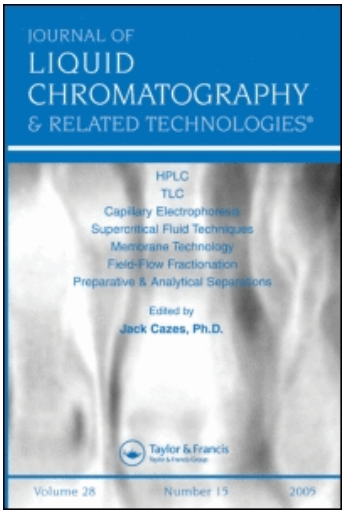

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Renata Gadzala-Kopciuch[a]; Michal Szumski[a]; Boguslaw Buszewski[a]
[a] Department of Environmental Chemistry and Ecoanalytics, Nicolas Copernicus University, Faculty of Chemistry, Torun, Poland

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Determination of Biotin in Pharmaceutical Preparation by Means of HPLC and/or MEKC

Renata Gadzala-Kopciuch, Michal Szumski, and Boguslaw Buszewski*

Department of Environmental Chemistry and Ecoanalytics,
Nicolas Copernicus University, Faculty of Chemistry,
Torun, Poland

ABSTRACT

This work describes the development of HPLC and micellar electrokinetic capillary chromatography (MEKC) methods, towards the selectivity for biotin analysis and its use for the analysis of pharmaceutical preparation (gel as a filling of a capsule). The isolation of biotin from this matrix was possible after dissolution of the gel from the capsules in hexane, and extraction of fat-soluble vitamins. The separation of the biotin extract by HPLC did not give good results. Therefore, separation of biotin from interfering substances was performed with high resolution by MEKC with diode-array detector (DAD). The optimization of composition of the buffer in MEKC was obtained for six different concentration of sodium

*Correspondence: Boguslaw Buszewski, Department of Environmental Chemistry and Ecoanalytics, Nicolas Copernicus University, Faculty of Chemistry, 7 Gagarin St., 87–100 Torun, Poland; E-mail: bbusz@chem.uni.torun.pl.

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dodecyl sulfate (SDS). The relative standard deviation (RSD) for migration time was 0.91% and for peak area the RSD was better than 2.5%. The detection limit ranged from 5 to 200 μg/mL. The limit of detection level for biotin was 0.15 μg/mL.

Key Words: Pharmaceutical analysis; Water soluble vitamins; Biotin; HPLC; MEKC; Liquid-liquid extraction.

INTRODUCTION

It is known that vitamins play a very important role in the development, growth, and healthy life of many organisms, including humans. At present, there are produced many pharmaceuticals containing vitamins as supporting media, as well as multivitamin products, which are very popular, to fulfil an organism's needs. Therefore, easy and reliable methods are required to determine vitamins during production steps and in the final products of food and pharmaceutical industries.[1]

In general, the vitamins are divided into fat and water-soluble. Water-soluble vitamins are usually determined using reversed-phase high performance liquid chromatography (RP-HPLC). As this group of vitamins is comprised of compounds characterized by different characters, from strongly ionic (e.g., B_1 or B_6) to hydrophobic (e.g., B_2 phosphate), it is not easy to determine them simultaneously.[1–5] Simultaneous determination of water- and fat-soluble vitamins has not been easy to be carried out so far. Usually, two separation runs have been needed—one for water-soluble species, the second one for fat-soluble compounds.[1,2]

For example, Dong et al.[3] applied ion-pairing technique for RP-HPLC analysis of water-soluble vitamins. They examined several alkylsulfonates (C_5–C_{10}) as ion-pairing media and other experimental parameters. The optimal parameters they reported were as follows: 4–7 mM 1-hexanesulfonate, pH = 2.8–3.2, 12.5–20% of methanol as an organic modifier, 1% acetic acid, 0.10–0.13% TEA (triethylamine), C_8 column was used and kept at 22–28°C.

Amin and Reusch[4,5] reported simultaneous separation of four B vitamins using 50/50 methanol/water mobile phase that had to be freshly prepared to provide good separation. Recently, Li and Chen[6] successfully separated nine water-soluble vitamins and 12 water- and fat-soluble vitamins in a single HPLC run. Wide ranges of linearity and low detection limits were found for all vitamins being separated. On the other hand the reported method of extraction of the vitamins from multivitamin tablets was characterized by high recovery

rates (>94%). However, the disadvantage of the method seems to be the long time of the analysis (ca. 40 min), despite the gradient elution was used.[7]

In capillary zone electrophoresis (CZE) charged species are separated due to the differences in their electrophoretic mobilities. Moreover, due to the presence of the electroosmotic flow in the capillary it is possible to separate positively and negatively charged compounds in a single run. Neutral species are not separated in such a system. In 1984 Terabe[8] introduced a mode of capillary electrophoresis—micellar electrokinetic capillary chromatography (MEKC), which is a technique in which the micelles formed in a buffer play a role of pseudo-stationary hydrophobic phase and, thus, allow separating hydrophobic compounds on the basis of their interactions with hydrophobic centers of the micelle. Simultaneously, the charged compounds can be separated on the basis of the electrophoresis. Using this technique, Nishi et al.[9] demonstrated a separation of water-soluble vitamins using 50 μm i.d. capillary and 20 mM phosphate–borate buffer (pH = 9.0) containing 50 mM of sodium dodecyl sulfate (SDS).

One of the water-soluble vitamins is biotin (vitamin H, hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-pentanoic acid; Fig. 1) classified as one of B-complexes, which is found in small quantities in all living cells. Biotin, as a coenzyme component, takes part in decarboxylation and deamination processes.[7]

This work presents the results of determination of biotin in a novel multivitamin preparation (containing biotin on ppm level) by HPLC and MEKC techniques. The determination of biotin in pharmaceutical products was difficult, taking into account small amounts of this vitamin in preparations in comparison to other vitamins (in some cases biotin can be masked by, for example, vitamin C during separation), as well as problems with UV-Vis spectrophotometric detection. Moreover, a novel multivitamin product has a gel-like consistence, which demanded elaboration of methods of sample preparation of the choice of suitable solvents for removal of matrix and extraction of fat-soluble vitamins. The preparation that was obtained from the pharmaceutical company in Poland has not been available on the market yet

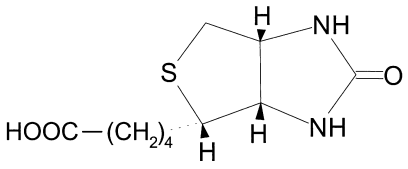

Figure 1. Structural formulae of biotin.

(development a new product), and was given to us for comparative determination of biotin. Therefore, we do not refer to certified amounts of the analyte in the preparation in comparison of the results with the certified values.

EXPERIMENTAL

Chemicals

Hexane, sodium carbonate, sodium dihydrogen phosphate, disodium hydrogen phosphate were analytical grade, methanol was HPLC grade; these chemicals were purchased from Merck (Darmstadt, Germany). Sodium tetraborate, SDS, biotin standard, and the multivitamin preparation were kindly gifted by Hasco-Lek (Wroclaw, Poland). Water, purified with a Milli-Q (Bedford MA, USA) purification system, was used for the preparation of mobile phases, buffers, and solutions.

Sample Preparation

Two g of the multivitamin preparation (which was a "gel" obtained after covering of capsule was removed) was put into a 200 mL beaker, 50 mL of hexane was added, and sonicated for 10 min. Fifty milliliter of phosphate buffer (36.4 g of $NaH_2PO_4 \cdot 2H_2O$ and 5.0 g of KH_2PO_4 dissolved in water and filled up to 1000 mL) was added and the mixture was sonicated for 5 min further. Then, the content of the beaker was transferred to the extraction funnel and shaken for 10 min. After that, the aqueous phase was collected in a trial tube and the hexane phase was extracted for 10 min with another portion (50 mL) of the buffer. The obtained two water extracts were combined and centrifuged at 4000 r.p.m. for 15 min. Then, the extract was passed through the filtration column (obtained from S. Witko—J.T. Baker, Lódz, Poland), collected in a 100 mL volumetric flask, filled up to the mark, and subjected to the analysis.

The standard solution was prepared by weighing 0.7240 g of biotin and dissolving it in a mobile phase (see below) in a 25 mL volumetric flask.

HPLC and MEKC Instrumentation

Liquid chromatographic separations were performed using a Shimadzu HPLC system equipped with a diode-array detector (DAD). A Supelco

(Bellefonte, USA) LC-18-DB (250 × 4.6 mm, $d_p = 5\,\mu m$) column was used. A mobile phase was a composition of phosphate buffer (pH = 2.5) and methanol in an 85/15 ratio. A buffer was prepared by dissolution of 2.76 g of $NaH_2PO_4 \cdot 2H_2O$ in 500 mL of water, adjusting it to pH = 2.5 with 85% phosphoric acid, and filling to 1000 mL in a volumetric flask. The flow rate of the mobile phase was 1.5 mL/min. Detection was performed at $\lambda = 200$ nm (Table 1).

Micellar electrokinetic capillary chromatography separations were performed using HP^{3D}Electrophoresis (Agilent Technologies, Waldbronn, Germany) system equipped with DAD and ChemStation software for instrument control and data collection. A 50 μm i.d. fused silica capillary was purchased from Composite Metal Services (Worcester, Great Britain). The capillary was prepared as follows: a 64.5 cm long piece was cut and a ca. 2–3 mm of polyimide coating was burned out at both ends. A detection window was created at 8.5 cm distance from the outlet end, also by gentle burning out several millimetres of the coating, and wiped with a tissue wetted with isopropanol. Then, the capillary, placed in the cassette, was put into the instrument and pretreated in the following way: 10 min of flushing with 0.1 M NaOH, than left for 5 min, rinsed with water for 20 min, and flushed with a running buffer for 10 min. Before each run was started, the capillary was flushed and refilled with fresh buffer to remove any residual. The running buffer was 20 mM sodium tetraborate pH = 9.4, containing 100 mM SDS. The sample was injected hydrodynamically 15 mbar × 20 s. The separations were performed at the voltage of 30 kV and at the temperature of 20°C. During the MEKC experiments, the detection wavelength was $\lambda = 198$ nm (Table 1).

Table 1. High performance liquid chromatographic and micellar selectrokinetic capillary chromatogarphic conditions of determination of biotin.

	HPLC	MEKC
Mobile phase	15/85 Methanol/phosphate buffer pH = 2.5	20 mM borate buffer pH = 9.4 + 100 mM SDS
Column	Supelcosil LC-18-DB (250 × 4.6 mm, $d_p = 5\,\mu m$)	Fused silica capillary, $L_{tot} = 64.5$ cm, $L_{eff} = 56$ cm, I.D. = 50 μm
Flow rate/voltage	1.5 mL/min	30 kV
Injection	20 μL sample loop	Hydrodynamic 15 mbar × 20 s
Detection	$\lambda = 200$ nm	$\lambda = 198$ nm
Temperature	Ambient (20–23°C)	20°C

RESULTS AND DISCUSSION

The UV spectrum of biotin presented in Fig. 2 clearly shows that in order to obtain a high signal to noise ratio, the detection should be performed at as low a wavelength as is possible. Hence, the detection was performed at $\lambda = 200$ nm during HPLC experiments. Detection at wavelengths below $\lambda = 200$ nm resulted in a large noise, due to the presence of methanol in a mobile phase (cut off at $\lambda = 205$ nm). On the contrary, during the MEKC separations, where no organic modifier was added to the running buffer, this problem did not occur, which allowed detecting biotin at $\lambda = 198$ nm. For example, the peak heights obtained at $\lambda = 210$, 205, and 198 nm were 17.6, 24.8, and 30.3 mAU, respectively.

The optimization of the mobile phase composition for HPLC (multicomponent hydro-organic buffer) and/or MEKC (buffer with SDS addition) was performed using the biotin standard solution. The effect of SDS concentration on the migration time is presented in Fig. 3. The migration time of biotin rises slowly with the increasing of SDS concentration, however, this parameter had a great influence on the resolution, yielding very good separation of biotin from the rest of peaks at the SDS concentration of 100 mM.

The example chromatograms obtained during HPLC and MEKC analyses are presented in Figs. 4, 5. It is clearly seen, that MEKC determination of biotin is much faster than HPLC, where the retention time is 20 min. (Fig. 4a, b). This fact is attributed to the complication of the matrix from

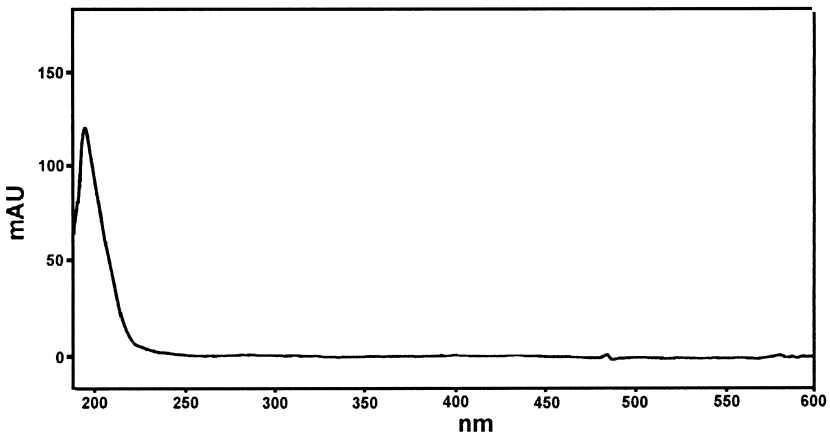

Figure 2. UV-VIS spectrum of biotin in eluent.

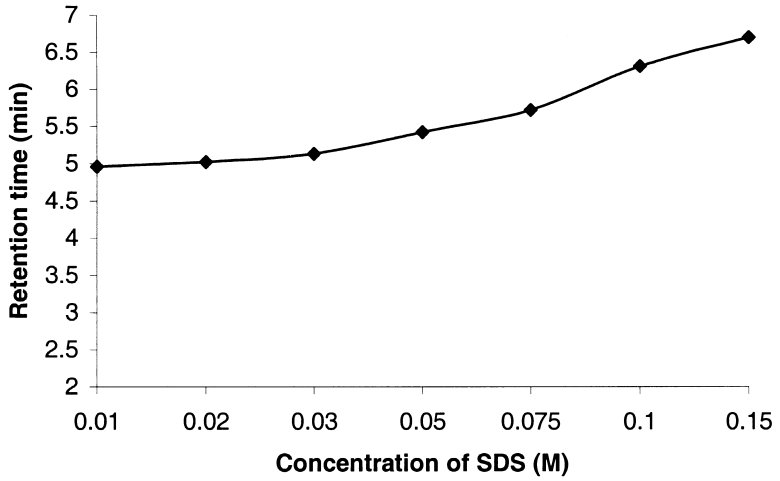

Figure 3. Effect of SDS concentrations on the retention time of biotin.

which the biotin was extracted and to the presence of many compounds. Li and Chen[6–7] demonstrated simple extraction (with ethanol and phosphate buffer) of the vitamins from tablets followed by filtration. The obtained chromatograms did not show any impurities. In our case, the sample that was a gel (or a pulp) placed in a capsule, gave a strong matrix effect easily seen on HPLC chromatograms. In MEKC, the addition of 100 mM of SDS gave the peak of biotin well separated from the other compounds (Fig. 5a, b). In our case, the high concentration of vitamin C in the pharmaceutical preparation can have a great influence on the HPLC separation and further quantitative interpretation of obtained results. Changing the buffer in the capillary after each analysis resulted in very reproducible parameters of the biotin peak (RSD of migration time 0.91%, RSD of peak area 2.5% for $n = 10$). The conditions of the HPLC and MEKC analyses are compared in Table 1.

As it is shown in Table 2, the retention and/or migration time were very reproducible, as well as, the linearity of calibration curves were obtained with the correlation coefficients $R^2 = 0.9997$ for HPLC and $R^2 = 0.9986$ for MEKC. The detection limits for both methods were similar (ca. 0.2 μg/mL).

The determination of biotin in a complicated multivitamin preparation can be easily done by HPLC and/or MEKC methods. However, the latter

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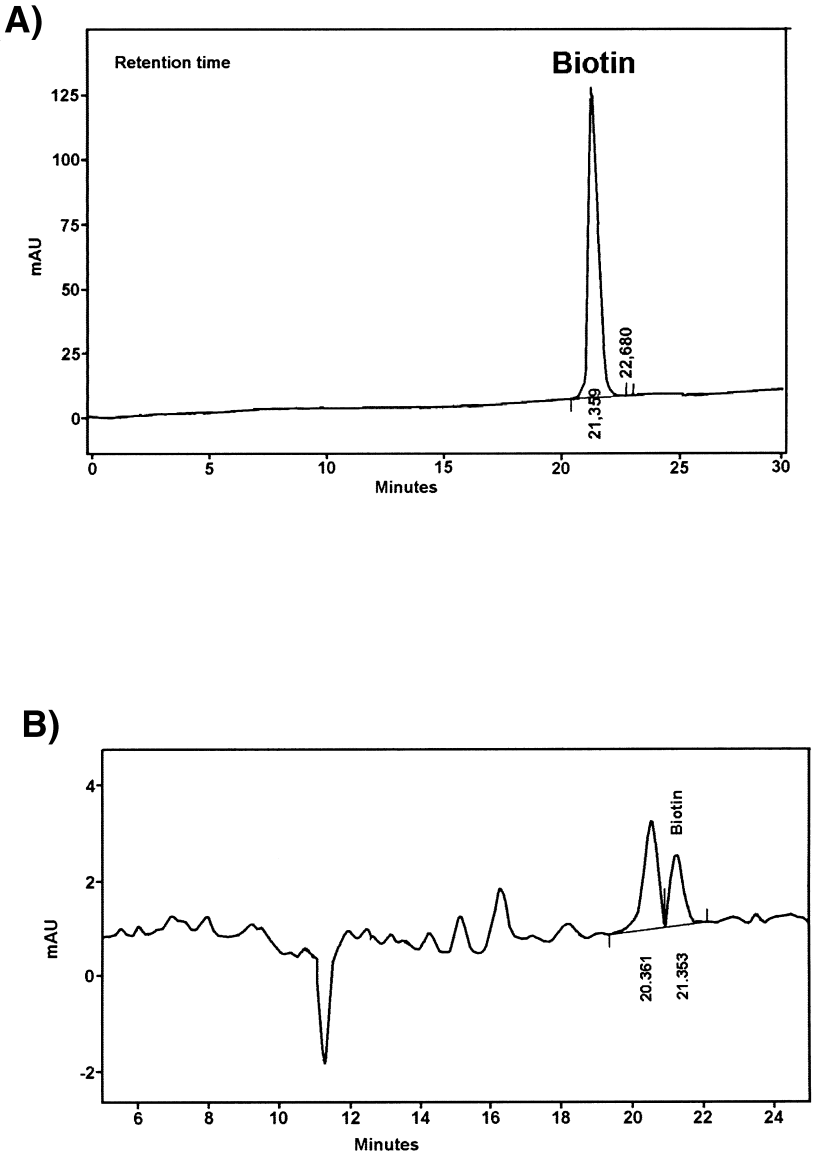

Figure 4. Chromatograms of biotin standard (A), and biotin extracted from multivitamin preparation (B).

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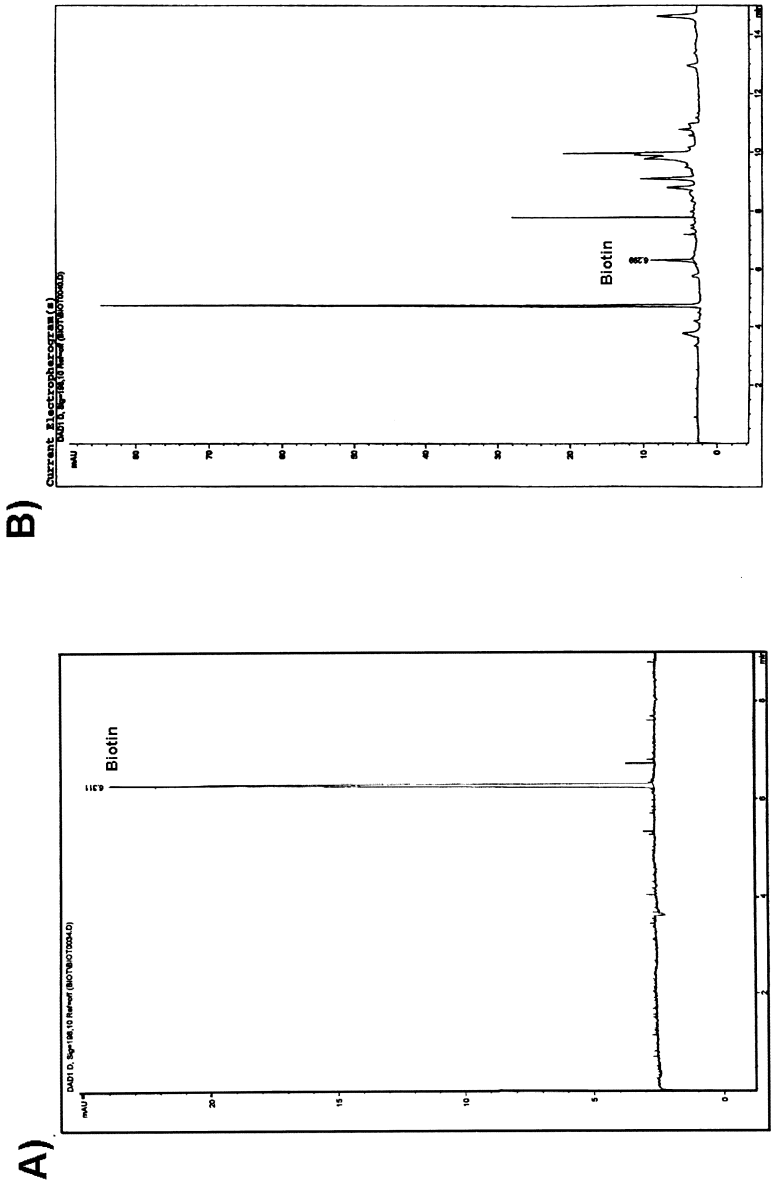

Figure 5. Electropherograms of biotin standard (A), and biotin extracted from multivitamin preparation (B).

Table 2. Linearity, detection limits, retention and migration time for the standard biotin determined by HPLC and MEKC.

Type of method	Retention/migration time (min)	R^2	Linearity range (μg/mL)	Detection limit (μg/mL)
HPLC	21.359	0.9997	10–200	0.20
MEKC	6.311	0.9986	5–200	0.15

technique is much faster, and the cost of single analysis is much lower in comparison to HPLC.

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