Journal of Chromatography, 505 (1990) 179-190
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 256

Analysis of aliphatic and phenolic carboxylic acids by capillary supercritical fluid chromatography-Fourier-transform infrared microspectrometry

MARK W. RAYNOR, KEITH D. BARTLE* and ANTHONY A. CLIFFORD
School of Chemistry, University of Leeds, Leeds LS2 9JT (U.K.)
JOHN M. CHALMERS
Wiiton Materials Research Centre, Imperial Chemical Industries PLC, P.O. Box 90. Wilton, Middlesbrough, Cleveland TS6 8JE (U.K.)
TAKAO KATASE
Department of Chemistry, Kanagawa Prefectural College of Nursing and Medical Technology, 50-1 Nakao, Asahi, Yokohama 241 (Japan)
and
CHRISTINE A. ROUSE, KARIN E. MARKIDES and MILTON L. LEE
Department of Chemistry, Brigham Young University, Provo, UT 84602 (U.S.A.)

SUMMARY

A mobile phase elimination interface and a microscope accessory are used to combine capillary supercritical fluid chromatography with Fourier-transform infrared spectrometry for analyzing carboxylic acids. Separations of a mixture of oligomers prepared by a self-condensation polymerization of 12-hydroxystearic acid and of a mixture of 16 phenolic carboxylic acids are achieved by using a well-deactivated fused-silica capillary column coated with an oligoethyleneoxide substituted methylpolysiloxane (glyme) stationary phase and carbon dioxide as the mobile phase. Components requiring identification are deposited, with elimination of the carbon dioxide, onto potassium bromide discs as compact spots, from the end of a heated restrictor, which is attached at the end of the column. Unique spectroscopic information is obtained by positioning the spots in the microscope and measuring their infrared spectra. Phenolic acids are unambiguously identified and the degree of polymerization of oligomers in the 12-hydroxystearic acid reaction mixture is indicated from the ratio of ester carbonyl to acid carbonyl absorptions.

INTRODUCTION

Combination of state-of-the-art Fourier-transform infrared spectrometry (FT-IR) and high-resolution capillary chromatography creates a powerful tool for the analytical chemist. Volatile and thermally stable compounds can be analyzed using

0021-9673/90/$03.50

gaseous mobile phases and higher-molecular-weight, thermally labile or polar material can be analyzed using supercritical mobile phases.

Carbon dioxide is the most commonly used supercritical mobile phase, because of its attractive chromatographic and spectroscopic properties. From a chromatographic point of view, the solvating power of carbon dioxide can be increased by programming the mobile phase from gas-like to liquid-like densities. Compounds which are not amenable to analysis by capillary gas chromatography (GC) can therefore be separated at low temperatures and with reasonable efficiency, because of the gas-like viscosity and high diffusivities of solutes in the mobile phase. From a spectroscopic point of view, carbon dioxide absorbs minimally in the mid-IR region and is gaseous under atmospheric conditions. Consequently capillary supercritical-fluid chromatography (SFC) has been coupled to FT-IR using flow-cell and mobile phase-elimination interfaces -a subject which has been reviewed in the literature recently'?

SFC-FT-IR microspectrometry makes use of the mobile phase-elimination approach, where the column effluent is depressurized from a heated restrictor at the end of the capillary column. Involatile analytes, at the low nanogram level, are deposited uncontaminated onto an IR-transparent support as the mobile phase evaporates away. The window is stepped so that the components may be spatially separated for analysis in an FT-IR microscope. An advantage of this interface over the flowcell is that it is compatible with polar or modified mobile phases which may be necessary for chromatography[3]. Further, once a compound is deposited, extensive signal averaging can be performed to improve the quality of the final spectra. The mobile phase elimination method was adapted for SFC-FT-IR by Pentoney and co-workers[4,5], who analyzed polysiloxane oligomers, vegetable oil shortening and a synthetic mixture of ter- and quaterphenyl isomers. Raynor and co-workers have used a similar system for analysing polymer additives[2,6], steroids' and polycyclic aromatic hydrocarbons.*.

The aim of this report is to demonstrate further the capability of SFC-FT-IR microspectrometry for the analysis of aliphatic and phenolic carboxylic acids. These compounds are frequently present in products of plant origin which are important economically. Free fatty acids for example are obtained from vegetable sources such as sunflower seed, and used in large amounts by the food and surface-coatings industries. Methods for analysing these types of materials by SFC[9] and packed-column SFC-FT-IR using a flow cell" are documented in the literature. Phenolic acids such as syringic, p-hydroxybenzoic and vanillic acids are commonly found in grape and other fruit juices", while distilled beverages such as whisky contain other acids such as ferrulic acid, which originate from the cereal used for fermentation[12]. These acids are polar and highly adsorptive solutes, but have appreciable solubility in supercritical carbon dioxide[13]. This indicates that direct analysis by SFC is possible without derivatization, provided that the capillary column is well deactivated and is coated with a stationary phase which has selectivity and capacity for polar solutes and does not interact strongly with acid and hydroxyl groups. For this purpose, Rouse *et al.*[14] have recently developed a 50% oligoethylene oxide-substituted methylpolysiloxane (glyme) stationary phase which has a resolving power similar to that of Carbowax 20M. A 50 μm I.D. fused-silica capillary column coated with an immobilized film of this phase has therefore been investigated for separating the acids.

EXPERIMENTAL

The experimental procedure and instrumentation required for SFC-FT-IR microspectrometry has already been fully described in the literature[6]. However, in order to analyze acidic compounds, various changes have been made to this methodology. These changes are itemized in this section.

Supercritical fluid chromatography

A Lee Scientific (Salt Lake City, UT, U.S.A.) 501 SFC syringe pump was used for density programming the mobile phase. The pump software was run on an IBM XT Model 286 personal computer (IBM, Portsmouth, U.K.). SFC-grade carbon dioxide (Air Products, Rotherham, U.K.) was passed through a basic alumina trap and a 2-μm filter prior to filling the pump. This was carried out to reduce hydrocarbon contamination in the mobile phase. The carbon dioxide was pressurized and delivered by the pump to a Valco C14W microvalve injector fitted with a 200-nl internal sample rotor (Valco Instruments, Houston, TX, U.S.A.). The injector was cooled to approximately 18°C prior to injection with a cooling jacket which was connected to the main water supply. The column was connected to the injector using an inlet splitter (SGE, Austin, TX, U.S.A.) which was adjusted to give a split ratio of approximately 1:4. Separations were performed on a 10 m × 50 μm I.D. fused-silica capillary column, which had been deactivated using a cyanopropyl hydrosiloxane procedure' [5] and coated with a 0.25 -μm film of the glyme stationary phase[14,16]. The stationary phase was cross-linked with azo-tert.-butane, rinsed with methylene chloride and conditioned prior to use[16].The capillary column was installed in a Carlo-Erba (Milan, Italy) Fractovap Series 2150 GC oven with a flame ionization detection (FID) system held at 400°C. The column effluent was split between a tapered capillary restrictor[17] in the FID system and a heated transfer line to the mobile phase-elimination interface by using a butt connector and a graphite ferrule (SGE, Milton Keynes, U.K.).

Mobile phase-elimination interface

The transfer line was a 50 cm × 50 μm I.D. piece of deactivated fused-silica tubing (SGE) with a tapered restrictor fabricated at the interface end". The transfer line was inserted into a 50 cm ×1/16 in. O.D. stainless-steel tube maintained at the column temperature. The end 10 cm of the tube was heated to approximately 200°C using a GC injector heating block. The column effluent was split fairly evenly between the FID system and the interface, by cutting the tapered restrictors back until each had a gaseous flow-rate of approximately 1 ml/min (measured at room temperature and at a column pressure of 150 atm). The end of the restrictor was positioned 50-100 μm above the surface of a potassium bromide window which was stepped manually for peak collection. Solutes were deposited with the window at room temperature and held stationary.

FT-IR microspectrometry

Spots on the potassium bromide discs were analyzed using a Spectra-Scope IR microscope accessory (Spectratech, Warrington, U.K.) and a Nicolet 60SX FTIR spectrometer fitted with an MCT detector (Nicolet Instruments, Warwick, U.K.).

The FT-IR sample compartment which housed the microscope was purged with nitrogen to reduce spectral interference from water vapour and carbon dioxide in the atmosphere. An IR spectrum of the background was measured using a clean area of the window. This spectrum was stored and subtracted from the spectra measured from deposited solutes. Spots to be analyzed were positioned in the beam focus of the microscope using the visible transmission viewing mode. After inspection, the IR beam was stopped down to the diameter of the deposit which was typically 100-200 μm for solids and less than 100 μm for liquids (see Results and Discussion section). On average 1000 spectra, with a resolution of 4 cm^{-1} per spot, were measured and co-added in about 5 min to obtain each final spectrum.

Materials

A mixture of oligomers of 12-hydroxystearic acid was obtained from ICI. The aromatic acids used in this study were donated from several sources. Samples were dissolved in analytical-grade methanol or dichloromethane (BDH, Liverpool, U.K.). Spectroscopic grade potassium bromide (BDH) was used for preparing windows for deposition of selected solutes.

RESULTS AND DISCUSSION

The objective of this work was to investigate capillary SFC with carbon dioxide as the mobile phase for separating a range of aliphatic and phenolic carboxylic acids and to assess the suitability of the mobile phase-elimination interface for their collection and subsequent FT-IR analysis.

Mixed oligomers of 12-hydroxystearic acid

The method was first applied to the analysis of a reaction mixture produced by a self-condensation polymerisation of 12-hydroxystearic acid. The reaction schematic in Fig. 1 shows how this material was prepared. At the start of the polymerization, the acid group of one molecule reacts with the hydroxyl group of another with the elimination of water to form an ester. This dimer can then react in the same way to form a trimer and so on. The resultant reaction product therefore consists of a complex mixture of acid oligomers of various chain lengths which are difficult to analyze by GC and high-performance liquid chromatographic (HPLC) methods without derivatization. Capillary SFC is, however, a particularly good technique for direct analysis of this sample as shown by the chromatogram in Fig. 2. For this separation, the mixture was dissolved in dichloromethane and injected into the chromatograph. The carbon dioxide mobile phase (held isothermally at 100°C) was programmed from 0.35 g/cm^3 to 0.76 g/cm^3 at 0.008 g/cm^3 per min after a 10-min isoconfertic period to elute progressively longer oligomer chains. Although oligomers with similar chain lengths have not been fully resolved, an efficientseparation has been achieved, which is more than adequate for collecting components at the interface, particularly as little difference was expected between the IR spectra of co-eluting material.

Components associated with each labeled peak in Fig. 2 were collected on the KBr window for subsequent FT-IR analysis. On microscopic examination, each spot was observed to be a collection of small globules which had been spread over an area of 300-500 μm in diameter by the expanding mobile phase leaving the restrictor.

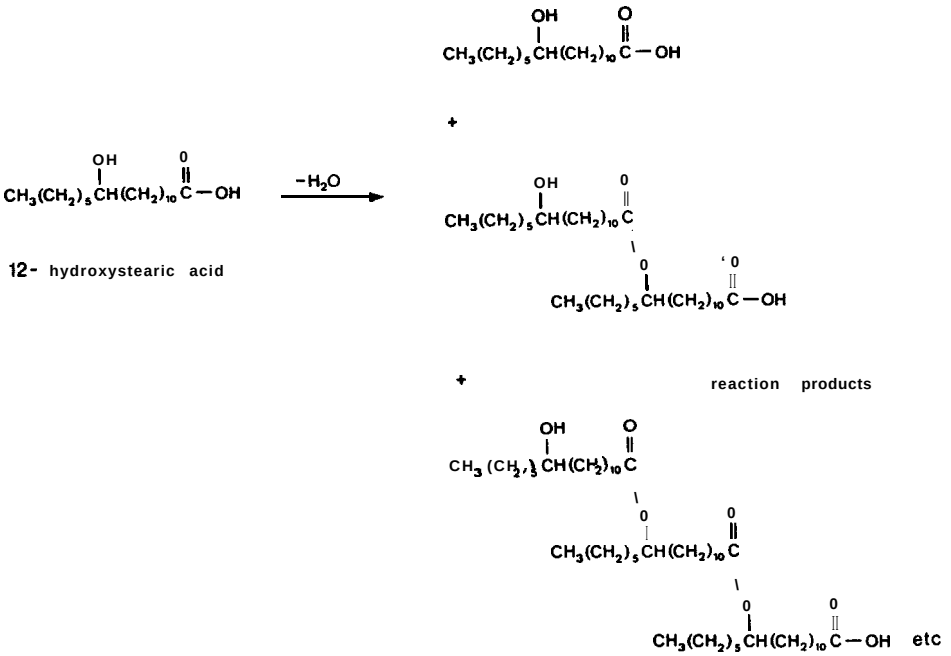

Fig. 1. Self-condensation polymerization of 12-hydroxystearic acid to form oligomers.

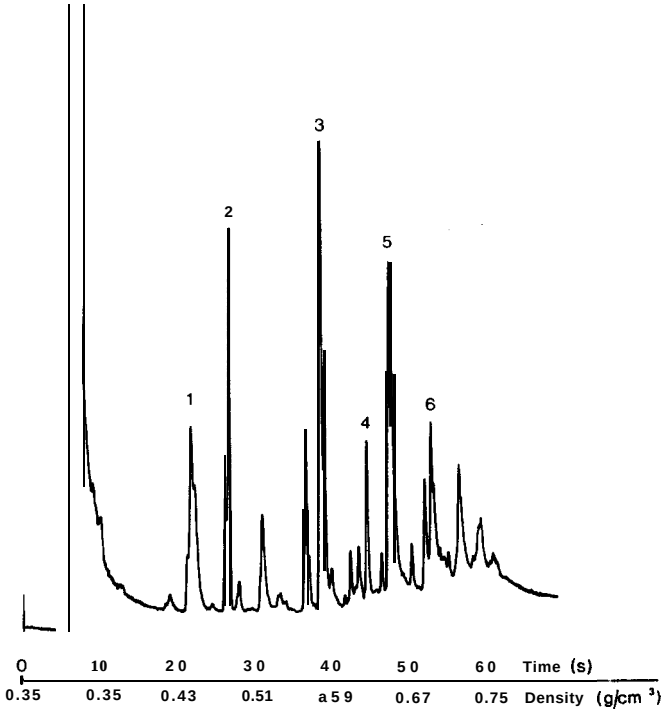

Fig. 2. Separation of 12-hydroxystearic acid oligomers by capillary SFC. Conditions: 10 m × 50 μm I.D. capillary column, glyme stationary phase (0.25 μm film thickness), CO, programmed from 0.35 g/cm³ (10 min) to 0.76 g/cm³ at 0.008 g/cm³ per min, 100°C, FID at 400°C.

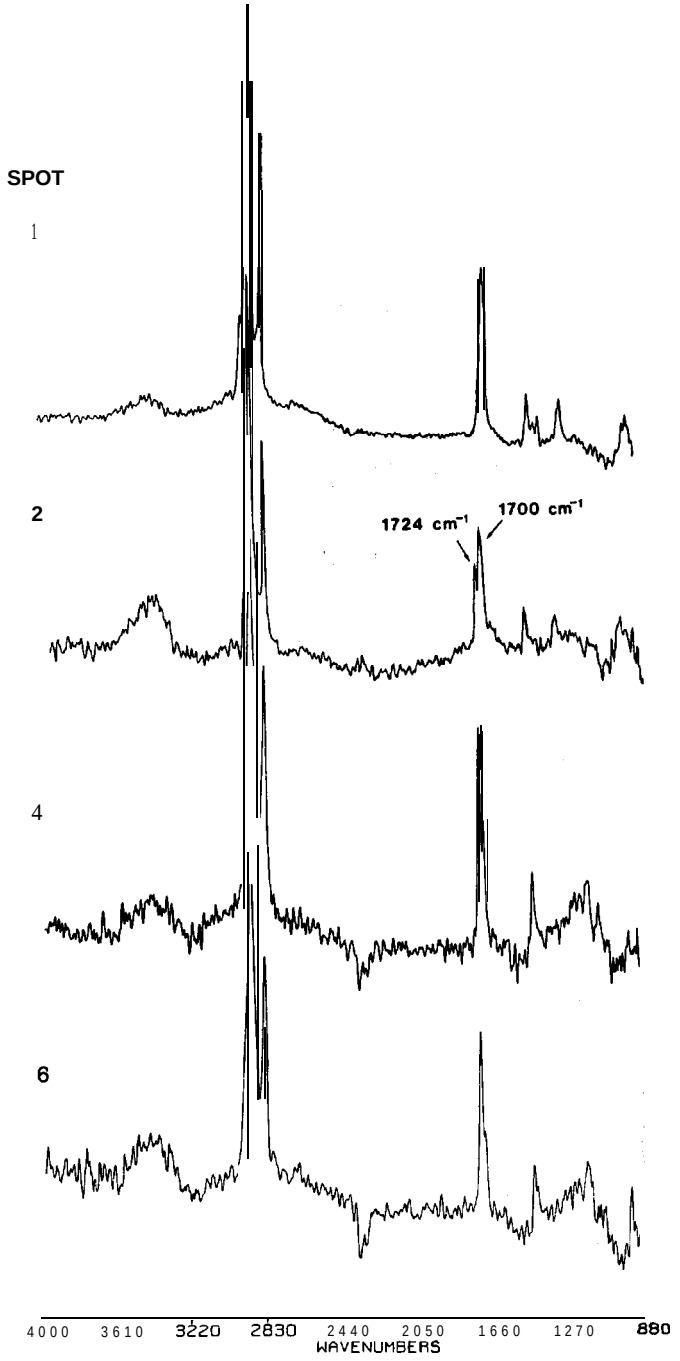

Fig. 3. IR spectra of 12-hydroxystearic acid oligomers separated by capillary SFC in Fig. 2.

Measurement of IR spectra from these samples was more difficult than for solid samples which tend to deposit as compact spots and therefore have greater pathlengths. Pentoney et *al.*[4] have shown that lowering the window temperature improves the deposition characteristics of liquids. However, to achieve this experimentally, the interface becomes significantly more complex and we have found that sufficient spectral information can still be obtained from samples deposited onto the window surface as liquids providing that they are involatile. The IR spectra from spots 1, 2, 4 and 6 shown in Fig. 3, were measured by focusing the microscope beam onto the largest globule of sample in each of the deposits. The beam aperture was stopped down to the approximate size of the globule (typically below 100 μm).

A certain amount of chemical information about the oligomers can be extracted from the 1600-1800 cm-' region of the spectra. The IR spectrum of spot 1 has one absorption at 1700 cm-' in this region. This is assigned to the carbonyl stretching vibration of the 12-hydroxystearic acid which exists (in the deposited state) as a dimer due to hydrogen bonding. The presence of an additional absorption at 1724 cm^{-1} in the IR spectrum of spot 2 indicates that an ester carbonyl is now present in this oligomer. The IR spectrum of spot 4 has a significantly more intense ester carbonyl absorption than acid carbonyl absorption which indicates that this oligomer has a greater chain length (the number of ester groups has increased whereas there is still only one terminal acid group on the end of the oligomer). The effect is further enhanced in the IR spectrum of spot 6, where the acid-carbonyl absorption is only just evident as a shoulder on the ester-carbonyl peak. Accompanying the relative decrease in carbonyl absorption at 1700 cm-', is the loss of absorption intensity at 1270 cm^{-1}, assigned to the νC–O of the acid group. Conversely, as the relative intensity of the ester νC = 0 increases this is accompanied by an increase in νC–O–C intensity in the 1210 cm-' region. The difference observed in the intensity of the νO–H near 3400 cm^{-1} between the spectra is due to the difference in the levels of absorbed water in the KBr support in regions of sample deposition compared to regions where background spectra were accumulated.

The above example demonstrates the capability of SFC with FID and FT-IR detection for monitoring reactions. For example, samples could be removed from a mixture at set times during a reaction and analyzed to determine the extent of polymerization. Unfortunately, IR spectroscopy does not give any molecular weight information, which may be important for further identification. However, there is a good possibility that a gold-coated moving-belt type interface could be constructed which would enable SFC to be coupled with both FT-IR and mass spectrometry (MS).

Phenolic acids

Phenolic acids are difficult compounds to analyze, particularly if FT-IR identification of underivatized material is required. As these compounds are solids they pose no potential problem for collection at the mobile phase-elimination interface, provided that they can be efficiently separated. Capillary SFC was investigated using a synthetic mixture containing approximately 500 ng/μl of benzoic acid, cinnamic acid, 4-chlorobenzoic acid, 3,5-dimethoxybenzoic acid, 4-chlorocinnamic acid, vanillic acid, 3,4-dimethoxycinnamic acid, syringic acid, ferrulic acid, sinapic acid, 4-hydroxyphenylpropionic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid,

cis-4-hydroxycinnamic acid, *trans*-4-hydroxycinnamic acid and 2,4-dihydroxybenzoic acid in methanol.

The chromatogram in Fig. 4 was obtained after optimizing the conditions for separation. The carbon dioxide mobile was density programmed at 100°C from 0.5 g/cm^3 to 0.74 g/cm^3 at 0.015 g/cm^3 per min after an initial 5-min isoconfertic period. The rapid density ramp was necessary to elute progressively more polar acids and to preserve the peak shapes of the last four eluting acids to some extent. These compounds eluted after the final density (i.e., maximum pressure setting of the pump) had been reached and therefore exhibited band broadening in an analagous fashion to the elution of low-volatile compounds after the final temperature of a GC temperature program has been reached. A more polar or modified mobile phase is necessary if further di- and trihydroxylated phenolic acids are to be chromatographed.

The glyme column showed good selectivity for the phenolic acids, although a certain amount of peak tailing occurred, particularly at temperatures above 130°C. Below 80°C, chromatographic efficiency dropped off considerably, which was probably due to the lower rate of solute diffusion between the mobile and stationary phases at high mobile phase densities. An oven temperature of 100°C was therefore used throughout the study.

The results indicate that the glyme phase has good solubility and diffusion characteristics for organic acids. The medium polarity of the phase ensures only moderate retention of polar solutes which is essential for their elution from the column, when a non-polar mobile phase like carbon dioxide is used. Further, through

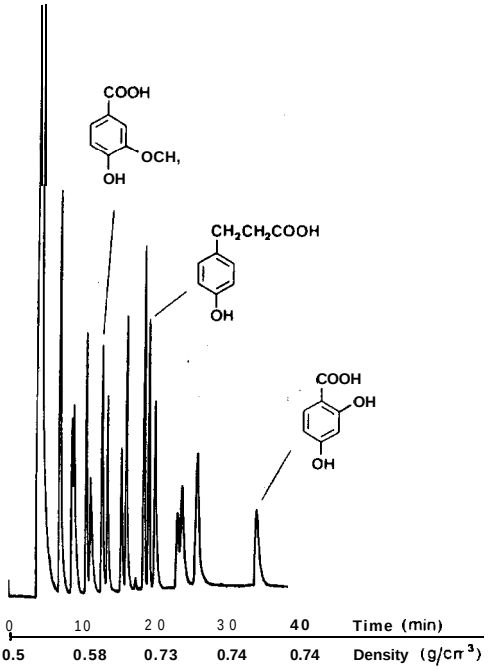

Fig. 4. Capillary SFC separation of phenolic acids. Conditions: 10 m × 50 μm I.D. capillary column, glyme stationary phase (0.25 μm film thickness), CO, density programmed from 0.5 g/cm^3 (5 min) to 0.74 g/cm^3 at 0.015 g/cm" per min, 100°C, FID at 400°C.

cross-linking and immobilization, the glyme phase is chemically and physically stabilized towards degradation. All of these advantages make the glyme phase an ideal stationary phase for polar isomeric solutes in SFC.

Deposition characteristics of the phenolic acids at the interface were investigated using a five-component mixture containing 4-chlorobenzoic acid, vanillic acid, 3,4-dimethoxycinnamic acid, 4-hydroxyphenylpropionic acid and 2-acetoxynaphth-7-oic acid in methanol. Approximately 100 ng of each component were introduced onto the column so that about 50 ng of each acid was delivered to the interface and also detected by FID (Fig. 5). The acids were all deposited as compact solid spots approximately 200 μm in diameter, from which good-quality IR spectra could be measured. Figs. 68 show the IR absorption spectra from spots associated with peaks 2, 4 and 5 in Fig. 5. These spectra are highly characteristic and compare well with those of reference compounds ground into potassium bromide, which indicates that unknown acids deposited at this interface could be positively identified using library-search facilities.

A significant amount of structural information can be obtained from the spectra. For example, phenolic acids have OH stretching absorptions due to both phenolic and carboxylic acid moieties. The phenolic OH stretching absorption is narrow and occurs from 3500-3400 cm-' (3470 cm-' in the case of vanillic acid, Fig. 6), while the OH stretching absorptions of the carboxylic acid are broad due to hydrogen

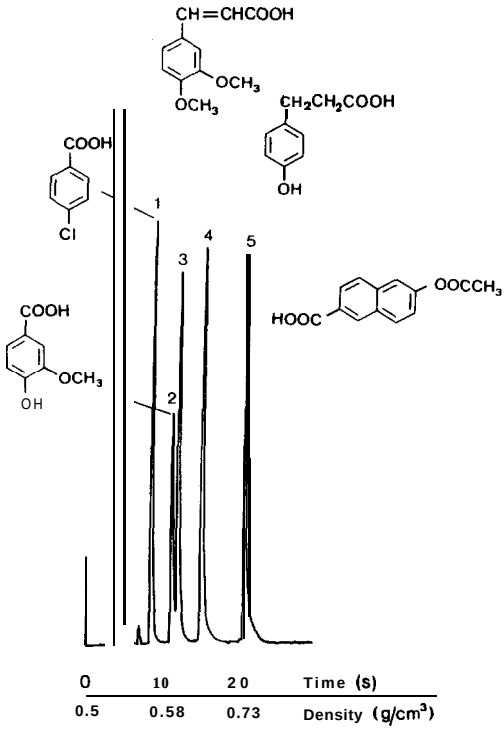

Fig. 5. Separation of 5-component mixture to demonstrate the mobile phase-elimination interface for deposition of phenolic acids. Conditions as in Fig. 4.

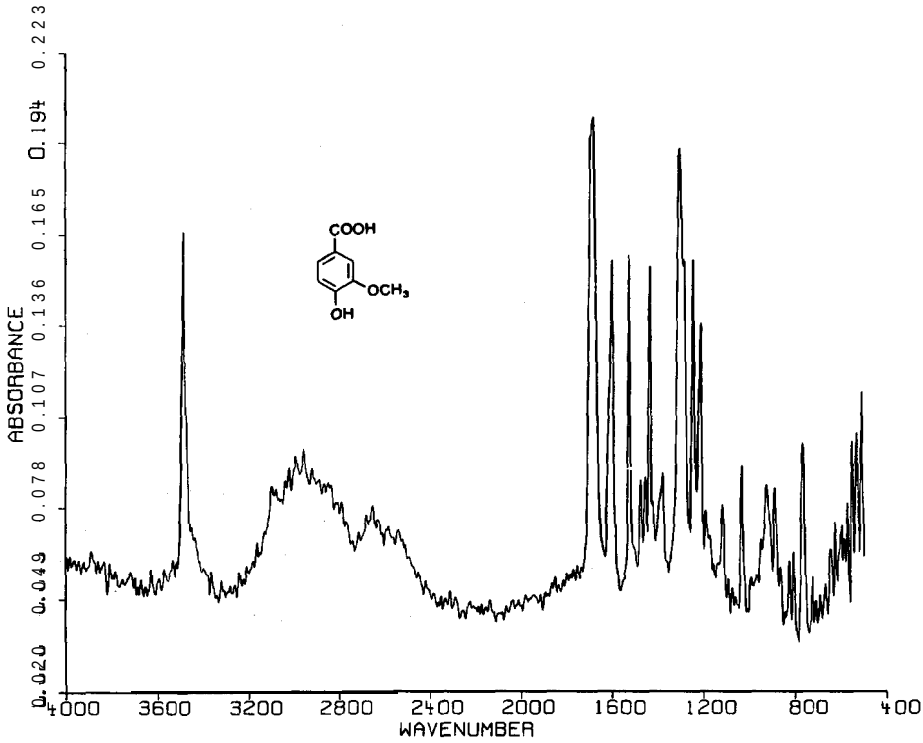

Fig. 6. IR spectrum of vanillic acid (peak 2 in Fig. 5).

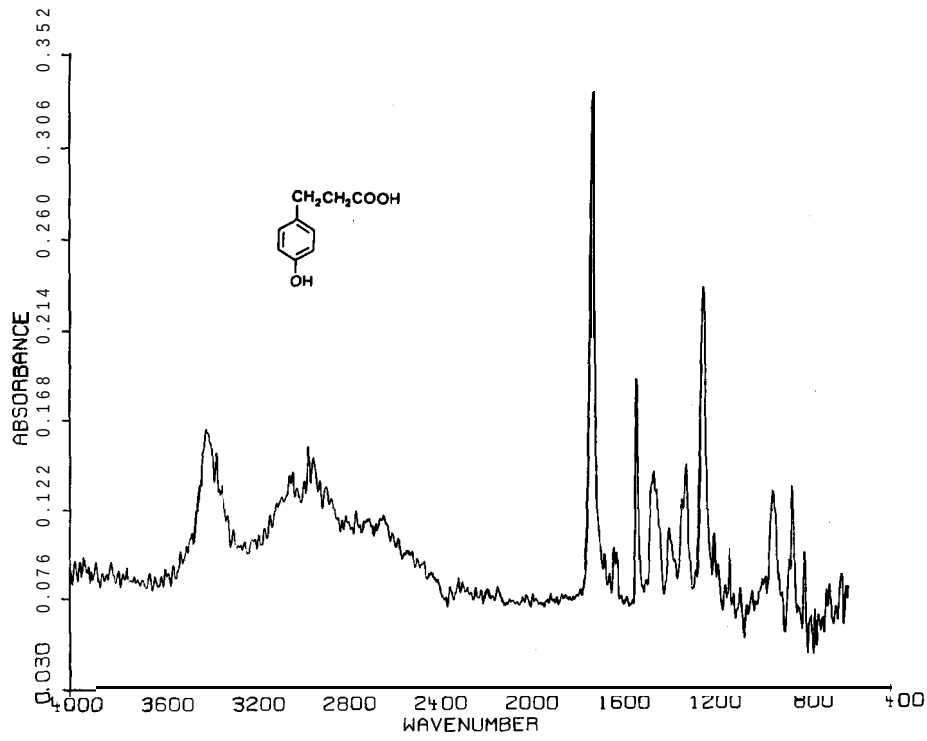

Fig. 7. IR spectrum of 4-hydroxyphenylpropionic acid (peak 4 in Fig. 5).

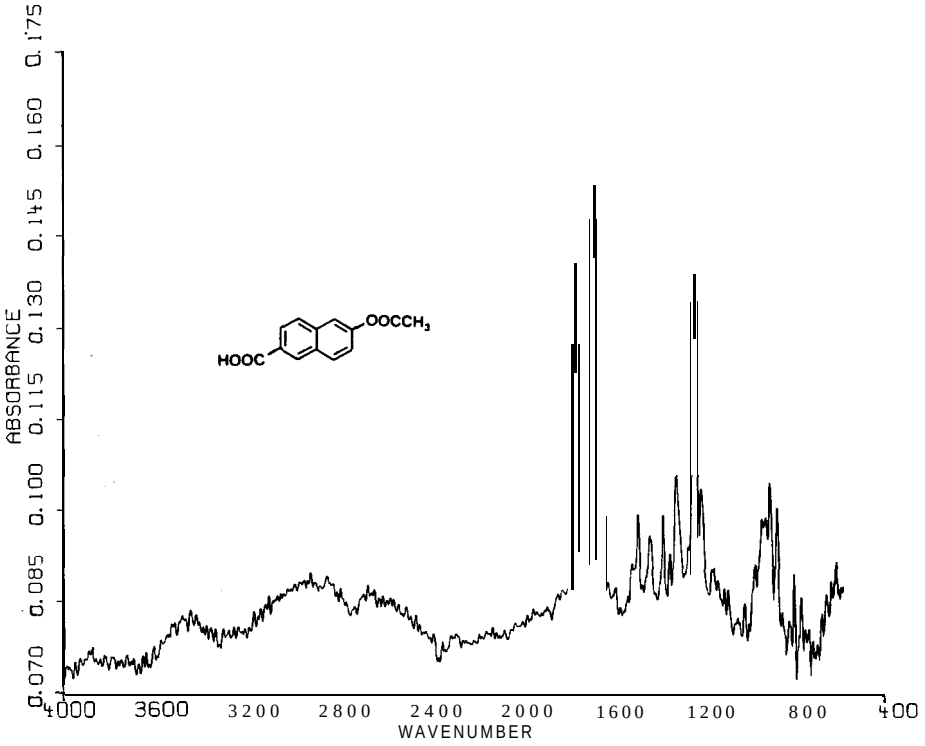

Fig. 8. IR spectrum of 2-acetoxynaphth-7-oic acid (peak 5 in Fig. 5).

bonding and occur from 3400-2500 cm-‘. This interaction also results in the acid carbonyl absorption occurring between 1710-1680 cm-’ (monomeric acid would be expected to give rise to an absorption near 1750 cm-‘). It occurs in the IR spectrum of 4-hydroxyphenylpropionic acid (Fig. 7) at 1701 cm^{-1} and in the IR spectrum of vanillic acid and 2-acetoxynaphth-7-oic acid (Fig. 8) at 1680 cm-‘. Acid carbonyl and ester-carbonyl absorptions, which in the case of 2-acetoxynaphth-7-oic acid occur at 1680 cm-’ and 1758 cm-‘, respectively, are therefore easily distinguished from one another.

ACKNOWLEDGEMENTS

The authors thank J. Piggott and B. Cook for supplying some of the phenolic acid samples, G. Dent and S. Westwood for supplying the hydroxystearic acid oligomers and N. Moore for assistance with the IR work. Financial support from the Science and Engineering Research Council (GR/E/00556) and ICI PLC is greatly appreciated.

REFERENCES

1 K. Jinno, *Chromatographia,* 23 (1987) 55.
2 K. D. Bartle, M. W. Raynor, A. A. Clifford, I. L. Davies, J. P. Kithinji, G. F. Shilstone, J. M. Chalmers and B. W. Cook, *J. Chromatogr. Sci., 27 (1989) 283.*

3 C. Fujimoto, Y. Hirata and K. Jinno, ***J. Chromatogr., 332 (1985) 47.***
4 S. L. Pentoney, Jr., K. H. Shafer, P. R. Griffiths and R. Fuoco, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 9*** (1986) 168.
5 S. L. Pentoney, Jr., K. H. Shafer and P. R. Griffiths, ***J. Chromatogr. Sci., 24*** (1986) ***230.***
6 M. W. Raynor, K. D. Bartle, I. L. Davies, A. Williams, A. A. Clifford, J. M. Chalmers and B. W. Cook, ***Anal.*** Chem., 60 (1988) 426.
7 M. W. Raynor, I. L. Davies, K. D. Bartle, A. Williams, J. M. Chalmers and B. W. Cook, ***Eur. Chromatogr. News,*** 1 (1987) 18.
8 M. W. Raynor, I. L. Davies, K. D. Bartle, A. A. Clifford, A. Williams, J. M. Chalmers and B. W. Cook, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 (1988) 766.
9 K. E. Markides, S. M. Fields and M. L. Lee, ***J. Chromatogr. Sci., 24*** (1986) 254.
10 J. W. Hellgeth, J. W. Jordan, L. T. Taylor and M. Ashaf-Khorassani, ***J. Chromatogr. Sci., 24*** (1986) 183.
11 F. Villeneuve, G. Abravanel, M. Moutounet and G. Alibert, ***J. Chromatogr., 234*** (1982) 131.
12 V. Pussayanawin and P. L. Wetzel, ***J. Chromatogr.,*** 391 (1987) 243.
13 I. Stahl, ***Angew. Chem., 90*** (1978) ***778.***
14 C. A. Rouse, A. C. Finlinson, B. J. Tarbet, J. C. Pixton, N. M. Djordjevic, K. E. Markides, J. S. Bradshaw and M. L. Lee, ***Anal. Chem., 60*** (1988) 901.
15 K. E. Markides, B. J. Tarbet, C. M. Schregenberger, J. S. Bradshaw, K. D. Bartle and M. L. Lee, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 8*** (1985) 741.
16 B. E. Richter, J. C. Kuei, N. J. Park, S. J. Crowley, J. S. Bradshaw and M. L. Lee, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 6*** (1983) 371.
17 M. W. Raynor, K. D. Bartle, I. L. Davies, A. A. Clifford and A. Williams, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 (1988) 289.