Journal of Chromatography, 161 (1978) 299–302

CHROM. 11,233

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF VARIOUS TOCOPHEROLS

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(Received April 11th, 1978)

SUMMARY

Chromatographic separation of the various molecular forms of tocopherols (Vitamin E) into the three fractions of α-, β- plus γ-, and δ-tocopherols, can be achieved using columns packed with pellicular materials such as Corasil II, Bondapak C_{18} Corasil and Bondapak Phenyl/Corasil. Such columns are very useful for the routine assay of a single component like alpha tocopherol in biological samples. A commercially available microparticulate column Partisil PXS10, 25 cm × 4.6 mm I.D., is far more efficient and can be used to separate all four tocopherols under the experimental conditions specified in this report.

INTRODUCTION

Several molecular forms of tocopherols (vitamin E) are known to occur in nature[1]. These compounds differ considerably from each other in their biological potencies and natural occurrence[2,3]. Therefore, analysis of biological materials for vitamin E ought to provide complete information on the levels of each of the various tocopherols. Chromatographic techniques are ideally suited for this purpose. A method of chromatographic separation of the various tocopherols using hydroxy-alkoxypropyl Sephadex columns and final assay based on spectrophotofluorometric detection has been reported by Thompson *et al.*[4]. Determination of free tocopherols in plant oils by liquid–solid chromatography was reported by Van Niekerk[5]. High-performance chromatography of tocopherols has also been the subject of a few recent reports[6,7].

This laboratory has published a study on the determination of alpha tocopherol in brain samples using high-performance liquid chromatography (HPLC) with fluorescent detection[8]. A relatively inexpensive modular system was used with Corasil II as the solid adsorbent. It was found that this system did not separate

beta and gamma tocopherols. Therefore, a study of the chromatographic separation of the various tocopherols was conducted using pellicular packing materials (both normal and reversed phase) as well as microparticulate silica gel. The results of these investigations are reported in this paper.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatograph was assembled in this laboratory (using commercially available components). Details of this operation have already been published[8].

Columns. Stainless-steel columns of various lengths and 1/8 in. O.D. and 2 mm I.D. were dry-packed with the following pellicular packings: Corasil II, Bondapak C_{18}/Corasil and Bondapak Phenyl/Corasil. These packings were obtained from Waters Assoc. (Milford, Mass., U.S.A.). A Partisil PXS10 column, 25 cm × 4.6 mm I.D. was purchased from Whatman (Clifton, N.J., U.S.A.). The Partisil column was preceded by a precolumn, 2 in. × 2 mm I.D., packed with Corasil II.

Mobile phase. Several mixtures of mobile phases were used with each column. Hexane modified by small, precise amounts of methanol was the most satisfactory mobile phase in normal phase operations of silica columns. The methanol concentration in hexane was varied from 0.3 to 0.8%. For reversed-phase chromatography the mobile phases ranged from 2.5% to 15% water in methanol.

Reagents

Nanograde hexane and methyl alcohol were obtained from Mallinckrodt (St. Louis, Mo., U.S.A.). The α-, β-, γ- and δ-tocopherols were purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.).

Procedure

Standard solutions of the various tocopherols were prepared in hexane and injected directly into the column. The mobile phases were always freshly prepared and degassed under vacuum just before using. In every case the flow-rates were determined by collecting the column effluent in a graduated cylinder for a known amount of time.

RESULTS AND DISCUSSION

With a Corasil II column it was possible to separate the various tocopherols into three fractions *viz.*, alpha, beta plus gamma, and delta while a mobile phase of small amounts of methanol in hexane was used. However, separation of beta and gamma tocopherols could not be achieved with the Corasil II columns under a variety of experimental conditions where concentrations of methanol in hexane, flow-rates and lengths of the columns were systematically changed. Substitution of methanol with other alcohols such as ethanol and isopropyl alcohol also failed to separate the β- and γ-tocopherols.

The reversed-phase pellicular columns, Bondapak C_{18}/Corasil and Bondapak Phenyl/Corasil, were quite comparable in their separation efficiency with Corasil II

TABLE I

COMPARISONS OF COLUMNS PACKED WITH VARIOUS PELLICULAR MATERIALS SUCH AS CORASIL II, BONDAPAK C_{18} CORASIL AND BONDAPAK PHENYL/CORASIL

Type of adsorbent	*Particle size of adsorbent (μm)*	*Length of column (cm)*	*Solvent*	*Flow-rate (ml/min)*	*Separations of β- and γ-tocopherols*	*Number of theoretical plates per meter*
Corasil II	37–50	175	0.8% methanol in hexane	0.43 at 200 p.s.i.	No separation	500
Bondapak C_{18}/ Corasil	37–50	175	2.5% water in methanol	0.56 at 400 p.s.i.	No separation	300
Bondapak Phenyl/ Corasil	37–50	100	85% methanol and 15% water	0.40 at 300 p.s.i.	No separation	310

columns and the tocopherols could be easily separated into α, β plus γ, and δ forms. The details of a comparison of chromatography of the various tocopherols on pellicular columns are given in Table I. The number of theoretical plates per meter obtained under the specified experimental conditions is rather low being of the order of three to five hundred. Relatively low pressures were used, and the flow-rates were in the range of 0.40 to 0.56 ml/min which is adequate for most chromatographic purposes. Columns derived from pellicular packings are very convenient for the routine assay of alpha tocopherol, the most biologically active form of all tocopherols[2]. The pellicular columns can be prepared in any laboratory by simple dry packing techniques[9].

As expected, a microparticulate column such as the Partisil PXS10 packed with silica gel particles of 10-μm average diameter was far more efficient than any of the columns with pellicular packings. The Partisil PXS10 column could be easily incorporated into our modularly constructed liquid chromatograph and could be operated at relatively moderate pressures. The data in Table II exemplify the superior efficiency and resolving power of the microparticulate column compared with a pellicular column. Under the experimental conditions specified in Table II,

TABLE II

COMPARISON BETWEEN A PELLICULAR COLUMN, CORASIL II, AND A MICROPARTICULATE COLUMN, PARTISIL PXS10

Column packing	*Average adsorbent particle size (μm)*	*Length of the column (cm)*	*Solvent*	*Flow-rate (ml/min)*	*Resolution for beta and gamma tocopherols*	*Number of theoretical plates per meter*
Partisil PXS10	10	25	0.3% methanol in hexane	2.39 at 400 p.s.i.	1.67	24.000
Corasil II	37–50	175	0.8% methanol in hexane	0.43 at 200 p.s.i.	0.44	500

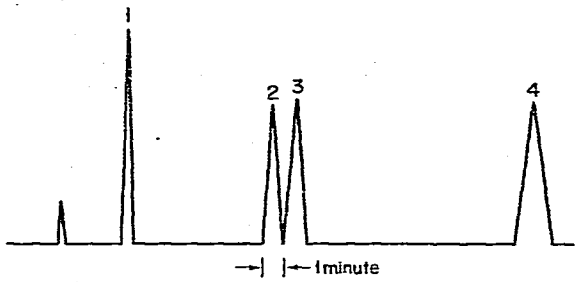

Fig. 1. Separation of a mixture of tocopherols on a Partisil PXS10 column (25 cm × 4.6 mm I.D.). Eluent, 0.3% methanol in hexane; flow-rate, 2.39 ml/min; pressure, 400 p.s.i.; fluorescent detection with excitation wave length 295 nm and emission wave length 340 nm. The peaks 1, 2, 3 and 4 correspond to α-, β-, γ- and δ-tocopherols, respectively.

a baseline separation of alpha, beta, gamma and delta tocopherols was obtained using a Partisil PXS10 column. Abtypical chromatogram with the Partisil PXS10 column is given in Fig. 1.

ACKNOWLEDGEMENTS

This study was supported by research funds from the Veterans Administration.

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