line drug given for tuberculosis. This drug exerts a beneficial effect on the disease. However, doses of PAS required are relatively large and the compound must be administered continuously. As PAS alone is of little value in the treatment of tuberculosis, it is generally given along with INH. PAS also interferes in the determination of INH.

Table 2
Determination of INH in presence of vitamin C

INH taken (μg)	Vitamin C taken (μg)	INH recovery (%)
100	300	97
100	400	101
100	500	103
100	1000	98

Table 3
Determination of INH in presence of rifampicin

INH taken (μg)	Rifampicin taken (μg)	INH recovery (%)
100	250	98
100	500	101
100	750	99
100	1000	100

Table 4
Determination of INH in presence of PAS

INH taken (μg)	PAS taken (μg)	INH recovery (%)
100	300	99
100	500	102
100	700	98
100	1000	101

PAS at different concentrations in combination with INH has been removed by selective extraction prior to the assay of INH, as follows.

PAS (0.1 g) (Aldrich Lab) was dissolved in saturated bicarbonate solution and made up to 100 ml. To 0.3–1 ml of this solution (300–1000 μg of PAS) in a separating funnel, 1 ml (100 μg) INH solution was added and diluted to 5 ml. This solution was then extracted with 5 ml isobutylmethylketone (MIBK) for 10 min. PAS is completely extracted into MIBK. The MIBK that was dissolved in the aqueous layer was removed by extraction with 5 ml ether, three times. To the aqueous layer, which contained only INH, 1 ml 0.5 N sulphuric acid and 2.5 ml RACAB solution were added. INH was determined as described earlier. The results are shown in Table 4. From Table 4 it can be seen that the recovery of INH is satisfactory.

The proposed method for the assay of INH is simple, rapid and quantities as low as 25 μg can be determined. The sensitivity of the method can be further enhanced by using radioactive chloramine-B with a high specific activity. The relative standard deviation was found to be 3% for 10 separate determinations containing 100 μg INH using the procedure described previously under *Calibration graph*.

Acknowledgements — M. Subhashini wishes to thank CSIR, New Delhi, and IIT, Madras, for providing financial assistance to attend the symposium.

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[*Received for review 16 May 1989; revised manuscript received 30 May 1989*]