ION-EXCHANGE CHROMATOGRAPHY ON ALGINIC ACID OF CERTAIN B-GROUP VITAMINS

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Quantitative separation of mixtures of organic bases into their components has been achieved by ion-exchange chromatography on alginic acid. This simplifies the routine control of nicotinamide and aneurine and pyridoxine hydrochlorides, when these are present in certain pharmaceutical preparations.

THE successful application of alginic acid to the separation of organic bases from non-basic material (Foster and Murfin, 1961) led to an attempt to extend the work to pharmaceutical preparations containing some vitamins of the B group. It was hoped to achieve fractionation and quantitative separation of pyridoxine, nicotinamide and aneurine when present in admixture with other materials, and subsequently to assay each of these separated components spectrophotometrically. This approach promised a simpler and quicker method of estimation than the official microbiological assays.

Whilst the formaldehyde treatment of alginic acid had proved moderately successful, material thus prepared proved difficult to free from matter absorbing in the ultra-violet region of the spectrum. Prolonged use, moreover, tended to lead to disintegration into "fines". An alternative means of preparing alginic acid for ion-exchange was therefore sought.

EXPERIMENTAL

Preparation of Alginic Acid Ion-Exchanger

The following method, based on that of Specker and Hartkamp (1953) was found suitable.

Dust alginic acid (40 g.) slowly on to a solution of sodium hydroxide (12 g.) in water (450 ml.) with vigorous stirring. Continue stirring until a glutinous, homogenous solution results. Add this dropwise through a nozzle approximately 1·5 mm. internal diameter to 10 per cent w/w hydrochloric acid, stirring continuously during and for 15 min. after addition. Decant the liquid from the precipitate through a muslin filter over the mouth of the vessel and wash the precipitate until the washings are neutral to litmus and chloride-free. Wash the alginic acid three times with acetone, steep in the same solvent overnight and dry at a temperature not exceeding 50°.

Powder the product—hard, translucent pellets, light yellow-brown in colour—in a mill with a high-speed rotating blade and select a mesh fraction suitable for use.

Preparation of Columns

Use Pyrex tubes of 2 cm. internal diameter. Soak the prepared alginic acid (4 g. 72–85 mesh B.S. per column) in water until swelling is complete

(~ 4 hr.), then pack in suspension into the tubes previously plugged with cotton wool. Allow the alginic acid to settle and place second plugs on top. Wash the columns with 2N hydrochloric acid until the washings have no measurable extinction at the wavelengths subsequently to be employed and then with water until the effluents are neutral to litmus.

When not in use, leave the columns saturated with water.

General Procedure

Place the sample (10 ml. of aqueous solution) on the column, at 1 ml./min. and wash in with small volumes of water at the same flow-rate. Wash the column through with water (200 ml.) at the full flow-rate of up

TABLE I

RECOVERIES OF ANEURINE, NICOTINAMIDE AND PYRIDOXINE

	Wt. of compound taken (mg.)	Eluant (HCl)		Wave-length (mμ) extinction measured	Per cent recovery		
		Normality	Quantity (ml.)		1	2	3
Nicotinamide	15	0·005	500*	261	99·7	99·4	100·0
Aneurine	2	2·0	150	246	100·0	99·8	100·0
Aneurine, after passing 500 ml. of 0·005N HCl	2	2·0	150	246	100·0	99·8	99·8
Separation of Aneurine and Nicotinamide:							
1. Nicotinamide	15	0·005	500*	261	100·0	100·0	100·0
Aneurine	2	2·0	150	246	100·2	100·4	100·2
2. Nicotinamide	15	0·005	500*	261	100·0	100·0	100·0
Aneurine	2	2·0	150	246	100·0	99·8	100·0
Pyridoxine	5	0·005	500	291	100·0	100·0	100·0

* Dilute to 1 litre with eluant for measurement

to 20 ml./min. and then elute each base with hydrochloric acid of suitable strength at 12 ml./min. Completeness of elution and cleanness of separation are verified by spectroscopic measurements on small extra volumes of eluate.

Calculate the recovery of each base from the extinction of the eluate using the parent solution as a standard. Make all measurements against the eluting acid using 1 cm. cells except where otherwise stated.

Separation of Aneurine, Nicotinamide and Pyridoxine

Recovery experiments were made on the vitamins singly and in admixture. In addition, the separations were followed by the examination of 20 ml. fractions. The details and results are given in Table I and Figs. 1 and 2.

The weights per fraction of nicotinamide and pyridoxine hydrochloride were calculated from a two-point procedure.

	246 mμ	261 mμ	291 mμ
Aneurine hydrochloride *E* (1 per cent, 1 cm.)	416	—	—
Nicotinamide *E* (1 per cent, 1 cm.)	—	411	2·24
Pyridoxine hydrochloride *E* (1 per cent, 1 cm.)	—	48·2	427

Although the ultra-violet absorption spectrum of pyridoxine is pH sensitive over part of the range (Stiller, Keresztesy and Stevens, 1939),

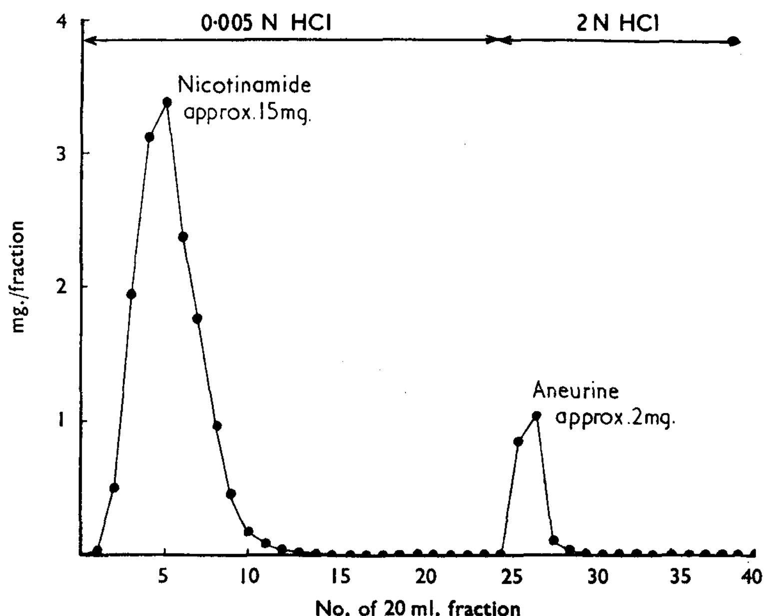

FIG. 1. Characteristic curves for the separation of nicotinamide (15 mg.) from aneurine HCl (2 mg.) Complete elution of nicotinamide, fractions 1–18; of aneurine, fractions 26–34. Elution of the first 25 fractions with 0·005 N HCl; the remainder with 2 N HCl.

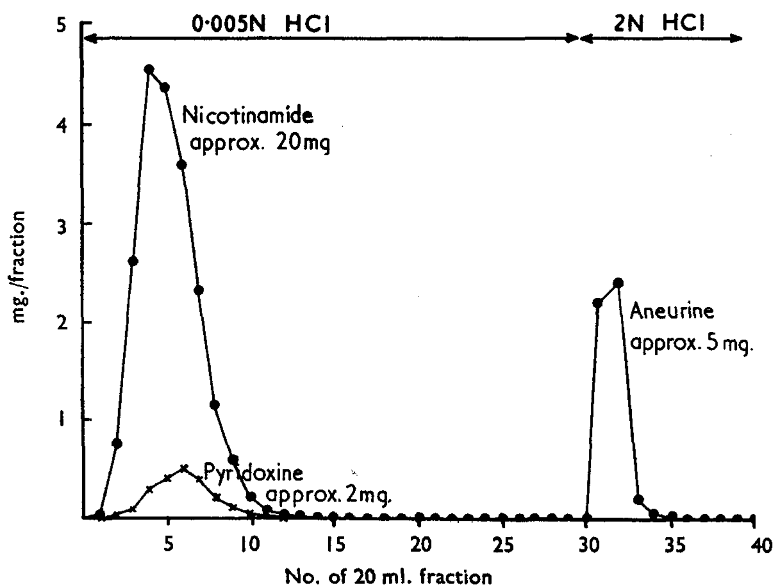

FIG. 2. Separation of nicotinamide (20 mg.) and pyridoxine HCl (2 mg.) from aneurine HCl (5 mg.) Loading in 10 ml. of 0·5 per cent w/w acetic acid. Complete elution of nicotinamide, fractions 1–17; of pyridoxine, fractions 1–14; of aneurine, fractions 31–36. Elution of the first 30 fractions with 0·005 N HCl; the remainder with 2 N HCl.

the extinctions at 261 and 291 mμ are constant in 0·004 to 0·006N hydrochloric acid.

Curves for nicotinamide and pyridoxine hydrochloride covering the wavelengths used are shown in Fig. 3.

Application to the Examination of Pharmaceutical Preparations

The procedures described are based on the assumption that no significant deterioration of aneurine has occurred, as may happen on long or unsuitable storage.

Tablets of Aneurine, Compound, B.P.C.

Take a sample of tablets and determine the average weight. Powder the sample and weigh accurately the equivalent of about seven

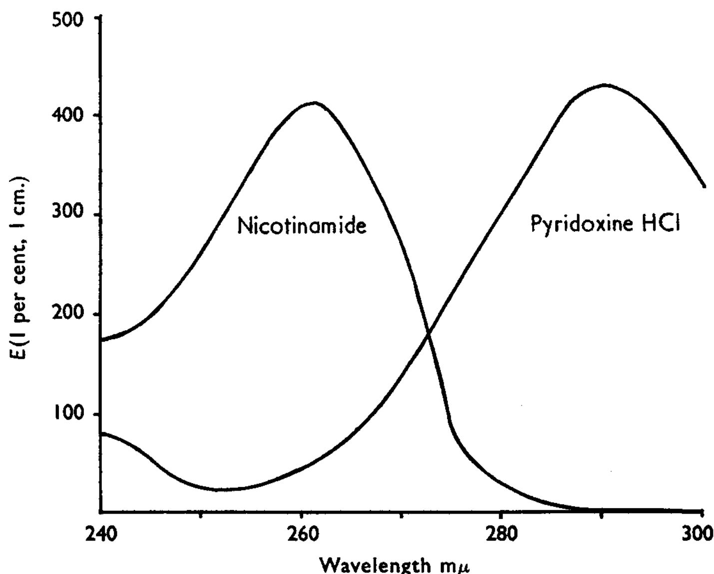

FIG. 3. Ultra-violet spectra of nicotinamide and pyridoxine HCl in 0·005 N HCl.

tablets. Extract the bases by agitating the powder continuously with 50·0 ml. of 0·5 per cent v/v acetic acid for 30 min. Filter (pH should lie between 3 and 4) and reject the first 10 ml. filtrate. Separate and estimate the nicotinamide and aneurine as detailed under general procedure and in Table I.

This procedure was carried out on a standard mixture of the tablet ingredients in which starch was used as the inert diluent, and then on three production batches of tablets. The results (Tables II and III) were compared with those obtained by the B.P.C. methods.

Tablets of Aneurine, Compound, Strong, B.P.C.

Slight modification of the assay above allows pyridoxine hydrochloride to be estimated in addition to the other two components. Use

100 ml. of 0·5 per cent v/v acetic acid for extracting the bases. Separate nicotinamide + pyridoxine from aneurine hydrochloride as detailed in general procedure, eluting the nicotinamide and pyridoxine with 500 ml. of 0·005N hydrochloric acid. Measure the extinction of this solution at

TABLE II

RECOVERIES FROM MIXTURES OF INGREDIENTS OF PHARMACEUTICALS

	Per cent recovery		
	1	2	3
Tab. Aneurine Co., B.P.C.			
Aneurine HCl	100·3	100·0	100·0
Nicotinamide	100·3	100·3	100·3
Tab. Aneurine Co. Strong, B.P.C.			
1. Aneurine HCl	99·7	99·1	—
Nicotinamide	99·9	99·9	—
2. Aneurine HCl	100·0	99·2	100·3
Nicotinamide	99·9	99·9	100·0
Pyridoxine HCl	99·0	99·0	99·0
Capsules of Vitamins, B.P.C.			
Aneurine HCl	99·7	100·0	100·4
Nicotinamide	100·3	100·2	100·2

291 mμ in a 4 cm. cell and the extinction of a twofold dilution at 261 mμ in a 1 cm. cell. Elute the aneurine with 250 ml. of 2N hydrochloric acid.

This modification was tried on a solution of aneurine, nicotinamide and pyridoxine and on two batches of tablets. An earlier method for aneurine and nicotinamide only, used 1 cm. cells throughout. This

TABLE III

ESTIMATION OF ANEURINE AND NICOTINAMIDE IN PRODUCTION SAMPLES

Product	Batch No.	Nicotinamide mg. per capsule or tablet				Aneurine hydrochloride mg. per capsule or tablet			
		Proposed method			B.P.C. method	Proposed method			B.P.C. method
		1	2	3		1	2	3	
Tabs. Aneurine Co., B.P.C.	1	14·8	14·8	14·8	14·7	0·93	0·93	0·93	0·89
		14·5	14·5	—		0·92	0·92	—	0·88
	2	14·9	14·9	—	14·8	1·03	1·03	—	1·01
	3	13·6	13·6	—	13·6	1·04	1·04	—	1·06
Tab. Aneurine Co, Strong, B.P.C.	1	18·9	18·9	—	19·0	5·05	5·05	—	5·3
	2	19·8	19·8	—	19·9	5·34	5·33	—	5·4
Capsules of Vitamins, B.P.C.	1	7·09	7·11	—	7·6	0·92	0·92	—	1·15
	2	7·07	7·07	—	7·3	1·01	1·01	—	1·14
	3	7·17	7·18	—	7·3	1·08	1·08	—	1·16
	4	7·05	7·06	—	7·6	1·00	1·00	—	1·20
	5	7·15	7·18	—	7·3	1·05	1·05	—	1·23
	6	7·07	7·07	—	7·5	1·09	1·09	—	1·07

method had been applied to a mixture of tablet ingredients, and to the same two batches of tablets.

The results are given in Tables II, III, IV.

Capsules of Vitamins, B.P.C.

Accurately weigh an amount of capsule contents equivalent to about 20 capsules. Add cyclohexane (20 ml.) and 0·15 per cent v/v acetic

acid (20 ml.). Warm and stir on a steam bath for 5 min. Transfer to a separator; wash the beaker out into the separator with alternate small volumes of cyclohexane and warm dilute acetic acid. Cyclohexane up to 60 ml. and dilute acetic acid up to 50 ml. may be used. Shake well and allow to separate. Filter the aqueous layer through a sintered-glass filter (No. 3 or 4 porosity) to remove undissolved riboflavine. Transfer the filtrate to a 100 ml. calibrated flask. Extract the cyclohexane layer with dilute acetic acid (3 × 15 ml.) using each extract to wash the filter and flask and adding each to the calibrated flask. Cool the flask and make up to volume with water. The pH of this solution should be 3–4. Place 10 ml. on an alginic acid column and carry out the separation and measurements for nicotinamide and aneurine by the general procedure using the details given in Table I.

TABLE IV

ESTIMATION OF ANEURINE, NICOTINAMIDE AND PYRIDOXINE IN PRODUCTION SAMPLES OF TABLETS OF ANEURINE CO. STRONG, B.P.C.

B. No.	Nicotinamide mg. per tablet				Aneurine hydrochloride mg. per tablet				Pyridoxine hydrochloride mg. per tablet		
	Proposed method			B.P.C. method	Proposed method			B.P.C. method	Proposed method		
	1	2	3		1	2	3		1	2	3
1	18·9	18·9	18·9	19·0	5·08	5·08	5·07	5·3	1·74	1·74	1·74
2	19·6	19·7	—	19·9	5·29	5·30	—	5·4	1·90	1·90	—

This procedure was applied to a standard mixture of constituents equivalent to the contents of about 20 capsules, then to six samples of production capsules. In the standard mixture Halibut Liver Oil, B.P., was used to supply the vitamin A requirement.

Attempts to modify the method so as to apply it to disintegrated whole capsules have not been successful.

The results obtained are shown in Tables II and III.

DISCUSSION

The procedure for capsules of vitamins is based on the assumption, inherent in the B.P.C. method for ascorbic acid, that there is negligible absorption of water-soluble vitamins into the shell of the capsule. Although our method leads to results for aneurine hydrochloride and nicotinamide lower than by the B.P.C. assays, the extraction procedure is efficient and does not destroy aneurine. In an earlier series of experiments, four standard mixes of capsule contents were examined, using formaldehyde-treated alginic acid. Recoveries of aneurine between 98·1 and 100·6 per cent and of nicotinamide between 99·7 and 100·5 per cent were obtained.

It seems likely that the absence of cross-linking in the exchanger ensures rapid and quantitative sorption and recovery of large organic molecules, whereas with synthetic cross-linked ion-exchangers quantitative recovery is often more difficult. This behaviour renders alginic

acid suitable for the resolution of a mixture of organic bases having different ionic charges.

Although the authors discarded formaldehyde-treated alginic acid, it should be mentioned that as an ion-exchanger it is not inferior to the precipitated material. Many of the experiments described above were made originally, on formaldehyde-treated alginic acid, with similar results.

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The paper was presented by MR. FOSTER. The following points were made in the discussion.

The alginic acid used was the normal commercial grade. The column was capable of an indefinite number of operations, but occasional re-packing might be necessary. The mesh size was not critical. A much wider range, 50–120 mesh, could be used but uniformity was necessary. The material might better be prepared by damping with aqueous alcohol of suitable strength and then granulating to the required mesh size.