Determination of Isoniazid in Drugs with 2-Iodoxybenzoate

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Isoniazid has been determined in pharmaceutical preparations by reaction with 2-iodoxybenzoate in acid medium followed by iodometric evaluation of the surplus reagent. 4-Aminosalicylic acid, reducing sugars, and other excipients of drugs do not interfere. Mixtures of isoniazid, 4-aminosalicylic acid, and vitamin C have been analyzed without their prior separation by a combination of three methods which involve oxidation with 2-iodoxybenzoate in the presence of (i) potassium iodide or (ii) potassium bromide or (iii) in the absence of these alkali halides.

Isoniazid (isonicotinyl hydrazide) finds wide application in the treatment of tuberculosis and has been the subject of considerable publications concerning its analysis (*1*). Many pharmaceutical preparations also contain 4-aminosalicyclic acid.

The hypoiodite oxidation of isoniazid (*2*) tends to produce variable results particularly with changes in temperature and time of reaction. This method is not applicable when lactose and other reducing carbohydrates are used in dispensing form. Other oxidizing agents have also been proposed (*3–10*) but removal of reducing substances is necessary. Reaction with bromine is more satisfactory. Carbohydrates do not interfere as long as the medium is acidic (*11–15*); however, both 4-aminosalicylic acid and vitamin C also react quantitatively with bromine. Modifications have been suggested involving measurement of nitrogen released in the reaction between isoniazid and *N*-bromosuccinimide (*16*), differential acidimetric titration of isoniazid after lowering the basicity of 4-aminosalicyclic acid by acetylation (*17, 18*), or thin-layer chromatographic separation of constituents followed by their densitometric analysis (*19*). Recently, isoniazid is titrated with sodium methoxide in dimethylformamide when alkali 4-aminosalicylate does not interfere (*20*); however, any free acid present, owing to poor preparation, will cause a positive error.

While studying procedures for the determination of mixtures of organic compounds by redox reactions without the need for preliminary separation of constituents (*21–26*), 2-iodoxybenzoate was observed to oxidize isoniazid, vitamin C, and 4-aminosalicytic acid as follows:

(i) Vitamin C can be titrated with 2-iodoxybenzoate in the presence of acidified potassium iodide and starch when isoniazid and 4-aminosalicylic acid do not interfere.

(ii) Isoniazid reacts quantitatively with 2-iodoxybenzoate in acid medium without any interference from 4-aminosalicylic acid. Vitamin C, if present, also reacts but a correction for it can be made by method (i).

(iii) 4-Aminosalicylic acid undergoes nuclear substitution with bromine generated in situ from acidified potassium bromide by 2-iodoxybenzoate. Vitamin C and isoniazid exhibit the same stoichiometry as in methods (i) and (ii) and thus their substraction from (iii) yields 4-aminosalicylic acid.

EXPERIMENTAL SECTION

Reagents. 2-Iodoxybenzoic acid was synthesized by the method of Banerjee et al. (*27*). A 0.1 N (0.025 M) reagent solution was prepared by dissolving 7.0 g of free acid in a slight excess (about 26 mL) of 1 M potassium hydroxide, diluting to 1 L, and standardizing iodometrically.

$$^{-}O_2CC_6H_4IO_2 + 4I^- + 5H^+ \rightarrow 2I_2 + HO_2CC_6H_4I + 2H_2O$$

A 0.02 N solution was made by diluting the stock solution with deionized water.

Sampling. A known number of tablets are weighed and finely ground. An accurately weighed amount of powder is treated with 10 mL of 10% sulfuric acid, stirred to effect salt formation, mixed with 50 mL of water with stirring and any insoluble portion (mostly, starch and lactose) being filtered off on Whatman No. 41 paper, and washed with 10 mL of 1% sulfuric acid. The combined filtrate and washings are made up to known volume with deionized water. Aliquots of syrups are mixed with 10 mL of 10% sulfuric acid and diluted appropriately.

(*i*) *Determination of Vitamin C.* A known aliquot of solution is mixed with 50 mg of potassium iodide, 1 mL of 1% starch, 2 mL of 10% sulfuric acid, and 25 mL of water and titrated with 0.02 N 2-iodoxybenzoate until the appearance of a blue color.

$$\text{amt of vitamin C (mg)} = 88VN$$

where V is volume of 2-iodoxybenzoate of normality N.

(*ii*) *Determination of Isoniazid.* A portion of sample solution is treated with 10 mL of 10% sulfuric acid and a measured excess (25 mL) of 0.1 N 2-iodoxybenzoate. The contents are shaken well and kept for 5 min. Thereafter, 1 g of potassium iodide is added and the liberated iodine is titrated with 0.04 N thiosulfate using starch near the end point. A blank determination is also run concurrently.

$$\text{amt of isoniazid (mg)} = 34.25VN$$

where V is volume consumed of thiosulfate of normality N.

If the sample also contains vitamin C, analysis is carried out by methods (i) and (ii) on equal but separate aliquots of solution. In the determination of vitamin C there is no interference from isoniazid but when the method for isoniazid is used, a total of two compounds is obtained, isoniazid is obtained by difference

$$\text{amt of isoniazid (mg)} = 34.25[(VN)^{ii} - (VN)^{i}]$$

where $(VN)^{ii}$ stands for volume of thiosulfate of normality N consumed in method ii, and $(VN)^{i}$ is volume of 2-iodoxybenzoate of normality N consumed in method i.

(*iii*) *Determination of 4-Aminosalicylic Acid.* To a measured volume of sample solution taken in a 250-mL flask of iodine there are added 10 mL of 10% sulfuric acid, a measured volume (25 mL) of 0.1 N 2-iodoxybenzoate, 25 mL of water and about 1 g of potassium bromide. The flask is stoppered and kept for 5 min for complete reaction. The residual bromine is evaluated by adding about 2 g of potassium iodide and titrating the liberated iodine with 0.04 N thiosulfate employing starch near the end point. A blank determination on the same volume of 2-iodoxybenzoate is also carried out.

$$\text{amt of 4-aminosalicylic acid (mg)} = 25.5VN$$

Mixtures that also contain isoniazid and/or vitamin C can be resolved by correcting the amount of 2-iodoxybenzoate used in the 4-aminosalicyclic acid titration for that used in the oxidation of the other components; that is

$$\text{amt of 4-aminosaliicylic acid (mg)} = 25.5[(VN)^{iii} - (VN)^{ii}]$$

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Table I. Determination of Vitamin C, Isoniazid, and 4-Aminosalicylic Acid in Pure Solutions and Synthetic Mixtures

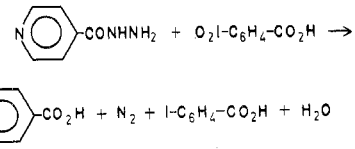
		amt taken, mg	amt found,[a] mg present method	CV	comparison method
vitamin C (I)		4.25	4.22	0.2	4.24[b]
		5.61	5.64	0.1	5.63
		6.98	6.93	0.3	7.00
		9.28	9.21	0.2	9.23
		10.25	10.19	0.3	10.22
isoniazid (II)		1.98	1.97	0.2	1.95[c]
		2.69	2.70	0.1	2.72
		4.26	4.27	0.3	4.30
		5.34	5.30	0.2	5.36
		6.25	6.28	0.3	6.29
4-aminosalicylic acid (III)		2.31	2.29	0.2	2.33[d]
		4.95	4.98	0.3	4.99
		6.21	6.17	0.1	6.12
		8.02	8.09	0.3	7.97
		9.88	9.81	0.3	9.80
mixt no. 1	I	8.67	8.62	0.3	
	II	2.51	2.48	0.5	
	III	3.34	3.27	0.6	
mixt no. 2	I	17.32	17.28	0.4	
	II	5.05	4.94	0.6	
	III	6.68	6.64	0.5	
mixt no. 3	I	21.62	21.58	0.4	
	II	6.25	6.17	0.7	
	III	8.34	8.28	0.8	
mixt no. 4	I	26.02	26.29	0.5	
	II	7.54	7.48	0.6	
	III	10.01	9.89	0.8	

[a] Average of six determinations; CV, coefficient of variation. [b] Titration with chloramine-T (*22*). [c] Titration with *N*-bromosuccinimide (*15*). [d] Bromimetry (*1*).

where $(VN)^{ii}$ and $(VN)^{iii}$ stand for volume of thiosulfate of normality N consumed in methods ii and iii, respectively.

RESULTS AND DISCUSSION

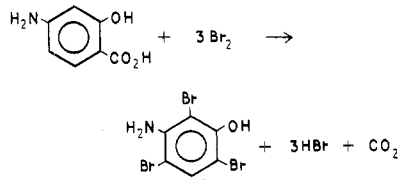
The determination of isoniazid and vitamin C is based on the reducing properties of the hydrazino and 1,2-enediol groups, respectively, which react as



$$2C_6H_8O_6 + O_2IC_6H_4CO_2H \rightarrow 2C_6H_6O_6 + IC_6H_4CO_2H + 2H_2O$$

4-Aminosalicylic acid undergoes nuclear substitution with bromine generated in situ from acidified potassium bromide by 2-iodoxybenzoic acid.

$$4HBr + O_2IC_6H_4CO_2H \rightarrow 2Br_2 + IC_6H_4CO_2H + 2H_2O$$



As in direct reaction with 2-iodoxybenzoate (methods i and ii), isoniazid and vitamin C show their four- and two-electron oxidation on reaction with bromine (method iii).

In Table I, results are given for the determination of isoniazid, vitamin C, and 4-aminosalicylic acid when present alone or in their synthetic combinations. The results are compared with those obtained by previously checked independent methods (*1*, *15*, *22*). Results on the determination of isoniazid in drugs are given in Table II, while Table III includes results for the analysis of isoniazid and 4-aminosalicylic acid.

In almost every oxidimetric procedure for isoniazid (*2–15*), 4-aminosalicylic acid interferes because it is susceptible to oxidation to an azo compound (*28*, *29*). Although only isoniazid gives nitrogen in its gasometric determination with *N*-bromosuccinimide (*16*), 4-aminosalicylic acid, which is often formulated in larger proportions, consumes large amounts of *N*-bromosuccinimide causing difficulty in maintaining its excess. 2-Iodoxybenzoate does not react with 4-aminosalicylic acid and is an oxidimetric reagent of choice. It may be reduced following a two-electron change to 2-iodosobenzoate which may undergo a further two-electron change to yield 2-iodobenzoate.

$$HO_2CC_6H_4IO_2 + 2H^+ + 2e^- \rightarrow HO_2CC_6H_4IO + H_2O$$

$$HO_2CC_6H_4IO + 2H^+ + 2e^- \rightarrow HO_2CC_6H_4I + H_2O$$

Table II. Determination of Isoniazid in Drugs

drug	isoniazid (mg) per tablet or 5 mL of syrup: makers' specif.	amt found[a]: present method	coefficient of variation	comparison method
Isonex	300	284.8	0.3	286.9
Isokin 300[b]	300	297.9	0.3	300.1
Isokin-T Forte[c]	300	293.0	0.4	295.6
Inapas[d]	25	25.2	0.6	
Uni-Thioben[e]	75	75.2	0.4	75.8
Thiocent[f]	150	147.0	0.5	148.5
Siozid[g]	280	261.5	0.6	265.3
Pasilin[h]	25	23.6	0.7	
Glutapasizid[i]	25	26.8	0.6	
Isokin 100	100	98.2	0.3	99.4

[a] Average of six determinations. [b] The excipient was vitamin B_6 (10 mg). [c] The excipient was thiacetazone (150 mg). [d] The excipient was sodium 4-aminosalicylate (834 mg). [e] The excipients were thiamine hydrochloride (1 mg), thiacetazone (37.5 mg), riboflavine (1 mg), niacin (10 mg), cholesterol USP (150 IU), and tetrazine as coloring matter. [f] The excipients were thiacetazone (75 mg), thiamine mononitrate (2 mg), pyridoxine hydrochloride (5 mg), riboflavin (2 mg), and nicotinamide (10 mg). [g] The excipients were cetylpyridinium chloride (1.313 mg), terpin hydrate (13.13 mg), sodium citrate (0.21 mg), citric acid (13.13 mg), vitamin B_6 (0.876 mg), and vitamin C (4.375 mg). [h] The excipients were sodium 4-aminosalicylate (750 mg), vitamin B_1 (2 mg), vitamin B_6 (3 mg), lysine hydrochloride (20 mg), and glutamic acid (50 mg). [i] The excipients were sodium 4-aminosalicylate (800 mg) and glutamic acid (50 mg).

Table III. Determination of Isoniazid and 4-Amionsalicylic Acid in Drugs

drug	amt, makers' specification, mg: isoniazid I	4-amino-salicylic acid[b] II	amt found,[a] mg: I	CV	II	CV
INH-PAS	25	601.2	22.4	0.6	593	1.0
Pazide	15	362.2	17.9	0.4	356	0.8
PAS opizyd	30	768.3	26.4	0.5	773	0.9
Isocadipas G	150	3622.0	158.9	0.6	3615	1.0
Isocadipas T	33.4	724.4	30.2	0.4	701	0.8
PAS with INH	17	384.1	15.6	0.4	376	0.6
Inapas	25	604.2	26.8	0.6	615	0.6

[a] Average of six determinations. [b] The amount of calcium and sodium salts converted to that of free acid.

The electrode potentials of the 2-iodoxy-/2-iodosobenzoate system at 25° are 1.33, 0.61 and 0.56 V at pH 1, 4 and 7 respectively. At the corresponding pH, the 2-iodoso-/2-iodobenzoate system has electrode potentials 1.21, 0.53 and 0.48 V.

Large amounts of glucose, maltose, sucrose, lactose, sodium formate, glycine, and alanine do not interfere with the determination of isoniazid; other materials that do not affect the analysis appear in the footnote of Table II.

LITERATURE CITED

(1) Garratt, D. C. "The Quantitative Analysis of Drugs"; Chapman & Hall: London, 1964; pp 360, 561.
(2) Balka, M.; Bode, D.; Rhodes, H. J. *J. Pharm. Sci.* **1974**, *63*, 1303.
(3) Pszonicka, M.; Skwara, W. *Chem. Anal. (Warsaw)* **1970**, *5*, 175.
(4) Rao, P. V. K.; Rao, G. B. B. *Analyst (London)* **1971**, *96*, 712.
(5) Pinazautl, S.; Dal Piaz, V.; La Porta, E. *Farmaco, Ed. Prat.* **1974**, *29*, 136.
(6) Eremina, Z. I.; Antonichik, J. A. *Farm. Zh. (Kiev)* **1972**, *27*, 79.
(7) Rao, K. S.; Rao, G. B. B.; Rao, P. V. K. *Chem. Anal. (Warsaw)* **1974**, *19*, 927.
(8) Sollman, R.; Belal, S. A. *J. Drug. Res.* **1974**, *6*, 7.
(9) Nair, V. R.; Nair, C. G. R. *Anal. Chim. Acta* **1971**, *57*, 429.
(10) Chateau-Gosselin, M.; Patniarche, G. J. *Anal. Chim. Acta* **1978**, *102*, 215.
(11) Nair, C. G. R.; Kumari, L. *Indian J. Chem., Sect. A* **1976**, *14A*, 115.
(12) Abramo, M. K. *Farmatsiya (Moscow)* **1975**, *24*, 80.
(13) Urbanyi, T.; Winchurch, R. *J. Assoc. Off. Anal. Chem.* **1973**, *56*, 1464.
(14) Bark, L. S.; Prachuabpaibul, P. *Anal. Chim. Acta* **1976**, *87*, 505.
(15) Barakat, M. Z.; Shaker, M. *Analyst (London)* **1966**, *91*, 466.
(16) Sarwar, M.; Yaqub, M.; Naeem, I.; Kazi, A. A. *Z. Anal. Chem.* **1977**, *285* 269.
(17) Kuhni, E.; Jacob, M.; Grossglauser, H. *Pharm. Acta Helv.* **1954**, *29*, 233.
(18) Devani, M. B.; Shishoo, C. J. *J. Pharm. Sci.* **1970**, *59*, 90.
(19) Pavlyuchenkova, L. P. *Farmatsiya (Moscow)* **1976**, *25*, 73.
(20) Das, A.; Boparai, K. S. *Talanta* **1982**, *29*, 57.
(21) Verma, K. K., *Mikrochim. Acta* **1980**, 211.
(22) Verma, K. K.; Gulati, A. K. *Anal. Chem.* **1980**, *52*, 2336.
(23) Verma, K. K.; Gulati, A. K. *Analusis* **1981**, *9*, 506.
(24) Verma, K. K.; Gupta, A. K. *Talanta* **1981**, *28*, 849.
(25) Verma, K. K. *Talanta* **1982**, *29*, 41.
(26) Verma, K. K.; Gupta, A. K. *Anal. Chem.* **1982**, *54*, 249.
(27) Banerjee, A.; Banerjee, G. C.; Bhattacharya, S.; Banerjee, S.; Samaddar, H. *J. Indian Chem. Soc.* **1981**, *58*, 606.
(28) Verma, K. K.; Bose, S. *Mikrochim. Acta* **1976**, 591.
(29) Verma, K. K. *Talanta* **1979**, *26*, 257.

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Separation of Tervalent Lanthanides from Actinides by Extraction Chromatography

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A procedure is described for the removal of milligram amounts of lanthanides from the tervalent actinides. The routine application of an earlier procedure resulted in low americium yields and poor resolution of the α spectrum, mainly due to inadequate removal of neodymium. The reported procedure offers many significant improvements and involves prepurification of the lanthanide–actinide fraction by precipitation as a hydroxide, removal of the lanthanides from the tervalent actinides by extraction on a column of bis(2-ethylhexyl)-phosphoric acid sorbed on Teflon powder, and carrying the actinides directly from the eluate on about 50 μg of cerium fluoride for α spectrometry. The actinide recovery from a mixture of 5 mg of cerium, 3 mg of lanthanum, and 2 mg of neodymium was 86% for californium and 96% for americium and curium, with less than 10 μg of the neodymium remaining. The average overall recovery of americium from routine analysis of 131 soil samples was 84 ± 6%.

The separation of the tervalent actinides from the lanthanides by elution with a buffered diethylenetriaminepentaacetate (DTPA) solution through a column of bis(2-ethylhexyl)phosphoric acid (HDEHP) sorbed on Teflon powder has been previously described by Filer (*1*). The routine application of this method to the americium fraction obtained from the analysis of 10-g soil samples (*2*) at the Radiological and Environmental Sciences Laboratory (RESL) resulted in frequent low yields and poor resolution of the α spectra of the subsequently electrodeposited americium (*3*). The poor americium yields and resolution of the α spectra were due to excess neodymium eluted with the americium. Filer studied other lanthanides but not neodymium. His conditions, though adequate for cerium and lanthanum, were not adequate for the separation of tervalent actinides from the amount of neodymium found in many soils (*4*). Also, other problems were encountered involving decreasing column efficiency with use, pH measurements, amounts of lanthanides greater than the 2 mg limit, initially impure lanthanide–actinide fractions, and incomplete dissolution of these fractions prior to loading on the column.

In the present method these problems have been reduced significantly. Most of the conditions affecting the column chromatographic separation have been studied and are re-