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Simultaneous Determination of Vitamins B_1, B_2, B_6, and Niacinamide in Multivitamin Pharmaceutical Preparations by Paired-Ion Reversed-Phase High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic procedure for the simultaneous determination of vitamins B_1, B_2, B_6, and niacinamide in multivitamin pharmaceutical preparations was developed and evaluated. The method uses paired-ion reversed-phase partition chromatography for baseline separation of the four water-soluble vitamins. This method was applied to the analysis of a multivitamin and multivitamin–multimineral tablets, and a technique was developed to reduce vitamin adsorption by the minerals. The results obtained by this method were compared with those obtained by the official methods. It was concluded that this method is fast, accurate, specific, and suitable for routine quality control use.

Keyphrases □ Vitamins, water soluble—simultaneous high-pressure liquid chromatographic analyses in multivitamin preparations □ High-pressure liquid chromatography—simultaneous analyses of various water-soluble vitamins in multivitamin preparations □ Multivitamin preparations—simultaneous high-pressure liquid chromatographic assay of various water-soluble vitamins

Progress in vitamin preparations is being impeded by methodological problems. Current official methods (1, 2) for the assay of water-soluble vitamins involve a complicated sample workup, tend to reproduce poorly because of the instability and pH sensitivity of the color development, and require that each vitamin be analyzed individually. These methods also are subject to interferences from various sources when analyzing samples with complex matrixes (3, 4).

High-pressure liquid chromatographic (HPLC) procedures for the simultaneous determination of water-soluble vitamins in pharmaceutical preparations have been described (5–7), but no mechanism has been developed to control vitamin adsorption by the minerals in multivitamin–multimineral tablets. This paper describes an HPLC procedure for the simultaneous determination of vitamins B_1, B_2, B_6, and niacinamide in multivitamin and multivitamin–multimineral tablets by paired-ion reversed-phase HPLC.

EXPERIMENTAL

Apparatus—The HPLC system included two solvent pumps[1], a solvent programmer[2], a UV absorbance detector[3] at 280 nm, and an autoinjector[4]. A μBondapak phenyl (10 μm) 30-cm × 3.9-mm i.d. column and a 10-mv full-scale recorder[5] were used. Peak areas were determined using a laboratory data system[6]. The column temperature was regulated at 30° by a constant temperature bath[7]. A high-speed centrifuge was used[8], and the samples were disintegrated in a shaker bath[9] set at 60°.

Materials and Reagents—Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, and niacinamide were obtained as USP reference standards. 1-Hexanesulfonic acid was used as received[10]. Anhydrous citric acid[11] was obtained commercially and used without further purification. Solvents, all distilled-in-glass[12] grade, were obtained commercially. Commercial multivitamin preparations were obtained from local pharmacies.

Preparation of Mobile Phases—The two pumps used 0.0025 *M* 1-hexanesulfonic acid in water and in methanol, respectively. Prior to use, the mobile phases were degassed by vacuum filtration through a 0.5-μm pore, 47-mm diameter filter[13]. With a linear solvent program[14], a gradient was run at a constant flow rate of 2.0 ml/min for 18 min. Solvent conditions varied from 0 to 80% methanol in water. Five minutes was allowed between sample injections.

[1] Model 6000A, Waters Associates, Milford Mass.
[2] Model 660, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford Mass.
[4] WISP model 710A, Waters Associates, Milford Mass.
[5] Omniscribe, Houston Instrument, Houston, Tex.
[6] Model 3353, Hewlett-Packard, Palo Alto, Calif.
[7] FK2, Haak Inc., Saddle Brook, N.J.
[8] Sorvall RC-5B centrifuge fitted with SS-34 motor, DuPont Instruments, Wilmington, Del.
[9] Magni-Whirl water bath 15-453-400, Fisher Scientific Co., San Francisco, Calif.
[10] B-6 PIC reagent, Waters Associates, Milford Mass.
[11] Matheson, Coleman and Bell, Norwood, Ohio.
[12] Burdick & Jackson, Muskegon, Mich.
[13] Type LS, 47 mm, Millipore Corp. Bedford, Mass.
[14] Curve 6 on model 660 solvent programmer.

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Table I—Effect of Ferrous Gluconate and Magnesium Oxide on the Recovery of Pyridoxine Hydrochloride in 100 ml of 0.1 *N* HCl Solutions

Sample	Solution Composition: Amount of B_6, mg	Ferrous Gluconate, mg	Magnesium Oxide, mg	B_6, % recovery[a]	*SD*	*RSD*, %
Pyridoxine	0.5	—	—	101	1.02	1.01
Ferrous gluconate + magnesium oxide	—	40	90	None	—	—
Pyridoxine hydrochloride + ferrous gluconate	0.5	40	—	80.43	1.01	1.26
Pyridoxine hydrochloride + magnesium oxide	0.5	—	90	59.00	5.31	9.00
Pyridoxine hydrochloride + ferrous gluconate and magnesium oxide	0.5	40	90	91.67	1.03	1.12

[a] $n = 5$.

Table II—Water-Soluble Vitamins Found in Multivitamin–Multimineral Tablets[a] by Various Modes of Extraction

Vitamin, Label Claim	Percent of Label Claim: 0.1 *N* HCl	Dimethyl Sulfoxide	1% Citric Acid in Dimethyl Sulfoxide
Thiamine hydrochloride, 2.1 mg/tablet	96.6 ± 1.94	108.5 ± 7.54	100 ± 1.25
Riboflavin, 2.4 mg/tablet	81.2 ± 0.90	104.2 ± 1.80	100 ± 1.75
Pyridoxine hydrochloride, 2.0 mg/tablet	88.3 ± 1.70	84.2 ± 0.78	100 ± 1.58
Niacinamide, 2.0 mg/tablet	101.9 ± 3.26	100.6 ± 0.85	100 ± 3.11

[a] Vita Lea, Shaklee Corp.; each value is the mean ± *SD* of five determinations.

Preparation of Standard Solutions—Stock standard solutions of thiamine hydrochloride, pyridoxine hydrochloride, and niacinamide (0.5 mg/ml each) were prepared separately by dissolving accurately weighed USP reference standards in 0.1 *N* HCl. Due to poor solubility, only the riboflavin working standard solution was prepared in a more diluted form; <0.1 g accurately weighed USP reference standard was dissolved in 1000 ml of 0.1 *N* HCl, heated, and constantly stirred in a low actinic volumetric flask.

Different aliquots from each stock standard vitamin solution were combined and diluted with the riboflavin working standard solution. This procedure resulted in a working standard solution containing all four water-soluble vitamins at the desired concentration.

Preparation of Sample Solutions—*Multivitamin Tablets*—A suitable number of tablets containing no more than 10 mg of riboflavin were placed in a low actinic glass-stoppered erlenmeyer flask. Exactly 100 ml of 0.1 *N* HCl was pipetted into the flask, and the flask was stoppered and shaken for 45 min in a constant-temperature bath at 60°. The sample was allowed to cool to room temperature and then was centrifuged at 44,000×*g* for 18 min. Then 10 μl of the clear supernate was injected into the liquid chromatograph.

Multivitamin–Multimineral Tablets—A suitable number of tablets containing no more than 10 mg of riboflavin were placed in a low actinic glass-stoppered erlenmeyer flask. Exactly 100 ml of dimethyl sulfoxide containing 1% citric acid (anhydrous) was delivered into the flask. The solution then was shaken for 45 min in a constant-temperature bath at 60°. It was allowed to cool to room temperature and then was centrifuged at 44,000×*g* for 18 min. Then 10 μl of the clear supernate was injected into the liquid chromatograph.

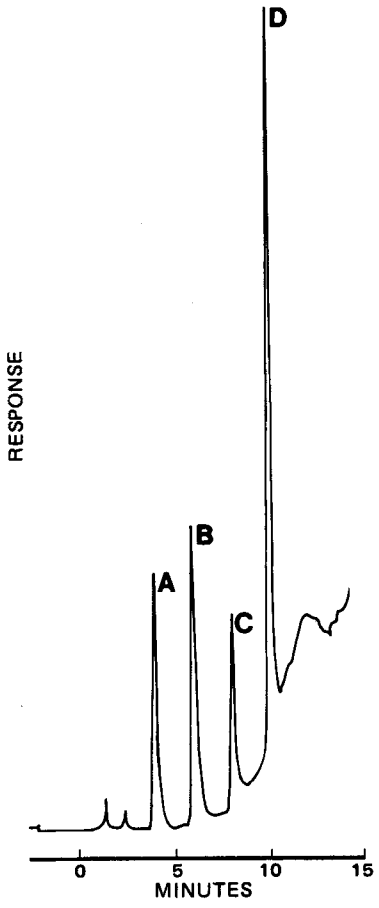

Figure 1—*Liquid chromatographic trace of a standard mixture of niacinamide (A), pyridoxine (B), thiamine (C), and riboflavin (D).*

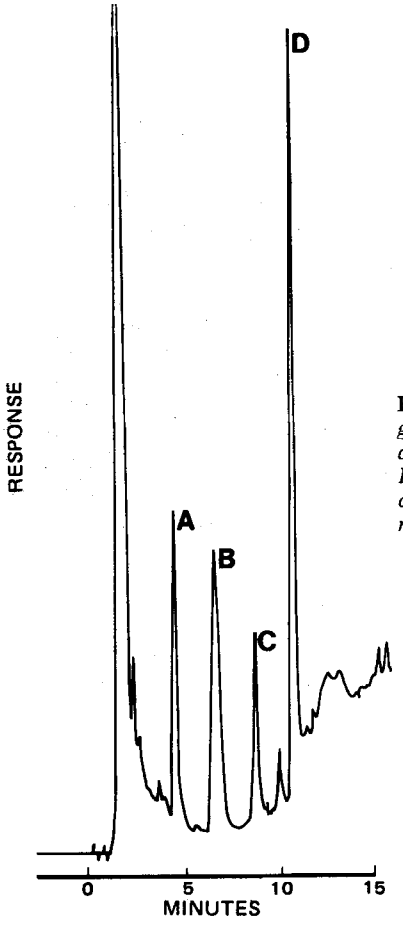

Figure 2—*Liquid chromatographic trace of the extract of a vitamin B complex tablet. Key: A, niacinamide; B, pyridoxine; C, thiamine; and D, riboflavin.*

Table III—Recovery Results on Vitamins B_1, B_2, B_6, and Niacinamide

Sample	Percent Recovery[a], mean ± *SD* (% *RSD*)			
	B_1	B_2	B_6	Niacinamide
Control multivitamin tablets[b]	None	None	None	None
Multivitamin tablets + added vitamins[c]	100.8 ± 2.23 (2.21)	102.1 ± 2.48 (2.43)	104.3 ± 6.7 (6.43)	102.1 ± 2.78 (2.72)
Control multivitamin–multimineral tablets[b]	None	None	None	None
Multivitamin–multimineral tablets + added vitamins[c]	101.6 ± 2.21 (2.18)	99.60 ± 2.01 (2.02)	99.62 ± 2.92 (2.93)	99.97 ± 1.45 (1.45)

[a] Each value is the mean ± *SD* (% *RSD*) of six determinations. [b] Control tablets contain the complete formulation of B-Complex (multivitamin tablets) and Vita Lea (multivitamin–multimineral tablets) without vitamins B_1, B_2, B_6, and niacinamide. [c] Same as control tablets but with vitamins B_1, B_2, B_6, and niacinamide added directly to the flask.

Table IV—Comparison of USP Method *versus* HPLC Method for the Analysis of Vitamins B_1 and B_2 in Multivitamin Products

Product[a]	Vitamin B_1					Vitamin B_2				
	Formulation Amount	Found[b]				Formulation Amount	Found			
		HPLC	*SD*	USP	*SD*		HPLC	*SD*	USP	*SD*
A	66.42 mg/g	65.91	0.56	66.5	1.83	70.72 mg/g	70.66	0.27	72.9	1.36
B	1.178 mg/tablet	1.151	0.02	1.248	0.04	1.143 mg/tablet	1.172	0.018	1.245	0.026
C	9.45 mg/tablet	9.63	0.35	8.73	0.90	8.415 mg/tablet	8.597	0.110	7.910	0.129
D	0.678 mg/tablet	0.687	0.003	0.695	0.009	0.718 mg/tablet	0.716	0.002	0.674	0.010
E	10 mg/tablet	10.5[c]	—[d]	—[d]	—[d]	10 mg/tablet	18.0[c]	—[d]	—[d]	—[d]
F	11.6 mg/tablet	11.9[c]	—[d]	—[d]	—[d]	15 mg/tablet	14.6[c]	—[d]	—[d]	—[d]
G	3 mg/capsule	3.1[c]	—[d]	—[d]	—[d]	3 mg/capsule	3.4[c]	—[d]	—[d]	—[d]

[a] Key: A, Vita Lea Premixes (Shaklee); B, Vita Cal (Shaklee); C, B-Complex (Shaklee); D, Vita Lea (Shaklee); E, Super Thera-Vite M (Nature's Blend); F, Stress Formula (Value Rite); and G, B-Complex Capsules (Nature's Blend). [b] Each value is a mean ± *SD* of five determinations. [c] Single determination. [d] Not determined.

RESULTS AND DISCUSSION

HPLC can provide a quick and accurate evaluation of multivitamin and multivitamin–multimineral tablets, and paired-ion reversed-phase HPLC is particularly attractive for determining water-soluble vitamins because polar constituents that dissolve in the aqueous mobile phase elute at or near the solvent front. Lipophilic constituents with very limited solubility in the aqueous mobile phase partition with the stationary phase, thereby eluting much later. Under these conditions, separation can be achieved. Thus, sample preparation time is greatly reduced and the accuracy of the method also is enhanced.

In the present study, 0.1 *N* HCl was used to extract the water-soluble vitamins from the multivitamin preparations. Dimethyl sulfoxide containing anhydrous citric acid was used to disperse the multivitamin–multimineral preparations since neither 0.1 *N* HCl nor dimethyl sulfoxide alone extracts pyridoxine completely. This may be due to adsorption of the vitamin by one or more of the minerals. Kwok *et al.* (8) established that vitamin A acetate adsorbs to ferrous gluconate and that a complexing agent can minimize this effect.

With a similar approach, it was demonstrated in these laboratories that, in 0.1 *N* HCl solution, pyridoxine hydrochloride adsorbs strongly to ferrous gluconate and magnesium oxide (Table I) and that a complexing agent like citric acid in dimethyl sulfoxide can minimize this effect (Table II). Thus, anhydrous citric acid was dissolved first in dimethyl sulfoxide to allow for the exposure to the minerals as soon as possible. Table I shows the effect of ferrous gluconate and magnesium oxide on pyridoxine hydrochloride in 0.1 *N* HCl solution. Table II demonstrates the percentage of recovery of all four water-soluble vitamins in multivitamin–multimineral tablets by different modes of extraction. It is obvious from Table II that 1% citric acid in dimethyl sulfoxide reduced the effect of ferrous gluconate and magnesium oxide to an insignificant level. The extraction of the other vitamins (thiamine, riboflavin, and niacinamide) was not impaired by the addition of the anhydrous citric acid in dimethyl sulfoxide.

Linearity of response over the range studied can be achieved using standard solutions. In these laboratories, the ratios of the concentration of the standard solution (expressed in nanograms per 10 microliters) to the area under the peak were consistent for all four water-soluble vitamins studied. These ratios were determined over 6 days, and their relative standard deviations were <1%. This finding indicates that the procedure can be used as a single-point standard for each vitamin.

Chromatographic traces of vitamin B standards, vitamin B complex, and the multivitamin–multimineral tablet extracts are presented in Figs. 1, 2, and 3, respectively. Using paired-ion reversed-phase HPLC, baseline

Table V—Comparison of USP Method *versus* HPLC Method for Analysis of Vitamin B_6 and Niacinamide in Multivitamin Products

Product[a]	Vitamin B_6					Niacinamide				
	Formulation Amount	Found[b]				Formulation Amount	Found			
		HPLC	*SD*	USP	*SD*		HPLC	*SD*	USP	*SD*
A	54.21 mg/g	54.33	1.40	54.9	1.24	None	None		None	
B	None	None		None		None	None		None	
C	11.97 mg/tablet	11.37	0.15	9.27	0.23	99.00 mg/tablet	98.60	3.31	97.20	4.40
D	0.670 mg/tablet	0.668	0.013	0.680	0.019	5.49 mg/tablet	5.54	0.06	5.18	0.30
E	5 mg/tablet	4.7[c]	—[d]	—[d]	—[d]	100 mg/tablet	101.5[c]	—[d]	—[d]	—[d]
F	5 mg/tablet	5.1[c]	—[d]	—[d]	—[d]	100 mg/tablet	90.8[c]	—[d]	—[d]	—[d]
G	0.5 mg/capsule	0.9[c]	—[d]	—[d]	—[d]	20 mg/capsule	26.0[c]	—[d]	—[d]	—[d]

[a] Key: A, Vita Lea Premixes (Shaklee); B, Vita Cal (Shaklee); C, B-Complex (Shaklee); D, Vita Lea (Shaklee); E, Super Thera-Vite M (Nature's Blend); F, Stress Formula (Value Rite); and G, B-Complex Capsules (Nature's Blend). [b] Each value is a mean ±*SD* of five determinations. [c] Single determination. [d] Not determined.

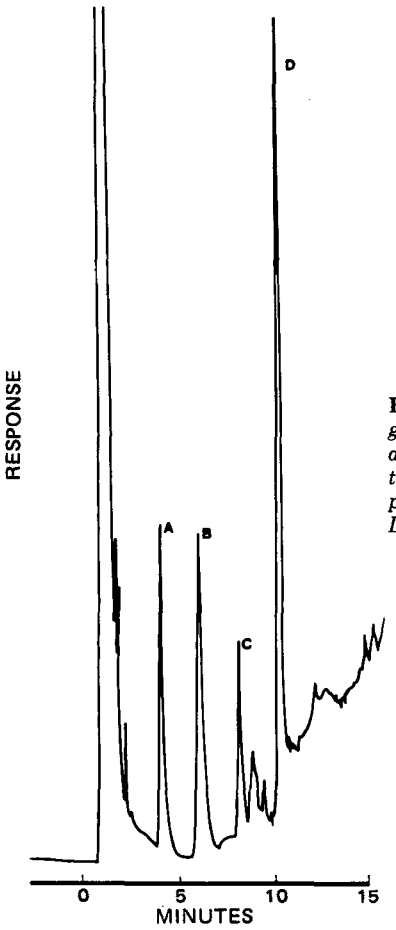

Figure 3—*Liquid chromatographic trace of the extract of a multivitamin–multimineral tablet. Key: A, niacinamide; B, pyridoxine; C, thiamine; and D, riboflavin.*

separation of all four water-soluble vitamins is possible (Figs. 1 and 2). There also is a baseline separation of the vitamins in the dimethyl sulfoxide extract (Fig. 3).

To validate the selectivity of the procedure, placebo formulations spiked with known amount of the vitamins were employed. The results are tabulated in Table III. All four vitamins were quantitatively recovered in both multivitamin and multivitamin–multimineral tablets.

To verify the applicability of this procedure further, various commercial multivitamin and multivitamin–multimineral tablets were assayed simultaneously by the HPLC and the USP methods (Tables IV and V). In each case, the precision of the HPLC procedure was greater than that of the current official procedure. This result may be attributed to the less complicated sample workup required by the former procedure. However, the results of the two methods compared favorably in terms of accuracy.

The described HPLC method appears to be attractive in terms of analysis time and should be adapted to various commercial products. The column lifetimes under these conditions of minimum sample preparation initially was a subject of concern. However, over 500 preparations were processed without change in the chromatographic characteristics of the system. This stability may be due in part to the small quantities of sample injected and in part to the proper care as recommended by the column supplier. The methodology for the HPLC determination of other vitamins is currently under investigation and will be published later.

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In Vivo–In Vitro Correlations with a Commercial Dissolution Simulator I: Methenamine, Nitrofurantoin, and Chlorothiazide

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Abstract □ Dissolution profiles were determined for nine methenamine, 14 nitrofurantoin, and six chlorothiazide dosage forms using a dissolution simulator. Various *in vivo–in vitro* correlations were examined. The best correlation for methenamine was between the maximum urinary excretion rate and the time for 15% dissolution. A good correlation for the 50-mg nitrofurantoin tablets was also found between cumulative percent of drug excreted in 12 hr and the percent dissolved in 1 hr. There were no significant correlations for the 100-mg nitrofurantoin dosage forms. Good correlations were also observed for the 250- and 500-mg chlorothiazide tablets between the percent of drug dissolved in 1 min or the time for 15% dissolution and the maximum excretion rate.

Keyphrases □ Dissolution—profiles for methenamine, nitrofurantoin, and chlorothiazide, dissolution simulator □ Dissolution simulator—profiles for methenamine, nitrofurantoin, and chlorothiazide □ Methenamine—dissolution profile using dissolution simulator □ Nitrofurantoin—dissolution profile using dissolution simulator □ Chlorothiazide—dissolution profile using dissolution simulator

Many methods developed to study the *in vitro* dissolution properties of dosage forms have been reviewed previously (1). The primary objective of most efforts to develop *in vitro* dissolution procedures is to provide data that can be related to the performance of oral dosage forms when administered to human subjects. One sophisticated

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