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Journal of Pharmaceutical and Biomedical Analysis
16 (1998) 1311–1315

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Liquid chromatographic determination of biotin in multivitamin-multimineral tablets

Anthony E. Ekpe *, Charlene Hazen

Consumer Care Division, Bayer Corporation, 36 Columbia Road, Morristown, NJ 07962, USA

Received 27 February 1997; accepted 19 May 1997

Abstract

A reproducible reverse phase high pressure liquid chromatography (RP-HPLC) method for the determination of biotin in multivitamin-multimineral tablets has been developed and validated. This method involves reverse phase separation of the component monitored by absorbance at 200 nm wavelength. The method has excellent precision and accuracy with S.D. 0.83 and 2.9%, respectively. The established linearity range was 0.5–2 μg ml^{-1} ($r^2 > 0.9999$). The recovery of biotin from spiked placebo was $> 97\%$ over the linear range. The extraction procedure is simple and the HPLC conditions separate biotin from its degradation products and excipients. The method has been successfully used in determining biotin content in 4 brands of commercially available multivitamin- multimineral tablets.

Keywords: Biotin; Multivitamin-multimineral; Reverse phase high pressure liquid chromatography; UV detection; Wavelength

1. Introduction

Biotin (*cis*-hexahydro-2-oxo-1 H-thieno-[3,4]-imidazoline-4-valeric acid), also known as vitamin H (Fig. 1), is a water-soluble vitamin which is essential to the metabolism of amino acids, carbohydrates and in some nucleic acid syntheses.

Many methods have been described for the determination of biotin in pharmaceutical products, using microbiological and chromatographic procedures. In general, microbiological assays are time consuming, with an average incubation time

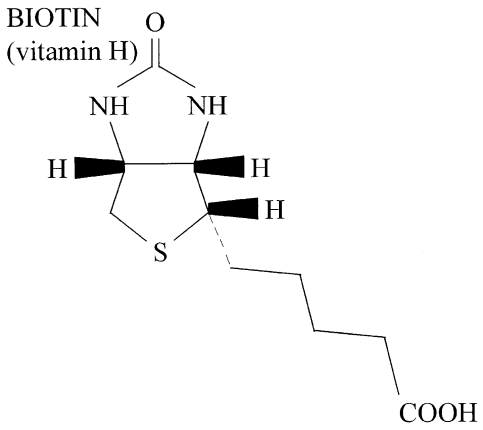

Fig. 1. Structure of biotin.

* Corresponding author. Tel.: +1 201 2545000; fax: +1 201 2544717; e-mail: Anthony.Ekpe@bayer.com

0731-7085/98/$19.00
PII S0731-7085(97)00143-X

of 18–24 h. A microbiological assay has been published for multivitamin tablets and capsules that do not contain multiminerals [1].

Several chromatographic procedures have been reported for multivitamin-multimineral pharmaceuticals [2] or for multivitamin products only [3–7]. Although these methods are an alternative to the microbiological method, there has been no published report of reverse-phase high performance liquid chromatographic (RP-HPLC) analysis of biotin in multivitamin-multimineral tablets using ultra-violet (uv) wavelength without sample clean-up process. Methods proposed for a multivitamin product with minerals relied on a sample clean-up process and column conditioning after every sample injection. Spike recovery did not conform to a linear model [2]. Other chromatographic methods relied on either reversed-phase (RP) ion-interaction HPLC [3], RP HPLC with electrochemical detection [4], RP HPLC with post-column fluorescence detection [5], micellar electrokinetic chromatography (MEC) [6], capillary zone electrophoresis (CZE) [7] or RP-HPLC analysis of B-complex tablets and vitamin premixes [8].

Compendial methods for the determination of biotin in pharmaceutical dosage forms [9] are often cumbersome and time consuming. Although, Method 1 is a RP-HPLC assay, in our experience, there is coelution of another component at the same retention time as biotin with multivitamin-multimineral tablets. Method 2 is a microbiological assay that requires 16–24 h incubation. This paper describes a rapid and reproducible RP-HPLC method for the determination of biotin in multivitamin-multimineral tablets without sample clean-up process. The proposed method uses C_8 reverse phase column, with detection in the uv region.

2. Experimental

2.1. Apparatus

(a) Liquid chromatograph: Perkin Elmer, consisting of Series 200 IC Pump, Series 200 Autosampler equipped with 150 μl loop, Series 235C Diode Array Detector with 600 Series Link and Laserjet 5 Printer (Perkin Elmer, Norwalk, CT).

(b) Column: Reverse phase C_8 YMC Octylsilane, 150 × 4.6 mm id, 3 μm particle size (YMC, Wilmington, NC).

(c) Mobile phase: A mixture of 95:5 of water (adjusted to pH 2.2 with phosphoric acid) and acetonitrile, filtered through 0.45 μm nylon filter membrane and degassed. The flow rate was 2.0 ml min^{-1} with the column temperature set at 50°C which gave a retention time of about 20.5 min.

Table 1
Spike recovery of biotin from placebo

μg added	μg recovered	Recovery (%)
62.3	60.7	97.4
56.1	59.2	105.6
62.8	64.7	103.1
93.3	96.0	102.9
99.0	97.2	98.2
95.2	99.2	104.3
126.7	127.0	100.2
126.0	125.8	99.8
114.5	114.7	100.3
154.0	156.1	101.3
144.9	144.2	99.5
159.9	169.4	106.3
187.1	185.3	99.1
181.7	177.0	97.4
168.5	165.0	97.9
Mean	—	100.9
% RSD	—	2.9

2.2. Chemicals

HPLC grade acetonitrile and reagent grade sulfuric acid were purchased from VWR Scientific Products (South Plainfield, NJ). Water was purified using a Milli-Q-filter system (Millipore, Milford, MA). Biotin (S.D. 1%) used for spiking placebo was purchased from Roche Vitamins and Fine Chemicals (Nutley, NJ). The USP reference standard used was Lot H.

2.3. Standard preparation

A stock solution was prepared by weighing 10 mg of biotin reference standard into a 250 ml low

actinic volumetric flask, dissolved and diluted to volume with water (adjusted to pH 2.2 with phosphoric acid). The stock solution was diluted to obtain a final concentration of 1.0 μg ml^{-1} with a solution mixture of 85:15 water (adjusted to pH 2.2 with phosphoric acid) and acetonitrile.

2.4. Sample preparation

Twenty tablets were finely ground in a mechanical grinder and a weighed portion equivalent to 100 μg biotin, was transferred into a 100 ml low actinic volumetric flask. About 60 ml of 1.5% phosphoric acid aqueous solution (v/v) was added to the flask and sonicated for 20 min using a water bath set at 50°C. The flask was shaken mechanically for 20 min. Fifteen milliliters of acetonitrile was pipetted into the flask, diluted to volume with 1.5% phosphoric acid aqueous solution and mixed. A portion of the sample was filtered through a 0.45 μm nylon filter membrane and injected.

2.5. Calibration

Aliquots of the standard stock solution of biotin were pipetted into different 100 ml low actinic volumetric flasks and diluted to mark with a solution mixture of 85:15 water (adjusted to pH 2.2 with phosphoric acid) and acetonitrile. The final concentrations of biotin were in the range of 0.53–3.20 μg ml^{-1}. Each solution was injected in duplicate. Peak areas were recorded for all the solutions.

Table 2
Results of assay of biotin in commercial multivitamin-multimineral tablets

Products[a]	Amount declared μg/tablet	Amount found μg/tablet
A	30	34.3
B	30	33.6
C	30	30.8
D	30	35.7

[a] Tablet from: (i) One a Day® 55 plus, Bayer Corporation; (ii) Advanced Formula Centrum, Lederle Consumer Health Division, American Cyanamid Company; (iii) Quintrem Advanced Formula, Thrift Drug Inc.; (iv) Theragran-M (Complete Formula with Antioxidants), Mead Johnson and Company

2.6. System suitability test

The system suitability was evaluated by making 6 replicate injections of standard preparation and recording peak responses. The system was deemed to be suitable for use if the coefficient of variation was $< 2.0\%$ and tailing factor was < 1.5.

2.7. Procedure

Six injections of standard preparation and 2 injections of sample preparation were chromatographed using an injection volume of 100 μl. The quantity of biotin per tablet was calculated by the following formula:

$$\mu cg/\text{tablet} = \frac{R_{sam}}{R_{std}} \times C \times F \times \frac{ATW}{W_{sam}} \quad (1)$$

where R_{sam} and R_{std} = average peak responses of sample preparation and standard preparation, respectively; C = concentration, μg ml^{-1}, of standard preparation; F = dilution factor; ATW = average tablet weight (mg); and W_{sam} = sample weight (mg).

3. Results and discussion

The proposed chromatographic method was assessed for specificity, linearity, precision, accuracy and stability.

3.1. Precision

The system precision was determined by chromatographing 6 injections of the standard solution and calculating the relative standard deviation (RSD) of the peak area responses. The method precision was established by assaying 6 replicates of authentic sample with the proposed chromatographic method. The RSD% for standard and samples were 1.01 and 0.83 respectively.

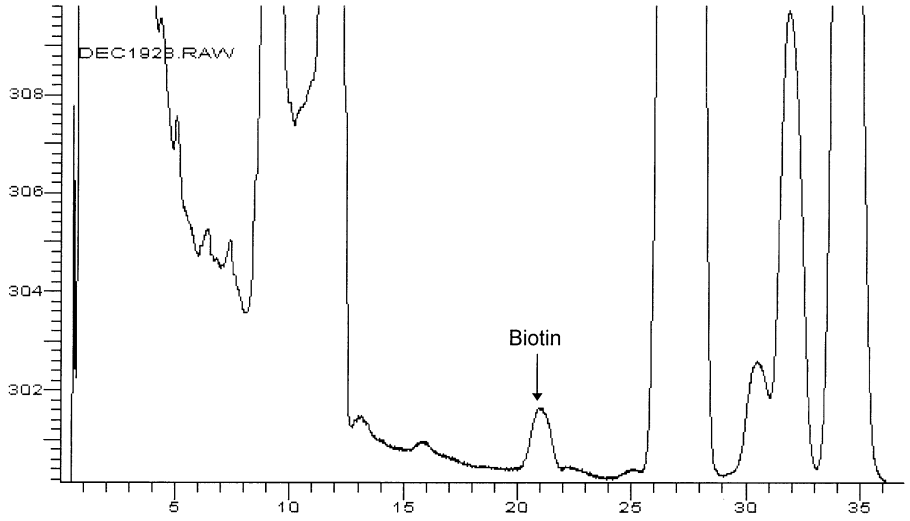

Fig. 2. Example chromatogram of sample stored at 40°C/75% relative humidity

3.2. Linearity of standard

Linearity of standard was determined by chromatographing 4 standard solutions spanning 50–200% of the expected working standard solution (1.0 μg ml^{-1}). Linear regression analysis of the peak area response (y) versus the theoretical concentration (x) gave the following equation: $y = 3092 + 51587x$, $r^2 = 1.0000$. The correlation coefficient demonstrated linearity of the method over the concentration range that was analyzed.

3.3. Accuracy

The recoveries of biotin from placebo were assessed by spiking placebo (containing 13 water-soluble vitamins, 4 fat soluble vitamins and 13 minerals) with 1% spray dried biotin. The spiking was done at 5 levels and in triplicates spanning 60–180% of biotin in the dosage form. The average recovery for the 5 levels was 100.9% with a coefficient of variation of 2.9%. Linear regression analysis of the average amount recovered (y) versus the average amount added (x) gave the following equation: $y = 3.25 + 0.98x$, with a correlation coefficient of 0.9986 (see Table 1).

Table 3
Results of stability sample

Condition/time	Amount declared μg/tablet	Percent initial
Initial	34.3	—
1 month stored @ 50°C	35.2	102.6
3 month stored @ 40°C/75% RH	32.8	95.6

3.4. Analysis of commercial pharmaceutical tablets

In order to establish the validity of the proposed HPLC method, 4 different brands of commercially available multivitamin-multimineral tablets were assayed in duplicate. The assay values are presented in Table 2. The difference between the amount declared and the amount found could be attributed to the fact that vitamins are usually formulated in excess of the declared amount.

3.5. Stability of sample

Sample that was stored at both 50°C for 1 month and at 40°C/75% relative humidity for 3 months was used to determine whether the pro-

posed HPLC method was able to separate any biotin degradation products/impurities. No extra peaks were observed in the vicinity of biotin retention time (Fig. 2). The stability of the sample solution at 20°C, 24 h after preparation was verified by re-assaying. There was no indication of any decomposition of biotin in the sample (Table 3).

3.6. Ruggedness and robustness

Preliminary experiments revealed that amongst the many operating parameters involved, the apparent pH of the sample was the most influential parameter on the repeatability of the method, when suitable precautions have been taken with regard to instrument set-up. The concentration of phosphoric acid (1.5%) in the extraction solution was enough to bring the apparent pH of the sample and standard to 2.2. The proposed HPLC method was used by analysts employing 2 different instruments to analyze same sample. The results showed no statistical differences between operators and instruments. The difference between the 2 analysts was 1.2%.

4. Conclusion

The described method was found to be rugged, linear, reproducible, specific, accurate and capable of separating biotin from its degradation products and excipients. The extraction of biotin from multivitamin-multimineral tablet is simple and biotin is stable in the extraction solution. The validated RP-HPLC has been utilized to determine biotin content in 4 brands of commercially available multivitamin-multimineral tablets.

Acknowledgements

The assistance of the product development division of Bayer Consumer Care in manufacturing the One A Day® tablets and Katie Ellis for her secretarial help are gratefully acknowledged.

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