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Note

Determination of biotin in multivitamin pharmaceutical preparations by high-performance liquid chromatography with electrochemical detection

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Biotin (vitamin H, coenzyme R) is widely distributed in animals and plants, and was first isolated from egg yolk[1]. It is one of the most active biological substances known, being a coenzyme essential in amino acid metabolism and in the maintenance of skin, hair and nerves, with an important rôle in growth, decarboxylation of amino acids and carbohydrate metabolism.

Microbiological methods are generally used to determine small amounts of biotin[2–7]. However, these methods require an incubation period of 18–24 h in addition to the sample preparation. The chemical methods reported comprise spectrophotometric assays based on the binding of a dye with avidin[8–10], oxidation with potassium iodate[11] or reaction with 4-dimethylaminocinnamaldehyde[12,13]. None is applicable to multivitamin pharmaceutical preparations because of a lack of sensitivity, or due to interferences from other water-soluble vitamins. Several gas chromatographic procedures have been reported[14,15], but all involve volatilizing biotin by silation. Therefore, other methods for the determination of biotin in multivitamin pharmaceutical preparations are needed.

It has recently been demonstrated that high-performance liquid chromatography (HPLC) is suitable for the analysis of many drugs, and numerous applications of this technique to multivitamin pharmaceutical preparations have been reported[16–21]. Biotin is difficult to determine by conventional HPLC techniques, because it does not have an adequate UV chromophore. It must be measured at a low UV wavelength when direct UV detection is applied. Thomas *et al.*[22] described a procedure for the quantitative analysis of biotin in pharmaceutical products by using a reversed-phase column, and an UV monitor operating at 230 nm. Kanazawa *et al.*[23] described a method for determining biotin as its 9-anthryl ester by HPLC with fluorometric detection.

We have applied HPLC with electrochemical detection (ED) to the determination of biotin in multivitamin pharmaceutical preparations, and describe here some factors affecting the analysis.

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EXPERIMENTAL

Chemicals

Biotin (Wako), acetonitrile (HPLC grade; Wako), ethanol (95%, v/v; Wako), potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid (analytical reagent grade; Wako) and deionized water were used.

Instrumentation

The HPLC apparatus consisted of a JASCO pump Model BIP-I (Japan Spectroscopic, Tokyo, Japan), a Rheodyne injector Model 7125 equipped with a 20-μl loop (Rheodyne, CA, U.S.A.) and an electrochemical detector Model VMD-101A (Yanagimoto, Kyoto, Japan). The potential of the detector was set at +1.4 V *versus* a silver–silver chloride reference electrode. Prepacked LiChrosorb RP-18 Hiber (7 μm, 250 × 4.6 mm I.D., Cica Merck) was used under ambient conditions. Acetonitrile–0.05 *M* KH_2PO_4 (adjusted to pH 2.0 with phosphoric acid) (15:85) was used as a mobile phase at a flow-rate of 1 ml/min. It was filtered through a Millipore membrane filter (0.45 μm; Millipore, Bedford, MA, U.S.A.) and degassed under vacuum prior to use. Samples of 5 μl were injected. The results were evaluated by the Shimadzu Chromatopac C-R1A digital integrator. The ultrasonic bath was from Kyoto Daichi Kagaku (Kyoto, Japan).

Sample preparation

A representative number (usually 20) of tablets were accurately weighed and finely powdered so as to pass through a 60-mesh sieve. A suitable amount of the powder was placed in a 50-ml volumetric flask. The final biotin concentration was *ca.* 50 μg/ml. Approximately 30 ml of 0.05 *M* KH_2PO_4 (pH 2) were added and the flask was placed in an ultrasonic bath for 10 min. After cooling to room temperature, the contents were diluted to the volume with 0.05 *M* KH_2PO_4 (pH 2). A portion of the sample solution was filtered through a 0.45-μm filter, discarding the first 10 ml of filtrate. A 5-μl volume of the filtrate was injected for HPLC.

RESULTS AND DISCUSSION

The relationship between the applied potential and sensitivity was examined first by changing the potential from +0.9 to +1.6 V *versus* the silver–silver chloride reference electrode, other conditions being held constant. The results are shown in Fig. 1. The sensitivity of the detector increased with increasing potential between +0.9 and +1.4 V, but was almost constant in the range +1.4 to +1.6 V. The large background current arising at high detector potentials led to baseline drift and higher noise. From these results, the potential was chosen a +1.4 V *versus* silver–silver chloride reference electrode.

The effects of the mobile phase pH and the concentration of KH_2PO_4 on the sensitivity were then examined. As is seen from Figs. 2 and 3, the sensitivity of the detector increased with decreasing mobile phase pH, and with increasing concentration of KH_2PO_4 between 0.005 and 0.05 *M*. The sensitivity was almost constant in the range 0.05–0.1 *M* KH_2PO_4. Therefore, pH 2.0 and 0.05 *M* KH_2PO_4 were adopted.

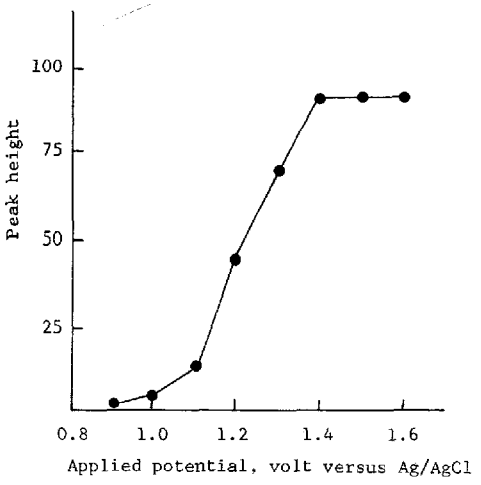

Fig. 1. Dependence of the electrochemical detector response upon the applied potential. Mobile phase: 15% acetonitrile–0.05 *M* KH_2PO_4 (pH 2.0); flow-rate, 1.0 ml/min.

The solvent had a very strong influence on the HPLC separation. Various mixtures of acetonitrile and 0.05 *M* KH_2PO_4 (pH 2.0) were used on the LiChrosorb RP-18 column. For sufficiently rapid elution a content of about 15% acetonitrile was needed. At lower acetonitrile percentages the retention time was increased.

The effects of the mobile phase pH and the concentration of KH_2PO_4 on the retention time are shown in Table I. The retention time decreased with increasing pH, and with increasing concentration of KH_2PO_4.

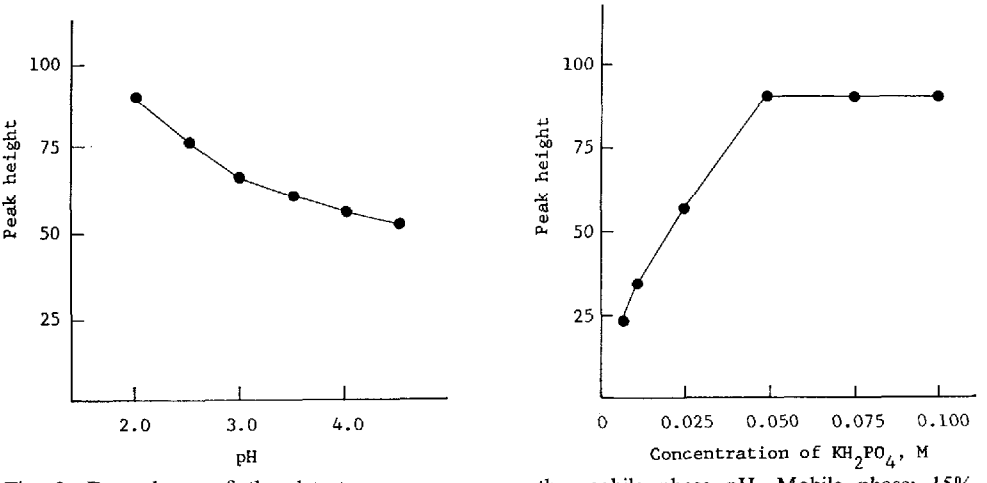

Fig. 2. Dependence of the detector response upon the mobile phase pH. Mobile phase: 15% acetonitrile–0.05 *M* KH_2PO_4, adjusted to the appropriate pH by titration with H_3PO_4; flow-rate, 1.0 ml/min. Electrode potential: +1.4 V *vs.* Ag/AgCl.

Fig. 3. Dependence of the detector response upon mobile phase ionic strength. Mobile phase: various concentrations of KH_2PO_4 (pH 2.0) with 15% acetonitrile; flow-rate, 1.0 ml/min. Electrode potential: +1.4 V *vs.* Ag/AgCl.

TABLE I

EFFECTS OF MOBILE PHASE pH AND CONCENTRATION OF KH_2PO_4 ON THE RETENTION TIME OF BIOTIN

Content of acetonitrile is 15%.

pH	*Concentration of KH_2PO_4 (M)*	*Retention time (min)*
2	0.01	9.60
2	0.05	8.92
2	0.10	8.57
3	0.05	8.23
4	0.05	7.27

The electrochemical detector was found to be more sensitive than an UV detector operating at 230 nm by at least a factor of ten. The limits of detectability for biotin range between 5 and 10 ng, based on twice the noise level. The linearity of the detector response to biotin was tested by injecting 5 μl of 5–50 μg/ml solutions of biotin. The plot of the peak height *versus* the amount of biotin was linear (correlation coefficient, 0.998). The precision of the chromatographic system was tested by five injections of the biotin: a standard deviation of 0.45% for 50 μg of biotin is typical. Thus this method can be used for quantitation.

Studies were conducted on the separation of six water-soluble vitamins which are widely used in multivitamin pharmaceutical preparations. The chromatographic behaviour of these compounds is summarized in Table II.

Thomas *et al.*[22] suggested that the HPLC determination of biotin in pharmaceutical products by direct injection without clean-up was difficult, because the coexisting riboflavin interfered with the determination. Our method was free from interference by riboflavin.

In order to investigate its applicability, the present method was applied to some multivitamin pharmaceutical preparations. A typical chromatographic separation of biotin is shown in Fig. 4. The chromatograms of all the other samples also showed a sharp peak for biotin with no interference from other substances. The

TABLE II

CHROMATOGRAPHIC BEHAVIOUR OF BIOTIN AND SOME WATER-SOLUBLE VITAMINS

Compound	*Retention time (min)*	
	ED	*UV 254 nm*
Biotin	8.92	8.89
Calcium pantothenate	–	3.24
Riboflavin	–	7.22
Niacinamide	–	2.52
Pyridoxine hydrochloride	2.68	2.57
Ascorbic acid	2.47	2.46
Thiamine hydrochloride	–	2.77

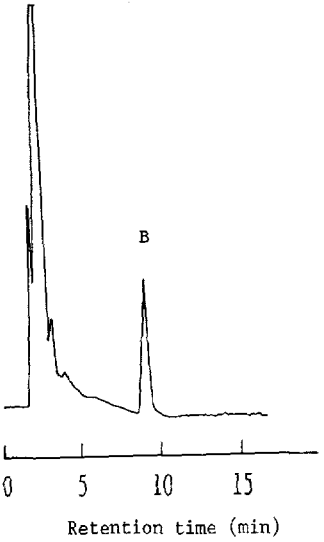

Fig. 4. Chromatogram of a multivitamin tablet extract. Peak B is biotin. Conditions as in the text.

results indicate that this method is suitable for the determination of biotin in multivitamin pharmaceutical preparations.

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