Determination of Pantothenic Acid in Multivitamin Pharmaceutical Preparations by Reverse-Phase High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic procedure was developed for the analysis of calcium pantothenate in nutritional supplements. The method involves a simple extraction using phosphate buffer and sonication. Chromatographic separation is obtained using an aminopropyl-loaded silica gel column in the reverse-phase mode. A UV detector set at 210 nm was used to monitor the effluent. Quantitative recoveries were obtained, and precision of the method is discussed. The method is applicable to multivitamin tablets, calcium pantothenate raw material, and yeast grown in the presence of high levels of calcium pantothenate. The results of the method are compared with results obtained from the USP microbiological method of analysis. It was concluded that the procedure is rapid, accurate, easily automated, and practical for routine quality control use.

Keyphrases □ Pantothenic acid—multivitamin preparations, HPLC □ Vitamins—pantothenic acid determination by HPLC □ High-performance liquid chromatography—pantothenic acid determination in multivitamins

Pantothenic acid is an important nutrient in the human diet and is a constituent of CoA, which is essential to the metabolism of both protein and fats. Since pantothenic acid can easily be destroyed by the prolonged heating often encountered in food and tablet processing, the more stable calcium salt is usually used in various fortified products.

Most chemical methods of analysis (1–4) require cleavage of the pantothenic acid molecule, reaction with a suitable derivatizing agent, and subsequent spectrophotometric measurement. The disadvantages associated with this approach include interferences from sugars and common pharmaceutical vehicles (4) as well as ascorbic acid and riboflavin. Considerable sample manipulation is required to remove these interferences, thus making the procedure very time consuming.

The traditional quality control procedure is the official microbiological assay (MBA) method (5). This method requires sample solution inoculation with *Lactobacillus plantarum*, incubation for 16–24 h, photometric measurement of the resulting turbidity, and interpolation of results using a standard growth response curve. This method suffers several disadvantages: it requires 24 h before data can be obtained and extensive manipulation by well experienced analysts to achieve reliable results. In addition, trace pantothenic acid contamination can easily cause erratic results since very small (<ppm) amounts are typically analyzed.

This report describes a high-performance liquid chromatographic (HPLC) procedure for the quantitative determination of pantothenic acid in multivitamin and multimineral tablets and raw materials.

EXPERIMENTAL

Apparatus—The HPLC system consisted of two solvent pumps[1], an autoinjector[2], an integrating recorder[3], and a UV absorbance detector[4] set at 210 nm. An aminopropyl-bonded silica gel column[5] was used and regulated at 25°C by a constant-temperature bath[6]. A high-output sonication device[7] was used to aid sample dissolution. The mobile phase was deaerated using a sonication water bath[8] and a vacuum pump[9]. All samples were filtered using a 0.45-μm filter unit[10].

Chemicals and Reagents—The solvents used were distilled in glass[11]. Potassium phosphate was HPLC grade[12] and calcium pantothenate was obtained as a USP reference standard[13]. Commercial vitamin preparations were obtained from local pharmacies and distributors.

Mobile Phase—A mixture of 0.005 M monobasic potassium phosphate (pH 4.5)–acetonitrile (13:87 v/v) was deaerated by sonication with vacuum and run at a flow rate of 2.0 mL/min using pump A. Pump B contained a mixture of water–acetonitrile (90:10) used as a final column washing step initiated after completion of an automated set of analyses.

Standard Preparation—An appropriate amount of calcium pantothenate reference standard was weighed and dissolved in 0.005 M potassium phosphate (pH 4.5) to obtain a concentration of 0.25 mg/mL.

Sample Preparation—A suitable number of weighed tablets were placed in a 200-mL volumetric flask. The final calcium pantothenate concentration was ~250 μg/mL. Approximately 100 mL of 0.005 M potassium phosphate solution (pH 4.5) was added, and the contents were sonicated at 75 W for 2 min. Thickly coated tablets required pulverization prior to sonication. The sample flask was diluted to volume with phosphate buffer after cooling to room temperature; a portion of the sample solution was filtered through a 0.45-μm filter unit, and 10 μL of the filtrate was injected into the liquid chromatograph.

Fortified yeasts, vitamin premixes, and raw material were treated in the same manner after appropriate sample amounts were selected.

RESULTS AND DISCUSSION

Pantothenic acid exhibits UV absorption between 200 and 225 nm, but no absorption in the 254–280-nm range, which is typically used for most HPLC analyses. Proper derivatization could provide a strong chromophore; however, this time-consuming step is impractical for the routine analyses of large numbers of samples. Analyses using UV detection <220 nm introduce problems caused by background UV absorption of mobile phase solvents and dissolved oxygen interference with a stable baseline (6). Solvents used for the mobile phase deaerated by sonication under vacuum were successful in this type of analysis. A problem associated with

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model 710B, Waters Associates, Milford, Mass.
[3] Model 730, Waters Associates, Milford, Mass.
[4] Model LCL75, Perkin-Elmer Corp., Norwalk, Conn.
[5] Hibar II LiChrosorb NH_2 (10 μm), 250 mm × 4.6-mm i.d., Alltech Associates, Los Altos, Calif.
[6] Model FK, Haake, Saddlebrook, N.J.
[7] Model 184, Bronson Sonic Power Co., Danbury, Conn.
[8] Model 52H, Bronson Sonic Power Co., Danbury, Conn.
[9] Model 290, Thomas Co., Sheboygan, Wis.
[10] Type Ha, Millipore Corp., Bedford, Mass.
[11] Burdick & Jackson, Muskegon, Mich.
[12] Fisher Scientific Co., Fair Lawn, N.J.
[13] U.S. Pharmacopeial Convention, Rockville, Md.

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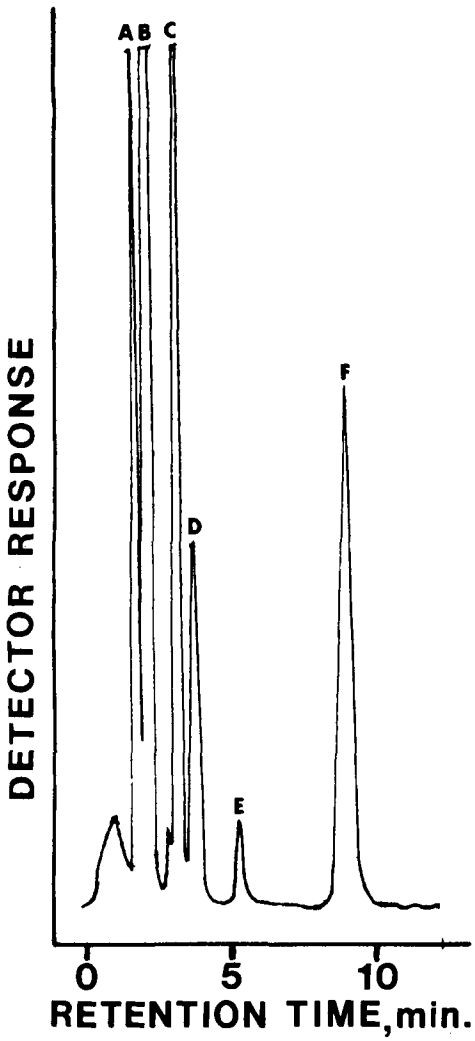

Figure 1—*Chromatogram of a multivitamin–multimineral tablet extract. Key: (A) niacinamide, (B) vitamin B_6, (C) vitamin B_2, (D) vitamin B_1, (E) unknown, (F) unknown, (G) pantothenic acid, (H) unknown.*

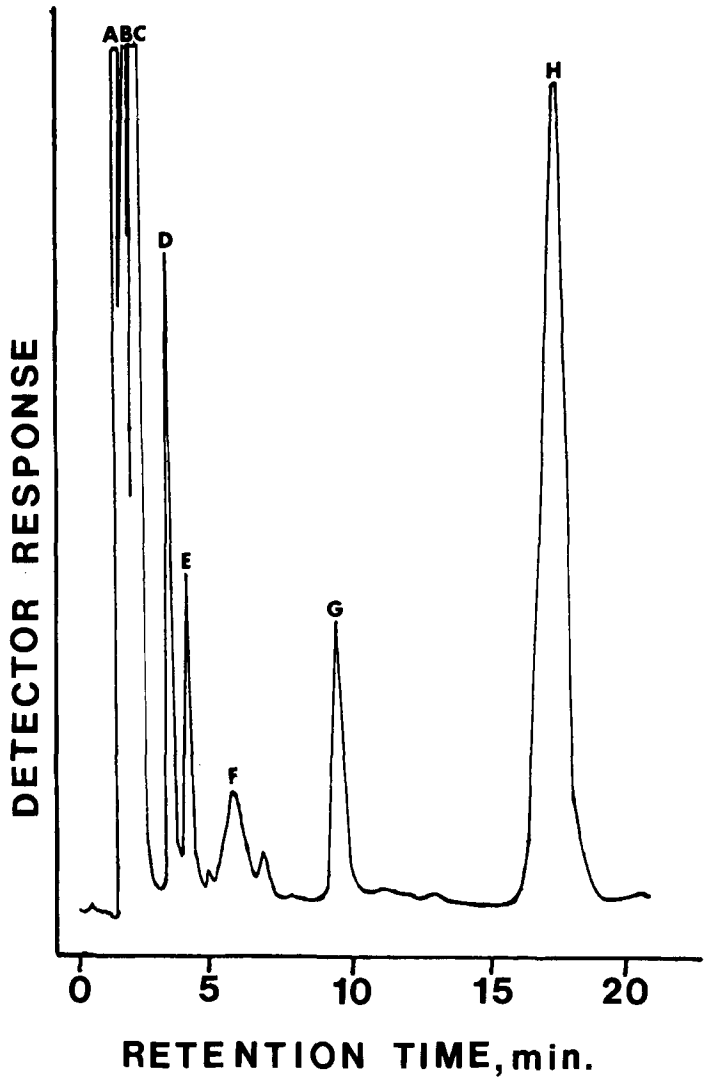

Figure 2—*Chromatogram of a high potency B complex tablet extract. Key: (A) niacinamide, (B) vitamin B_6, (C) vitamin B_2, (D) vitamin B_1, (E) unknown, (F) pantothenic acid.*

using the phosphate–acetonitrile solvent system is the potential for phosphate precipitation in the HPLC column. Considerable precipitation was encountered when the phosphate level was >0.15 M or the amount of acetonitrile was >95% in the mobile phase. A water–acetonitrile (90:10 v/v) column wash was very effective for maintaining column life. Over 500 routine samples were injected in duplicate using an automated system within a 3-month period with no appreciable loss in column performance. This is probably the result of using a small injection volume and performing the column washing step after every set of automated analyses.

Typical chromatographic separations of pantothenic acid in a multivitamin–multimineral tablet and in a B-complex tablet are shown in Figs. 1 and 2, respectively. The sample peak corresponding to the retention time of the pantothenic acid reference standard was recycled through the column four times with no further separation taking place. This technique is useful to determine the presence of any coeluting substances. The identity of the peak as pantothenic acid was confirmed by collecting the peak fractions from several sample injections, removing the acetonitrile portion of the mobile phase, and analyzing the peak material using the microbiological assay method.

A linearity study (ratio of concentration to peak area) showed complete linearity within the concentration range selected, 0.050–0.50 mg/mL, and a correlation coefficient of 0.998. Accuracy of the procedure was determined by spiking a placebo formulation and various samples with known concentrations of standard, while precision was determined by analyzing eight identical samples on different days. Recoveries of 96.4, 96.9, 96.8, and 100.1% and relative standard deviations (*RSD*) of 1.5, 2.2, 1.6, and 0.79% were obtained for multivitamin tablets, fortified yeast, vitamin premix, and raw material, respectively. A comparison of results from the HPLC method described and the USP microbiological method using the same sample is shown in Table I. Low levels of calcium pantothenate (<3 mg/tablet) were more precisely analyzed by the HPLC procedure (1.42% *RSD*) than the USP microbiological method (4.59% *RSD*). This may be related to the increase in sample size in the HPLC preparation and less sample manipulation than required in the microbiological assay method.

The HPLC method described can accurately and precisely determine calcium pantothenate or pantothenic acid in nutritional products. The procedure is rapid (~30 min for sample extraction and chromatography), easily automated with the use of an autoinjector, practical for routine

Table I—Comparison of Pantothenic Acid Results From the USP Microbiological Assay and the High-Performance Liquid Chromatographic Assay

Product[a]	Label Amount, mg/tablet	Amount Found[b], mg/tablet, ±*SD*	
		High-Performance Liquid Chromatographic Assay	Microbiological Assay
A	3.0	3.65 ± 0.075	3.73 ± 0.176
B	3.4	3.81 ± 0.099	3.88 ± 0.178
C	2.0	2.87 ± 0.041	2.66 ± 0.188
D	40.0	46.78 ± 0.823	47.0 ± 2.85
E	64.0	68.77 ± 2.077	67.84 ± 2.76
F	70.0	67.96 ± 1.990	66.47 ± 3.66
G	80.0	86.69 ± 1.830	87.41 ± 4.01

[a] Multivitamin-multimineral tablets: A, Vita Lea Chewable; B, Neo-Jr.; C, Vita Lea B-Complex tablets; D, Shaklee B-Complex; E, B-Guard; F, Generic B; G, stress formula B-Complex tablet, Hi Bee. [b] Average of five determinations.

quality control use, and the results compare favorably with the current official procedure.

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Detection Limits for a GC Determination of Methanol and Methylene Chloride Residues on Film-Coated Tablets

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Abstract □ A GC assay was developed that quantitates methanol and methylene chloride at the lowest detectable levels for this mode of analysis. A statistical limit of detection was determined for both methanol and methylene chloride. This method is sensitive and reliable for detecting possible residues of these solvents on film-coated tablets.

Keyphrases □ Methanol—determination of detection limits for a GC method, methylene chloride, residues on film-coated tablets □ Methylene chloride—determination of detection limits for a GC method, methanol, residues on film-coated tablets □ Detection limits—determination, GC method for methanol and methylene chloride residues on film-coated tablets □ Film-coated tablets—determination of detection limits for a GC method, methanol and methylene chloride residues

Organic solvents are frequently used to dissolve film-coating materials such as methylcellulose and ethylcellulose to facilitate application onto compressed tablets. These tablets are subsequently air dried for varying periods of time until constant tablet weight is achieved. The assumption was made that drying removes all the organic solvents from the finished product. However, there are a scarcity of data documenting the actual organic solvent levels that may be found in the coated tablet.

Patt and Hartmann (1) studied the effect of tablet core porosity, spraying techniques, drying condition, and evaporation qualities of the solvents used during the film-coating process. The residual levels of organic solvents in the tablet cores and film coats were determined by a GC method. In the present study, a similar GC system was used to determine the levels of methanol and methylene chloride in tablets at various times during the film-coating process. These levels were monitored during a 24-h period of air drying after the film coating was complete. The analytical data were then evaluated by a method similar to Hubaux and Vos (2) to determine a statistically based limit of detection for this method. This technique is similar to the statistical methods defined by Parsons (3) and Currie (4).

EXPERIMENTAL

Materials—A GC[1] equipped with a flame-ionization detector was used with a 1.8-m × 3-mm coiled-glass column containing 80–100 mesh porous polymer packing[2]. Reagents included isopropyl alcohol (AR), chloroform, methanol, and methylene chloride; all reagents were glass distilled[3].

Tablet Coating—A solution of ethylcellulose and methylcellulose in methanol and methylene chloride was sprayed[4] onto the compressed tablets in a heated, rotating coating pan[5]. Uniform coating was achieved when a coating of ~3% of the tablet weight was deposited.

Analytical Procedure—Standard stock solutions of methanol and methylene chloride at concentrations of 24 and 40 ppm were prepared in chloroform, and dilutions were injected onto the GC at the lowest attenuation (where the baseline noise did not exceed 2 mm). The volume of injection of these dilutions was 2.5 μL. Quantitation of the peaks was done with isopropyl alcohol as the internal standard. At an oven temperature of 160°C and a gas flow rate of 60 mL/min, the methanol eluted at ~1–2 min, the methylene chloride at 3–4.5 min, and the isopropyl alcohol at 4–5 min. The limit of detectability, as determined by the instrument at a slope sensitivity of 1 and an attenuation of 8, was ~6 ppm for methanol and 10 ppm for methylene chloride. A calibration curve was prepared by injecting a series of six dilutions which varied fourfold in concentration. The range for methanol was 6–24 ppm, and for methylene chloride the range was 10–40 ppm.

A series of six spiked placebos at concentrations of 0–24 ppm for methanol and 0–40 ppm for methylene chloride were prepared to determine the linearity and recovery over the range of interest. A placebo was defined as a tablet manufactured with no organic solvents in the film-coating formulation.

Coating Studies—Once the lower limit was approximated and a range of linearity established, an experiment was designed to detect methanol and methylene chloride on tablets during the film-coating process. The film-coating formulation consisted of the following ingredients: hy-

Table I—Residual Methanol and Methylene Chloride During and After Film Coating

Sample	Concentration, ppm per tablet[a] Methanol	Methylene Chloride
Uncoated tablet (control)	0	0
5-min coating process	173	52
10-min coating process	312	74
15-min coating process	252	66
Immediately after coating	242	55
5-min heat drying	143	37
15-min air drying	118	31
30-min air drying	115	30
24-h air drying	—[b]	—[b]

[a] Average tablet weight is 760 mg. [b] — No detectable levels.

[1] Hewlett-Packard Model 5830A.

[2] Poropak R packing; Waters Associates, Milford, Mass.

[3] Glass-distilled solvents; Burdick and Jackson, Muskegon, Mich.

[4] Spray gun apparatus; Devilbiss, Toledo, Ohio.

[5] Erweka AR 400 coating pan.

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