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Note

Direct determination of D-panthenol in pharmaceutical preparations by ion-pair chromatography

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D-Panthenol, 2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl butanamide, is a constituent of numerous liquid multivitamin preparations, such as drops or ampuled solutions. D-Panthenol has as much biological activity as pantothenic acid and is also more stable, especially in aqueous solution. Pantothenic acid is a constituent of coenzyme A, which is known to have an important role in the metabolism of carbohydrates, fats and nitrogen compounds.

The structure of D-panthenol is shown in Fig. 1. Only the dextrogyre forms are physiologically active (D-panthenol, calcium D-pantothenate, sodium D-pantothenate).

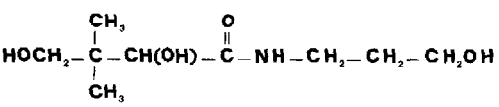

Fig. 1. Structure of D-panthenol.

Owing to its chemical structure, traditional methods almost require cleavage of the molecule by acid or alkaline hydrolysis. The cleavage products can be determined either by microbiological assay (*Acetobacter suboxydans*)[1] or by chemical assay using colour reactions (1,2-naphthoquinone-4-sulphonate[2], hydroxylamine[3], concentrated sulphuric acid[4], ninhydrin[5] or iodine[6]).

To improve specificity of the assays, sample pretreatment using chromatographic methods have been employed. Thin-layer chromatography has been used to separate D-panthenol in multivitamin preparations; the compound was decomposed by heating and detected with ninhydrin[7]. Ester and silylated derivatives of panthenol have been determined by gas–liquid chromatography in pharmaceutical preparations[8]. The system requires derivatization of D-panthenol. More recently, liquid chromatographic determination of D-panthenol in bulk and pharmaceutical preparations has been described[9]. The method was tedious, involving acid hydrolysis of D-panthenol, derivatization of the products with fluorescamine, and chromatographic assay.

This paper describes a simple, rapid, and very selective technique for the direct

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determination of D-panthenol in multivitamin preparations. Pharmaceutical samples were diluted to the working standard solution, then injected for panthenol assay by ion-pair chromatography with linear gradient elution.

MATERIALS AND METHODS

Apparatus

A Hewlett-Packard 1084B liquid chromatograph equipped with a Hewlett-Packard variable-wavelength UV detector was used. The column)stainless steel, 250 × 4.6 mm I.D.), packed with LiChrosorb RP-18 (particle size 10 μm) (Merck, Darmstadt, F.R.G.), was obtained from Knauer (Berlin, F.R.G.). A guard column (30 × 4.6 mm I.D.) (Brownlee, Santa Clara, CA, U.S.A.) packed with LiChrosorb RP-18 (10 μm) preceded the analytical column.

Reagents and standard

Methanol (HPLC grade S) was purchased from Rathburn(Walkerburn, U.K.). Hexanesulphonic acid sodium salt was obtained from Eastman Kodak (Rochester, NY, U.S.A.). Ammonia solution (25%) and phosphoric acid (85%) from Merck were reagent grade. Potassium dihydrogen phosphate was obtained from Fluka (Buchs, Switzerland). Doubly distilled, deionized water was used to prepare all solutions.

D-Panthenol (Fluka) was dried for 4 h at 60°C over phosphorus pentoxide. A standard solution of 0.3 g/l in water was used.

Mobile phase A was prepared by dissolving 0.95 g of hexanesulphonic acid in water, adding 0.4 ml of ammonium hydroxide and adjusting the pH to 2.7 with 85% phosphoric acid. The solution was diluted to 1 l with water and filtered through a 0.45-μm Millipore filter (Bedford, MA, U.S.A.).

Mobile phase B was prepared by dissolving 0.95 g of hexanesulphonic acid in methanol, adding 0.4 ml of ammonium hydroxide and 0.4 ml of 85% phosphoric

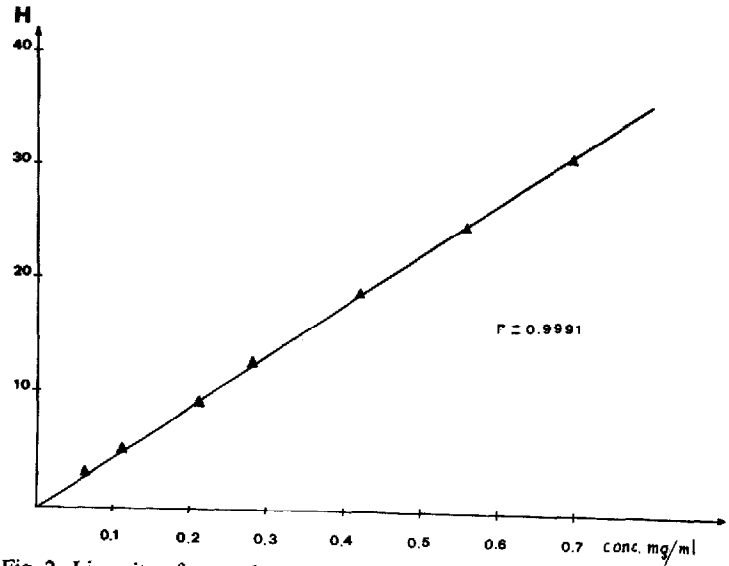

Fig. 2. Linearity of D-panthenol content with peak height (*H*) measured with UV at 230 nm.

acid. The solution was diluted to 1 l with methanol and filtered through a 0.45-μm Millipore filter.

Sample preparation

Liquid multivitamin preparations were diluted with water to yield a nominal 0.3 mg/l. The mixture was filtered through a 0.45-μm Millipore filter. A 30-μl portion of this solution was injected.

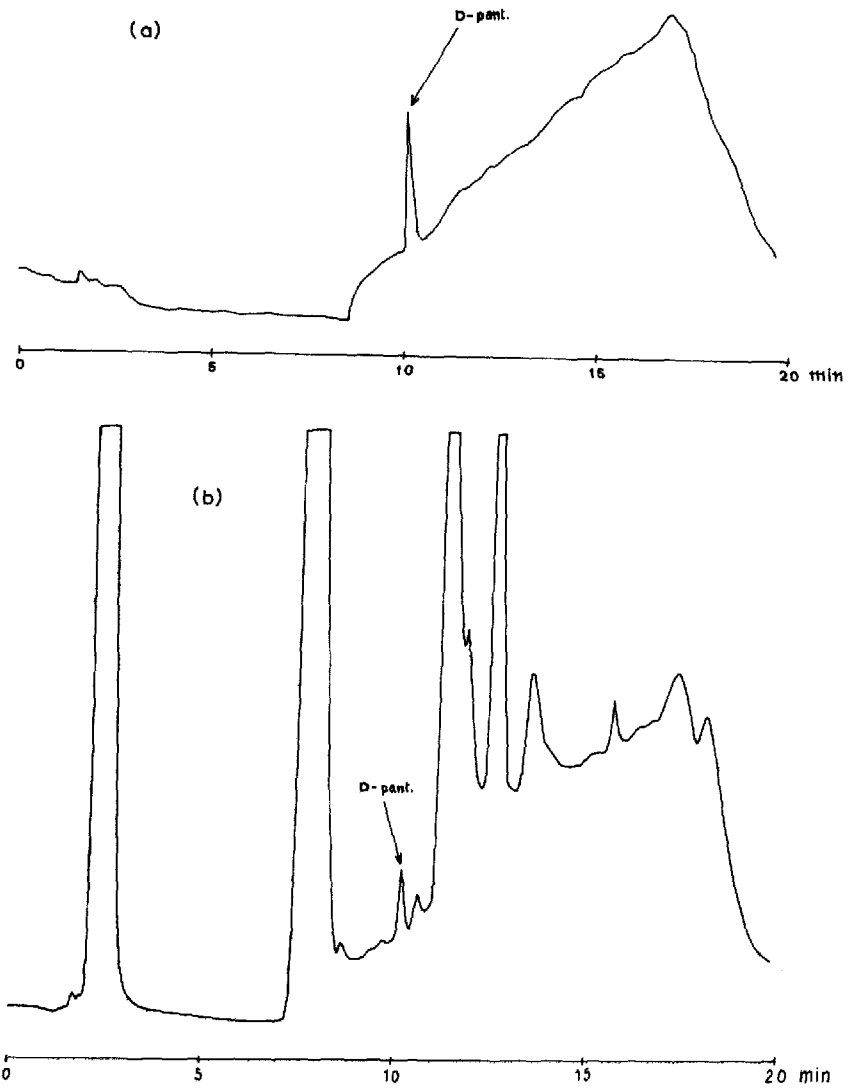

Fig. 3. Chromatograms of (a) D-panthenol reference compound and (b) D-panthenol in a multivitamin syrup. For chromatographic conditions and mobile phase gradient see Materials and methods. D-pant. = D-panthenol.

Chromatographic conditions

A linear mobile phase gradient was used for the separation of D-panthenol in multivitamin preparations. The system was equilibrated with mobile phase A. After injection, mobile phase A was maintained for 2 min, before mobile phase B was introduced and varied linearly from 0% to 40% in 15 min, then from 40% to 0% in 2 min. The flow-rate was 2 ml/min. The oven temperature was set at 40°C. The absorbance at 230 nm was monitored at a chart speed of 1 cm/min.

RESULTS AND DISCUSSION

The variation in the retention time of D-panthenol over twenty injections during the same day was less than 1%. Using the same chromatographic conditions on different days, these variations were found to be less than 5%.

Quantification was based on peak heights using external standard method. A linear response of the UV detector was observed from 2 to 20 μg of panthenol (Fig. 2). The lower detection limit was *ca.* 300 ng.

A chromatogram of D-panthenol reference compound, and that of a multivitamin preparation, are presented in Fig. 3. With an identical linear mobile phase gradient, but without the ion-pair reagent, the capacity factor (k') of D-panthenol remains virtually unchanged, but D-panthenol was not separated from other water-soluble vitamin compounds in the multivitamin preparation. Hexanesulphonic acid as pairing ion is a main factor in increasing the selectivity of other water-soluble vitamin compounds, and consequently of D-panthenol too. Identification was based on retention behaviour and cochromatography with the reference compound.

Four pharmaceutical preparations from different manufacturers were analysed by this procedure. The results obtained by ion-pair chromatography were in close agreement with the theoretical amount of panthenol claimed in the various samples. They were also comparable with those obtained by colorimetric and microbiological assays (Table I).

TABLE I

ANALYSES OF D-PANTHENOL-CONTAINING SAMPLES

Sample	*Theoretical*	*HPLC, found*	*Colorimetric determination**	*Microbiological assay***
"Multivitamin" drops	1.5 mg/ml	1.45 mg/ml	1.40 mg/ml	1.47 mg/ml
"Kiddi" multivit. syrup	10 mg/15 ml	11.1 mg/15 ml	10.7 mg/15 ml	11.5 mg/15 ml
"Becozyme" 2 ml vials	6 mg/2 ml	6.3 mg/2 ml	5.9 mg/2 ml	
"becozyme" drops	3 mg/ml	2.8 mg/ml	2.7 mg/ml	
"Alvityl" multivit. syrup	43 mg/100 ml	48.0/100 ml	45.5 mg/100 ml	
"Multisanasol" syrup	0.03 g/100 ml	0.032 g/100 ml	0.027 g/100 ml	0.029 mg/100 ml

* Colorimetric determination with 4-dimethylaminobenzaldehyde.
** Microbiological assay with *Acetobacter Suboxydans* ATCC 621 H.

Recovery of D-panthenol from "spiked" pharmaceutical preparations was 99% (Table II).

The direct measurement of D-panthenol by ion-pair chromatography is suitable for liquid multivitamin preparations containing from 0.3 to 1.5 mg/ml of D-panthenol. The detection limit was found to be 300 ng injected (*i.e.* 30 μl of a solution of 10 μg/ml).

TABLE II

RECOVERY OF D-PANTHENOL FROM "SPIKED" PHARMACEUTICAL PREPARATIONS

Sample	*Found (mg/ml)*	*Final dilution (mg/ml)*	*Added (mg/ml)*	*Recovered (mg/ml)*	*Recovered (%)*
"Becozyme" 2-ml vials	3.18	0.32	0.25	0.56	98
"Becozyme" drops	2.84	0.28	0.25	0.53	100
"Alvityl" multivitamin drops	0.48	0.24	0.25	0.50	102

Although less sensitive than the microbiological assay, the method described here is rapid, selective, and less time-consuming than chemical technique, gas chromatography or liquid chromatography. Because of its rapidity, it is particularly interesting for control analyses.

A very high column efficiency is required for the separation. A daily clean-up of the column with water (50 ml), followed by methanol (50 ml), is recommended.

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