

Spectrophotometric determination of isoniazid in excess *p*-aminosalicylate

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The presence of a large excess of *p*-aminosalicylic acid prevents the direct application of Vierordt's spectrophotometric method to the determination of isoniazid in tablets. The method can, however, be applied after the bulk of the *p*-aminosalicylic acid has been removed with ether. The extinction of the aqueous phase containing all the isoniazid and some of the *p*-aminosalicylic acid is then determined at two wavelengths and the concentration of isoniazid calculated therefrom.

THE determination of isoniazid in mixtures with sodium *p*-aminosalicylate has received much attention and methods involving precipitation, gasometry, non-aqueous titrations, polarographic and colorimetric procedures have been critically reviewed by Mitchell, Haugas & McRoe (1957). Lee & Ho (1962) found the method of Mitchell & others (1957), which involved the reduction of isoniazid with a zinc-copper couple, to give erratic results when applied to tablets. In their modification of this method, Lee & Ho removed interfering tablet material with hydrochloric acid and obtained better results for tablets. However, this modified method is more involved and the authors have not reported the recovery of small amounts of isoniazid in *p*-aminosalicylate (about 5 mg of isoniazid in 0.5 g of *p*-aminosalicylate which was found by Mitchell & others to give smaller recoveries due to errors in small titre differences).

In a search for a method which did not involve reduction, distillation, colorimetry, extraction and weighing, the application of the spectrophotometric analysis to a two-component mixture was considered. At the onset it was evident that because of the large excess of *p*-aminosalicylate associated with isoniazid in commercial samples a direct spectrophotometric method would be of no avail. Investigations with extraction procedures revealed that with mixtures of *p*-aminosalicylate-isoniazid about 50:1, the bulk of *p*-aminosalicylate could be removed by ether from an acid solution of the mixture. The aqueous phase then contained some *p*-aminosalicylate and all of the isoniazid. The application of the spectrophotometric analysis for the estimation of isoniazid in the presence of small quantities of *p*-aminosalicylate in this aqueous phase was found to give satisfactory results.

Glenn (1960) derived equations for the concentration of two components in a mixture, viz

$$C_A = E_1/\alpha_1 \left[\frac{b - m}{b - a} \right] \dots \dots \dots (1)$$

$$C_B = E_2/\beta_2 \left[\frac{b(m - a)}{m(b - a)} \right] \dots \dots (2)$$

where the subscripts "1" and "2" refer to wavelengths, *E* denotes extinction of a 1 cm layer of the solution of a mixture of A and B, *C_A* and

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DETERMINATION OF ISONIAZID IN EXCESS *p*-AMINOSALICYLATE

C_B are concentrations of A and B, α and β are their respective extinction coefficients, and

$$m = E_2/E_1, \quad a = \alpha_2/\alpha_1, \quad b = \beta_2/\beta_1$$

where m refers to the mixture, a to substance A and b to substance B.

The condition for the application of the above equations is that both A and B must be absorbing substances which do not interact with each other, and the ratio b/m should be outside the limit 0.1–2.0. These conditions were found to be fulfilled for the acid solution after extraction with ether. Swietoslawska (1956) has derived expressions for calculating the analytical error for variable a , b and concentration of C_A and C_B . For maximum accuracy of C_A and C_B , Berry (1945) has shown that α_1/β_1 should be a maximum and α_2/β_2 should be a minimum. By drawing a graph of α/β against wavelength it is possible to choose a maximum and minimum of the plot as the two best wavelengths 1 and 2. For isoniazid and *p*-aminosalicylate, these wavelengths were found to be 255 $m\mu$ ("1") and 305 $m\mu$ ("2"). For the present purpose "A" was chosen to refer to isoniazid and "B" to *p*-aminosalicylate.

The extinction coefficients, E (1%, 1 cm) of isoniazid (α_1, α_2) and *p*-aminosalicylate (β_1, β_2) in 0.01 N hydrochloric acid were determined at 255 and 305 $m\mu$ from which the a and b values calculated were used for all the determinations. However, it must be pointed out that the a and b values can simply and rapidly be determined from solutions of "A" and "B" of unknown concentration. For the first assay using the proposed method it would, of course, be more convenient to calculate the a and b values from extinctions of the standard solutions. C_A and C_B would be in g/100 ml solution.

Specific example: Commercial sample claiming 15 mg of isoniazid and 0.5 g of sodium *p*-aminosalicylate per tablet. Average weight of one tablet, 0.5995 g. Weight of powdered tablet taken for analysis, 0.1982 g. Acid solution after extraction with ether, diluted to 400 ml.

$$\begin{aligned} E_1 &= 0.477; & E_2 &= 0.268; \\ m &= E_2/E_1 = 0.5618; \\ \alpha_1 &= 329; \alpha_2 = 64.5; & a &= \alpha_2/\alpha_1 = 0.1960; \\ \beta_1 &= 112; \beta_2 = 378; & b &= \beta_2/\beta_1 = 3.375; \\ & & b/m &= 6.0 \end{aligned}$$

$$\begin{aligned} C_A &= E_1/\alpha_1 \left[\frac{b-m}{b-a} \right] = \frac{0.477}{329} \left[\frac{3.375-0.5618}{3.375-0.1960} \right] \\ &= 0.001283 \text{ g/100 ml} \end{aligned}$$

i.e. concentration of isoniazid is 0.001283 g/100 ml of solution. Therefore,

$$\begin{aligned} \text{isoniazid content per tablet} &= 0.001283 \times 4 \times 1000 \times \frac{0.5995}{0.1982} \\ &= 15.52 \text{ mg.} \end{aligned}$$

$$\begin{aligned} C_B &= E_2/\beta_2 \left[\frac{b(m-a)}{m(b-a)} \right] = \frac{0.268}{378} \left[\frac{3.375(0.5618-0.1960)}{0.5618(3.375-0.1960)} \right] \\ &= 0.0004901 \text{ g/100 ml} \end{aligned}$$

i.e. concentration of *p*-aminosalicylate is 0.0004901 g/100 ml of solution. Therefore *p*-aminosalicylate content per tablet (in aqueous solution)

$$= 0.0004901 \times 1000 \times 4 \times \frac{0.5995}{0.1982} = 5.930 \text{ mg}$$

This calculation is intended merely to show the small quantity of *p*-aminosalicylic acid left in the aqueous phase, indicating the efficiency of the ether extraction process.

Experimental

Extinction coefficients. 0.001% solutions of isoniazid and *p*-aminosalicylate in 0.01N hydrochloric acid are prepared and their extinctions measured at 255 and 305 m μ . These extinctions multiplied by 1000 gives the extinction coefficients *E* (1%, 1 cm) at the two wavelengths of each of the two substances. These extinction coefficients together with the ratios *a* and *b* should be revised from time to time. Since the ratios *a* and *b* do not depend on the concentrations, their revision merely requires preparation of solutions of unknown concentration and measuring the extinctions at 255 m μ and 305 m μ with the proviso that the extinctions be within a reasonable range (e.g. 0.2–1.0).

Procedure. Weigh and powder 20 tablets. Accurately weigh an aliquot of powder containing about 4 mg of isoniazid, transfer to a separating funnel. Add 0.1N hydrochloric acid (20 ml) and shake the funnel for about 2 min. The mixture is then extracted with 4 \times 30 ml ether. The combined ether extracts are washed with water (5 ml) and the washings transferred to the aqueous phase. The combined aqueous solutions are diluted to 200 ml. Dilute 25 ml of this solution to 50 ml with 0.01N hydrochloric acid. Determine the extinction of this solution as soon as possible at 255 and 305 m μ (*p*-aminosalicylic acid in solution undergoes decomposition on prolonged standing). The concentrations of isoniazid and *p*-aminosalicylate in the aqueous phase are then calculated from equations (1) and (2).

Results

TABLE 1. ESTIMATION OF ISONIAZID IN THE PRESENCE OF 0.1 g AMOUNTS OF SODIUM *p*-AMINOSALICYLATE

Weight of isoniazid taken (mg)	Isoniazid recovered	
	mg	%
1	1.00	100.0
2	1.97	98.5
3	2.97	99.0
4	3.96	99.0
5	4.93	98.9
6	5.93	98.9
7	6.97	99.6
8	8.07	100.9

DETERMINATION OF ISONIAZID IN EXCESS *p*-AMINOSALICYLATE

The method was applied to synthetic mixtures of varying quantities of isoniazid in the presence of sodium *p*-aminosalicylate and the results obtained are given in Table 1.

TABLE 2. ESTIMATION OF ISONIAZID IN TABLETS

Weight per tablet of sodium <i>p</i> -aminosalicylate (g) as stated	Weight per tablet of isoniazid (mg)		
	Stated	Found	
		(mg)	%
0.5	15	15.6	104
0.5	15	15.5	103
0.5	15	15.6	104
0.5	15	15.5	103
0.5	15	15.6	104

Five commercial samples in tablet form were analysed by the proposed method and the results are given in Table 2.

Discussion

Recoveries of isoniazid from tablets obtained by the proposed method (Table 2) are higher than those obtained by Lee & Ho (1962). Recoveries of isoniazid from synthetic mixtures (Table 1) containing as much as 100 times more *p*-aminosalicylate are excellent. The proposed method is therefore not sensitive to error for small amounts of isoniazid with *p*-aminosalicylate as in the method of Mitchell & others (1957).

Using equation (2), the quantity of *p*-aminosalicylate remaining in the aqueous phase was found to vary between 1 and 6 mg both for synthetic mixtures and tablets.

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