ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 435–439

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Application of capillary zone electrophoresis for analyzing biotin in pharmaceutical formulations—a comparative study

J. Schiewe, S. Göbel, M. Schwarz, R. Neubert*

Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther-University, Weinbergweg 15, D-06120 Halle/Saale, Germany

Received for review 27 March 1995; revised manuscript received 20 July 1995

Abstract

The detection limit and reproducibility of capillary zone electrophoresis (CZE) measurement of biotin were compared to those of a spectrophotometric method and those of the determination of the sulphur content by combustion of the biotin sample followed by coulometric titration of the formed SO_2. Drug analysis showed that all three methods gave consistent results and were suitable for the determination of biotin. CZE was found to be the best method for the determination of pharmaceutical formulations containing biotin because of its high separation efficiency, short analysis time, ease of instrumentation and sample preconditioning, and good precision.

Keywords: Biotin analysis; Capillary zone electrophoresis; Spectophotometry; Determination of sulphur content; Drug analysis; Pharmaceutical formulations

1. Introduction

Biotin is one of the lesser known water-soluble vitamins of the B-group. Interest has been focused on biotin since it can be successfully applied for the treatment of skin conditions, as well as hair and nail defects. Therefore, pharmaceutical formulations containing 2.5 or 5.0 mg biotin, as a single drug component have been developed.

A survey of analytical methods for biotin was conducted by Friedrich [1]. Bioassays with biotin-requiring strains of bacteria or yeasts as indicators or protein-binding assay methods were used for quantification of biotin in biological media [2]. Other methods were also described such as potentiometry with ion-selective electrodes [3], polarography [4], TLC [5] and HPLC [6,7]. However, these methods have advantages and disadvantages. UV detection which is mainly employed for HPLC suffers from the low absorbance of biotin above 210 nm. This is a problem because most of the conventionally used solvents strongly absorb radiation themselves above 210 nm. Therefore, derivative and complex sample preparation is necessary in most cases for HPLC analysis.

* Corresponding author.

SSDI 0731-7085(95)01641-4

Recently, a method for biotin analysis in mixtures of multiple water-soluble vitamins by free CZE and UV detection at 200 nm was reported [8]. The aim of this study was to find suitable analytical methods for routine standardization of the content and the stability of biotin. This paper compares the detection limit and the reproducibility of CZE with those of the spectrophotometric method described by Shimada et al. [9] and those of the determination of the sulphur content by combustion of the biotin sample followed by coulometric titration of the formed SO_2 [10].

2. Experimental

2.1. Chemicals

For CZE, thiamine hydrochloride, nicotinamide, nicotinic acid of BioChemika grade and L-ascorbic acid, L-cysteine, and D-(+) biotin of analytical grade were obtained from Fluka (Buchs, Switzerland). Ethanol, HPLC grade (Laborchemie Apolda GmbH, Apolda, Germany) and 4-(dimethylamino)-cinnamaldehyde of analytical grade (Merck, Darmstadt, Germany) were used in the spectrophotometric measurements.

2.2. Capillary zone electrophoresis

A Hewlett-Packard (Waldbronn, Germany) ^{3D}CE system fitted with a 600(515) × 0.05 mm (total length (length to detector) × i.d.) fused silica capillary and an on-column diode-array detector (190–600 nm) was used for capillary zone electrophoresis. The capillary was preconditioned for 10 min with 1.0 M NaOH (Fluka) before the first run and then for 2 min with 0.1 M NaOH and 3 min with run buffer prior to each following run. The separation conditions were: −30 kV voltage (detection end); 200 mbar s pressure injection; 25°C capillary temperature; and 20 mM phosphate run buffer pH 8.0 (Fluka). The vitamin standards were dissolved in double distilled water to produce concentrations in the range 1–200 μg ml^{-1}. In the case of L-ascorbic acid 10 mM L-cysteine was added for stabilization. The solid preparation containing biotin was dissolved in double-distilled water and filtered. All samples and the buffer were filtered through a 0.2 μm syringe filter before injection. The buffer was degassed by ultrasound for at least 10 min.

2.3. Spectrophotometry

A Shimadzu UV-120-02 spectrophotometer (Duisburg, Germany) equipped with 9.97 mm lightpath glass cuvettes was employed for the spectrophotometric measurements. The biotin content of the samples was determined using the following procedure: 0.5 ml of the sample was diluted with 2 ml of ethanol, and 0.1 ml of the concentrated hydrochloric acid (Fluka) was added. The solution was then mixed with an ethanolic solution of 4-(dimethylamino)-cinnamaldehyde containing 4 g l^{-1} (for 1–10 μg ml^{-1} biotin) or 1 g l^{-1} (for 10–100 μg ml^{-1} biotin). The intensity of the developed red colour was measured at 531 nm within 20–25 min after the addition of the cinnamaldehyde.

2.4. Determination of sulphur content

Combustion of the sample dissolved in water was performed on a quartz furnace in an argon–oxygen (10:1, v/v) atmosphere at 1100°C and a flow rate of 250 ml min^{-1} (gases from Linde AG, Höllriegelskreuth, Germany). The combustion gases containing SO_2 and SO_3 were trapped on containers filled with γ-Al_2O_3 (Merck) at room temperature. These containers were then pyrolyzed in a pure argon atmosphere at 1100°C and a flowrate of 150 ml min^{-1} to desorb the trapped gases and to convert the formed SO_3 into SO_2 which then was passed into the detection cell. The sulphur content of the sample was determined by iodometric titration of the formed SO_2. The titrant, iodine, was generated electrochemically. The point of inflection was detected by amperometric detection using the square-wave polarization method described by Hahn et al. [11]. Coulometric titration was performed automatically with a compatible personal computer. The electrolyte in the titration cell was composed of a 9.078 g l^{-1} KH_2PO_4/11.876 g l^{-1} Na_2HPO_4 buffer (pH 6.8) and 0.1 M KI (all Fluka); the electrodes were made of Pt.

3. Methods and results

3.1. Capillary zone electrophoresis

CZE has the advantage of high separation efficiency, short analysis time, and ease of instrumentation and sample preconditioning compared to the other two methods. Fig. 1 shows the electropherogram of a standard mixture of biotin together with vitamin B_1 (thiamine), vitamin C (ascorbic acid); nicotinamide and nicotinic acid (niacine) [8].

A pH 8.0 phosphate run buffer was selected where biotin, ascorbic acid and nicotinic acid form anions where as thiamine occurs as a cation. The separtion is then based on the different migration velocities of the ions in the applied electric field. In that medium nicotinamide is a neutral species and cannot be separated from other neutral sample ingredients. The pH also influences the magnitude of the electro-osmotic flow that is directed towards the negative end of a fused silica capillary. For the simultaneous determination of cations and anions in free zone electrophoresis in a reasonable analysis time, it was necessary to force an electro-osmotic flow (EOF) towards the detector (negative end) whose magnitude exceeds the migration velocity of the analyte anions. Therefore, a phosphate buffer of pH 8.0 was selected where separation run time did not exceed

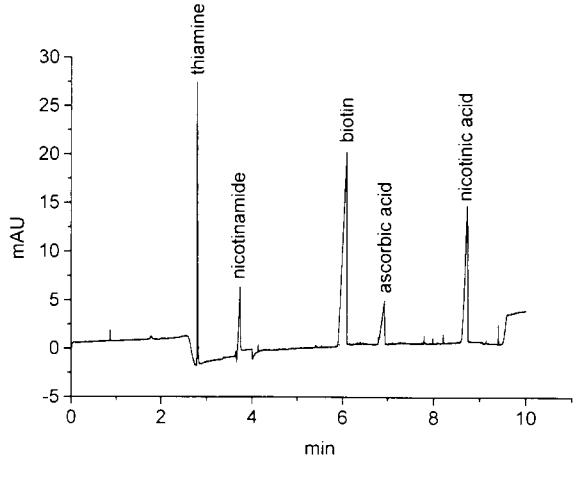
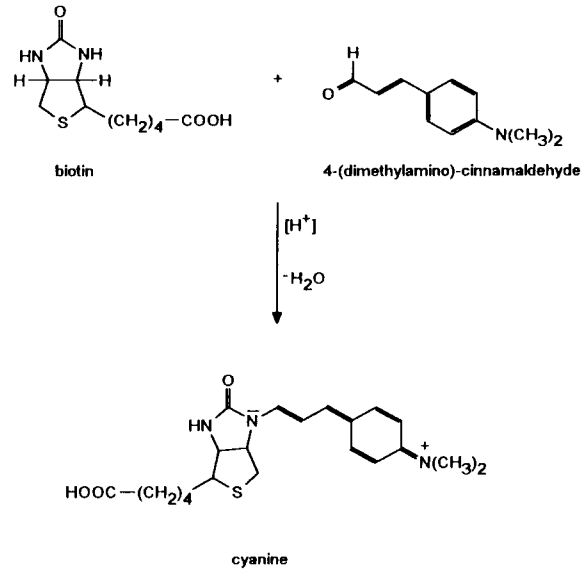


Fig. 1. Electropherogram of a mixture of five vitamins. Experimental details as given in text. Detector signal at 200 nm.



Scheme 1.

10 min and an optimal separation could be achieved.

Since the separation could be performed in an aqueous medium biotin could be detected by UV detection at 200 nm without further derivatization, which is a great advantage of this method. The lower detection limit was 3.37 μg ml^{-1} biotin and the linearity range was 3–200 μg ml^{-1} (correlation coefficient 0.998).

3.2. Spectrophotometry

The method is based on the reaction of the biotin amino-group with 4-(dimethylamino)-cinnamaldehyde in an acid medium to form a red cyanine with a λ_{max} value of 531 nm (Scheme 1). This reaction is stereospecific and invloves the less sterically hindered N atom of the ureido portion of the molecule. Addition of 4-(dimethylamino)-cinnamaldehyde to the sample dissolved in ethanol and hydrochloric acid immediately gives an intense red colour that fades slightly with time. The maximum absorbance was constant between 15 and 35 min (Fig. 2). Therefore, all spectrophotometric data were collected at 531 nm within 20–25 min after addition of the cinnamaldehyde.

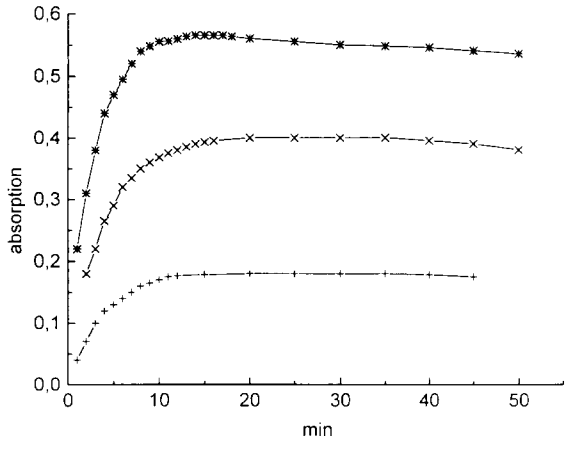


Fig. 2. Colour development at 531 nm. +, 160 μg ml^{-1} biotin; ×, 120 μg ml^{-1} biotin; *, 480 μg ml^{-1} biotin.

3.3. Determination of sulphur content

Biotin was quantified using oxidative methods for analyzing the total sulphur content in liquid and solid samples, from the field of environmental analysis. In the first furnace the dissolved sample is pyrolyzed in a mixture of argon and oxygen to form CO_2, SO_2, SO_3 and water. Argon is added to maintain combustion conditions. According to the thermodynamic equilibrium and the kinetics of sulphur oxide generation, a small amount of SO_3 is formed depending on the reaction temperature, reaction time and the partial pressure of oxygen (Scheme 2). This SO_3 must be reconverted into SO_2 prior to the detection. For this reason the sulphur oxides were trapped on γ-Al_2O_3 and afterwards desorbed at 1100°C in an oxygen-free atmosphere in the second furnace. In this way quantitative formation of SO_2 from the sample sulphur content could be maintained. The quantification of the formed SO_2 was performed by iodometric titration according to the Bunsen reaction (Scheme 3). Iodine is formed electrochemically from iodide. The electrolyte in the detection cell, therefore, consisted of sodium iodide and a phosphate buffer (pH 8.0) to maintain a low H_3O^+ concentration. The point of inflection was determined by amperometric detection. The amount of iodine necessary for the titration was calculated from the transferred charge using Faraday's law (Scheme 4). The coulometric titration is an accurate working method for the absolute determination of small sample amounts (parts per million to parts per billion range).

$$SO_2 + 1/2\ O_2 \longrightarrow SO_3 \quad \Delta H^\circ = -95.6\ kJ/mol$$

Scheme 2.

$$SO_2 + 6\ H_2O + I_2 \longrightarrow SO_4^{--} + 2\ I^- + 4\ H_3O^+$$

Scheme 3.

$$Q = i \cdot t = n \cdot F \cdot z$$

Scheme 4.

4. Results

Table 1 lists the calculated analytical parameters of all three methods. As represented by the standard deviations and the lower limits of detection, the method of derermination of the sulphur content permits the measurement of absolute values. Therefore, no calibration is necessary. In contrast, CZE and spectrophotometry are relative methods. CZE has the advantage of good reproducibility, but the limit of detection is rather high. The

Table 1
Analytical parameters, of the three methods

Method	Standard deviation (replicates)	Lower limit of detection
CZE	0.29 μg ml^{-1} ($n = 8$)	3.37 μg ml^{-1}
Spectrophotometry	1.8 μg ml^{-1}[a] 0.45 μg ml^{-1}[b] ($n = 8$)	4.1 μg ml^{-1}[a] 1.22 μg ml^{-1}[b]
Sulphur content	0.12 μg ($n = 5$)	0.75 μg[c]

[a] 1 g l^{-1} 4-(dimethylamino)-cinnamaldehyde (10–100 μg ml^{-1} biotin).
[b] 4 g l^{-1} 4-(dimethylamino)-cinnamaldehyde (1–10 μg ml^{-1} biotin).
[c] Three times the standard deviation of the zero value.

Table 2
Comparison of the results of biotin determination in Gabunat® by the three methods

Method	Result (mg)
CZE	4.63 ± 0.03[a]
	4.79 ± 0.03
Spectrophotometry	3.8 ± 0.3
	4.4 ± 0.3
Sulphur content	4.46 ± 0.11
	4.52 ± 0.08

[a] 95% Confidence intervals ($n = 5$).

biotin content of the pharmaceutical preparation Gabunat® (Wölfer GmbH, Bovenau, Germany) that contains the single drug was determined using all three methods (Table 2).

5. Summary

All three methods gave consistent results and are suitable for determination of biotin. CZE gave the best results and allows the analysis of biotin in complex sample matrices, because it is a separation method. In contrast, the spectrophotometric method is more sensitive but is less reproducible. Moreover, the quantification of unknown samples is affected by bathochromic or hypsochromic shifts depending on the sample matrix. The coulometric method is strictly limited by the fact that none of the other sample ingredients can contain sulphur; however, this technique has the advantage of being an absolute method. For these reasons, CZE is the best method for the determination of pharmaceutical formulations containing biotin.

Acknowledgement

The authors wish to thank the Deutsche Forschungsgemeinschaft for financial support of the project NE 427/4-1.

References

[1] W. Friedrich, Handbuch der Vitamine, Urban & Schwarzenberg, Munich, 1987.
[2] E.A. Bayer and M. Wilchek, Methods Biochem. Anal., 26 (1980) 1–45.
[3] C.R. Gebauer and G.A. Rechnitz, Anal. Biochem., 103 (1980) 280–284.
[4] A. Serna, J. Vera and D. Marin, J. Electroanal. Chem. Interfacial Electrochem., 45 (1973) 156–158.
[5] K. Gröningsson and L. Jansson, J. Pharm. Sci., 68 (1979) 364–366.
[6] P.-L. Desbene, S. Coustal and F. Frappier, Anal. Biochem., 128 (1983) 359–362.
[7] E. Röder, U. Engelbert and J. Troschütz, Fresenius' Z. Anal. Chem., 319 (1984) 426–427.
[8] J. Schiewe, Y. Mrestani and R. Neubert, Application and optimization of capillary zone electrophoresis in vitamin analysis, poster contribution, 7th Int. Symp. on HPCE, 29 January–2 February 1995, Würzburg, Germany.
[9] K. Shimada, Y. Naganese and U. Matsumoto, Yakugaku Zasshi, 89 (1969) 436–441.
[10] M. Schwarz, Diplomarbeit, Martin-Luther-Universität Halle, 1992.
[11] M. Hahn, H.H. Rüttinger, H. Matschiner and N. Lenk, Fresenius' Z. Anal. Chem., 340 (1991) 22–26.