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APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF VOLATILE OILS

MALCOLM STEWART FRANK ROSS

Pharmacognosy Department, Welsh School of Pharmacy, U.W.I.S.T., King Edward VII Avenue, Cardiff (Great Britain)

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SUMMARY

Because of the limitations inherent in the commonly available detectors for high-performance liquid chromatography (HPLC) this technique has received little attention for the analysis of volatile oils and it is envisaged that gas–liquid chromatography (GLC) with flame ionisation detection will remain supreme in this field. However, a number of volatile oils contain, often as their major constituents, compounds which may be readily assayed by HPLC with UV detection. In these cases HPLC provides a quicker analysis method and, because of the selectivity of the UV detector, a much simpler chromatogram than that obtained by GLC. For certain volatile oil constituents fluorescence detection is of value.

The use of derivatisation for the detection of compounds possessing poor chromophoric properties is demonstrated.

INTRODUCTION

Many volatile oil constituents cannot be analysed by high-performance liquid chromatography (HPLC) with UV detection because of the lack of chromophoric groups in compounds such as the monoterpene hydrocarbons and alcohols. Thus HPLC cannot normally be considered for the total analysis of volatile oils but rather for quality control of oils where acceptability may be determined by reference to certain compounds with good chromophoric properties.

This approach has been successfully used for the determination of the phototoxic furocoumarin, bergapten, in bergamot[1] and other citrus oils[2]. We have reported the HPLC analysis of cinnamon and cassia oils together with a quality control method based on eugenol and cinnamaldehyde determination[3].

Volatile oils have been used as models to examine the potential of particular techniques, for example orange oil to demonstrate the use of incremental gradient elution[4]. Application notes on a number of essential oils are available[5] and the volatile oils from *Lindera umbellata* and *L. sericea* have been examined by reversed-phase HPLC using both UV and refractive index (RI) monitoring. It is noteworthy that more peaks were detected by UV which would seem to indicate that RI detection

will not be particularly useful in analysis of these types of compounds[6]. The same oils were also chromatographed on μStyragel. Elution with tetrahydrafuran allowed the separation of monoterpene hydrocarbons from alcohols and acetates on a molecular weight basis. In all these latter examinations the identity of the eluted compounds is not reported.

We describe the use of HPLC in the analysis of a number of commercial volatile oils. In all cases the HPLC method is simpler than gas–liquid chromatography (GLC) because of the selectivity of the detector and the analysis times are appreciably shorter than those required for conventional temperature-programmed GLC.

EXPERIMENTAL

Materials

Eugenol, isoeugenol, methyl salicylate, benzaldehyde, benzyl acetate, methyl anthranilate, thymol and citral were obtained from B.D.H., Poole, Great Britain. *p*-Methylanisole, carvone and menthone were obtained from Aldrich, Gillingham, Dorset, Great Britain.

n-Heptane and Analar methanol were from B.D.H. and HPLC grade acetonitrile from Rathburn Chemicals, Walkerburn, Peeblesshire, Great Britain.

Equipment

Analyses were performed on 25 cm × 4.6 mm I.D. stainless-steel columns packed with either Partisil-5 or Hypersil-SAS (Jones Chromatography, Llanbradach, Mid-Glamorgan, Wales, Great Britain). The eluent flow-rate was 2.0 ml/min in all cases and was generated by a Model 6000M solvent delivery system (Waters Assoc., Milford, Mass., U.S.A.) and injections were made using a septum injector. An UV spectrophotometer (Cecil Instruments CE 212 with modified flow cell) was used as detector. Where fluorescence detection was used the fluorescence detector (Aminco Fluoro-Monitor, irradiation Blacklight, G.E. F4TF/BL with a Corning 7-51 filter and a Wratten No. 8 filter for emission) was connected after the UV detector.

The composition of eluents for the various analyses is described in the text. In all cases the oils and standards were dissolved in a solvent of the same composition as that used for elution. Injections were made by the stopped flow technique. Analysis of Woodward's gripe-water (concentrated dill water 3.6%, sodium bicarbonate 1.0%, ginger tincture 1.25%) was achieved by injection of an extract (2 ml gripe-water with 1 ml eluent) in acetonitrile–*n*-heptane (1:99). 2,4-Dinitrophenyl hydrazine (2,4-DNP) derivatives of citral, menthone and carvone were prepared by normal chemical procedures and purity was checked by thin-layer chromatography (TLC) and melting point. For analysis of the volatile oils 1.0 μl neat oil was mixed with 10.0 μl of 2,4-DNP reagent (500 mg 2,4-dinitrophenylhydrazine + 1.0 ml conc. sulphuric acid made up to 25 ml with methanol), 500 μl of acetonitrile–*n*-heptane (1:99) added and 5 μl used for injection.

RESULTS AND DISCUSSION

Aromatic compounds

Although eugenol analysis in cinnamon oils has been reported[3] the separation

on Corasil II did not give rise to the sort of results expected from the new generation of columns. The suitability of Hypersil-SAS for the reversed-phase HPLC of phenolic compounds led us to investigate the use of this material. Elution with methanol–water (1:1) proved effective for the separation of a number of phenolic compounds found in volatile oils and detection was achieved by UV monitoring at 260 nm (Fig. 1). In addition, methyl salicylate could be detected by fluorescence monitoring. This system was applied to the determination of eugenol in pimenta and clove oils and methyl salicylate in gaultheria oil. For both eugenol and methyl salicylate (using UV detection) calibration curves of peak height *versus* concentration were linear over the range 0.01–0.5 μl (10 μl injection). With the exception of one sample of clove oil the assays indicated the samples to be of satisfactory quality[7]. The one exception was an old sample of clove oil the chromatogram of which indicated a eugenol content of 84.1%. The chromatogram also revealed a small peak just before the eugenol peak which was provisionally identified as isoeugenol by co-chromatography. The presence of isoeugenol in small amounts does not interfere with the assay of eugenol based on peak height and as isoeugenol is a much better chromophore than eugenol itself, peak height ratios are deceptive. GLC analysis of this sample of clove oil confirmed the presence of isoeugenol and gave an assay figure for eugenol as 83.7%. The oils so far discussed have a common feature in that they contain essentially one aromatic constituent in amounts far greater than any other component. The same HPLC system was then applied to ylang-ylang oil. This oil, derived from the flowers of *Cananga*

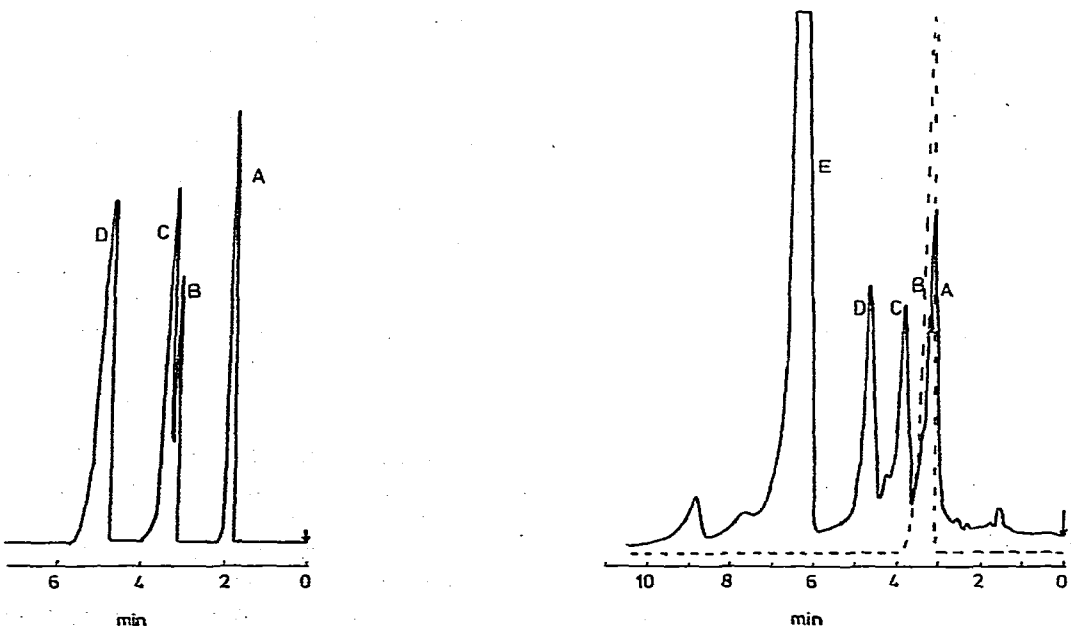


Fig. 1. Separation of phenolic constituents of volatile oils on Hypersil SAS. Mobile phase, methanol–water (1:1). A, Vanillin; B, methyl salicylate; C, eugenol; D, thymol.

Fig. 2. Separation of ylang-ylang oil. Conditions as Fig. 1. A, Benzaldehyde; B, methyl anthranilate; C, benzyl acetate; D, *p*-methylanisole; E, unidentified. ———, UV detection; – – –, fluorescence detection.

odorata, is rich in aromatic constituents and presents a much more complex picture. Identification of the peaks was by co-chromatography with standards together with trapping of eluting components followed by UV scanning. This technique gives a positive advantage over GLC in that without modification of the equipment it is possible to obtain additional physico-chemical data to confirm assignments based on retention data. It was found that the system described here did not completely resolve benzaldehyde and methyl anthranilate; however, the use of fluorescence monitoring for methyl anthranilate in grape juice[8] suggested that coupled UV/fluorescence monitoring would prove effective for the determination of this compound in ylang-ylang oil (Fig. 2). It should be noted that the major aromatic constituent in ylang-ylang oil is benzyl acetate[9] which is a relatively poor chromophore and, in the sample of oil studied, although producing a relatively minor peak actually represents 26.2%. Conversely, the major peak in the chromatogram does not correspond to any previously reported compound isolated from ylang-ylang oil, nor could it be observed in GLC analysis. UV analysis indicated a maximum at 250 nm, with a very small shoulder at 278 nm. Attempts to obtain a mass spectrum from the eluted fraction failed and the compound remains unidentified. We assume that this compound is an exceptionally good chromophore and is actually present in very low amounts, thus explaining the inability of GLC to detect it.

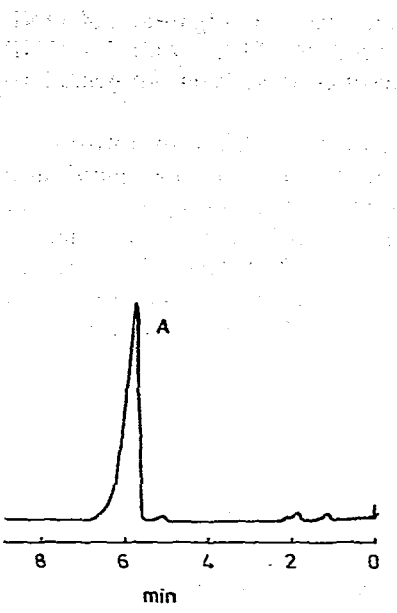
Enones

A number of important volatile oil components have good chromophoric properties by virtue of possessing an enone moiety. Foremost amongst these are the compounds carvone, neral and geranial, the last two being geometrical isomers, normally found together. The *cis–trans* mixture is known as citral and, often in analyses, composition is quoted as total citral content. It is normally assumed that the GLC-flame ionisation detection response of the two isomers is the same and a total figure for citral is derived by summation. The situation is complicated by the fact that it is difficult to obtain analytically pure neral and geranial as standards. Detailed GLC analysis methods for citral determinations have been described[10,11]. The analysis by GLC may be further complicated by co-chromatographing compounds, particularly monoterpene esters. A recent report discusses the problems of GLC analysis of citral and describes HPLC on Corasil II with UV monitoring at 254 nm as a means of detecting citral with no separation of the isomers[12].

The enones were readily detectable by UV monitoring at 242 nm and elution with acetonitrile–*n*-heptane (1:99) on a 5-μm Partisil column afforded a simple and rapid method for the determination of carvone in spearmint (Fig. 3) and dill oils. Neral and geranial are incompletely resolved in this system although sufficiently separated to allow accurate peak height determination. Allowance would have to be made for the different extinction coefficient of the *cis* and *trans* isomers. As a demonstration of the efficiency of this method, a commercial gripe-water containing small amounts of concentrated dill water and ginger tincture in small amounts (ginger oil contains between 30 and 50% citral) was subjected to analysis (Fig. 4).

2,4-Dinitrophenylhydrazine derivatives

An important constituent in peppermint oil is the saturated monoterpene ketone, menthone. As this class of compounds has unsatisfactory chromophoric



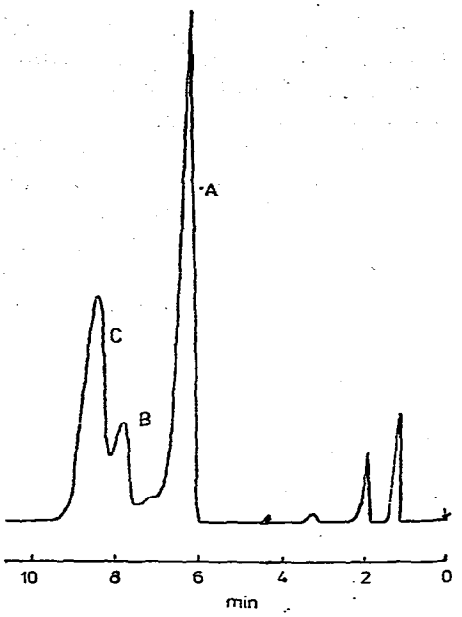
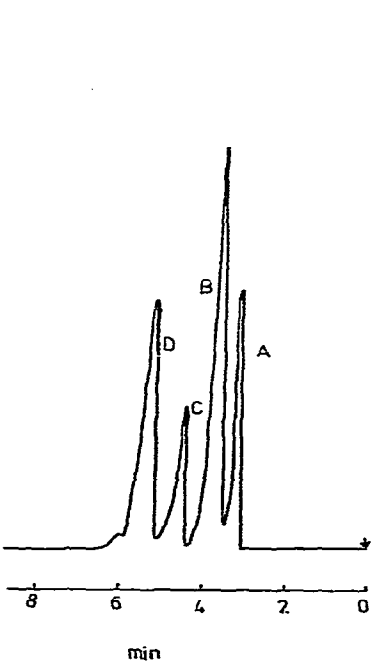


Fig. 3. Analysis of carvone in spearmint oil B.P. using Partisil-5 with acetonitrile–*n*-heptane (1:99) as mobile phase. A, Carvone.

Fig. 4. Separation of extract of gripe-water. Conditions as Fig. 3. A, Carvone; B, neral; C, geranial.



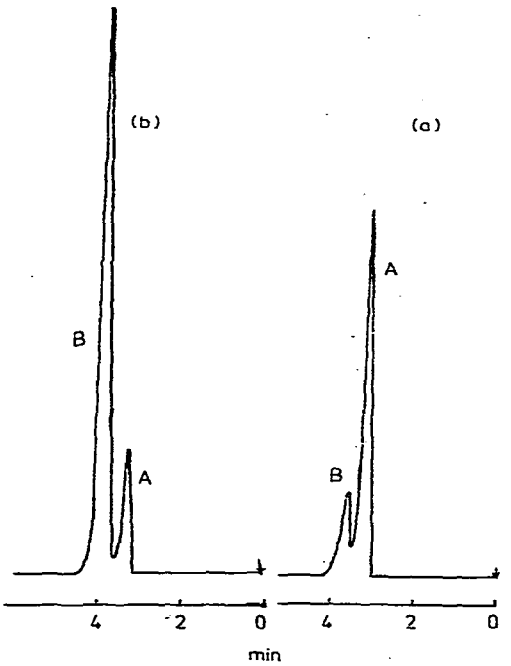


Fig. 5. Separation of 2,4-DNP derivatives of monoterpene ketones on Partisil-5 with ethyl acetate–*n*-heptane (1:19) as mobile phase. A, Menthone; B, carvone; C, neral; D, geranial.

Fig. 6. (a) Separation of derivatised peppermint oil (American WJB). (b) Separation of derivatised spearmint oil (*Mentha viride*). Conditions as Fig. 5. A, Menthone 2,4-DNP; B, carvone 2,4-DNP.

potential the possibility of pre-column derivatisation was investigated. 2,4-DNP derivatives of monoterpene ketones have been analysed by TLC[13] and 2,4-DNP derivatives of both simpler[14] and more complex[15] ketones have been subjected to HPLC analysis with success.

Ethyl acetate–*n*-heptane (1:19) on a Partisil-5 column and UV monitoring at 370 nm allowed the separation and detection of menthone, carvone, neral and geranial as their 2,4-DNP derivatives (Fig. 5). Pre-column derivatisation of mint oils followed by HPLC (Fig. 6) allowed the determination of menthone and carvone.

An additional bonus was found in the use of 2,4-DNP derivatives for the determination of neral/geranial ratios as, at 370 nm, the extinction is due to the derivatising moiety and independent of the orientation of the double bond in the parent molecule.

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