Gas-Liquid Chromatography of Niacin and Niacinamide

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A procedure has been developed for the qualitative and quantitative determination of niacin and niacinamide by gas-liquid chromatography. Niacin was determined as its ethyl ester (ethyl nicotinate) or as *N*-ethylnicotinamide. Niacinamide was determined as niacinamide or converted to either ethyl nicotinate or *N*-ethylnicotinamide. An immobile biphase of 2.5 percent NPGS–10 percent SE-30 was the most satisfactory immobile phase evaluated. The results revealed that, in general, this sensitive gas-liquid chromatographic method could detect 5, 8, and 10 ng. of ethyl nicotinate, *N*-ethylnicotinamide, and niacinamide, respectively, using the hydrogen flame-ionization detector. The β-argon ionization detector (Ra_{226}) detected 15, 20, and 25 ng. of each compound, respectively. It was found that increasing quantities of ethyl nicotinate, *N*-ethylniacinamide, and niacinamide gave linear responses.

NIACIN (nicotinic acid) and its analog niacinamide (nicotinic acid amide) are important members of the B-complex vitamins. Niacinamide is included as an ingredient in decavitamin capsule and tablet composition requirements of the USP (1).

Chemical and biological methods are now widely used. The 10th edition of "Official Methods of Analysis" of the A.O.A.C. (2) includes both a chemical and biological method; the USP method (1) is based on a chemical technique. The chemical methods generally utilize the König reaction (3, 4) in which pyridine and its derivatives react with cyanogen bromide and an aromatic amine. In this reaction polymethine dyes are formed and are measured spectrophotometrically at 450 mμ. However, the chemical method is undesirable because the color development is pH sensitive and has low stability, which makes it difficult to reproduce results.

Biological methods provide the most specific quantitative assay presently available. The dog and chicken have been used for biological assay for total niacin activity. However, microbiological methods have been more popular and are far more practical from the standpoint of sensitivity, time, and expense. The A.O.A.C. method (2) uses *Lactobacillus plantarum*, which measures total niacin activity and can be used to specifically assay nicotinic acid in the presence of nicotinamide if the latter is first converted to 3-aminopyridine (6). Johnson has used *Leuconostoc mesenteroides* to measure nicotinic acid (5).

A polarographic method (7) and a thin-layer chromatographic method (8, 9) are available but have not been widely accepted.

Although a number of satisfactory methods are available for their separation and identification, a more rapid and simple method specific for niacin and niacinamide is needed. Since previous work from this laboratory (10, 11) has shown that vitamin B_6 of the water-soluble vitamin series is amenable to gas-liquid chromatography (GLC), it was felt that niacin and its analogs could also be measured by GLC. The present report is a summary of the feasibility of utilizing the gas chromatograph for the analysis of niacin and niacinamide.

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EXPERIMENTAL

Instrumentation—A model 5000 series Barber-Colman gas chromatograph (Barber-Colman, Rockford, Illinois) fitted with a high temperature hydrogen flame-ionization detector (FID) and a β-argon ionization detector (AID) with a 56 μc Ra_{226} foil was used with a 5-mv., 2-sec., 28-cm.-(11-in.) strip-chart recorder.

Materials—Niacin, niacinamide, and ethyl nicotinate were purchased from Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York. *N*-Ethylnicotinamide was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. Pure grade *n*-hexane was obtained from Phillips Petroleum Co., Special Products Division, Bartlesville, Oklahoma. All other reagents were A.R. grade and used without further purification. Whatman No. 4 filter paper was used for all filtrations.

Preparation of Derivatives—Ethyl nicotinate was prepared by two methods (12): (*a*) direct action of ethanol and sulfuric acid on either niacin or niacinamide; (*b*) interaction of niacin with thionyl chloride followed by the reaction of the nicotinyl chloride hydrochloride product with ethanol and subsequent neutralization. Two methods of preparing niacinamide were used: (*a*) treatment of ethyl nicotinate with concentrated ammonia (12); (*b*) action of thionyl chloride on niacin followed by treatment of the resulting acid chloride with ammonia (13). *N*-Ethylnicotinamide was prepared by the following reactions: (*a*) action of thionyl chloride on niacin and treatment of the resulting acid chloride with ethylamine hydrochloride; (*b*) direct action of ethylamine on niacin in the presence of phosphorus pentoxide; (*c*) action of an aqueous solution of ethylamine on ethyl nicotinate.

Standard Solutions—Niacinamide and *N*-ethylnicotinamide were dissolved in enough ethanol and ethyl nicotinate in enough *n*-hexane so that a constant injection volume of 2 μl. was maintained throughout the investigation. Calibration standards were prepared from commercially obtained materials.

Gas-Liquid Chromatography—Three different column packings were used in this study. Column

1: 183 cm. × 3 mm. i.d. packed with a biphase mixture of 2.5% neopentyl glycol succinate (NPGS) and 10% SE-30 (w/w) on 100/120 mesh Gas Chrom P (silanized). Operating conditions: ethyl nicotinate: column, 180°; detector, 230°; flash heater, 220°; carrier gas, 50 ml./min. Niacinamide and *N*-ethylnicotinamide: column, 230°; detector, 280°; flash heater, 270°; carrier gas, 50 ml./min. Column 2: 244 cm. × 3 mm. i.d. packed with 5% NPGS (w/w) on 100/120 mesh Gas Chrom P (silanized) for the analysis of ethyl nicotinate. Operating conditions: column, 180°; detector, 260°; flash heater, 250°; carrier gas, 80 ml./min. Column 3: 244 cm. × 4 mm. i.d. packed with 2% neopentyl glycol sebacate (NPGSeb) (w/w) on 100/120 mesh Gas Chrom P (silanized) for the analysis of niacinamide and *N*-ethylniacinamide mixtures. Operating conditions: column, 210°; detector, 250°; flash heater, 230°; carrier gas, 60 ml./min.

All columns were U-shaped Pyrex glass and were preconditioned 24 hr. at 235° with carrier-gas flow rate of 60 ml./min. Argon was used as the carrier gas throughout the study. Compressed air and hydrogen flow rates were 460 and 36 ml./min., respectively, with the FID. A d.c. cell voltage of 900 was used at all times with the AID. Electrometer output was 1×10^{-14} amp. when FID was used and 3×10^{-9} amp. when the AID was used. The electrometer output is stated as full-scale output into a 5-mv. recorder unless otherwise stated in the text. Peak areas were determined by triangulation (width at half-height × height = peak area).

RESULTS AND DISCUSSION

The applicability of gas chromatography to niacin is limited by the presence of an acid group in the niacin molecule, its insolubility in most organic solvents, and the fact that niacin sublimes. It was therefore necessary to convert niacin to a derivative volatile enough for GLC. Ethyl nicotinate and *N*-ethylnicotinamide were chosen because of their relatively low boiling points, ease of preparation, and stability. Although niacinamide has an amide group, it is soluble in some organic solvents, has a low melting point, and is suitable for GLC analysis.

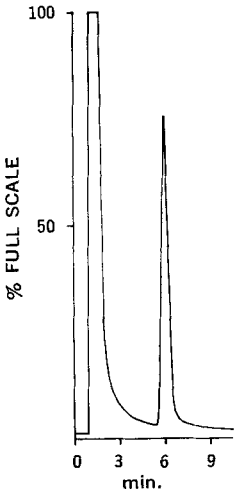

Fig. 1—Chromatograms of 1.0 mcg. ethyl nicotinate in n-hexane on a 2.5% NPGS–10% SE-30 biphase column at 180°.

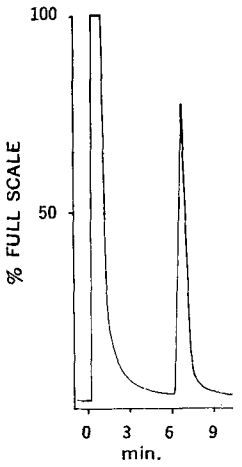

Fig. 2—Chromatogram of 2 mcg. niacinamide in ethanol on a 2.5% NPGS–10% SE-30 biphase column at 230°.

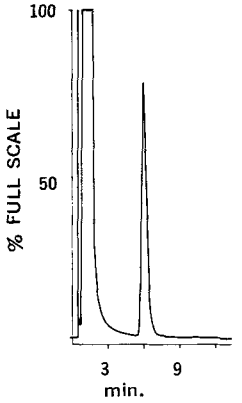

Fig. 3—Chromatogram of 1.25 mcg. ethyl nicotinate in n-hexane on a 5% NPGS column at 180°.

Derivative Preparation Efficiency—The yields of three derivatives were in good agreement with existing literature values (12, 13). GLC scans of the prepared derivatives indicated no contaminants present. The structures of the reaction products were confirmed by IR and UV spectroscopy.

Effect of Immobile Phase—The elution peaks of the three niacin analogs were typical adsorption isotherms on SE-30, QF-1, and biphase mixtures of NPGS–SE-30 when the ratio of NPGS to SE-30 was less than 1:4. The preceding immobile phases were rejected on the basis of their tailing adsorption isotherms. The 2.5% NPGS–10% SE-30 column at 180° gave a satisfacory elution peak for ethyl nicotinate (Fig. 1). When the temperature was increased to 230°, niacinamide produced a usable elution peak exhibiting slight tailing (Fig. 2). On this column, *N*-ethylniacinamide was found to precede niacinamide. The results suggest that by varying the column temperature and carrier solvents, GLC may be a potential means of screening for analogs of the vitamin.

Ethyl nicotinate exhibited a nearly symmetrical elution peak on the 5% NPGS immobile phase column (Fig. 3) whereas the other two analogs gave

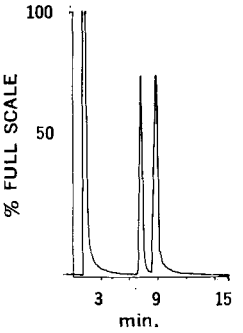

Fig. 4—Chromatogram of a mixture of 1.0 mcg. N-ethylniacinamide (7.6 min.) and 1.2 mcg. niacinamide (9.2 min.) on a 2% NPGSeb column at 210°.

broad tailing elution peaks at much longer retention times. The 2% NPGSeb column eluted *N*-ethylnicotinamide ahead of niacinamide (Fig. 4). At 210°, ethyl nicotinate was eluted along with the carrier solvent. This column will resolve all three niacin analogs at 180° but the retention times for *N*-ethylniacinamide and niacinamide are extended and the elutions peaks were quite broad and were, therefore, less desirable for quantitative analysis.

Effect of Quantity Injected—Response data for ethyl nicotinate, *N*-ethylnicotinamide, and niacinamide using the biphase column of 2.5% NPGS and 10% SE-30 are given in Table I. It can be seen from these data that as the quantity of compound increased the response was linear. The response lines for *N*-ethylnicotinamide and niacinamide are almost identical but the response line for ethyl nicotinate was not closely related to those of the other two analogs.

Sensitivity—The GLC method for niacin and niacinamide is very sensitive. By using the FID, an attenuator setting of 1, an electrometer output of 1×10^{-10} amp., and a 0–1-mv. recorder, 5 ng. of ethyl nicotinate and 10 ng. of niacinamide were detectable. The AID, with an attenuation of 1 and an electrometer output of 3×10^{-9} amp., detected 15, 20, and 25 ng. of ethyl nicotinate, *N*-ethylnicotinamide, and niacinamide, respectively. Quantitation at these low levels, however, is difficult because of the noise-to-signal ratio and because there is less control over the quantity injected.

CONCLUSIONS

It has been established that it is possible to detect and analyze three analogs of the vitamin niacin, *i.e.*, nicotinic acid as the ethyl ester, nicotinamide, and *N*-ethylnicotinamide. Studies are currently underway on applying the GLC analysis to pharmaceuticals containing niacin and niacinamide. Preliminary results indicate that the GLC analysis of the vitamin niacin is adaptable to pharmaceutical products.

TABLE I—RESPONSE OF ETHYL NICOTINATE, *N*-ETHYLNICOTINAMIDE, AND NIACINAMIDE[a]

	Peak Area,[b] mm.²		
mcg.	Ethyl Nicotinate	*N*-Ethyl-Nicotinamide	Niacinamide
0.5	25.0 ± 0.4	18.9 ± 0.5	9.8 ± 0.2
1.0	40.0 ± 1.4	31.3 ± 0.8	21.3 ± 0.2
2.0	70.0 ± 1.5	62.1 ± 1.3	45.0 ± 1.0
5.0	190.0 ± 2.5	124.2 ± 1.4	117.2 ± 1.3
10.0	384.0 ± 3.4	250.3 ± 2.9	245.4 ± 3.1
15.0	572.5 ± 4.4	362.5 ± 4.3	364.0 ± 2.7
20.0	757.5 ± 4.2	473.2 ± 4.7	477.8 ± 3.8

[a] 10 × attenuation, electrometer output of 1×10^{-10} amp. hydrogen flame detector, and a 2.5% NPGS–10% SE-30 column. [b] Mean of six analyses ± standard deviation.

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Keyphrases

Niacin—analysis
Niacinamide—analysis
Ethyl nicotinate, *N*-ethyl nicotinamide—derivatives
GLC—analysis