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Capillary Supercritical Fluid Chromatography–Mass Spectrometry Using a "High Mass" Quadrupole and Splitless Injection

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Most capillary supercritical fluid chromatography–mass spectrometry (SFC–MS) has been done on instruments of mass range of less than 1500 Da, though SFC is able to elute species of molecular masses in the thousands of daltons. In addition, splitting injection is usually employed in SFC, which hampers quantitation. This work addresses both these problems by using a 3000-Da mass range quadrupole in combination with splitless injection. A solution of poly(dimethylsiloxane) in carbon dioxide is introduced to the ion source by supercritical fluid injection for tuning purposes. Poly(dimethylsiloxanes) and derivatized oligosaccharides are transported intact through the SFC–MS interface. Simple ammonia chemical ionization spectra, dominated by the ammonium adduct ion, are seen to the mass limit of the instrument. A signal-to-noise ratio of approximately 4 is measured for the ammonium adduct ion of 0.8 ng of methyl arachidate.

Capillary supercritical fluid chromatography (SFC) is an important analytical separation method, complementary to both gas chromatography (GC) and high-performance liquid chromatography (HPLC). SFC separations may be performed at temperatures as low as 35 °C with CO_2 mobile phase, thus allowing the determination of thermally labile species (*1*). Mixtures containing solutes of molecular mass of several thousand daltons have been successfully analyzed by SFC with flame ionization detection (SFC–FID) (*2*).

Mass spectrometry (MS) has been shown to provide sensitive, universal, and specific detection for SFC (*3–5*). Consistent progress in understanding the SFC–MS interface and in applications of SFC–MS has been reported by Smith, Wright, and co-workers (*6–12*). Significant contributions have also been made by the groups guided by Henion (*13, 14*) and Voorhees (*15, 16*).

Most SFC–MS work has been done with quadrupole mass analyzers that have mass ranges of less than 1500 Da. However, many potential SFC–MS applications involve solutes of mass greater than 1500 Da. Progress has been made in performing SFC–MS on an extended-mass-range, double-focusing magnetic instrument (*17*). Early attempts were slowed by high-voltage breakdown in the ion source. SFC–MS using Fourier transform ion cyclotron resonance mass spectrometry (FTMS) has also been attempted (*18*). The high mass capibilities of FTMS were not demonstrated, however. This lack of success at high mass is probably due to difficulties in maintaining supercritical conditions throughout the long transfer line to the FTMS cell and to high pressure within the cell (*18*).

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Here we describe SFC–MS using an extended mass range (3000 Da) quadrupole instrument. The low-voltage ion source of the quadrupole simplifies the interface design and facilitates the direct introduction of the chromatographic effluent. Since our purpose was the evaluation of the SFC–MS interface and of the mass spectrometer itself, test mixtures were chosen that are chromatographically well-behaved. In particular, instrumental performance was evaluated in terms of signal-to-noise ratio (S/N) near the limit of detection, mass range, and sensitivity as a function of mass resolution.

A successful interface for microbore and high-flow-rate capillary column SFC–MS has been reported using splitless injection (*12*). For high chromatographic efficiency using narrow (<100 μm i.d.) capillary columns in SFC and SFC–MS, mobile phase linear velocities must be slower than those for which this interface is designed. Injection volumes must also be very small. Thus most work in capillary SFC and SFC–MS has been done using split injection techniques. The dependence of split ratios on temperature, pressure, and molecular weight can be a severe hindrance to quantitative analysis (*19*). This study demonstrates that splitless injection with conventional 50-μm capillary hardware can be used in SFC–MS at linear velocities similar to those commonly used in capillary SFC while maintaining reasonable chromatographic resolution.

EXPERIMENTAL SECTION

The instrument used in these experiments is similar in design to that described in previous publications (*5*). In overview, a microcomputer controls a high-pressure syringe pump modified for pressure control (*20*). The pump delivers the mobile phase (liquid carbon dioxide in these experiments) through an internal loop injection valve to a capillary SFC column. Eluent from the chromatographic column is introduced into the mass spectrometer ion source via a custom-built interface probe that fits the direct-insertion-probe vacuum lock.

The capillary SFC system has been described previously (*20*). One modification was made to the standard configuration: the split-injection "tee" and vent assembly were removed. Splitless injection was accomplished by directly coupling a 50 cm × 50 μm i.d. retention gap to the electrically actuated injection valve (EC14W.06, Valco, Houston, TX) via an internal reducing union (IZR1.5, Valco). Care was taken to insert the retention gap only 1.3 cm into the reducing union, flush with the end of the ferrule, to avoid the formation of an unswept dead volume inside the reducing union. Low dead volume unions (ZU.5, Valco) were used to connect the column (DB-1, 10 m, 50 μm i.d., 0.2-μm film thickness, J&W Scientific, Inc., Folsom, CA) to the mass spectrometer interface.

Figure 1 shows the SFC–MS interface probe. The heart of the probe is an 80-cm length of uncoated 25 μm i.d., fused-silica tubing (25VS-025ID, Scientific Glass Engineering, Inc., Austin, TX). The end of this tube has been tapered, sized, and cut to form a restrictor in the manner previously described (*20*). The tapered end narrows to an aperture of approximately 3.4–4 μm diameter over a taper length of 2–4 cm. This restrictor is housed inside

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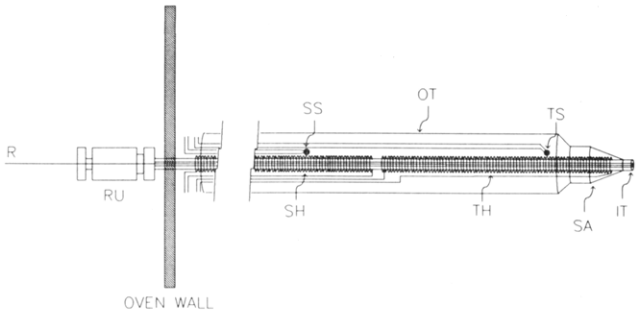


Figure 1. SFC–MS interface probe: R, flow restrictor; RU, reducing union; SH, stem heater; SS, stem sensor; OT, outer tube; TH, tip heater; TS, tip sensor; SA, source adaptor; IT, inner tube.

the "inner" tube, a 70 cm length of 0.25 mm i.d. × 1.59 mm o.d. stainless steel tubing (3005, Alltech Associates, Deerfield, IL).

The inner tube is divided into two heated regions. A 5 cm length at the tip of the probe has been wrapped with a layer of glass tape and a 60 cm length of 4 Ω/ft nichrome wire (Pelican Wire Co., Naples, FL) to form a tip heater. This heater is connected to a 22-W dc power supply (PAT 15-1.5, KEPCO, Flushing, NY) by 22-guage, Teflon-insulated leads which have been silver soldered to the nichrome wire. The temperature of the tip heater is measured by an all-glass insulated, 30-gauge, iron–constantan thermocouple (Omega Engineering, Stamford, CT). This thermocouple and the entire tip heater are potted in a high-temperature ceramic adhesive (AREMCO Products, Inc., Ossining, NY). The remaining 65 cm of the inner tube is wrapped with glass tape and 1.6 Ω/ft nichrome wire (Pelican) to form a stem heater. The temperature of the stem heater is measured by a second, identical, iron–constantan thermocouple. This heater is powered by a variable ac powerstat (10B, Superior Electric Co., Bristol, CT). The entire stem heater is encased in braided ceramic sleeving (XC-14, Omega Engineering). The SFC-oven end of the inner tube is coupled to a reducing union (ZRU1.5, Valco). This union forms a vacuum seal between the restrictor and the inner tube.

The outer diameter of the interface probe and the shape of its tip are dictated by the mass spectrometer's direct insertion port and ion source inlet. The probe's outer tube is a 28-cm length of 7.9 mm o.d. × 6.5 mm i.d. stainless steel tubing (Microgroup Inc., Medway, MA). The tip of the probe, or source adaptor, was custom-machined from 316 stainless steel. It is 1.3 cm long and is silver soldered to both the inner tube and the outer tube to form a vacuum seal. The conical section at the probe tip was fabricated with a 25° angle, with respect to the inner tube, to mate with the mass spectrometer source.

The interface probe required between 8 and 12 man-hours to construct. Under normal use, probe lifetime is indefinite. The flow restrictor plugged after the initial supercritical fluid injection experiments. A second restrictor was used for all the SFC-MS work.

A VG 30-250 automated quadrupole mass spectrometer (VG Masslab, Altrincham, England) was used for this work. The VG 250-11J data system was used to control the instrument and collect the data.

The standard EI/CI ion source was operated in CI mode, using anhydrous grade ammonia (Union Carbide Corp., Linde Division, Danbury, CT) as the CI reagent gas. Source manifold pressure was monitored with an uncalibrated Bayert-Alpert ion gauge. The base pressure in the source housing was 2×10^{-7} Torr. The source manifold pressure with only ammonia CI reagent added was typically $(5–6) \times 10^{-5}$ Torr. With the SFC–MS interface in place and the CO_2 pressure in the chromatographic system at 200 atm, the source manifold pressure was $(1–2) \times 10^{-4}$ Torr. During pressure-programmed chromatographic runs the analyzer manifold pressure reached $(2–3) \times 10^{-6}$ Torr. The base pressure in the analyzer manifold region was approximately 1×10^{-7} Torr.

A 3% solution of poly(dimethylsiloxane) (DC200, Contour Chemical Co., North Reading, MA) in SFC Grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was prepared for supercritical fluid injection as described elsewhere (*21*). This solution was introduced directly into the ion source through the heated interface probe. It supplied a steady ion current for tuning, calibration, and studying mass resolving power vs sensitivity throughout the instrument's mass range under ionization conditions similar to those encountered during SFC–MS (*21*).

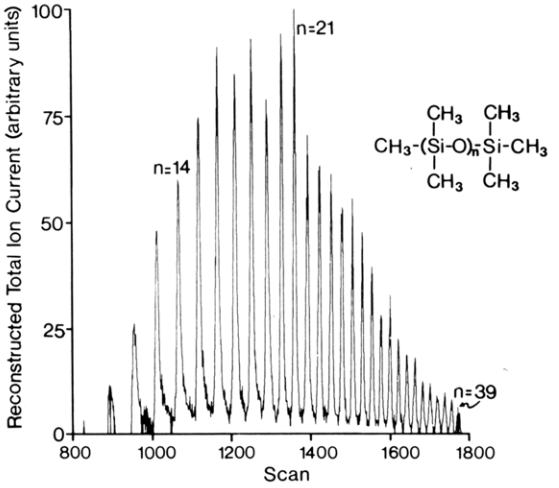


Figure 2. Reconstructed total-ion-current chromatogram of SFC–MS run of DC-200 poly(dimethylsiloxane).

Typical MS-tuning parameters for SFC–MS runs included: electron emission currents of 70–100 μA, electron energy at 200 eV, ion repeller at 7–12 V, ion energy at 4–15 V, ion energy ramp of 10 mV/Da, ion extraction at 0 V, ion focus at 20–30 V, and a multiplier voltage of 1500–1700 V. The sensitivity of the mass spectrometer for high mass ions (>1500 Da) was improved at least 10-fold by decreasing the instrument's mass resolving power. The resolving power was adjusted such that the valley between adjacent ions in the isotope multiplets above m/z 1500 was roughly 90% the height of the peak corresponding to the less abundant ion. The scan rate was 2 s/scan over a range of m/z 500–3000 except where indicated otherwise. The temperature of the CI source was held between 270 and 300 °C.

Chromatographic separations were typically performed by using linear pressure gradients of SFC grade CO_2 (Scott Specialty Gases) from 100 to 300 atm. The SFC oven temperature was set between 80 and 100 °C, the stem heater on the SFC–MS interface was held at 90 °C, and the tip heater was maintained at 350 °C.

Solutions of poly(dimethylsiloxane) (DC200), tristearin (NU-CHEK-PREP, Inc., Elysian, NJ), sucrose octaoleate (synthesized in-house), and methyl arachidate (Alltech Associates, Deerfield, IL) were prepared in methylene chloride (American Burdick and Jackson, Muskegan, MI) prior to chromatographic runs. Maltrin 100 (Grain Processing Corp., Muscatine, IA), a commercial corn syrup solid, was silylated prior to SFC–MS: approximately 10 mg of this material was added to 0.2 mL of 5/1 *N*-(trimethylsilyl)imidazole (TMSI) (Pierce Chemical Co., Rockford, IL) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce Chemical Co.) and heated at 80 °C for 1 h. After cooling, the resulting solution was diluted to 1 mL with dry methylene chloride (American Burdick and Jackson). All other solvents were ACS reagent grade (Fisher Scientific, Fair Lawn, NJ).

RESULTS AND DISCUSSION

SFC–MS of Poly(dimethylsiloxane). Figure 2 is a plot of the reconstructed total ion current (RTIC) chromatogram obtained during an SFC–MS run of the DC 200 poly(dimethylsiloxane). Peaks from oligomer number (n) 11 through 39, corresponding to ammonium adduct ions of m/z (most abundant isotope) 920 through 2998, are shown. Higher molecular weight oligomers were not detected since the instrument's mass range is limited to 3000 daltons.

During runs of high molecular weight oligomeric series such as the poly(dimethylsiloxanes), the resolving power of the instrument was purposely reduced as described in the Experimental Section and the peak detection parameters were adjusted such that the isotopic multiplets corresponding to the major ions of the tuning mixture merged into single, broad

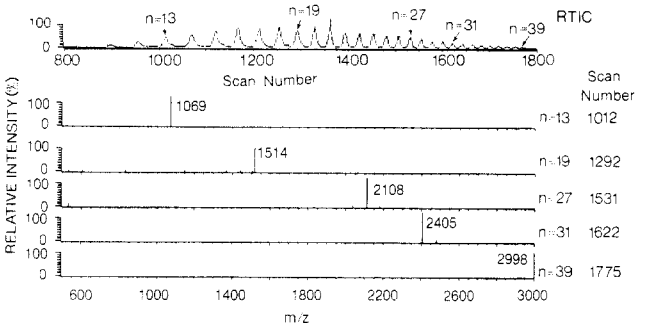


Figure 3. Reconstructed total-ion-current chromatogram (top) and selected ammonia CI spectra from SFC–MS run of poly(dimethylsiloxane).

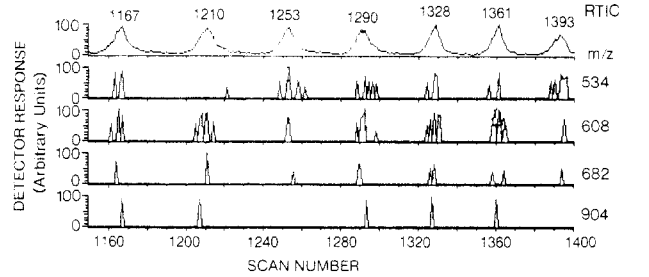


Figure 4. Reconstructed total-ion-current chromatogram (top) and selected mass chromatograms showing structurally related ions produced during NH_3 CI SFC-MS run of poly(dimethylsiloxane).

Table I. Monoisotopic Masses and Most Abundant Isotopes of Selected Ammonium Adducts of Poly(dimethylsiloxane) Oligomers

degree of polymerization (n)[a]	monoisotopic mass,[b] Da	most abundant isotope mass,[b] Da
9	772	772
13	1068	1069
19	1512	1514
23	1808	1811
27	2104	2108
29	2252	2256
31	2400	2405
33	2548	2553
39	2992	2998

[a] Refer to structure in Scheme I. [b] Rounded to nearest integral mass.

peaks. The instrument was mass calibrated such that the centroid of the broad peak was near the most abundant isotope (±1 Da) of the cluster. This dramatically improved the signal-to-noise ratio of the measurements. The reduction in resolving power increased the signal intensity by a factor of roughly 10 at m/z 2000 as directly measured on the oscilloscope during the tuning process.

Figure 3 illustrates representative spectra collected during the SFC–MS run of the poly(dimethylsiloxanes), the TIC of which is shown in Figure 2. All of the oligomers show $[M + 18]^+$ ammonium adduct ions. The centroid m/z values are within one unit of the expected most abundant isotope. As an aid to the reader, Table I lists the degree of polymerization ("n" value) associated with representative siloxane oligomers, the monoisotopic mass of the ammonium adduct, and the mass of the most abundant adduct isotope. Most spectra also show a weak signal corresponding to the protonated molecule. The largest chromatographic peaks in the series also show very weak ions at m/z 534, 608, 682, 756, 830, and 904. This is illustrated in Figure 4. The ions are separated by intervals of 74 Da, corresponding to the oligomeric unit, SiC_2H_6O. It

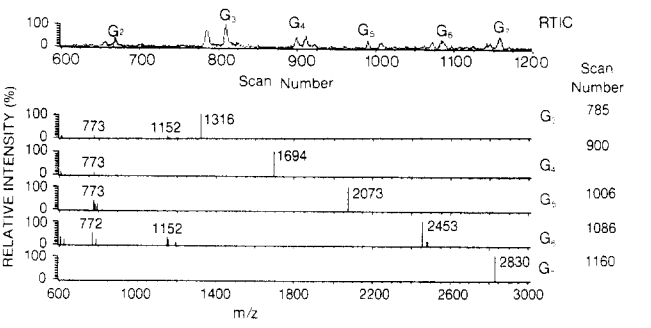


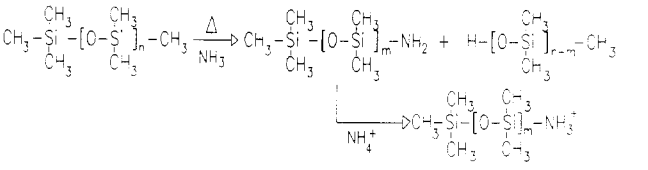
Figure 5. Reconstructed total-ion-current chromatogram (top) and selected spectra from NH_3 CI SFC-MS run of derivatized Maltrin 100.

Table II. Monoisotopic Masses and Most Abundant Isotopes of Selected Ammonium Adducts of Derivatized Oligosaccharides

no. of glucose units ("G" value)	monoisotopic mass,[a] Da	most abundant isotope mass,[a] Da
3	1314	1316
4	1692	1695
5	2070	2073
6	2448	2452
7	2826	2830

[a] Rounded to the nearest integral mass.

Scheme I



appears that a similar series has been previously observed in NH_3 CI, though the spectrum is not shown and the m/z values are not listed (*22*). The structure of these ions cannot be determined unambiguously from our data. A possible scheme yielding ions corresponding to these m/z values is shown in Scheme I. These structures may thus be due to ammonolysis of the poly(dimethylsiloxanes), where neutral ammonia reacts with an oligomer to form a new chemical species which is subsequently ionized. This type of behavior has been previously observed in NH_3 CI of oligomeric compounds (*23*, *24*). The amine-containing fragment (1) would be preferentially ionized over the hydroxy species (2) by the ammonium ion due to the difference in their proton affinities. An experiment with nitrogen-labeled ammonia would confirm or refute this hypothesis.

SFC–MS of Oligosaccharides. The trimethylsilyl derivatives of oligosaccharides can be easily eluted with unmodified CO_2 mobile phase in capillary SFC over a wide molecular weight range (*2*). The oligomeric series elutes as a series of irregularly spaced doublets. The corn syrup solid used, once derivatized, more than adequately covers the mass range of the VG instrument and is chromatographically well-behaved. Analyzing this mixture by SFC–MS not only allowed us to test our SFC–MS interface and the VG quadrupole but also allowed us to confirm the assignment of the doublets as anomeric forms of the reducing end of the oligosaccharide (*25*).

Figure 5 illustrates the TIC chromatogram for the oligosaccharides containing two through seven glucose units (G2–G7). The molecular weights of the oligosaccharides G8 and above were beyond the mass range of the mass spectrometer. Figure 5 also illustrates representative spectra collected during this NH_3 CI run. The mass spectral peaks were centroided,

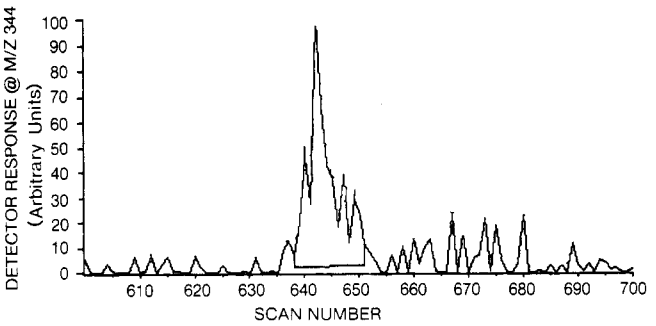


Figure 6. Mass chromatogram of m/z 344, ammonium adduct ion of methyl arachidate, about elution time of 0.8 ng run by NH_3 CI SFC–MS.

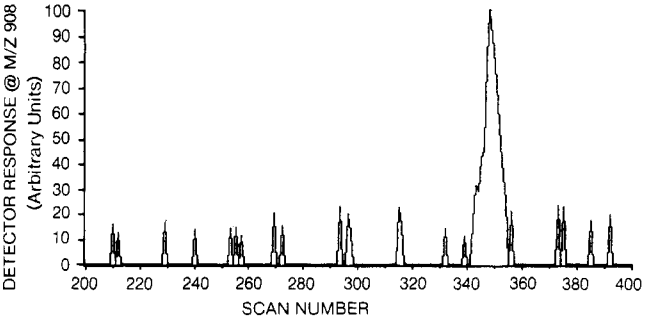


Figure 7. Mass chromatogram of m/z 908, ammonium adduct ion of tristearin, about elution time of 12 ng run by NH_3 CI SFC-MS.

as described above. Each oligosaccharide displays a base peak within 1 mass unit of that expected for its ammonium adduct ion. Table II lists the glucose unit number, the monoisotopic mass, and the "centroided" mass of the ammonium adduct ions of selected oligosaccharides.

The more intense spectra usually displayed fragment ions at or near m/z 773 and m/z 1152 as shown in Figure 5. These fragment ions were sometimes sporadic and always of low relative abundance (<10%). The difference in mass between these two ions of 379 Da is near the mass of the repeating unit of the derivatized oligosaccharide (378 Da). These ions may thus be related to the structure of the oligosaccharide. Such fragments are not unexpected given previous studies of the NH_3 CI of oligosaccharides (*23*, *24*). The data collected are insufficient to unambiguously determine the structure of these ions.

Evaluation of the Signal-to-Noise Ratio (S/N) Near the Detection Limit. Evaluation of the S/N near the detection limit in capillary SFC–MS is complicated by the customary use of splitting injection, standard in SFC–FID. Split ratios vary with temperature, pressure, and molecular weight (*19*, *26*). To avoid these problems, the retention gap was directly linked to a nominal 0.06-μL injection valve without splitting. The lower mass scan limit was above any ions produced by the broad solvent front, which we expected from previous SFC–FID experience. The column flow rate was not directly measured, but the robot-pulled tapered flow restrictor was typical (4.0 cm taper length, 3 μm aperture), and retention times of standards were near those expected. This mode of splitless injection in SFC–MS worked better than anticipated. The detection of early eluting peaks did not seem to be adversely affected by the solvent front. All the work discussed in this publication was performed in this mode of splitless injection, with adequate chromatographic resolution, as shown in the reconstructed TIC chromatograms. These separations were developed using a similar SFC instrument equipped with conventional *split* injection and flame ionization detection. A drop in chromatographic peak resolution was expected in moving from the split SFC–FID runs to the splitless SFC–MS runs, and such a drop was observed. For example, the seventh and eighth peaks in Figure 2, corresponding to oligomers with n = 16 and 17, are separated with a resolution of 2.1 in the SFC–MS run while the same peak pair in an SFC–FID run was separated with a resolution of 4.2.

Three standards were used to evaluate the S/N of this SFC–MS combination near the detection limit. All three were well-behaved chromatographically. One was methyl arachidate, with a molecular weight of 326. It has often been used to evaluate S/N in GC–MS. The second was tristearin, molecular weight 890. The third was sucrose octaoleate, with a molecular weight of 2454. The instrument was tuned to "unit" resolution (10% valley definition) before the S/N measurements were made.

Figure 6 is the mass chromatogram of m/z 344, $[M + NH_4]^+$ for methyl arachidate, produced upon injection of 0.8 ng of the ester in dichloromethane. The scan range was m/z 100–500 with a 1-s scan cycle time. The S/N is approximately 4. The contribution to this particular run of methyl arachidate of carry-over from previous runs was negligible. Sensitivities at least an order-of-magnitude higher have been reported for substituted biphenyls in SFC–MS using CH_4 CI (*7*). The authors have measured limits of detection (S/N of 3) in the tens of picograms for naphthalene using CH_4 CI SFC–MS (scanning over a 100 dalton or greater mass range) (*27*). The S/N is affected by the method of ionization chosen, the nature and molecular weight of the species in question, the chromatographic peak width, and the transmission/detection efficiency of the mass spectrometer.

While the methyl arachidate runs were performed with a relatively clean ion source, the tristearin runs were performed after about 60 h of nearly continuous NH_3 CI SFC–MS operation. Removal of the ion source after the tristearin runs revealed that the source was very dirty. This is the most probable cause of the lower signal levels and higher background noise levels for the oligosaccharides and tristearin runs. Figure 7 is a mass chromatogram of m/z 908, $[M + NH_4]^+$ for tristearin, collected after a splitless injection containing 12 ng of tristearin. The scan range was from m/z 500 to 1000 with a scan cycle time of 1.1 s. The S/N is roughly 5.

This work was performed over a 3-day period and involved equipment from two distant sites. We were not able to repeat the tristearin S/N experiments under optimal conditions due to these logistical constraints. However, we feel that these preliminary data are useful in at least establishing a "worst case" S/N figure obtained with no real attempt at optimization. In previous SFC–MS work it was shown that a simple cryopumping attachment dramatically reduces the ion source manifold pressure (by a factor of 5 under typical operating conditions) and improves signal levels (by a factor of 2 to 3) in SFC–MS (*26*). The VG instrument used in these experiments had no such means of additional pumping.

The beneficial effects of additional pumping are especially important at high mobile phase pressure (pressures of 300–400 atm are often present at the end of a pressure-programmed run). This may explain the poor results obtained during runs of the relatively high-mass S/N standard, sucrose octaoleate. The elution pressure of this species is 370 atm under the SFC conditions chosen. The ion source manifold pressure was over 3×10^{-4} Torr at this elution pressure. We observed no ions related to sucrose octaoleate under the NH_3 CI conditions used. This may also be due to nucleation and precipitation of the sucrose octaoleate in the tapered flow restrictor due to insufficient solubility in the unmodified CO_2 mobile phase (*10*). However, if this were the case, one would expect to also see detector "spiking" when this species is analyzed by SFC–FID. Such spiking has not been observed.

This work demonstrates that species of molecular weights up to at least 3000 can be introduced intact to the ion source

as solutes in supercritical CO_2 through a simple interface probe and ionized by traditional CI mass spectrometry. Ammonium adduct ions of the poly(dimethylsiloxanes) were observed to the mass limit of the spectrometer by supercritical fluid injection. The only limit to mass range in this case appears to be the upper mass limit of the mass analyzer itself. Spectra were obtained which were indicative of both molecular weight and structure in the case of the poly(dimethylsiloxanes) and of the oligosaccharides.

The mode of splitless injection used in this work is a step toward making SFC–MS a quantitative, trace analysis technique. Injection technology being developed in a number of laboratories which will allow the splitless injection of solutes present in microliter volumes will strengthen the standing of SFC and SFC–MS in this area. Capillary supercritical fluid chromatography–mass spectrometry using an unmodified CO_2 mobile phase as presented here is limited to relatively nonpolar solutes. The use of modified and more polar mobile phases should extend the range of solute polarities which SFC–MS can handle (*28*). As the high-mass performance of quadrupole mass analyzers improves and as problems in interfacing SFC to mass spectrometer ion sources operating at high voltage are overcome (*17*) SFC–MS and SFI–MS will become even more powerful tools in characterizing mixtures containing high molecular weight, less volatile species.

ACKNOWLEDGMENT

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Registry No. Methyl arachidate, 1120-28-1; tristearin, 555-43-1.

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Average Mass Approach to the Isotopic Analyses of Compounds Exhibiting Significant Interfering Ions

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The recently proposed average mass approach to isotopic analysis is extended to the analyses of materials for which the molecular ion groups are distorted by the presence of one or more overlapping species. The method is simple and direct and could prove to be more general and accurate than conventional approaches to these problems.

Mass spectrometry is the method of choice for the quantification of stable isotope labels in organic and inorganic materials (*1*) with only a few possible exceptions (*2*). The conventional approach to deducing the isotopic content of a sample from its mass spectral pattern is to construct a set of simultaneous equations which describe the molecular ion distribution in terms of the various isotopic forms comprising it. One then solves this set of simultaneous equations for the relative proportions of the labeled species in the sample. If the labeled material is comprised of elements that are predominantly monoisotopic in their natural forms (e.g., C, H, N, O, F, etc.) and gives rise to a "pure" molecular ion (i.e., there are no interfering fragment ions), then the set of simultaneous

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