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PHARMACEUTICAL ANALYSIS

Determination of Calcium Pantothenate in Chewable Multivitamin Tablets

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Abstract □ A hybrid procedure was developed for the determination of calcium pantothenate in a chewable multivitamin tablet. Applicable operations and techniques were adopted from different published sources. The resulting procedure incorporates solvent extraction, chromatography, hydrolysis, and color development. This method proved to be more accurate and precise than any published method investigated.

Keyphrases □ Calcium pantothenate in chewable multivitamin tablets—analysis, procedure using various analytical techniques □ Multivitamin tablets, chewable—analysis of calcium pantothenate, procedure using various analytical techniques □ Pantothenate, calcium, in chewable multivitamin tablets—analysis, procedure using various analytical techniques

In this laboratory, the determination of calcium pantothenate by wet methods has led to many difficulties. It has been necessary to modify and revise existing procedures continually to obtain reliable results on various dosage forms, as well as on similar dosage forms containing different pharmaceutic aids.

Most often the ninhydrin method (1) or an adaptation proved to be suitable. This method, however, could not be made applicable to certain chewable multivitamin tablet formulations containing relatively high levels of natural sweeteners. The most serious objection to this method was the pronounced variability. A statistical analysis showed the coefficient of variation to be over 6%. Analysis of variance studies indicated significant differences in the variability between replicates, days, and operators. Therefore, this method was considered unapplicable.

The iodine method (2, 3) was the next to be investigated. The lack of precision was also a problem with this procedure (3). Some developmental work was done to improve the precision. Subsequently, a statistical evaluation was undertaken which yielded a high coefficient of variation of 8.4%.

Finally, the naphthoquinone method (4) was tried. In this case, the chromatographic separation achieved by Panalaks and Campbell could not be duplicated. The pantothenate was partially eluted with the boric acid wash and tailed badly during the elution step. Consequently, poor results were obtained.

A search of the literature did not reveal any alternative procedures that were considered compatible with techniques and equipment available in this laboratory. However, during this in-depth study of available methods, it became evident that certain techniques involving sample cleanup and detection employed by various authors were very effective. The possibility that a hybrid procedure possessing combined advantages could be developed seemed promising. Such a procedure did, in fact, evolve from the combination of carefully selected existing steps and techniques.

This procedure, when applied to the product mentioned, supplied results significantly more accurate and precise than those provided by any published method tried. No special apparatus is required. Puri-

Table I—Hydrolysis of Calcium Pantothenate *versus* pH at 100°

pH	Percent Remaining after 1 hr
11.0	47
12.0	23
13.0	0.57
13.5	None detected

fication of reagents and solvents is not necessary, and analysis time is comparable to most other existing wet methods.

The purpose of this paper is to describe this method. It is hoped that it will provide an additional alternative to anyone encountering similar assay problems.

EXPERIMENTAL

Reagents—The following were used: ion-exchange resin[1], 100–200 mesh; and activated magnesium silicate[2], 60–100 mesh.

Boric Acid—Dissolve 100 g boric acid in distilled water and dilute to 2 liters.

Naphthoquinone Solution—Dissolve 250 mg sodium 1,2-naphthoquinone-4-sulfonate in distilled water and dilute to 50 ml. This reagent should be prepared fresh daily.

Formaldehyde Solution—Mix three parts 6 *N* hydrochloric acid, four parts acetic acid, and four parts 0.6 *M* formaldehyde.

Thiosulfate Solution—Dissolve 2.5 g sodium thiosulfate pentahydrate in water and dilute to 100 ml with water.

Standard Solution—Dissolve 120 mg calcium pantothenate in water and dilute to 100 ml with water.

Procedure—Slurry pack 5 cm of ion-exchange resin over 5 cm activated magnesium silicate in a chromatographic tube, 1.5 cm i.d., fitted with a stopcock. Place pledgets of glass wool at the bottom and top of the column and between the two chromatographic materials. Just prior to using the columns, wash with 10 ml 0.1 *N* HCl and then with 100 ml of water.

Next, prepare an aqueous extract of the sample containing about 1.2 mg calcium pantothenate/ml. Transfer 10.0 ml of sample and standard to 40-ml centrifuge tubes (Oak Ridge) containing 13 g sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$). Shake the tubes on a mechanical shaker for 15 min. The shaker used was of the oscillating type with a 4.5-cm travel and 30 cpm. To each tube, add exactly 20 ml of benzyl alcohol and shake another 15 min. After centrifugation, transfer 15.0 ml of the clear upper benzyl alcohol layer to another centrifuge tube. Add 10.0 ml toluene and 12.0 ml water to each tube and again shake 15 min. Centrifuge to obtain a clear lower layer.

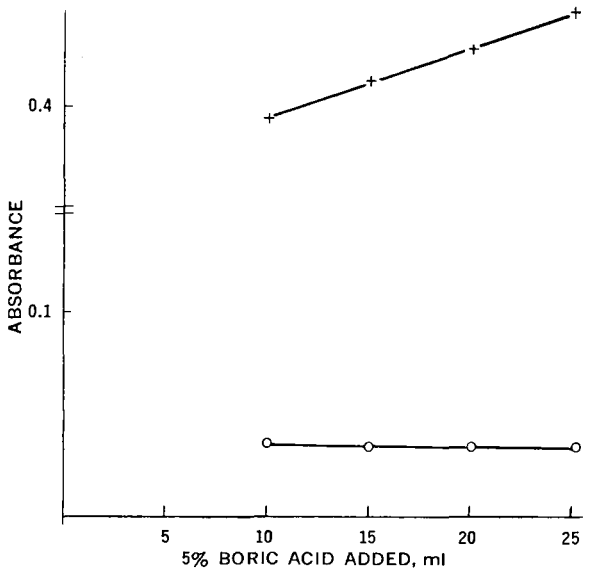

Figure 1—*Effect of boric acid concentration on assay sensitivity. Key: +, standard; and O, reagent blank.*

[1] 50W-X4, BioRad Labs, Richmond, Calif.

[2] Florisil, Fisher Scientific Co., Fair Lawn, N.J.

Table II—Effect of Cooling Conditions on Precision

Cooling Conditions		
After Hydrolysis	After Color Development	Coefficient of Variation, %
Ice bath	Ice bath	0.64
Bench top, room temperature	Ice bath	1.57
Ice bath	Bench top, room temperature	1.15

Pipet 10-ml aliquots of the lower aqueous layer onto the previously prepared chromatographic columns and allow to percolate through at full flow. Collect all column effluents in 100-ml volumetric flasks. Wash the calcium pantothenate through the column with six 5-ml portions of water. To each receiver plus a reagent blank (40 ml water in 100-ml volumetric flask), add exactly 25 ml boric acid solution and 8 ml of 3.5 *N* NaOH. The pH of the solutions at this point should be about 13.5. Heat the flasks at 100° for 1 hr and then cool to near room temperature in an ice bath.

Adjust each solution to pH 8.0 by adding a predetermined volume of 3 *N* HCl. After adding 4.0 ml of the naphthoquinone reagent, heat the flasks at 100° for 10 min and cool in an ice bath as before. To each flask, add exactly 4 ml each of the formaldehyde and thiosulfate reagents and let stand 15 min. Bring to volume with water, mix, and determine the absorbance on a suitable spectrophotometer[3] *versus* a reagent blank at the maximum wavelength, at about 465 nm, in a 1-cm cell.

RESULTS AND DISCUSSION

Color Reaction—The detection method was selected first and the remainder of the procedure was designed to accommodate it. The naphthoquinone color reaction as carried out by Panalaks and Campbell (4) was chosen. Experience had shown this technique to be highly precise and accurate, and it is relatively convenient. The various parameters, *e.g.*, heating conditions, pH, and concentration of reagents, associated with the color development were investigated and confirmed as optimum. The color is stable for at least 1.5 hr.

Chromatography—β-Alanine, a degradation product of pantothenate, is the substance actually involved in the color reaction. Any of this material present due to degradation must be removed to render the method specific for intact pantothenate. The technique chosen employs the dual column proposed by Schmall and Wollish (1). Considerable experience with this column had revealed it to be consistently effective. The free β-alanine is removed by the ion-exchange portion of the column. Although its full potential was not determined, the resin has a demonstrated capability of removing an amount of β-alanine equivalent to 6 mg calcium pantothenate. Therefore, the stability of the products could be followed to a 50% degradation level. In addition, some interfering tablet ingredients, *e.g.*, riboflavin, are removed by the activated magnesium silicate portion.

Several authors (2, 3, 5) recommended slurrying to remove fines from chromatographic materials to increase precision. This practice was, therefore, incorporated into the presented procedure.

Solvent Extraction—Interfering materials that were not removed by the column necessitated the addition of the benzyl alcohol extraction step. A 65% reduction in response was noted when this step was omitted. This technique, proposed by Panalaks and Campbell (4), effectively eliminates interferences and was adopted without modification. Since the problem was solved in this manner, no attempt was made to determine the source of

[3] Beckman DU.

Table III—Statistical Evaluation of Procedure

Synthetic Sample	Calcium Pantothenate, mg		Recovery, %	Mean Recovery, %	*SD*	Coefficient of Variation, %
	Added	Found				
I	10.18	10.10	99.2			
		10.10	99.2			
		10.14	99.6			
		9.92	97.4			
		—	—	98.8	0.9848	0.97
II	7.49	7.59	101.3			
		7.59	101.3			
		7.65	102.1			
		7.69	102.6			
		7.57	101.1	101.7	0.6419	0.65
III	4.98	5.06	101.6			
		5.08	102.0			
		5.04	101.2			
		4.94	99.1			
		5.04	101.2	101.0	1.128	1.07

the interference. It is possible, therefore, that some formulations may not require this treatment.

Hydrolysis—The rate of hydrolysis of pantothenate increases uniformly with pH up to about 13, above which it tends to level off (Table I). A pH of 13.5 was chosen for the hydrolysis medium to reduce analysis time. The blank value in this case was unaffected by variations in pH.

The half-life of calcium pantothenate at 100° and pH 13.5 is approximately 5 min. The hydrolysis is at least 99.9% complete after 50 min.

Heating and Cooling Conditions—To provide the 100° heating conditions necessary for both hydrolysis and color development, several flasks were placed on a screen over an opening of a steam bath and a bell-shaped cover fashioned of aluminum foil placed over them. This technique provides very uniform and homogeneous heating conditions and eliminates bumping problems. Ice bath cooling conditions were employed not only for expediency but also to improve precision (Table II).

Boric Acid—Originally the boric acid was added only to facilitate adjustment and preservation of pH. It became evident, however, that the intensity of the measured color was directly proportional to the concentration of this reagent. The relationship of response *versus* the amount of boric acid present is shown in Fig. 1; the absorbance of the reagent blank was independent of boric acid concentration. Therefore, to achieve maximum sensitivity, the boric acid concentration was adjusted as high as practical. Consequently, to achieve precise results, analytical precision is required when adding this reagent.

Accuracy and Precision—To evaluate the method for accuracy and precision, synthetic samples were assayed. These samples were prepared by adding accurately known amounts of calcium pantothenate to a placebo, containing all tablet ingredients with the exception of calcium pantothenate. Three levels of pantothenate were selected. The first was the level present in the product of interest and the remaining two were equal to 75 and 50%, respectively, of the first. Thus, the stability-indicating ability of the assay was included in the evaluation. As mentioned, the ion-exchange column was effective in removing free β-alanine over this range of degradation (Table III). It is evident from these data that the assay adheres to a linear model. That is, similar recoveries were obtained at all three levels. The assay is stability indicating, as well as adequately accurate and precise.

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