THE DETERMINATION OF ISONIAZID IN PHARMACEUTICAL PREPARATIONS CONTAINING SODIUM *p*-AMINOSALICYLATE

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With the increased use of combined therapy in the treatment of tuberculosis there are now a number of preparations containing small quantities of isoniazid in combination with other antituberculosis drugs of the aminosalicylic acid type. The difficulty of determining isoniazid in the presence of aminosalicylic acid is obvious when reviewing the methods at present available. Most of the macromethods deal with pure product and the majority, including the B.P.C. method[1] are based on the oxidation of the hydrazine group by various oxidants[1–11]. Under these conditions aminosalicylic acid is also oxidised.

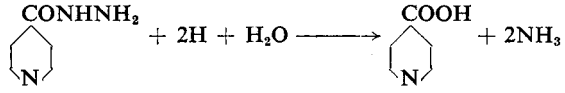
An attempt to separate isoniazid from calcium aminosalicylate was first made by Biffoli[12] who precipitated the free aminosalicylic acid with concentrated hydrochloric acid and titrated the filtrate with potassium iodate in the presence of chloroform or carbon tetrachloride. However, when the quantity of isoniazid is small, the separation is not complete, since either quantities of the isoniazid are retained by the voluminous precipitate of aminosalicylic acid hydrochloride or, on prolonged washing with acid, small amounts of the aminosalicylic acid hydrochloride are dissolved and high results obtained. Strickland and Hentel[13] used a gasometric micromethod, oxidizing hydrazine derivatives to nitrogen with sodium iodate in alkaline solution in the presence of aminosalicylic acid. This method has been tested by the authors as a macrodetermination in the presence of fifty times as much aminosalicylic acid and found to give satisfactory results, although subject to the usual errors of a gasometric estimation. Various other methods have been described for the estimation of isoniazid, including non-aqueous titrations, polarographic and colorimetric procedures, but these have not been investigated by the authors since the methods were either not sufficiently accurate or required the use of special equipment and, in many cases, the presence of aminosalicylic acid would have caused interference.

It would appear, therefore, that the determination of isoniazid in the presence of aminosalicylic acid and its derivatives must be carried out either by separation of the isoniazid and assaying it by one of the known methods, e.g., the B.P.C. assay procedure, or by direct estimation of the isoniazid, using a method which is not affected by large quantities of aminosalicylic acid. Separation from isoniazid by the use of ion exchange resins was considered, but preliminary experiments were unsuccessful and, since the high aminosalicylic acid content of commercial preparations would necessitate frequent regeneration of the resin, this line of investigation was not pursued. Attention was, therefore, concentrated on producing a simple method of assay that would be unaffected by large

quantities of aminosalicylic acid. In particular, the possibility of developing a method based on the reduction of the hydrazine group was investigated, since the aromatic amino group in aminosalicylic acid would not be affected.

In discussing the properties of hydrazine, Ephraim[14] states that it is decomposed in alkaline solution to give ammonia, nitrogen and water in varying proportions, depending on the catalyst used. Suzuki[15] distinguishes between hydroxylamine and hydrazine by reducing both to ammonia with ferrous sulphate in a glucose solution, the first reaction occurring in the presence of sodium carbonate and the second requiring sodium hydroxide. The possibility of estimating isoniazid by reducing the hydrazine group quantitatively to ammonia, followed by distillation into standard acid was, therefore, considered since, under these conditions, aminosalicylic acid and its derivatives would be stable and should not produce interference. It is interesting to note that Sanchez[16] describes a colorimetric assay in which isoniazid is reduced to an aldehyde with zinc powder and hydrochloric acid, the aldehyde being subsequently reacted with phenylhydrazine.

To obtain a quantitative reduction of isoniazid to ammonia according to the reaction



various reducing agents, including ferrous sulphate, powdered zinc, Devardas alloy, powdered tin, and zinc-iron, tin-copper, and iron-copper couples were tested in alkaline solution, but all gave less than 75 per cent of the theoretical yield of ammonia. Encouraging results were obtained with a zinc-copper couple in potassium hydroxide solution, recoveries being about 95 to 100 per cent and subsequent investigations were, therefore, restricted to the use of this reducing agent. It was found that, in the absence of sodium aminosalicylate, recoveries were occasionally above the theoretical. These anomalous results were due to traces of what appeared to be γ-picoline produced during the reaction, since the distillate, which had the odour characteristic of pyridine bases, gave a positive reaction with 1-chloro-2:4-dinitrobenzene[17,18]. In the presence of sodium aminosalicylate, however, the results were dependent only upon the zinc-copper ratio and the alkalinity of the solution. In addition, large quantities of zinc salts inhibit the reaction and the following optimum conditions have been found for the reduction of isoniazid in the presence of aminosalicylic acid:

1. The zinc-copper couple prepared from zinc powder and copper sulphate has to be washed thoroughly to remove all the zinc sulphate.
2. The ratio of zinc powder to copper sulphate should be about 4:1.
3. The quantity of zinc powder used should be between 4–10 g.
4. The normality of the potassium hydroxide solution should be between 0·1 and 0·5N.

5. The volume of the mixture at the beginning of the reaction should be 400 to 500 ml. and should contain about 5 g. sodium aminosalicylate.

The following procedure is recommended:

Reagents. Zinc powder, 25 per cent w/v aqueous copper sulphate solution, 2·5 per cent w/v aqueous potassium hydroxide solution (approximately 0·4N), 0·1N sulphuric acid and 0·05N sodium hydroxide.

TABLE I

THE ESTIMATION OF ISONIAZID IN THE PRESENCE OF 5 G. QUANTITIES OF SODIUM AMINOSALICYLATE

Weight of isoniazid taken (mg.)	Titre ml. 0·1N H_2SO_4	Isoniazid recovered	
		mg.	per cent
25	3·53 3·49	24·23 23·88	96·92 95·52 } 96·2
50	7·43 7·36	50·94 50·44	101·88 100·88 } 101·4
75	10·93 10·96	74·92 75·13	99·88 100·16 } 100·0
100	14·56 14·62	99·78 100·10	99·78 100·10 } 99·9

Procedure. About 10 g. of zinc powder, 25 ml. water and 10 ml. of 25 per cent copper sulphate solution are shaken in a 1-litre round flask until the supernatant liquid is nearly colourless. The liquid is decanted and the residue washed three times with 25 to 50 ml. portions of water, decanting each washing. About 5 g., accurately weighed, of the sodium aminosalicylate-isoniazid sample, containing 50 to 150 mg. of isoniazid, is placed in the flask, together with 400 ml. of 2·5 per cent potassium hydroxide solution. The mixture is distilled into 25 ml. of 0·1N sulphuric acid until 100 to 150 ml. has been collected (15 to 30 minutes). The distillate is back titrated with 0·05N sodium hydroxide solution, using methyl red screened with methylene blue as an indicator.

Each ml. of 0·1N sulphuric acid used is equivalent to 0·006858 g. of isoniazid.

TABLE II

THE ESTIMATION OF ISONIAZID IN SODIUM AMINOSALICYLATE/ISONIAZID CACHETS

Weight of sodium aminosalicylate per cachet (g.)	Weight of isoniazid per cachet (mg.)	
	Stated	Found
1·50	33	33·13 32·50 } 32·8
1·25	25	25·12 25·63 } 25·4
1·50	50	49·28 50·84 } 50·1
1·25	25	24·86 24·86 } 24·9
1·50	33	32·66 33·12 } 32·9

The above method was used with varying quantities of isoniazid in the presence of 5 g. of sodium aminosalicylate; the results obtained are given in Table I.

The greater error in the lower concentrations of isoniazid is probably due to the small titre difference. In general, when reasonable quantities of isoniazid are employed, a satisfactory accuracy is obtained.

Six commercial samples of isoniazid/sodium aminosalicylate cachets, containing various quantities of isoniazid, were examined and the isoniazid contents obtained are given in Table II.

Summary

A rapid and accurate method for the determination of isoniazid in the presence of sodium *p*-aminosalicylate has been developed. The hydrazine group of the isoniazid is quantitatively reduced by a zinc-copper couple in potassium hydroxide solution to give ammonia, which is titrated after distillation into standard acid.

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