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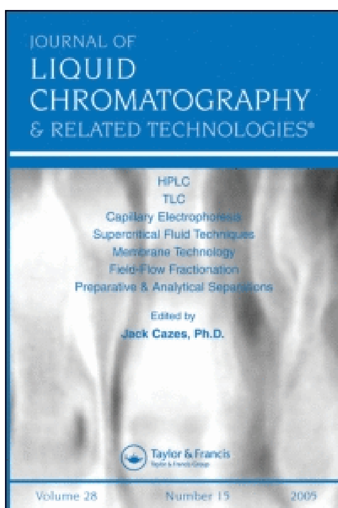

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PREPARATIVE HPLC OF THE LIPID FRACTION OF TEUCRIUM CANADENSE L.

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ABSTRACT

The petrol extract of higher plants usually afford phytosterols, triterpenes, hydrocarbons and triglycerides as the major components. The separation of these by conventional column chromatography can be very time-consuming. The use of HPLC methods can effect a considerable reduction in analysis time.

INTRODUCTION

In the examination of plant constituents the first step is usually a defatting process in which the dried plant material is extracted with petrol. The petrol extract usually consists predominantly of phytosterols, triterpenes, hydrocarbons and triglycerides although the exact composition will vary from plant to plant.

Analysis of these components is most often achieved by gas liquid chromatography (GLC) but this cannot be carried out directly on the plant extract as there may be overlap between the longer

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chain hydrocarbons, phytosterols and the simpler triterpenes. The triglycerides are normally saponified and characterised by their fatty acid composition (GLC as methyl esters).

The commonest method for obtaining fractions suitable for GLC analysis consists of simple adsorption chromatography and, because of the nature of plant extracts, this process may take several days and often then requires further fractionation.

Similar problems have been solved by the use of preparative HPLC for the analysis of mineral oils (1) and the products of chemical synthesis (2). We report the use of preparative HPLC in the analysis of plant lipid fractions. The technique is demonstrated on the petroleum extract of _Teucrium canadense L._

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MATERIALS AND METHODS

Teucrium canadense, whole herb, was authenticated by the Royal Botanic Gardens, Kew, Richmond, Surrey. It was extracted (500g) for 72 hr in a Soxhlet apparatus. using Petrol B.Pt. 40 - 60° affording 12.101g of dry extract. 6.0g was slurry coated onto Silica Gel (TLC grade without additives, Whatman Ltd., Maidstone, Kent, No. 0022).

Initial preparative HPLC was carried out on a 4 ft x 1 in I.D. steel column with swagelock fittings and packed with TLC silica (210 g). Elution was carried out using reagent grade n-hexane and ethyl acetate (B.D.H., Poole, Dorset) programming linearly to 50% v/v ethyl acetate/n-hexane in 5 hr. after holding in pure n-hexane for 1 hr. Solvent flow and gradient programming was produced by two reciprocating piston pumps, (Model 6000A, Waters Ass. Milford, Mass). and a gradient programmer (Model 660 solvent programmer, Waters Ass.) operating to produce a flow rate of 8.0ml/min. 100ml. fractions were collected and monitored by TLC.

Further preparative HPLC was performed isocratically (10% ethyl acetate in n-hexane; Analar, B.D.H.) using a 25cm x 1.0cm I.D. Partisil-10 column (Jones Chromatography, Llanbradach,

Glamorgan, U.K.). The flow rate, generated by a 6000A reciprocating pump (Waters Ass.) was 4.0ml/min with the samples applied using a valve loop injector (Specac, Cambridge, U.K.) with a 500μl loop.

GLC analysis was carried out using a Perkin-Elmer F-17 gas chromatogram (Beaconsfield, U.K.) with FID. Hydrocarbons were analysed on a 1m x 1/8 in SE 30 column (10% on chromsorb G) at 255°C, N_2 flow rate 40ml/min with C_{18}, C_{22} and C_{32} as standards. Steroids and triterpenes were analysed using a 1m x 1/8 in OV 1 (2½% on Chromosorb G) column at 235°C with N_2 flow at 40ml/min. They were converted to their silyl ethers with Tri-Sil (Pierce, Rockford, Illinois) prior to analysis.

Triglycerides were saponified and the fatty acids produced were methylated with an ethereal solution of diazomethane. GLC of the esters was carried out on a 1m x 1/8 in OV1 (2½% on Chromosorb G) column at 150°C, N_2 flow 40ml/min.

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DISCUSSION AND RESULTS

The constituents under discussion are all poor chromophores and are not suitable for UV detection particularly as the eluants commonly used for their separation (petrol, ethyl acetate, chloroform etc.) prohibit the use of low wavelength monitoring. RI monitoring is excluded as gradient elution is essential to deal with the range of compounds present. Further more such plant extracts are often extremely gummy oils and direct injection is certain to result in a very short life for the HPLC columns.

As a consequence the traditional HPLC approach of high efficiency column packings with synchronous detection appeared inappropriate. However an initial separation using a 4ft x 1 in. I.D. steel column packed with TLC silica proved effective. The resolving power of such a column is poor compared to ordinary HPLC columns and the eluant composition is monitored by TLC but the use of TLC silica, a high flow rate and gradient rather than

stepwise elution resulted in a much more efficient separation than that achieved with an ordinary silica column. The reduction in time for the separation was approximately twenty-fold.

One point worthy of note is the method of sample application. It was found that ordinary injection via syringe or large loops was not possible as the material invariably solidified during application unless applied as a very dilute solution. The method finally adopted consisted of dry packing the column, pumping through with hexane to displace air and then repacking the top of the column with silica which had been previously coated with the plant extract and dried. Gradient elution allowed the rapid separation of the material into four main fractions.

The hydrocarbon fraction was analysed by GLC (3) giving a range of alkanes from C_{17} - C_{33} (Table 2).

The impure triterpene and sterol fractions could not be immediately analysed by GLC as the triterpene β-amyrin obscures the clear separation of the common phytosterols (Fig. 1a). However conventional preparative HPLC using a Partisil-10 column with 10% v/v ethyl acetate in hexane allows the complete separation of the sterols from the triterpene. The conditions for the separation were first established using a mixture of dotriacontane, sitosterol and β-amyrin, the elution pattern being monitored by TLC to determine the retention times (hydrocarbon, 3½ - 5min; sitosterol, 15 - 18.5 min; β-amyrin, 27 - 31 min). The impure sterol and triterpene fractions were then chromatographed, the appropriate elution volumes collected and, after drying and silylation, subjected to GLC (Fig 1b and 1 c). A small amount of a triterpene eluting from the HPLC column after β-amyrin was detected by TLC and on TLC it co-chromatogrammed with oleanolic acid but insufficient material was available for confirmatory analysis.

The triglyceride fraction was saponified and the fatty acid composition determined by GLC of the methyl esters (4).

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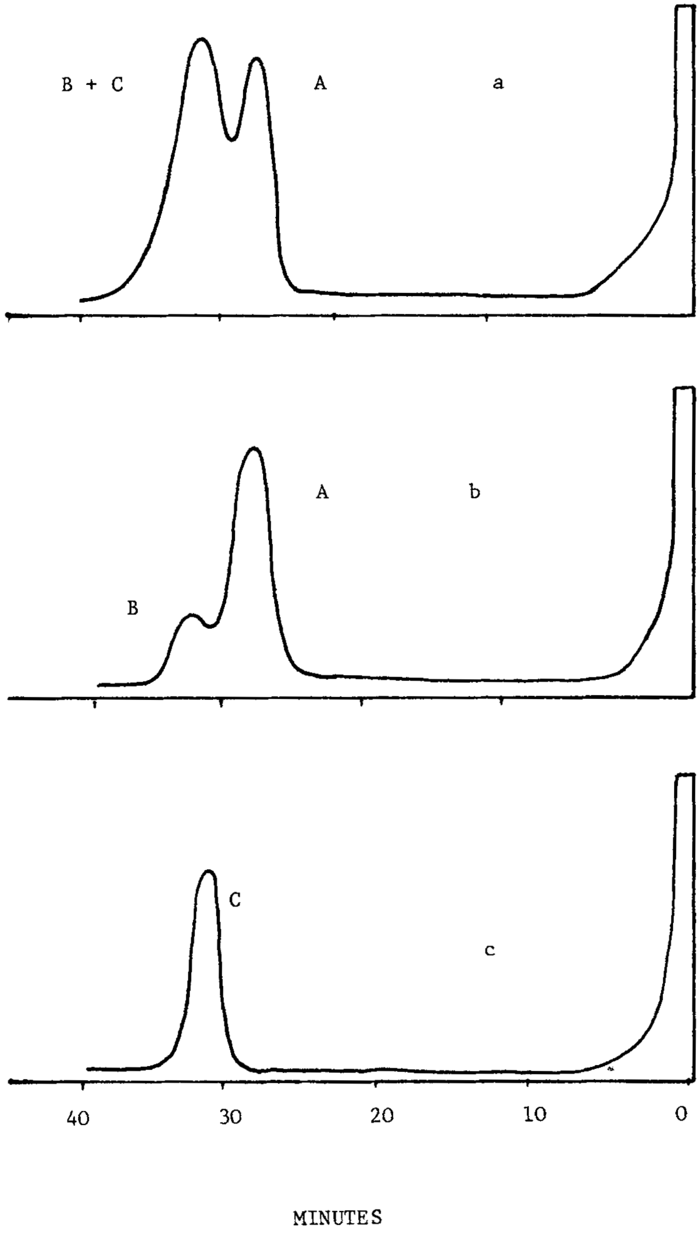

FIGURE 1

GLC separation of sterol -β-amyrin mixture (a) before HPLC separation, (b) sterol fraction, (c) β-amyrin. A=stigmasterol, B = sitosterol, C= β-amyrin.

TABLE 1

Preparative HPLC of *Teucrium* Extract

Fraction No.	Wt (g)	Composition	Eluant (% EtAc in Hex)
1 - 6	1.32	Hydrocarbon	0 - 2.5
7 - 8	2.97	β-Amyrin + trace Hydrocarbon & Sterol	2.5 - 10.0
9 - 11	0.68	Sterol + trace β-Amyrin & Oleanolic acid	10.0 - 15.0
12 - 38	0.50	Triglyceride	15.0 - 25.0

Table 2

Hydrocarbon Composition in T.Canadense (for GLC conditions see text).

Hydrocarbon	Rt min	% composition
C_{18}	1.6	5.1
C_{19}	2.2	3.07
C_{20}	2.8	0.64
C_{21}	3.8	0.24
C_{22}	5.0	0.82
C_{23}	7.0	0.30
C_{24}	9.0	0.25
C_{25}	12.0	1.28
C_{26}	15.6	0.25
C_{27}	20.0	6.92
C_{28}	27.0	0.51
C_{29}	34.2	9.40
C_{30}	46.4	3.20
C_{31}	59.6	12.30
C_{32}	77.6	9.48
C_{33}	100.0	46.15

The main fatty acids present were Lauric (5.2%), Myristic (12.0%) and Palmitic (45.8%).

Although the approach to the analysis of the lipid fraction from higher plants has not been altered, the use of preparative HPLC, both in a crude form to effect initial

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separation and, in a more sophisticated form, to allow clean-up of samples before GLC analysis, results in a greatly reduced analysis time.

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