Simultaneous High-Pressure Liquid Chromatographic Determination of Niacin and Niacinamide in Multivitamin Preparations: Reversed-Phase, Ion-Pairing Approach

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Abstract □ A high-pressure liquid chromatographic procedure for the simultaneous determination of niacin and niacinamide in multivitamin preparations was developed and evaluated. Reversed-phase liquid chromatography with dioctyl sodium sulfosuccinate in the mobile phase is utilized for the analysis. The accuracy and precision of the procedure were compared with those of the currently official procedure.

Keyphrases □ Niacin and niacinamide—simultaneous analysis, reversed-phase high-pressure liquid chromatography, multivitamin preparations □ High-pressure liquid chromatography, reversed phase—simultaneous analysis, niacin and niacinamide in multivitamin preparations □ Vitamins—niacin and niacinamide, simultaneous analysis, reversed-phase high-pressure liquid chromatography, multivitamin preparations

Currently, procedures for the determination of niacin and niacinamide in multivitamin preparations suffer from several disadvantages. An involved sample workup often is required (1, 2). Niacin is either ignored entirely in the determination (3) or included in the results as "niacinamide" due to hydrolysis of the sample prior to determination (4, 5). In addition, reagents required for analysis are often caustic and unstable.

These objections have been considerably reduced in procedures (6) where a solution of the vitamin product is chromatographed, the appropriate migration zones are eluted, and the eluate is determined spectrophotometrically. However, these procedures are somewhat tedious. Therefore, a related method for the simultaneous analysis of niacin and niacinamide, utilizing reversed-phase high-pressure liquid chromatography (HPLC), was developed and evaluated.

EXPERIMENTAL

Apparatus—A liquid chromatograph[1] equipped with a high-pressure pump and an injector was used, and effluents were monitored with the 254-nm detector. Peak areas were determined using an electronic digital integrator[2]. The column[3], 30 cm × 4 mm i.d. stainless steel, was packed with totally porous silica particles of 10-μm average size, chemically bonded to a monomolecular layer of octadecyltrichlorosilane. The flow rate was 2.5 ml/min.

Materials and Reagents—Niacin and niacinamide were obtained as USP reference standards. Dioctyl sodium sulfosuccinate, camphorsulfonic acid, and sodium hexanesulfonate were obtained from commercial suppliers and used as received. Commercial multivitamin preparations were obtained from local pharmacies.

Preparation of Mobile Phase—Solvent systems were prepared by dissolving the salt (2.0×10^{-3} *M*) in distilled water (300 ml), adjusting the pH to 2.5 with 10% formic acid solution, and combining the resulting solution with methanol (300 ml). The mobile phase was deaerated prior to use by the brief application of a vacuum.

Standard Solutions—Solutions of niacinamide in pH 6.0 phosphate buffer were prepared to contain 1.0, 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, and 6.0 mg/100 ml. Niacin standard solutions were prepared to contain 0.5, 1.0, 1.5, 2.0, and 2.5 mg/100 ml.

Sample Solutions—The homogenized vitamin preparation (one dosage unit) was placed in a volumetric flask (100 ml) with pH 6.0 phosphate buffer (50.0 ml) and shaken for 5 min. Buffer was added to volume, the flask was shaken, and the particulate matter was allowed to settle. The sample was filtered when required, and the first 10–15 ml of filtrate was rejected. An aliquot of the remaining filtered solution was then diluted to a concentration of 2.5 mg/100 ml based on the label claim of niacinamide or 0.75 mg/100 ml based on the label claim of niacin.

Calibration Curves and Sample Analysis—Calibration curves were prepared by mixing niacinamide standard solutions (2.0 ml) with niacin solutions (2.0 ml) and injecting an aliquot (20 μl) of the mixture into the chromatographic system. Peak areas, determined using the digital integrator, were plotted against the analyte concentration for preparation of calibration curves.

Niacinamide and niacin were determined by injecting an aliquot (20 μl) of the sample solution, determining the resulting peak areas, and referring to the previously derived calibration curves.

RESULTS AND DISCUSSION

The complex matrixes in which the water-soluble vitamins are often found make chromatographic techniques a logical choice for their routine analysis. Of the quantitative chromatographic techniques, HPLC is particularly attractive since derivatives need not be formed prior to separation so sample preparation time may be reduced significantly. However, the choice of an appropriate stationary phase is critical in maximizing this advantage.

Methods using ion-exchange resins have been employed for separating niacin (7) from other constituents of vitamin products but suffer from poor reproducibility (8) and limited column life (9). Normal-phase chromatographic systems also may be used (10), but several constituents (*e.g.*, coloring and flavoring agents and diluents) are present in multivitamin preparations that are more polar than the vitamins. Methodologies based on these systems consequently require substantial sample workup or long analysis time.

Reversed-phase chromatographic systems appear to offer significant advantages in the determination of water-soluble vitamins in multivitamin preparations. Polar diluents, fillers, *etc.*, that dissolve in an aqueous dispersion of the multivitamin product elute at or near the solvent front while lipophilic constituents, such as the oil-soluble vitamins, have very limited solubility in the aqueous dispersion. Under such conditions, retention volumes are reproducible, column lifetimes are long (11), and sample preparation time is short. The latter advantage is not only important in terms of technician time but also in the precision of the method.

In such a chromatographic system, however, niacinamide and niacin have little affinity for the lipophilic stationary phase and also move through the system with the solvent front (Table I). However, it was shown previously (12) that addition of an appropriate salt to the mobile phase results in retention of ionic analytes in the reversed-phase system. For example, anionic analytes are retained in a reversed-phase system in which a small quantity of a lipophilic qua-

[1] ALC/GAS 202 equipped with an M6000 pump and a U6K injector, Waters Associates, Milford, Mass.
[2] Model 450, Varian Associates.
[3] μBondapak C_{18}, Waters Associates, Milford, Mass.

Table I—Retention Characteristics of Niacinamide and Niacin

Solute[a]	Niacin[b]	Niacinamide[b]
	1.8	1.8
Sodium heptanesulfonate	3.0	2.8
Sodium camphorsulfonate	6.2	5.6
Dioctyl sodium sulfosuccinate	10.5	9.0

[a] The salt (2×10^{-3} *M*) was dissolved in distilled water (300 ml), the pH was adjusted to 2.5 with 10% formic acid solution, and methanol (300 ml) was added. [b] Retention volume in milliliters with a flow rate of 3.0 ml/min.

ternary ammonium compound is added to the mobile phase. Conversely, cationic analytes in a mobile phase containing lipophilic sulfonate salts are dramatically retained when compared to the same systems not containing these salts. The advantages of such systems over ion-exchange procedures also were discussed (12).

In the current study, the pH of the aqueous component of the mobile phase was adjusted to 2.5, at which pH the heteronitrogens of niacin and niacinamide are protonated and carboxylate ionization (of niacin) is suppressed. Addition of the appropriate sulfonate salt to the mobile phase then results in retention of both niacin and niacinamide, with niacin less strongly retained because of its greater polarity. The retention characteristics of such ionic analytes in this system appear to be explicable in terms of the rate of formation and relative lipophilicity of the ion-pair formed within the system. This technique has been referred to as the reversed-phase, ion-pairing approach to HPLC; its use here allows sample preparation time to be held to a minimum.

By using this chromatographic system (Fig. 1), standard curves were prepared daily for 10 days. The standard deviations of the slope of the curve for niacinamide and niacin were 1.57 and 1.69%, respectively. The average correlation coefficient for niacinamide calibration curves was 0.996; for niacin, it was 0.998. The average *y*-intercepts

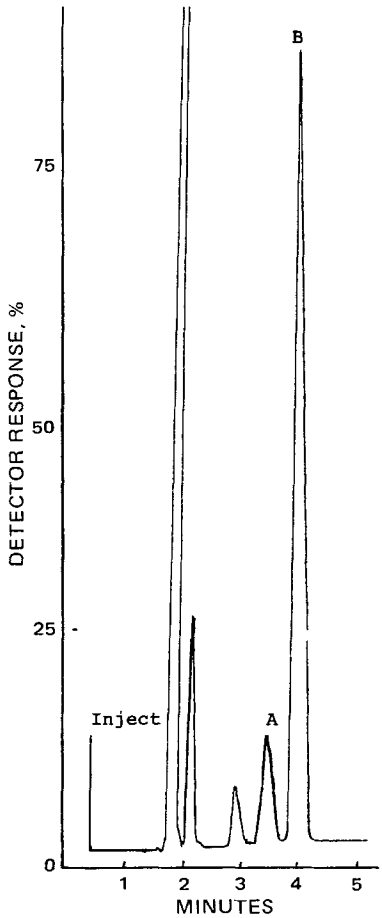

Figure 1—*Chromatographic trace of a multivitamin product found to contain niacin (A, 0.5 mg/dosage unit) and niacinamide (B, 9.3 mg/dosage unit).*

Table II—Analysis of Laboratory-Prepared Samples[a] by HPLC and the Cyanogen Bromide Procedure

	Niacin		Niacinamide		Total	
Sample	Amount Added, mg	Percent Recovered[b], HPLC	Amount Added, mg	Percent Recovered, HPLC	Amount Added, mg	Percent Recovered, USP
1	1.0	100.3 (1.46)	2.0	98.9 (1.55)	3.0	98.0 (3.14)
2	5.0	99.7 (1.74)	20.0	100.4 (1.76)	25.0	97.7 (2.46)
3	1.0	98.6 (1.85)	50.0	101.2 (1.94)	51.0	96.8 (3.44)
4	10.0	98.9 (1.40)	100.0	99.9 (1.63)	110.0	103.2 (2.16)
5	50.0	100.8 (1.21)	2.0	100.5 (1.40)	52.0	100.4 (2.96)
6	100.0	101.2 (1.81)	20.0	101.2 (1.84)	120.0	101.2 (2.84)
7	50.0	98.2 (1.64)	50.0	100.5 (1.77)	100.0	99.2 (3.10)

[a] Prepared according to the formula for decavitamin capsules USP. [b] Values in parentheses are percent standard deviation; $n = 8$.

Table III—Analysis of Niacinamide and Niacin in Multivitamin Products

				Amount Found[a]		
				Niacinamide		
Sample	Dosage Form	Labeled Components	Labeled Niacinamide, mg/Dosage Unit	USP	HPLC	Niacin[b], HPLC
1	Capsule	Nine vitamins, six minerals	100	101.6 (3.98)	94.5 (2.56)	5.4 (2.27)
2	Chewable tablet	Eight vitamins, artificial flavors, colors	10	103.4 (4.64)	97.6 (1.87)	3.2 (2.69)
3	Liquid	Eight vitamins, eight minerals, preservative, artificial flavors, colors	10	98.4 (3.60)	84.6 (2.20)	14.2 (2.90)
4	Drop	Eight vitamins	6	108.2 (3.14)	102.6 (1.94)	4.6 (2.20)
5	Capsule	Six vitamins	50	105.4 (3.90)	102.6 (2.27)	2.16 (1.86)
6	Tablet	Nine vitamins	50	99.8 (3.64)	96.4 (2.00)	5.46 (1.45)

[a] Percent of labeled amount (% *SD*); $n = 8$. [b] Expressed as percent of labeled niacinamide content.

were −3.24 integrator units for niacinamide and +4.70 integrator units for niacin. These data indicate that the procedure is amenable to use of a single-point standard.

The accuracy and precision of the proposed chromatographic procedure were investigated by analysis of laboratory-prepared samples (Table II), and results were compared with those of the USP procedure (4). In each case, the precision of the HPLC procedure was greater than that of the currently official procedure, and this result may be attributed to the less complicated sample workup required by the former. However, the two methods compared quite favorably in terms of the determination of the combined quantity of niacin and niacinamide.

Data regarding the suitability of the proposed procedure in the analysis of commercial multivitamin preparations are presented in Table III. Standard deviations of the HPLC procedure were again lower. Furthermore, niacinamide content, as determined by the proposed procedure, was less in each case. However, when the sum of niacinamide and niacin content was compared with that determined by the USP procedure, results were again comparable. Niacin was found in each sample examined, in quantities as high as 14.2% of the labeled niacinamide concentration.

This type of analysis is very attractive in terms of analysis time, since a trained technician can readily process 10 samples/hr with a minimum of active involvement. Column stability under these conditions of minimum sample preparation was a subject of concern initially. However, over 300 preparations have been processed with no change in the chromatographic characteristics of the system. This stability may be attributed in part to the small quantities of sample injected into the chromatographic system and in part to the daily flushing recommended by the column supplier.

In summary, the reversed-phase, ion-pairing approach to the simultaneous analysis of niacin and niacinamide appears to result in an accurate and precise procedure. These features, in combination with the simple sample preparation and short analysis time, should make the procedure attractive to pharmaceutical analysts. The application of this approach to the other water-soluble vitamins is currently under investigation.

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Solubility Profiles and Thermodynamics of Parabens in Aliphatic Alcohols

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Abstract □ The solubility of a series of compounds was determined in a wide polarity spectrum of normal aliphatic alcohols over a limited temperature range. The solutes chosen were the methyl through *n*-butyl *p*-hydroxybenzoates, several of these being useful preservatives. Solubility profiles were determined for these compounds, and the effect of temperature upon their solubility behavior was investigated. The solubility of the solutes is expressed in several concentration notations; mole fraction plots showed a variable twin peak array as a function of the dielectric constant for the solutes studied. Since heats of fusion were available and data were generated as a function of temperature, thermodynamic parameters for these systems could be calculated. The relationship of these parameters to multiple solubility peak array is discussed.

Keyphrases □ Parabens, alkyl—solubility and thermodynamics in aliphatic alcohols, effect of temperature □ Solubility—alkylparabens in aliphatic alcohols, effect of temperature □ Thermodynamics—alkylparabens in aliphatic alcohols, effect of temperature □ Alcohols, aliphatic—solvents for series of alkylparabens, effect of temperature □ Structure–activity relationships—alkylparabens, solubility and thermodynamics in aliphatic alcohols □ Antifungal agents—alkylparabens, solubility and thermodynamics in aliphatic alcohols

The effect of temperature upon the solubility of four normal alkyl *p*-hydroxybenzoates (parabens) was determined over a wide polarity spectrum of aliphatic alcohols from methanol to 1-decanol. The effect of decreasing polarity with an increase in size for the first four *n*-alkyl members for this ester series was also considered.

Previous solubility work (1–5) considered several of these compounds at ambient temperatures. It was believed that the extension of this work in pure solvents over a temperature range and polarity range would be instructive. Additionally, since several of these solutes gave multiple peak solubility isotherms, it was useful to determine if solubility was linear as a function of temperature. The temperature range used was 25–40° in four steps; the polarity range *via* dielectric constants was from 32 for methanol to eight for 1-decanol at 25°, utilizing seven in-