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Technical Note

A Reversed-Phase High-Performance Liquid Chromatographic (HPLC) Assay for the Determination of Biotin in Multivitamin–Multimineral Preparations

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INTRODUCTION

Biotin (*cis*-hexahydro-2-oxo-1H-thieno-[3,4]-imidazoline-4-valeric acid; vitamin H) is essential to the metabolism of amino acids and carbohydrates. Despite biotin's important biological activity, little has been published on its analysis in multivitamin–multimineral finished products.

Various methods for the determination of biotin using microbiological assays, thin-layer chromatography, and gas chromatography have been assembled (1). In general, microbiological assays are laborious, with an 18 to 24-hr incubation time required. A microbiological assay has been published for tablets and capsules; although the detection limit is more than adequate, the actual recovery and precision data on finished products are not mentioned (2). In addition, several high-performance liquid chromatographic (HPLC) procedures have been reported over the past 2 years (3–6). While these methods offer an alternative to the present microbiological methods, there has been no assessment of these methods on a finished multivitamin product (3,4). Of the HPLC methods, Hudson *et al.* (5) claim applicability only to multivitamin samples containing at least 150 μg biotin. Moreover, their work was done on B-complex tablets and vitamin premixes rather than a finished multivitamin–multimineral product (5). This report describes an HPLC method for the quantitative determination of biotin in multivitamin–multimineral products.

MATERIALS AND METHODS

The HPLC system was equipped with the following components: an automatic sample injector (Model 834, DuPont Co., Wilmington, Del.), a fixed-loop injector fitted with a 200-μl loop (Model 7126, Rheodyne Inc., Cotati, Calif.), a reciprocating piston pump (Model 110A, Altex Scientific, Berkeley, Calif.), and a variable-wavelength UV detector (Model 773, Kratos Analytical Instruments, Ramsey, N.J.), operating at a 200-nm wavelength. A 15-cm × 4.6-mm-i.d. analytical column (DuPont Zorbax ODS) was used, preceded by a column inlet filter (Rheodyne).

The mobile phase was a 77:23 (v/v) mixture of 0.25 *M* sodium phosphate (monobasic) buffer (adjusted to pH 3.5 with phosphoric acid) and methanol. The flow rate was 1.0 ml/min with the column temperature ambient, which gave a retention time for biotin of about 10.5 min.

A stock standard was prepared by weighing 25 mg of biotin reference standard into a 200-ml volumetric flask and diluting to volume with water. The stock solution was diluted to obtain a final concentration of the working standard of 0.0025 mg/ml.

Ten tablets were ground in a water-cooled mechanical grinder and approximately a one-tablet-weight portion, equivalent to 100 μg of biotin, was transferred into a 50-ml polypropylene centrifuge tube. Twenty milliliters of 0.02 *N* sodium hydroxide was pipetted into each tube and placed on a reciprocating shaker for 30 min at about 240 oscillations/min. The sample was centrifuged at about 14,000 rpm for 5 min. Ten milliliters of the supernate was pipetted onto a pretreated Waters SEP-PAK Florisil column (the columns are prewashed with about 100 ml of water) and eluted with 10–12 ml of 0.02 *N* sodium hydroxide into a 25-ml volumetric flask with the aid of a SEP-PAK cartridge rack and a vacuum pump. The sample was diluted to volume with 0.02 *N* sodium hydroxide. A portion of the sample solution was filtered through a 0.45-μm filter unit and injected. A methanol injection was made after each sample injection to decrease the retention times of late eluters and keep a constant autosampler sequence. A typical chromatogram is shown in Fig. 1.

A Hewlett–Packard 3357 laboratory automation system was used for integration.

The amount of biotin per tablet is calculated from

$$\mu g/tab\ biotin = \frac{A_{sam}}{A_{std}} \times C_{std} \times F_{sam} \times \frac{ATW}{W_{sam}}$$

where A_{sam} is the peak area response of biotin in the sample solution, A_{std} is the average peak area response of biotin in the standard solution, C_{std} is the concentration of the

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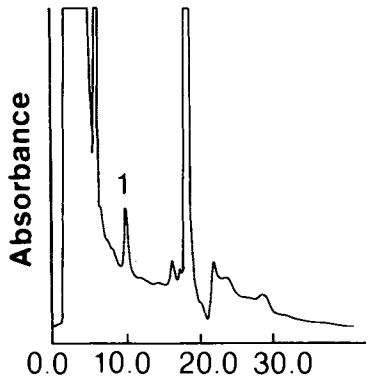

Fig. 1. Typical chromatogram of a multivitamin/multimineral tablet extract. (1) Biotin.

working standard, F_{sam} is the dilution factor, ATW is the average tablet weight, and W_{sam} is the weight (mg) of the sample.

RESULTS AND DISCUSSION

Applications literature from the DuPont Co. supplied a reversed-phase HPLC system for the separation of water-soluble vitamins including biotin (7). A 15-cm Zorbax ODS column was substituted for the 25-cm Zorbax ODS column to decrease analysis times. The ratio of 0.25 *M* sodium phosphate buffer and methanol was adjusted to 77:23 to resolve the biotin from the excipient materials. A 200-μl injection volume was used to maximize detectability without distorting chromatographic separation. Although the DuPont literature suggested detection at 210 nm, a UV spectrum of biotin standard prepared in the mobile phase gave maximum absorbance near 203 nm. Thus, a wavelength of 200 nm was used with a good detector response to biotin and no baseline noise interference.

Two modifications of the sample preparation used in a chemical method (8) for calcium pantothenate were applied to biotin. The original cleanup was modified slightly. The disposable Florisil column alone was found to give sufficient sample cleanup without affecting the recovery of the biotin.

Initial recovery studies using a sample extraction in water gave an average recovery of 90% on spiked placebos. The literature (8) states that biotin is only slightly soluble in water but easily dissolves in dilute alkali solutions. Extraction solvents including the mobile phase, 0.25 *M* sodium phosphate buffer, and various strengths of sodium hydroxide ranging from 0.01 to 0.10 *N* were tried in an effort to increase recovery on spiked placebo. Placebo background checks and spiked placebos (at the 100% of theory level) done in duplicate using 0.02, 0.03, and 0.04 *N* sodium hydroxide as the extraction solvent were assayed. The recoveries increased as the strength of the sodium hydroxide increased.

Sodium hydroxide normality	% of theory recovery
0.02	95, 94
0.03	101, 94
0.04	102, 101

However, the placebo background checks showed interfering peaks from the tablet matrix at the retention time of biotin when 0.03 *N* sodium hydroxide and 0.04 *N* sodium hydroxide were used as extraction solvents. The placebo background check using 0.02 *N* sodium hydroxide showed no interfering peaks and therefore was chosen as the extraction solvent.

The optimum extraction procedure used a reciprocating shaker set at about 240 oscillations for 30 min.

Chromatographic background checks of fresh biotin placebo and biotin placebo stored at 80°C for 3 weeks were carried out. No peaks were observed in the vicinity of the biotin retention time. Biotin raw material stored at 80°C for 3 weeks showed no extra peaks in the chromatogram, indicating no degradation of the biotin even under extreme conditions.

The sample recovery/linearity study consisted of spiking placebo with biotin raw material at five levels covering 50 to 150% of theory of the dosage form. The average recovery for all five levels was 94.5%, with a coefficient of variation (CV) of 4.9%. However, the average recovery of the 100, 125, and 150% levels was 97.8%, while the average recovery of the 50 and 75% levels was 89.6%, a considerable decrease. Since the data did not conform to a linear model, the difference in recovery at lower levels may be an indication of an incomplete extraction of an absolute amount of the biotin.

The sample preparation above is applicable to at least two different multivitamin–multimineral products. The assay value obtained on a competitor's commercial vitamin product was 44.9 μg/tablet, which represented 99.8% of their label claim.

A collaborative study was conducted to determine the precision of the method. Five collaborators ran four samples of multivitamin–multimineral product. Statistics on the five analysts' data were calculated. For example, see Ref. 9. The average assay value was 110.5 μg/tablet (110.5% of the claim). The CV within days was calculated to be 1.9%. The CV between days was calculated to be 2.4%. The CV of the overall method was calculated to be 3.1%, which showed a good precision.

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