THE ESTIMATION OF *ISO*NICOTINYL HYDRAZIDE

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THE introduction of the antitubercular substance *iso*nicotinyl hydrazide into pharmacy has necessitated an investigation of methods for its assay. The oxidation of the material by bromine and iodine has formed the basis of these studies and a method based on the oxidation by standard iodine solution has recently been described by Canbäck.[1] Having chosen for routine estimation a bromimetric method of assay and having obtained consistent figures for assaying the drug both as the pure substance and in the form of tablets, it is of interest to report a comparison of this method with that described by Canbäck.

In the case of the pure material, the bromimetric assay gave results which were significantly higher than those obtained by the iodimetric procedure. A more detailed study showed that whereas the iodimetric assay varied with the reaction time, the bromimetric assay remained virtually constant with reaction times greater than 10 minutes. These results are summarised in Table I.

TABLE I

EFFECT OF REACTION TIME ON THE IODIMETRIC AND BROMIMETRIC ASSAYS

Time minutes	Iodimetric assay		Bromimetric assay	
	Titre difference ml.	Assay per cent.	Titre difference ml.	Assay per cent.
5	25·12, 25·20	92·52	26·88, 26·89	99·49
10	25·83, 25·77	94·88	26·98, 26·99	99·93
15	25·90, 25·93	95·31	26·95, 26·90	99·71
20	26·47, 26·54	97·48	26·94, 26·97	99·84
40	26·86, 26·85	98·77	26·92, 26·94	99·74
	Blank titre = 46·90 ml. Factor of 0·05N $Na_2S_2O_3$ = 1·066 Weight of drug/titre = 49·71 mg.		Blank titre = 46·88 ml. Factor of 0·05N $Na_2S_2O_3$ = 1·067 Weight of drug/titre = 49·42 mg.	

TABLE II

THE ESTIMATION OF *iso*NICOTINYL HYDRAZIDE IN TABLETS (50 mg.)

Source of tablet	Iodimetric assay		Bromimetric assay	
		Average mg.		Average mg.
A (British	51·3, 51·1	51·2	50·1, 50·1	50·1
B (British)	50·2, 50·3	50·3	49·9, 49·9	49·9
	49·4, 50·1	49·7	49·4, 49·4	49·4
C (Swiss)	50·8, 50·6	50·7	50·5, 50·6	50·6

However, when the two methods were compared using various brands of 50 mg. tablets of the drug, it was found that the two assays agreed quite closely, the iodimetric assay tending to be slightly higher. Table II summarises the results obtained on tablets from three different sources.

It seemed most probable that the cause of the increased iodimetric assay of the drug in tablet form was due to the excipient used in tabletting the drug. For the 3 types of tablet examined, the excipient consisted mainly of lactose and starch and the effect of the reducing sugar lactose on the 2 assays was then investigated. Since the tablets contained 20 per cent. of drug, and in the case of two varieties of tablets the content of lactose was about 60 per cent., mixtures of *iso*nicotinyl hydrazide and lactose in the ratio 1:3 were assayed by the two methods. Table III shows the effect of lactose on the assay of *iso*nicotinyl hydrazide obtained from three different manufacturers.

TABLE III

THE EFFECT OF LACTOSE ON THE ESTIMATION OF *iso*NICOTINYL HYDRAZIDE

Source of drug	Iodimetric assay		Bromimetric assay	
	Normal	+150 mg. of lactose	Normal	+ 150 mg. of lactose
1 (German)	98·17 ± 0·1	101·5 ± 0·5	99·65 ± 0·1	99·80 ± 0·1
2 (British)	98·65 ± 0·07	100·35 ± 0·05	99·70 ± 0·02	99·75 ± 0·07
3 (Italian)	98·76 ± 0·18	101·8 ± 0·3	99·86 ± 0·02	99·88 ± 0·01

A comparison of the effect of the sugars glucose, lactose and sucrose on the assay procedures was then carried out in the absence of *iso*nicotinyl hydrazide. It appears that the alkaline medium used in the iodimetric procedure is necessary for the oxidation of glucose and lactose since iodination in the presence of excess acid (similar to the conditions used in the bromimetric assay) resulted in practically no oxidation of these sugars. The figures are only comparative, since in the presence of *iso*nicotinyl hydrazide the quantity of excess halogen is rapidly reduced. The results are summarised in Table IV.

TABLE IV

THE OXIDATION OF SUGARS BY BROMINE AND IODINE

Sugar (150 mg.)	Iodine in bicarbonate solution		Bromine in acid solution		Iodine in acid solution	
	Observed titre difference ml.	Equivlaent as drug mg.	Observed titre difference ml.	Equivalent as drug mg.	Observed titre difference ml.	Equivalent as drug
Glucose	6·78 6·40	12·4 11·7	0·04 0·04	0·07 0·07	0·00 −0·03	nil
Lactose	4·41 5·08	8·1 9·3	0·01 0·01	0·02 0·02	−0·05 −0·08	nil
Sucrose	0·13 0·18	0·2 0·3	−0·01 0·00	nil	−0·07 −0·02	nil

During the course of these investigations, it appeared that the iodimetric assay was susceptible to temperature changes and a series of estimations were carried out on a sample of *iso*nicotinyl hydrazide using both assay methods at various temperatures. Whereas the iodimetric assay increased from 92·1 per cent. to 98·8 per cent. between 10° and 30° C., the bromimetric assay only showed an increase from 98·7 per cent. to

99·5 per cent. in the same temperature range. The results are given in Table V.

TABLE V

EFFECT OF TEMPERATURE ON THE ESTIMATION OF *iso*NICOTINYL HYDRAZIDE

Temperature °C.	Iodimetric assay per cent.		Bromimetric assay per cent.	
		Average mg.		Average mg.
10	91·64, 92·45	92·1	98·88, 98·47	98·7
20	97·52, 97·81	97·7	99·33, 99·56	99·4
30	98·79, 98·72	98·76	99·40, 99·61	99·5

The mechanism of the reaction between *iso*nicotinyl hydrazide and bromine appears to be complete oxidation of the hydrazide group and formation of *iso*nicotinic acid. We have found that under the conditions used in the bromimetric assay, one mole of nitrogen is evolved per mole of *iso*nicotinyl hydrazide oxidised. The presence of *iso*nicotinic acid in the oxidised mixture can be demonstrated by treating the hydrazide in acid solution with bromine water and evaporating to dryness. The hydrobromide obtained is dissolved in water, neutralised and then brought to *p*H 3 with hydrochloric acid when *iso*nicotinic acid is precipitated (melting point = 319° to 320° C. undepressed when mixed with authentic *iso*nicotinic acid).

It is interesting to note that in the determination of the drug in biological fluids Rubin *et al.*[2] first oxidise the hydrazide to *iso*nicotinic acid with potassium permanganate. The fact that in both assay procedures 4 equivalents of halogen are used, suggests that the mechanism is the same in both cases. The reactions can, therefore, be expressed by the equations:—

$$RCONHNH_2 + 2I_2 + 4NaHCO_3 \rightarrow RCOOH + N_2 + 4NaI + 4CO_2 + 3H_2O$$

$$RCONHNH_2 + 2Br_2 + H_2O \xrightarrow{H^+} RCOOH + N_2 + 4HBr.$$

We suggest that the bromimetric assay should be used for the estimation of *iso*nicotinyl hydrazide, particularly when in the form of tablets since the presence of lactose has been shown to interfere with the iodimetric assay.

EXPERIMENTAL

Iodimetric Assay (according to Canbäck[1])

About 0·5 g. of pure *iso*nicotinyl hydrazide, accurately weighed, was dissolved in water and made up to exactly 250 ml. 25 ml. of this solution was pipetted into a 250-ml. iodine flask, and 1 g. of sodium bicarbonate together with 25 ml. of water added. 25 ml. of 0·1N iodine solution were pipetted into the mixture and the flask stoppered and allowed to stand 15 minutes. 10 ml. of 5N hydrochloric acid were added slowly and the excess iodine titrated with 0·05 sodium thiosulphate. A blank titration was carried out at the same time.

Percentage of *iso*nicotinyl hydrazide =

$$\frac{\text{Titre Difference} \times \text{Factor of 0·05N } Na_2S_2O_3 \times 1·715}{\text{Weight of sample taken in g.}}$$

Bromimetric Assay

About 0·4 g. of the hydrazide, accurately weighed, was dissolved in water and made up to 250 ml. 25 ml. of this solution was pipetted into a 250-ml. iodine flask together with 25 ml. of 0·1N potassium bromide/potassium bromate solution. 5 ml. of concentrated hydrochloric acid was added, the flask stoppered immediately and allowed to stand for 10 to 15 minutes. 5 ml. of 20 per cent. potassium iodide solution was slowly admitted to the flask (care is needed since there is a slight positive pressure in the flask due to the formation of nitrogen). The liberated iodine was then titrated with 0·05N sodium thiosulphate, a blank titration being carried out at the same time.

Percentage of *iso*nicotinyl hydrazide =

$$\frac{\text{Titre Difference} \times \text{Factor of 0·05N } Na_2S_2O_3 \times 1{\cdot}715}{\text{Weight of sample taken in g.}}$$

The Estimation of isoNicotinyl Hydrazide in Tablets

All samples examined were 250 mg. tablets containing 50 mg. of drug. 10 tablets were weighed and powdered as finely as possible. About 2·0 g. of the powder accurately weighed, was suspended in water and diluted to exactly 250 ml. After shaking thoroughly, the insoluble matter was allowed to settle for ½ hour and 25 ml. of the supernatant liquid pipetted into an iodine flask. The estimation was completed as described above, using 0·1N iodine or 0·1N potassium bromide/bromate solution.

Weight of drug in mg. per tablet =

$$\frac{\text{Titre Difference} \times \text{Factor of 0·05N } Na_2S_2O_3 \times 17{\cdot}15 \times \text{Average weight of Tablet}}{\text{Weight of sample taken in g.}}$$

The Effect of Sugars on the Estimation

Aqueous solutions containing 0·6 per cent. of glucose, lactose and sucrose respectively were prepared (25 ml. ≡ 150 mg. of sugar). Estimations were carried out as follows:—

Iodine in bicarbonate solution. In the iodimetric assay procedure, 25 ml. of the hydrazide solution was replaced by 25 ml. of the requisite sugar solution.

Bromine in acid solution. In the bromimetric assay procedure, 25 ml. of the hydrazide solution was replaced by 25 ml. of the requisite sugar solution.

Iodine in acid solution. The procedure used was the same as for iodine in bicarbonate solution except that 1 g. of sodium bicarbonate was replaced by 5 ml. of concentrated hydrochloric acid and 5 ml. of 20 per cent. potassium iodide solution.

Iodimetric assay of the drug and lactose. In the iodimetric assay procedure, 25 ml. of water was replaced by 25 ml. of 0·6 per cent. lactose solution.

Bromimetric assay of the drug and lactose. 150 mg. of lactose powder was added to the flask before the addition of 5 ml. of concentrated hydrochloric acid.

The Effect of Temperature on the Estimation

3 water baths were maintained at the temperatures required (10° C., 20° C. and 30° C.) by addition of ice or slight warming. In the case of the iodimetric assay, the titration flask and contents were placed in the bath 10 minutes before the addition of the 0·1N iodine. The reaction was then allowed to proceed for 15 minutes in the bath. For the bromimetric assay, the flask and contents were placed in the bath 10 minutes before adding the concentrated hydrochloric acid, and then left a further 10 minutes for the reaction to proceed.

Estimation of Nitrogen formed in the Reaction

0·6857 g. of *iso*nicotinyl hydrazide was dissolved in water containing 1·4 g. of potassium bromate and 7·0 g. of potassium bromide and diluted to 100 ml. The reaction bulb of a micro Van Slyke apparatus was filled with 5N hydrochloric acid and the gas burette and connecting tubes filled with water. 2 ml. of the hydrazide/bromate/bromide solution were admitted to the reaction bulb and the contents shaken mechanically for ½ hour. The gas evolved was driven over into the burette and the volume measured at atmospheric pressure. 3 estimations were carried out giving the following results.

Weight of drug per estimation = 13·71 mg. = 0·1 millimole.
Pressure = 762 mm. Temperature = 25° C.

Estimation	1	2	3
Volume of nitrogen	2·43 ml.	2·40 ml.	2·49 ml.
Mg. of nitrogen	2·71	2·67	2·77
Moles of nitrogen/mole drug	0·97	0·95	0·99

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REFERENCES

1. Canbäck, *J. Pharm. Pharmacol.*, 1952, **4**, 407.
2. Rubin *et al.*, *Dis. Chest.*, 1952, **21**, 439.

DISCUSSION

The two papers on *iso*nicotinyl hydrazide were discussed together.

The first paper was presented by MR. P. G. W. SCOTT, and the second by MR. B. W. MITCHELL.

DR. C. H. HAMPSHIRE (London), in a written contribution, drew attention to the international non-proprietary name isoniazid, and pointed out that, if the drug fulfilled the high expectations entertained, pharmacopœial monographs would be required. He quoted a letter from Dr. T. Canbäck, who had published a suggested assay and had seen the proofs of both of the papers, in which he stated that a study of the time function of the reaction had shown that 10 minutes was enough for completion of the oxidation with iodine in slightly alkaline solution.

Dr. Canbäck had repeated the time function studies and quoted figures which suggested that a second reaction started after about 35 minutes. The figures led to the conclusion that the sample examined contained about 97 per cent. of isoniazid and a few per cent. of a second compound which reacted with the iodine after 30 to 40 minutes. The same sample, tested by the authors' bromimetric method, gave 95·7 per cent. after 10 minutes, rising to 98·5 per cent. after 18 minutes, results which again suggested that there was a time factor in the bromimetric procedure and that there was present a second compound which was more difficult to oxidise. The compound used was not absolutely pure. For practical purposes both methods were, he thought, acceptable, but he hoped, for scientific reasons, that both methods would be further investigated. For tablets containing excipients it was never safe to rely more on bromine in acid solution than on iodine in slightly alkaline solution.

DR. G. E. FOSTER (Dartford) said he believed that the bromimetric method was the better one. He gave details of the old and more recent methods of preparing standard bromine solutions, and asked Mr. Mitchell whether he had tried the bromimetric assay using both solutions and, if so, whether he had obtained similar results. In his experience with other substances the solutions could give different results.

DR. E. M. BAVIN (Welwyn) asked Mr. Scott whether he had used the chlorodinitrobenzene method for the estimation of *iso*nicotinyl hydrazide in biological fluids. He wondered whether that reagent could be used, as difficulties were encountered with the *p*-dimethylaminobenzaldehyde method and with the old method using cyanogen bromide.

MR. N. L. ALLPORT (London) said he thought that Mr. Scott's claim of an error of not greater than $\pm$ 3 per cent. was optimistic. He would have thought a figure of $\pm$ 5 per cent. more likely.

MR. P. G. W. SCOTT, in reply, said that he had not applied the chlorodinitrobenzene method to the determination of *iso*nicotinyl hydrazide in biological fluids, but hoped to do so. It was not likely that many substances would be found in biological fluids which would interfere with that colorimetric determination. He felt that $\pm$ 5 per cent. was unduly wide, and that by tightening up the conditions of determination it should be possible to obtain $\pm$ 2 per cent.

MR. W. MITCHELL, in reply, referred to the fact that Dr. Canbäck used rather impure material, and said that it was essential that an assay method should be capable of giving good results with impure as well as pure material. He had used commercial material from various sources and had obtained results of over 99 per cent. with the bromimetric method. He could not comment on the variations with time using the bromimetric method, but was pleased to learn that the subject was being investigated. In answer to Dr. Foster, he had not investigated bromine solution prepared with potassium hydroxide and bromine. In his method excess of bromine was present all the time and appeared to have no deleterious effect.