Supercritical Fluid Chromatography with Infrared Spectrometric Detection

LARRY T. TAYLOR*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

ELIZABETH M. CALVEY

Division of Contaminants Chemistry, Food and Drug Administration, Washington, D.C. 20204

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Contents

I. Introduction

Supercritical fluid chromatography (SFC) has received a great deal of attention during the last several years. Supercritical fluids possess many of the attributes necessary for high-performance chromatography (HPLC). These include low mobile phase viscosity, high analyte diffusivity, and good solubility for a wide range of analytes. More importantly, by changing the density of the mobile phase with a change in temperature and/or pressure, one can significantly change the observed chromatographic characteristics in an SFC separation. Thus a single, supercritical mobile phase can be used to afford a wide variety of separations without the time-consuming column equilibration necessary in HPLC when mobile-phase composition is changed. Carbon dioxide is by far the most common mobile phase used in SFC. Packed columns described for use in HPLC and capillary columns initially employed in gas chromatography (GC) can be used.

Detection has been one of the major instrumental problems in SFC. Both conventional HPLC and GC detectors have proven to be compatible with SFC, given various modifications. For example, optical detector cell volumes must be fairly small and able to withstand high pressures. Ultraviolet detection[1] with packed columns, which permit the injection of more material on the column, has been the most popular mode of detection, since many SFC mobile phases are transparent in the UV region and most analytes studied thus far contain one or more UV chromophores. With capillary columns, flame ionization detection[2] has proven popular. These two detection systems provide for essentially universal detection rather than specific detection.

Various efforts have been made to couple spectrometric detectors with chromatographic systems in order to gain more specific information regarding eluting components. The most successful and widely used systems that are also commercially available couple GC with both mass spectrometry (GC/MS)[3] and Fourier transform infrared spectrometry (GC/FT-IR).[4] While the ease of coupling to these spectrometric systems has permitted the development of sensitive, information-rich detectors for GC, this has not been the case for other separation methods. For HPLC or SFC, the interfaces of these high-information detectors have not reached an advanced state of sophistication, although the development of LC/MS and SFC/MS systems has been and continues to be extensively investigated.[5,6] The development of a useful FT-IR detector for LC and SFC has received less attention.[7] For these two separation modes, the FT-IR detector is constrained by two major problems: mid-IR absorption by most chromatographically compatible mobile phases and relatively low FT-IR sensitivity compared to some other more established detectors. In order to minimize these problems, various ingenious interface designs have been explored. These designs appear to vary greatly, but they can be classified into two approaches: solvent elimination coupled with transmission or reflectance IR, and flow cell with transmission or attenuated total reflectance IR. Each approach has a unique set of characteristics that makes it attractive.

Although the interfacing of LC with FT-IR has been investigated for a longer period of time, it appears that SFC/FT-IR has attained a higher level of sophistication. A recent review by Jinno[8] discusses the various types of interfaces being developed for SFC/FT-IR and essentially covers the available literature from the early 1980s to late 1986. The purpose of our review is to briefly describe the types of interfaces available for SFC/FT-IR, including modifications, and the sensitivity studies that have appeared in the literature since the last review and to report recent applications of this technique.

II. Flow Cell Approach

Two types of interfaces based on those developed for HPLC are being actively investigated for SFC/FT-IR: flow cell[7] and solvent elimination.[9] Interest in interfacing SFC to on-line FT-IR via a flow cell initially arose from two basic concepts: (1) supercritical CO_2 transmits infrared radiation over a larger frequency range than many LC solvents, and (2) since the solvating characteristic of the supercritical fluid is a function of density, spectral subtraction of the mobile phase during density programming would be easier than with an LC mobile-phase gradient system.[10]

The IR transparency of CO_2 is very good, with only those regions from 3475 to 3850 cm^{-1} and from 2040 to

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Larry T. Taylor, a native of South Carolina, joined the VPI&SU faculty as an Assistant Professor in 1967. He spent $2^1/_2$ years as a National Institutes of Health Postdoctoral Fellow at The Ohio State University. In 1978 Dr. Taylor was promoted to Professor of Chemistry. During the summers of 1976 and 1984 he was a NASA–ASEE summer faculty fellow at the Langley Research Center, Hampton, VA. He received the Sporn Award for excellence in freshman teaching in 1977. He has authored more than 120 refereed technical publications plus numerous technical reports for the Department of Energy, Electric Power Research Institute, National Aeronautics and Space Administration, National Institutes of Health, Aluminum Company of America, Standard Oil Company of Ohio, etc. Dr. Taylor is currently the coauthor of four patents in the areas of analytical and polymer chemistry. He currently serves as Associate Editor for the *Journal of Chromatographic Science*. He organized the "HPLC–FT–IR" 1986 Summer Symposium for the ACS Division of Analytical Chemistry. Professor Taylor's research interests include the development and application of hyphenated (chromatography–spectroscopy) analytical techniques for the identification of polar material in complex matrices (e.g., GC–FT-IR, SFC–FT-IR, HPLC–FT-IR).

Elizabeth Madigan Calvey, originally from Tinton Falls, NJ, received her B.S. in Biochemistry in 1982 and M.S. in Chemistry in 1984 from VPI&SU, Blacksburg, VA. *While an undergraduate, she* participated in the Cooperative Education Program offered by the university. Since 1984 she has been a chemist in the Natural Products and Instrumentation Branch, Division of Contaminants Chemistry, Center for Food Safety and Applied Nutrition, FDA, Washington, DC. Her research interests have centered on the application of supercritical fluid technologies in the analysis of foods. In 1987, Elizabeth was awarded a long-term appointment from Health and Human Services to pursue her research interests *in cooperation with Prof. Larry T. Taylor. She is currently com*pleting the requirements for a Ph.D. in Chemistry.

2575 cm^{-1} completely lost because of strong absorption by CO_2 (Figure 1). Another area of the spectrum where information is potentially lost or reduced is between 1200 and 1400 cm^{-1}, where increased absorption by CO_2 is caused by Fermi resonance whose magnitude is a

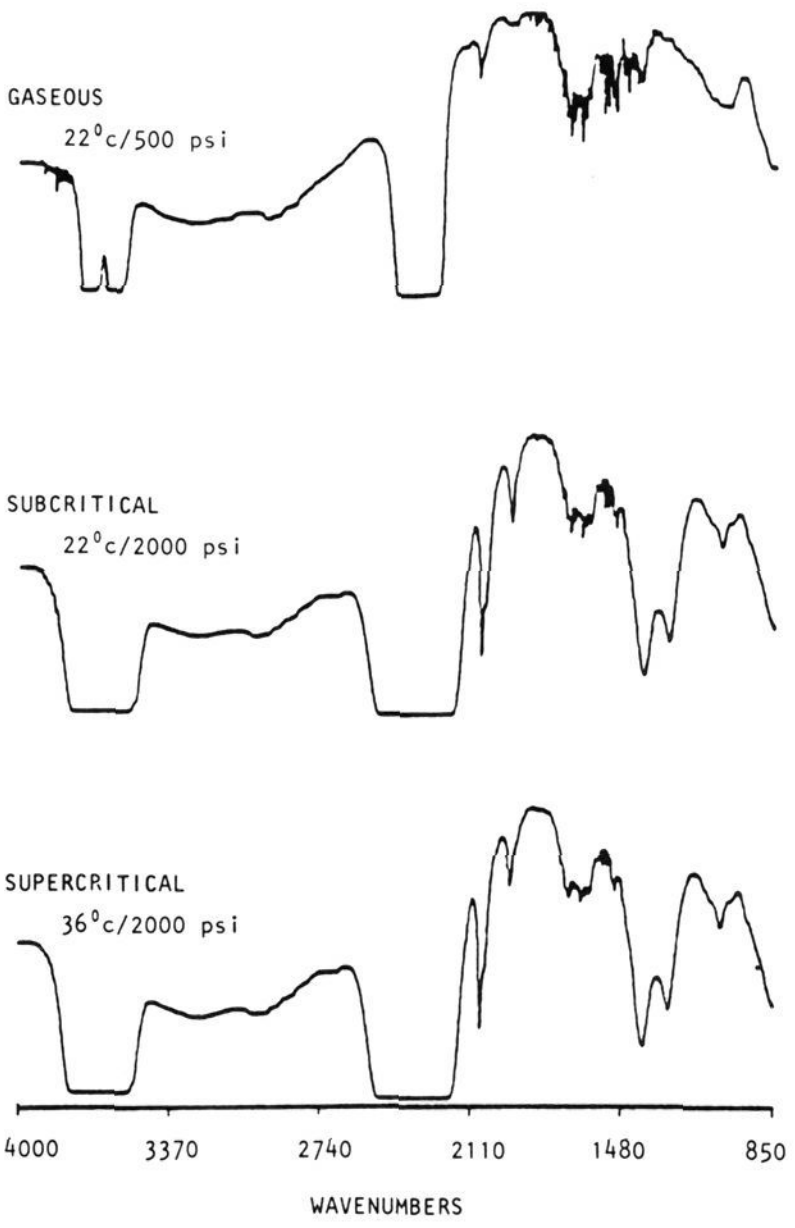

Figure 1. Single-beam spectra of gaseous, supercritical, and subcritical CO_2. Reprinted with permission from ref 10; copyright 1985 Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.

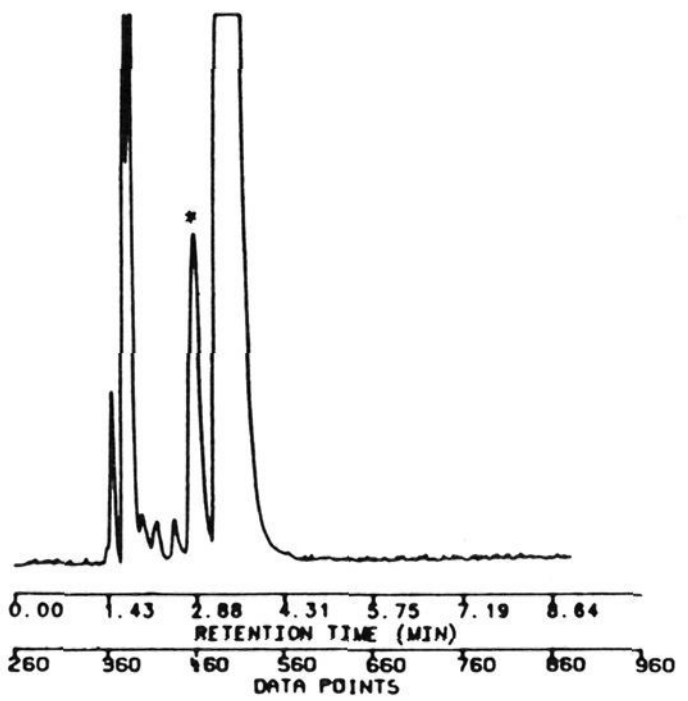

Figure 2. Gram–Schmidt reconstructed chromatogram of citrus oil test mixture. Mobile phase, supercritical CO_2; flow rate, 1 mL/min; isobaric, column head pressure = 1250 psi, back pressure maintained at 1400 psi; injection, 0.5 μL; column, 4 mm i.d. × 15 cm, 5 μm dp PRP-1; oven temperature, 50 °C. Reprinted with permission from ref 12; copyright 1988 American Chemical Society.

function of CO_2 density. The increase in absorptivity of this region that occurs as density increases causes severe base-line drift, which may mask solute peaks. Because of this phenomenon, many of the initial flow cell studies with on-line FT-IR involved isobaric conditions and essentially provided increased support for the solvent elimination approach, which could easily

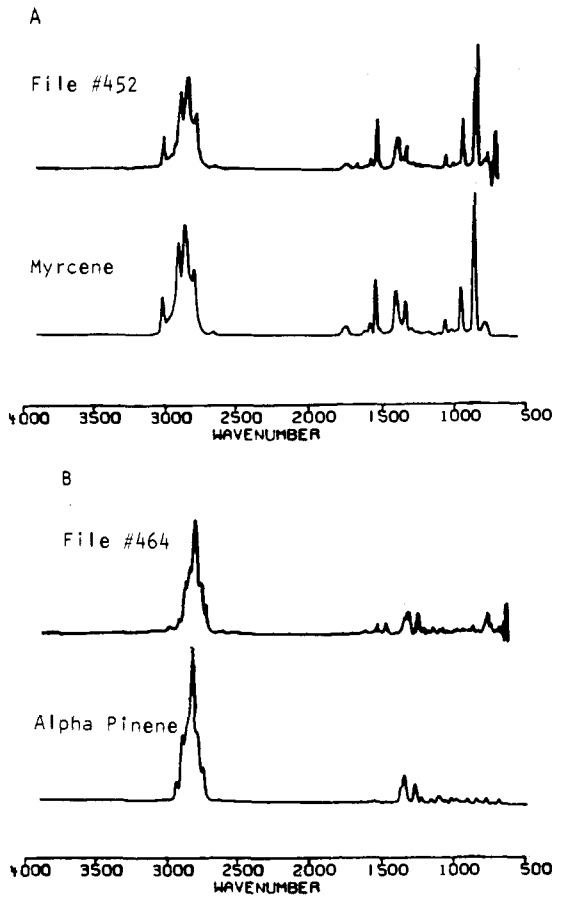

Figure 3. On-line FT-IR spectra of (A) leading edge and (B) trailing edge of starred chromatographic peak in Figure 2 with the best matched spectral search library reference spectra. Conditions: 8-μL flow cell, 8-cm^{-1} resolution, 8 scans coadded per file, 2.27-s time resolution between files. Reprinted with permission from ref 12; copyright 1988 American Chemical Society.

accommodate a variety of density programs. Other mobile phases such as supercritical xenon that do not exhibit any infrared absorption bands are viable for flow cell interfaces as previously demonstrated by French and Novotny.[11]

Wieboldt and Smith[12] recently published results of the analysis of volatile citrus oil components using a HP 1082B liquid chromatograph modified for SFC. The system was limited to isobaric conditions, and the UV flow cell was modified for IR detection by replacing the standard quartz windows with ZnSe windows. Figure 2 shows the Gram–Schmidt reconstruction (GSR) of a 0.5-μL aliquot of a citrus oil test mixture chromatographed on a PRP-1 analytical column (4.6 mm i.d. × 15 cm; 5 μm dp) at 50 °C. The column head pressure was 1750 psi, the column back pressure was maintained at 1400 psi, and the CO_2 flow was 1 mL/min. Figure 3 shows the spectra from the leading and trailing edges of the starred chromatographic peak in Figure 2 along with library reference spectra. This application is an important example of the value of resolving components spectrometrically when their chromatographic separation is not optimized.

Wieboldt and Hanna[13] overcame the undesirable base-line rise, due to increased absorptivity as a function of a density increase, in supercritical fluid chromatograms by using Gram–Schmidt orthogonalization with an augmented basis vector set. As shown in Figure 4, the addition of a vector from the high-density region

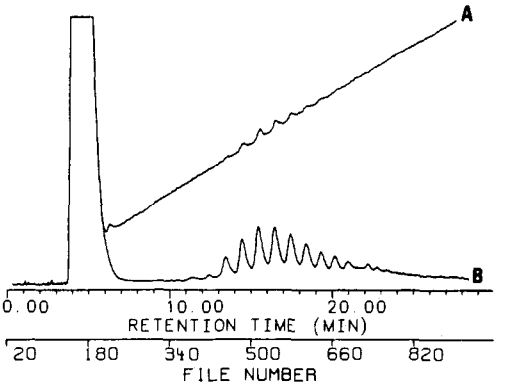

Figure 4. Compensation for carbon dioxide density gradient in SFC/FT-IR. (A) Gram–Schmidt reconstructed chromatogram using 10 basis vectors from start of run; (B) same data with an additional basis vector taken from file 900 (29.12 min) added to the basis set. Reprinted with permission from ref 13; copyright 1987 American Chemical Society.

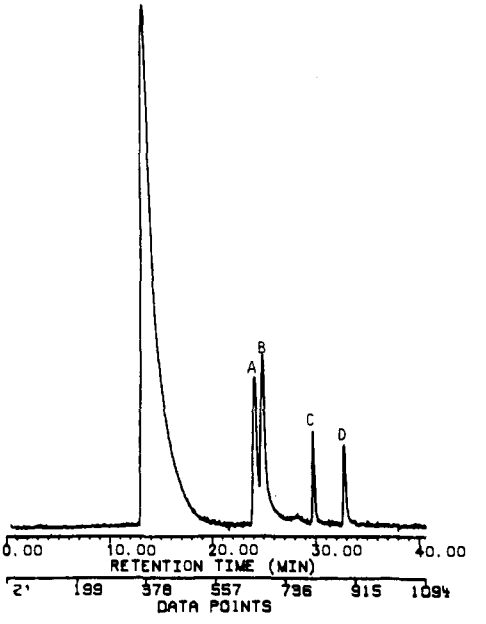

Figure 5. Separation of a carbamate pesticide mixture by SFC/FT-IR. Mobile phase, supercritical CO_2; linear velocity, ~1.4 cm/s; density program, 6.0-min hold at 0.180 g/mL, then to 0.360 g/mL at 0.010 (g/mL)/min, then to 0.600 g/mL at 0.040 (g/mL)/min, followed by 10.0-min hold; injection, 200 nL; split ratio, 22:1; column, 10 m × 100 μm SB-Methyl-100 capillary column; oven temperature, 100 °C. Peaks: (A) aldicarb, (B) methomyl, (C) captan, (D) phenmedipham. Reprinted with permission from ref 14.

of the chromatogram deconvolutes the chromatographic peaks (e.g., paraffin wax mixture) from the base-line drift caused by the density program and enhances detection of the chromatographic peaks.

Initial demonstration of this modified method of data treatment was provided by the separation of a methylene chloride mixture of four pesticides (e.g., Aldicarb, methomyl, captan, and phenmedipham) on a poly(methylsiloxane) capillary column (10 m × 100 μm) with density programming at 100 °C.[14] FT-IR spectra were recorded at 8-cm^{-1} resolution with 8 scans coadded per file. Figure 5 shows the chromatogram generated from approximately 50 ng of each component injected that was reconstructed from the total IR response. Figure 6 is the IR spectrum of the component eluting in the first peak, Aldicarb, obtained by coadding 96 scans. Several chemical features are immediately apparent

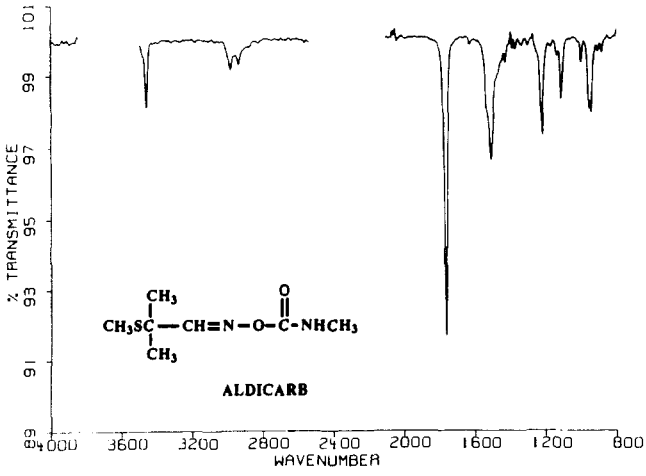

Figure 6. On-line SFC/FT-IR spectrum of Aldicarb (peak A in Figure 5). Conditions: 8-cm^{-1} resolution, 8 scans coadded per file, 12 files coadded. Reprinted with permission from ref 14.

from the spectrum. The strong band at 1762 cm^{-1} is caused by the carbonyl C=O stretch. The presence of the C–O stretching band at 1217 cm^{-1} indicates an ester functionality. The band at 3460 cm^{-1} is definitive evidence for a secondary N–H stretch. The additional band at 1507 cm^{-1} indicates that the nitrogen is part of an amide group. The two blank portions of the spectrum are the regions in which the supercritical CO_2 mobile phase absorbs all the available IR energy.

Wieboldt et al.[15] recently described the requirements for the optimized flow cell design for capillary SFC that was employed in the above work. This same cell design is applicable to packed-column SFC and in terms of chromatographic performance should perform better because peak volumes and cell volumes are more compatible. The dimensions of the flow cell are 0.60 mm i.d. × 5 mm path length, which provides a cell volume of 1.4 μL. The transfer lines from the chromatographic column and to the restrictor are made from fused silica (0.5 m × 50 μm i.d.). The flow cell design was a compromise between the conflicting requirements of an absorbance detector (longer path length) and a chromatographic detector (small cell volume). The flow cell was designed with a cell volume 5 times greater than the theoretically allowable detector cell volume for a 20 m × 100 μm i.d. capillary column, with a plate height of 0.6 d_c (internal column diameter) and a k' value of 1. Thus the design results in a loss of chromatographic resolution greater than 1%. The optics of the detector system dictated the cell diameter; therefore, any changes in the cell volume could only be achieved at the expense of detector path length and sensitivity (i.e., shorter path length, less sensitivity; longer path length, less throughput). The optical path length is dependent on the mobile phase. In this case the flow cell was optimized for SF–CO_2 because it is the most widely used mobile phase for SFC. Due to the increased absorption of the Fermi bands in CO_2, a 5-mm path length was found to be the maximum practical length when working at high densities. The flow cell design has a separate temperature control for the transfer line and the flow cell. These areas were independently heated because an improvement in peak shape was expected when the flow cell was at a lower temperature due to peak compression as the density of the carrier fluid will increase within the cell.

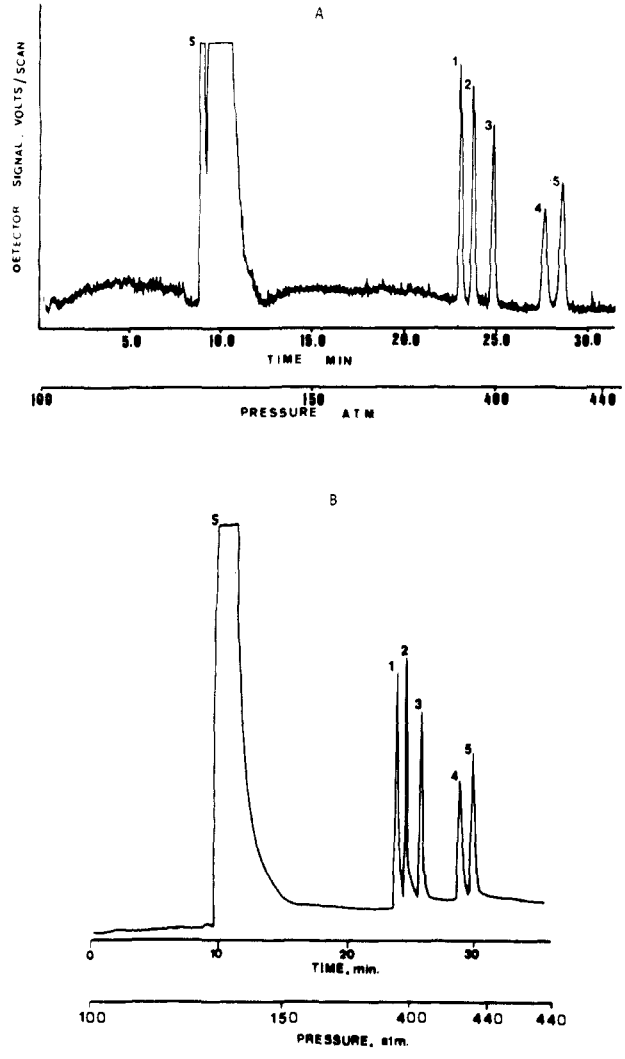

Figure 7. Separation of model steroid mixture (A) by SCF/FT-IR and (B) by SCF/FID (post FT-IR). Separation performed on SB-cyanopropyl-25 column (10 m × 100 μm i.d.) at 60 °C with 100% CO_2. S = CH_2Cl_2, 1 = progesterone, 2 = testosterone, 3 = 17-hydroxyprogesterone, 4 = 11-deoxycortisol, 5 = corticosterone. Reprinted with permission from ref 16; copyright 1988 Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.

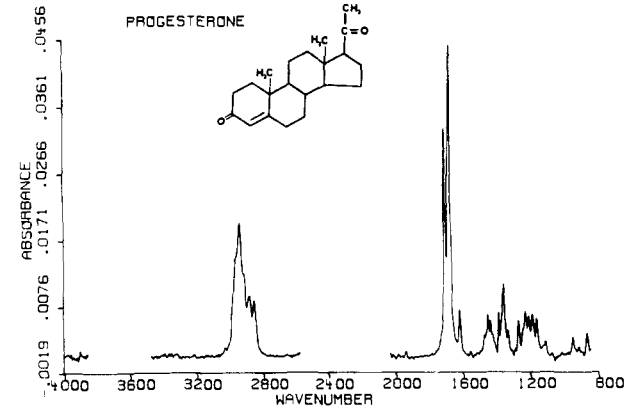

Figure 8. On-line SFC/FT-IR spectrum (5 coadded files) of progesterone (peak 1 in Figure 7). Conditions: 8-cm^{-1} resolution, 4 scans/file, 1 file/s. Reprinted with permission from ref 16; copyright 1988 Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.

Recently,[16] a mixture of five steroids was examined by using the previously described flow cell. A cyanopropyl polysiloxane capillary column was employed. Sequential detection via flame ionization after passage

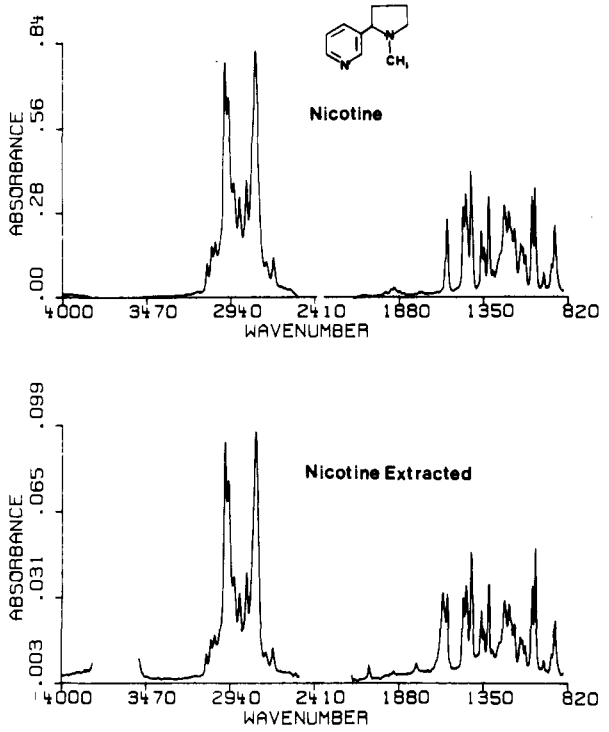

Figure 9. On-line SFC/FT-IR spectrum of neat nicotine (5 coadded files) and CO_2-extracted nicotine (10 coadded files) from a commercial tobacco product. SFE conditions: 100% CO_2, 60 °C, 350 bar. SFC conditions: 100% CO_2, 125 °C, Deltabond Methyl packed column (250 × 1 mm, 5 μm). Pressure program: 120 atm for 3 min, 120–300 atm at 25 atm/min, 300–400 atm at 10 atm/min. FT-IR conditions: 8-cm^{-1} resolution, 4 scans/file, 1 file/s.

through the FT-IR flow cell yielded similar chromatographic traces for this mixture (Figure 7). The file spectrum of 200 ng of injected progesterone (peak 1) is shown in Figure 8. Because of the CO_2 absorption, the hydroxyl stretches of the steroids were not observed, but the unique carbonyl stretch provided a means of identifying the individual components of the mixture.

Hedrick and co-workers[17] demonstrated the utility of this flow SFC/FT-IR interface by coupling it with supercritical fluid extraction (SFE). They were able to identify nicotine in a CO_2-extracted tobacco product by using packed-column SFC. Figure 9 compares the infrared spectrum of authentic nicotine with the spectrum obtained on-line. Retention time comparisons between the extract and a nicotine solution in methylene chloride were not conclusive for identification because the methylene chloride solvent slightly altered the chromatographic elution.

Wieboldt et al.[12,18] further demonstrated the utility of this flow cell interface by examining pyrethrins, naturally occurring esters with insecticidal activity, using capillary SFC/FT-IR. For example, a 20% pyrethrin extract (54 mg) was dissolved in 1 mL of methanol and chromatographed on a methyl polysiloxane capillary column (10 m × 10 μm; 5-μm film thickness). Separation was achieved via density programming at 100 °C. Figure 10 shows the FT-IR spectra generated on-line for Cinerin II and Pyrethrin II with their structures. These compounds are diesters giving rise to multiple C–O stretching absorbances between 1300 and 1100 cm^{-1}. The Pyrethrin II was differentiated from Cinerin II by the out-of-plane C–H deformation at 912 cm^{-1}.

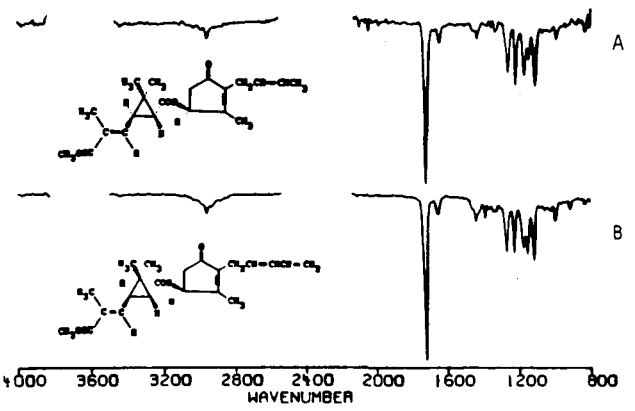

Figure 10. On-line FTIR spectra of (A) Cinerin II and (B) Pyrethrin II. Conditions: 1.4-μL flow cell, 8-cm^{-1} resolution, 8 scans coadded per file, 2.27-s time resolution between files. Reprinted with permission from ref 12; copyright 1988 American Chemical Society.

The addition of polar modifiers to increase the solvent strength of CO_2 or to deactivate the stationary phase also reduces the applicability of on-line FT-IR for obtaining identifiable spectra. Jordan and Taylor[19] showed that with a 5-mm path length cell, the addition of as little as 0.2% methanol reduced the accessible IR windows to 3400–2900, 2800–2600, 2100–1500, and 1200–1100 cm^{-1}. They concluded, however, that the FT-IR detector could still be used as a selective detector, thereby monitoring specific frequencies such as the carbonyl region, which remained transparent in the presence of methanol as a modifier. Morin and coworkers,[20] using a 10-mm path length cell with an 8-μL volume, studied the IR transparency of CO_2 with the addition of various polar modifiers under subcritical conditions. While the addition of polar modifiers caused a severe loss of available IR windows, specific frequencies could still be selectively monitored. For example, the carbonyl and carbon–carbon double-bond stretching regions always remained transparent with methanol and acetonitrile as modifiers. The use of CD_3CN as a modifier permitted monitoring of the C–H stretching region (2900–3100 cm^{-1}), and with <9% CD_3CN added, the aliphatic CH_2 and CH_3 bending region (1600–1400 cm^{-1}) could also be monitored. While Morin reported the available IR windows when using polar modifiers at levels between 0 and 80%, the analyst must be aware of the limited solubilities of various modifiers in the supercritical medium and whether a single-phase or two-phase system is present. Denyszyn[21] recently reported the solubilities of modifiers in supercritical fluids and stated that the solubility of acetonitrile in CO_2 is only approximately 3% by weight.

Other considerations when deciding which type of interface is viable for a particular analytical need are the minimum identification limit (MIL), defined as[22] the quantity of compound required for identification by spectral interpretation or computer search, and the injected minimum detectable quantity (IMDQ), defined as[23] the quantity of material that must be injected onto the column of choice to yield an infrared response 3 times the noise level. In contrast to solvent elimination methods, in which the number of scans can be increased to reduce spectral noise, flow cell methods provide only a few scans per peak since spectral acquisition is performed in real time. Maximum sensitivity will not be

realized if data are taken only at the peak maximum (or at a fixed time), since peaks with higher k' values will be broader and a smaller fraction of the total analyte will be sampled. Consequently, in order to achieve maximum sensitivity for all analytes, a method to optimize the signal-to-noise ratio (S/N) of the infrared spectrum generated in the flowing experiment must be adopted. Both from a theoretical treatment[24] and from experimental data[23] it has been shown that maximum S/N is realized when ±1.37 standard deviations of the chromatographic peak (~75%) are sampled. Certain spectroscopic parameters also affect detectability. If the molar absorptivity of the vibrational mode is very large, detection limits will be significantly lower, provided the noise level is invariable.

Various detection limits have been reported in the literature for the flow cell approach. Jordan and Taylor[19] reported a detection limit study employing *N*-methylaniline as the analyte and CO_2 as the mobile phase under isobaric conditions. The separation was performed on a 5-μm phenyl-derivatized silica column (25 cm × 4.6 mm i.d.) at an average pressure of 3000 psi and an oven temperature of 60 °C. The IR band of interest was the aromatic C=C stretch at 1608 cm^{-1}. For each cell, 4 scans/file (scan time, 0.45 s/scan) were collected, and coaddition was performed over 1.37σ of the Gram–Schmidt reconstructed peak. The same number of scans of background spectra (CO_2 only between 1650 and 1550 cm^{-1}) were coadded, and an average peak-to-peak noise value was found over the different injected quantities. The IMDQ of *N*-methylaniline with the 5-mm path length cell was found to be 470 ng (i.e., 3 times noise peak-to-peak, $3N_{p-p}$). Identical injections using the 10-mm cell yielded an IMDQ of 360 ng. Morin and co-workers[25] reported detection limits of 250 and 70 ng for benzonitrile and methyl benzoate, respectively, using CO_2 as the mobile phase. A 10-μm octadecyl bonded phase silica packed column (15 cm × 4.6 mm i.d.) was employed at 40 °C. Detection was defined as twice the S/N via monitoring the aromatic ring stretching vibration of benzonitrile and the carbonyl stretch of methyl benzoate.

A recent article by Shah and co-workers[16] indicated that for a strongly absorbing compound such as caffeine, the IMDQ is approximately 2 ng. This much lower detection limit was made possible by using a capillary column and the data manipulation approach and flow cell interface of Wieboldt and Hanna.[13] Caffeine was eluted with 100% CO_2 from a 25% cyanopropyl polysiloxane column in approximately 17.5 min with a k' of 0.97. A wide range of quantities of caffeine (250, 50, 25, 5, and 2.5 ng) was injected, and the absorbance of the intense carbonyl peak was correlated with the amount injected. A GSR was obtained for each injection from which 12 files (48 scans) were coadded across the caffeine peak to acquire the IR spectrum of greatest S/N. An S/N (peak to peak ±50 cm^{-1} from the reference peak) of >3 was achieved for as little as 2.5 ng injected. Figure 11 illustrates a portion of the on-line FT-IR spectrum generated under these conditions.

With spectral detectors such as FT-IR, identification limits may be more useful than detection limits. Wieboldt et al.[15] using the previously described optimized flow cell design determined that the MIL for methyl palmitate was 10 ng (Figure 12) on column. The

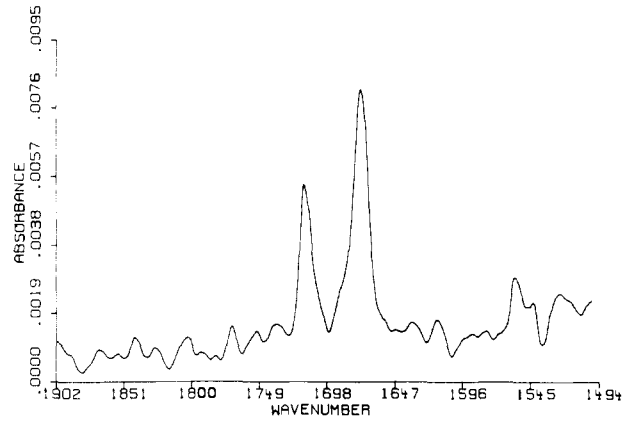

Figure 11. On-line SFC/FT-IR spectrum of caffeine (2.5 ng injected): 12 coadded files, 4 scans/file, 1 file/s. SFC conditions: SB-cyanopropyl-25 column (10 m × 100 μm i.d.) at 60 °C with 100% CO_2; linear pressure programming (100–175 atm/15 min, 175–400 atm/5 min). Reprinted with permission from ref 16; copyright 1988 Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.

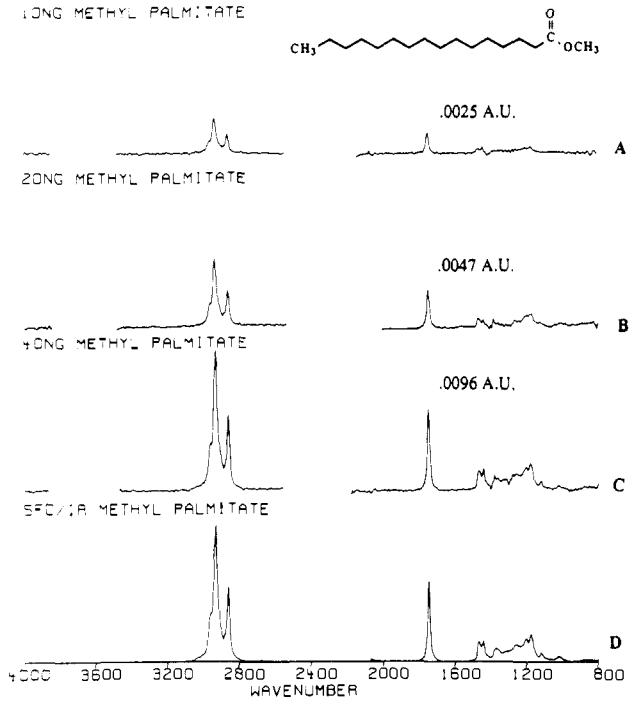

Figure 12. On-line FT-IR spectra of (A) 10, (B) 20, and (C) 40 ng of methyl palmitate delivered to column; (D) SFC/FT-IR reference spectrum of methyl palmitate. Conditions: 1.4-μL flow cell, 8-cm^{-1} resolution, 8 scans coadded per file, 1.1-s time resolution between files. Reprinted with permission from ref 15; copyright 1988 American Chemical Society.

spectral files collected across the chromatographic peak were coadded as necessary to obtain the best S/N in the final spectrum.

III. Solvent Elimination Approach

Research in interfacing SFC to FT-IR via the solvent elimination technique arose from two basic concepts: (1) the solvents encountered most commonly in SFC are gases at room temperature, thus reducing the heating requirements needed for HPLC solvents, and (2) the need to use polar modifiers to improve the chromatographic separation does not render a loss of IR spectral windows.

In the solvent elimination technique the column effluent is deposited onto a stepped or continuously

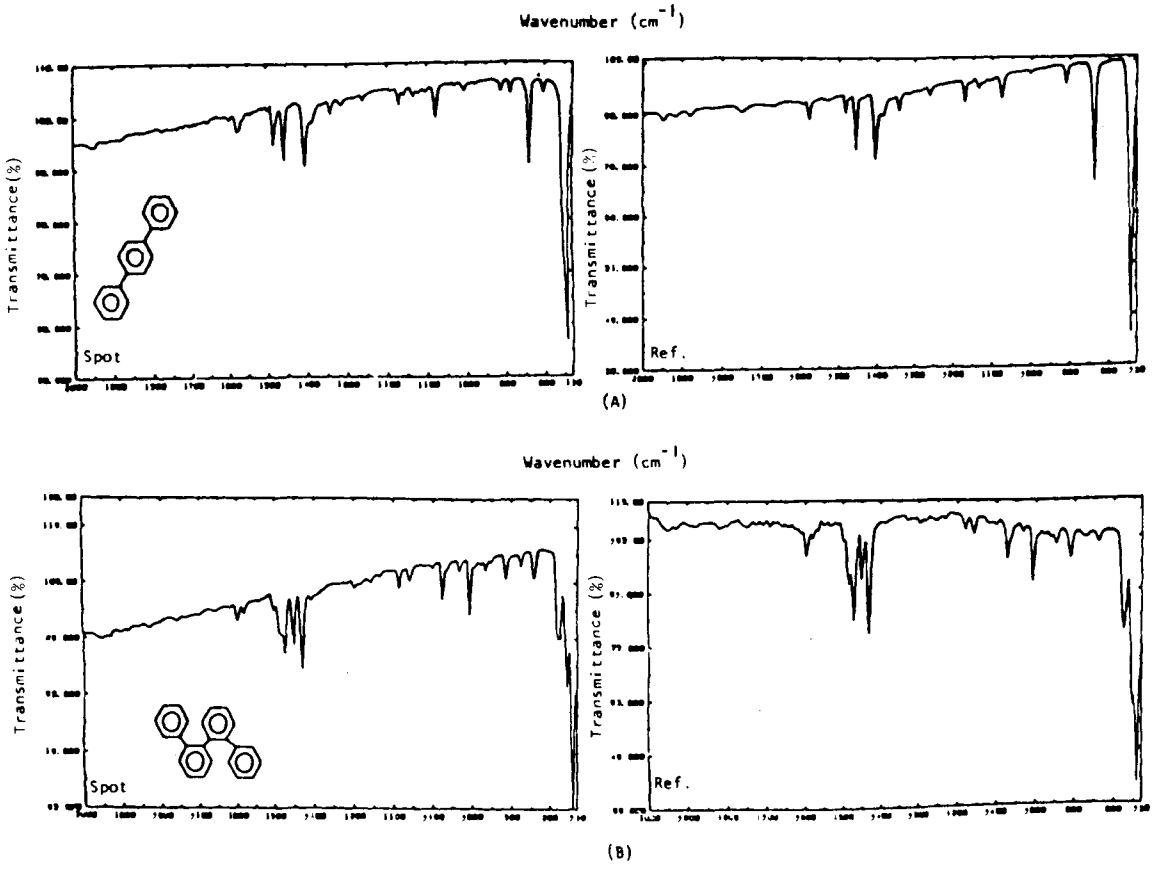

Figure 13. Spot and reference infrared spectra of (A) *p*-terphenyl and (B) *o,o'*-quaterphenyl. Reference spectra were obtained by syringe deposition from CH_2Cl_2 solution. Spectra were collected after 64 coadded scans at a resolution of 4 cm^{-1}. Reprinted with permission from ref 28; copyright 1986 Preston Publications Inc.

moving substrate. The type of IR method used for detection depends on the substrate employed. Fujimoto and co-workers[26] used a continuously moving potassium bromide crystal and obtained IR spectra by using beam condenser optics. Shafer and co-workers[27] deposited the effluent from a microbore packed column onto a potassium chloride powder strip and performed diffuse reflectance IR spectrometry (DRIFT). Since the substrate was not continuously moving, a secondary detector, in this case a UV detector, was necessary to signal when the KCl strip needed to be moved.

In order to extend the method to a capillary column, Pentoney and co-workers[28] have recently used a microscope accessory to match the IR beam size to the area occupied by the column effluent. This feature was found to be desirable since the sensitivity of the solvent elimination method is maximized by depositing the column effluent over as small a spot area as possible. With a microbore packed column, Griffiths et al.[9] reported a 1-mm spot diameter, while a spot of approximately 250-μm diameter was observed with a capillary column. Raynor and co-workers[29,30] used a capillary column (100 μm i.d.) and obtained spot sizes <300 μm in diameter with the end of the restrictor approximately 50 μm above the surface of a potassium bromide window. The sensitivity of the solvent elimination method has been shown to improve if the solute of interest can be deposited as a solid as opposed to a liquid because smaller spots result in the former case. In some situations this is made possible by cooling the substrate. Pentoney and co-workers[28] were able to reduce the spot size of *m*-terphenyl, which is a liquid at room temperature, on a ZnSe window by simply cooling the window to −4 °C. The lower temperature permitted *m*-terphenyl to be deposited as a solid.

Both Pentoney et al.[28] and Raynor et al.[29] used flame ionization as a secondary detector to ascertain when the substrate needed to be moved in order to accommodate the elution of another component. The use of this detector required that the column effluent be split between the FT-IR and the flame ionization detectors. This split was usually in a 1:1 ratio. Raynor indicated that this splitting could cause a compromise in chromatography because in some cases column overload was necessary in order to obtain enough analyte for acquisition of IR data. He suggested that a UV cell in-line could reduce the quantity of solute injected onto the column by eliminating the splitting requirement. If a UV detector is used, however, compounds without chromophores could be codeposited unless a continuously moving crystal was used to collect the column effluent.

Use of the IR–microscope interface by Pentoney and co-workers[28] was demonstrated by the separation of a synthetic mixture of *o*-terphenyl, *m*-terphenyl, *p*-terphenyl, *o*-quaterphenyl, and *m*-quaterphenyl prepared in methylene chloride. Approximately 280 ng of each component was injected onto the column, while, the quantity delivered to the interface for FT-IR analysis was approximately 135 ng. The microscope module was purged with dry air, and the ZnSe window was cooled to −2 °C. Spectra of high S/N were reported between 2000 and 700 cm^{-1} after the IR beam aperture was adjusted down to a diameter of approximately 100 μm and

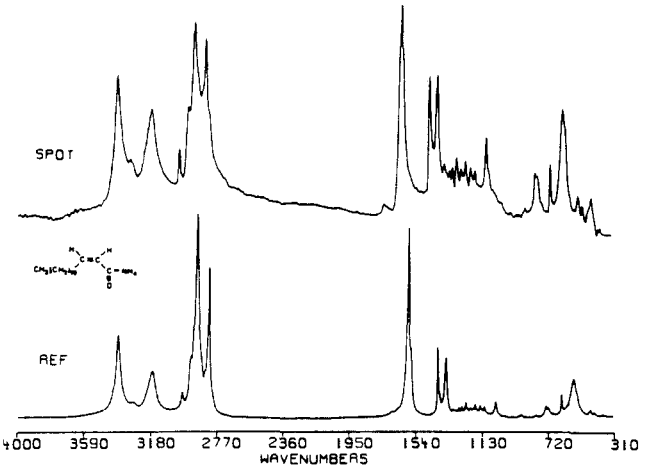

Figure 14. Spot and reference infrared spectra of erucamide. Reference material was ground into a potassium bromide disk. Spectra were collected after 1000 coadded scans at a resolution of 4 cm^{-1}. Reprinted with permission from ref 29; copyright 1988 American Chemical Society.

64 scans were coadded. Figure 13 shows the spot and reference spectra of two of the components, *p*-terphenyl and *o,o'*-quaterphenyl, of this synthetic mixture. Three of the components were completely resolved.

With the aid of a microscope, Raynor and co-workers[29,30] were also able to obtain identifiable spectra for several separated polymer additives with the deposition of 100 ng of each component. Figure 14 indicates the spot and reference spectra of erucamide (i.e., a long chain monoolefin primary amide). The spot spectra represented 1000 scans measured with a resolution of 4 cm^{-1} and accumulated in 4 min. For the two most retained compounds in this study (Irqanox 3114 and 1010) the aliphatic C–H stretching absorption in the 3100–2800-cm^{-1} region was stronger than that obtained for reference spectra. This increase in absorption was explained by the presence of hydrocarbon impurities in the CO_2.

Although the solvent elimination technique permits the use of modifiers in the supercritical fluid, in many cases the modifier is a liquid at room or lower temperature. Thus it appears that a compromise needs to be made between maintaining spot size to increase sensitivity by cooling the substrate and removing the modifier by possible heating of the substrate to eliminate spectral interferences.

Griffiths and co-workers[31] recently described an interface that should be applicable to all three types of chromatography—gas, liquid, and supercritical fluid. The interface involves mobile-phase elimination while condensing the eluting components in a small area onto a moving substrate. The spectra of these compounds are then measured with a FT-IR microscope. The design of the interface requires that the SFC/FT-IR interface operate at room temperature while the GC/FT-IR interface be cooled. Since the current review deals only with SFC/FT-IR, the discussion by Griffiths specifically dealing with SFC will be included. The current work involved using CO_2 or CF_2Cl_2 as the mobile phase. The infrared transparency of CF_2Cl_2 is poor and, therefore, it is not a viable mobile phase using the flow cell technique. A reconstructed chromatogram of four substituted indoles deposited after chromatographic separation using supercritical CO_2 onto a moving ZnSe window is shown in Figure 15. Each point on the chromatogram is computed from a single-scan spectrum. The MIL of indole-3-acetic acid was reported to be 1.4 ng. Figure 16 shows the spectra obtained from 1.4 ng of indole-3-acetic acid signal averaged for 1000 scans. Another advantage of the solvent elimination technique is that standard condensed-phase reference spectra usually can be used.

As mentioned previously, the solvent elimination technique provides a means to investigate other less infrared-compatible supercritical fluids as potential mobile phases. Figure 17 shows the transmittance

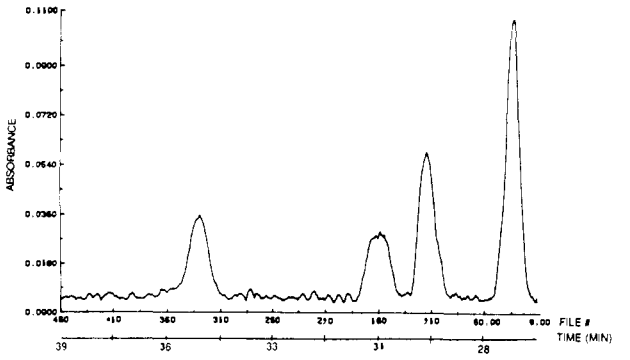

Figure 15. SFC/FT-IR reconstructed chromatogram from single-scan spectra integrated over 1600–1700 cm^{-1}. The separation represents 40 ng each of four substituted indoles deposited on moving ZnSe window. Mobile phase, supercritical CO_2; column, 20 m × 100 μm i.d., 5% phenylsilicone + 95% methylsilicone (0.4 μm thick). Reprinted with permission from P. R. Griffiths.

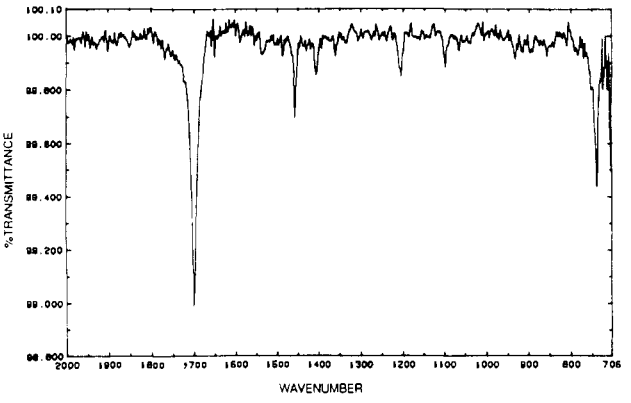

Figure 16. FT-IR spectrum of 1.4 ng of indole-3-acetic acid signal-averaged for 1000 scans. Conditions: solvent elimination deposited on a ZnSe window. Reprinted with permission from P. R. Griffiths.

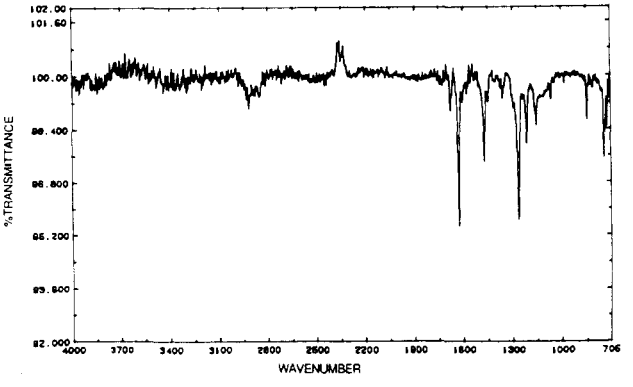

Figure 17. Transmittance spectrum of 1,8-dihydroxyanthraquinone after elution from SFC capillary column using CF_2Cl_2 as the mobile phase and deposited onto a ZnSe window. Reprinted with permission from P. R. Griffiths.

spectrum of 1,8-dihydroxyanthraquinone after elution from a capillary column using CF_2Cl_2 as the solvent. By elimination of the solvent, absorbance bands in the fingerprint region can be easily observed, although interferences from impurities in the mobile phase may interfere.

Recently, Griffiths and co-workers[32] reported on an evacuable variable-temperature GC/FT-IR and SFC/FT-IR interface. The interface was essentially developed as an alternative to the GC matrix isolation interface. The work involves a modification of the system described above to permit switching from GC/FT-IR to SFC/FT-IR measurements. Griffiths cautions that this is not a trivial matter due to the temperature requirements stated above.

Raymer and co-workers[33] have recently interfaced a capillary SFC with matrix isolation Fourier transform infrared (MI-FT-IR) detection. Neat CO_2 and CO_2 doped with 0.5 mol % carbon tetrachloride were used as mobile phases. The column effluent was split between the flame ionization detector (FID) and the Mattson Cryolect. Frit restrictors were used, requiring that the deposition tip be maintained at 120 °C. The Cryolect disk was maintained at 150 K to ensure that CO_2 did not deposit onto the disk. While the carbon tetrachloride did increase the base-line level and noise of the FID, analyte peaks were still observed at the nanogram levels. IR spectra were obtained above 900 cm^{-1}. The MI-FT-IR spectrum of approximately 81 ng of 1,4-naphthoquinone obtained by coadding 16 scans was reported. A peak at 2337 cm^{-1} was observed to be due to CO_2 trapped in the carbon tetrachloride matrix. Efforts were made to evaluate the system with pure CO_2. Although all six components in a test mixture were chromatographed as evidenced by the FID response, only two components, 1-octanol and *N,N*-dimethylaniline, were isolated on the disk in sufficient amounts to obtain usable spectra. The 1-octanol spectra with and without the carbon tetrachloride matrix were not provided but were described as being identical except below 1500 cm^{-1}, where the spectral peaks were broader without the matrix. The authors also indicated that the OH stretching region was broad in both cases, suggesting that there was incomplete isolation of the analyte within the matrix.

IV. Summary

Since the introduction of the first commercially available SFC system in the early 1980s, the development of the SFC/FT-IR technique has attained a high level of sophistication. The many types of interfaces that are being actively investigated for SFC/FT-IR are based on those developed for HPLC. Although the designs may appear to vary greatly, they can be classified into two approaches: flow cell (FC) and solvent elimination (SE). Each basic approach has advantages and disadvantages.

The advantages for the SE approach are that (1) it can accommodate a variety of mobile phases, including CO_2 modified with polar solvents such as methanol, (2) it permits the use of standard condensed-phase reference spectra, and (3) since the effluent from the chromatographic column is deposited onto a solid substrate, the number of scans can be increased to reduce spectral noise and increase sensitivity.

The advantages for the FC approach are that (1) the entire effluent stream is monitored so all sample components are detected intact, (2) other detectors such as the mass spectrometer may be placed in series after the FT-IR, and (3) the interface is mechanically simple.

The disadvantages of the SE approach are that (1) interface designs tend to be mechanically complex, (2) removal of the mobile phase in some instances can drive off volatile components, and (3) mobile phase impurities may be codeposited onto the substrate and cause interferences in the collected spectra.

The disadvantages of the FC approach are that (1) separate spectral libraries may be required for various mobile phases since complete spectra were not feasible, (2) usable mobile phases are limited when identifiable spectra are desired, and (3) lower sensitivity can be expected in most cases.

This review has attempted to provide an overview of SFC/FT-IR by discussing the advantages and disadvantages of the various approaches and discussing recent applications. The coupling of an SFC system to the information-rich FT-IR detector provides a more powerful technique for chemical analysis than either system alone.

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