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INDUSTRIAL APPLICATIONS OF SUPERCRITICAL-FLUID CHROMATOGRAPHY–MASS SPECTROMETRY INVOLVING OLIGOMERIC MATERIALS OF LOW VOLATILITY AND THERMALLY LABILE MATERIALS

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SUMMARY

Three recent applications of supercritical-fluid chromatography with flame ionization (SFC–FID) and with mass spectrometric (SFC–MS) detection are discussed. These applications involve thermally labile and/or relatively non-volatile materials. Specifically, they involve ethoxylated alcohols, inositol triphosphate and peroxides. Ammonia chemical ionization (CI) and methane CI SFC–MS were used to deconvolute complex chromatograms and to confirm postulated identities. The SFC–MS "electron ionization like" spectrum of the trimethylsilyl (TMS) derivative of inositol triphosphate was shown to be very similar to the library electron ionization spectrum of TMS-inositol diphosphate. SFC–MS was shown to be particularly suitable for the analysis of thermally labile peroxides using ammonia CI SFC–MS. All three applications discussed here would have been difficult, if not impossible, by traditional gas or liquid chromatographic methods.

INTRODUCTION

Industrial analytical chemists encounter separation problems involving widely differing types of materials. It is not uncommon that these problems are difficult to address by traditional methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC). This is the case when the mixture in question contains components which are too low in volatility or thermal stability for GC, and which simultaneously lack a good functional group for sensitive HPLC detection or are members of a mixture too complex for traditional HPLC separation[1].

Capillary supercritical-fluid chromatography (SFC) has experienced widespread growth and commercialization since it was reported in 1981[2,3]. Though it has been proposed as a general screening tool for non-polar samples[4], most SFC work has served to fill the "gap" between GC and HPLC described above. Applications involving thermally labile compounds, such as azo-compounds[5,6], and complex mixtures of low volatility, such as oligosaccharides[7], have been reported. Mass spectrometry (MS) has been shown to provide sensitive, universal, and specific detection for SFC[8–15]. Though our laboratory has been involved in supercritical-fluid

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chromatography–mass spectrometry (SFC–MS) for a number of years[16,17], we only recently acquired a mass spectrometer dedicated to SFC detection. This report discusses three of our first applications using this dedicated instrument. They involve ethoxylated alcohol mixtures, inositol triphosphate, and peroxides. In their own way, each is a good illustration of how SFC and SFC–MS can be used to solve problems which are difficult to address by traditional GC and HPLC.

EXPERIMENTAL

The SFC system is identical to that previously described[17] except for the incorporation of a stainless-steel tubing 10 cm × 0.127 mm (0.005 in.) I.D. × 1.6 mm (0.063 in.) O.D. just before the Valco injection valve in the carbon dioxide line from the syringe pump. This small addition dramatically reduced the solvent front tail in the direct (on-column) injection mode used in all the SFC–MS work.

Other modifications include the use of an Upchurch (Oak Harbor, WA, U.S.A.) fitting with an F226 fused-silica adapter and F140 nut to link the 1 m × 50 μm I.D. retention gap directly to the injection valve. SGE MVSU-004 zero dead volume unions (Scientific Glass Engineering, Austin, TX, U.S.A.) were used to link the retention gap to the column and the column to the restrictor. A fused-silica capillary sleeve (Polymicro Technologies, Phoenix, AZ, U.S.A.) with an O.D. of 375 μm and an I.D. of 200 μm was inserted into each SGE union to reduce the inner diameter. The column was a 10 m × 50 μm I.D. SB-Methyl 100 (Lee Scientific, Salt Lake City, UT, U.S.A.) with a 0.5-μm film thickness. The tapered-flow restrictor was fashioned from a 50 cm × 50 μm I.D. 180 μm O.D. fused-silica tubing (Polymicro Technologies) in the manner previously described[18]. It tapered to an aperture of approximately 3 μm over a 3.2-cm length.

The SFC–MS interface probe houses the flow restrictor and is similar to one previously described[17]. The tip of the interface probe was machined to fit the rear of the ion source ion volume of a Finnigan-MAT TSQ-70 triple quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA, U.S.A.). The aperture of the flow restrictor was positioned just inside the interface probe tip, as described previously[17]. The probe stem was held at the same temperature as was the SFC oven, and the probe tip was held at roughly 300°C, unless otherwise indicated.

The first and third quadrupoles (Q1 and Q3, respectively) of the TSQ-70 were operated in the "low-mass-range mode" (m/z 4–2000) with Q1 and Q2 transmitting all ions. Q3 was used as the mass filter. The instrument was tuned and calibrated using perfluorotributylamine and tris(perfluorononyl)-s-triazine (PCR Research Chemicals, Gainsville, FL, U.S.A.) with automatic tuning software in the customary manner. The ion source temperature was held at 150°C unless otherwise indicated and the analyzer manifold was held at 70°C. Ammonia chemical ionization (CI) was performed with 1% ammonia in methanol ("Matheson" purity, 99.99% minimum, Matheson Gas Products, Dayton. OH, U.S.A.). Matheson purity methane was used for methane CI (Matheson). Isobutane CI was performed with "Instrument Grade" isobutane (99.5% minimum, Matheson). The indicated "ion source" pressure required to achieve proper CI conditions was approximately 1.3 kPa (10 Torr) for both ammonia and methane CI, and approximately 360 Pa (2.7 Torr) for isobutane CI. However, these measurements are performed using an uncalibrated Pirani gauge in the

CI reagent gas supply line and do not provide an accurate measurement of the actual ion source pressure. Electron ionization (EI) (with possible contribution from EI-like carbon dioxide charge exchange ionization) was performed by simply exchanging the "CI probe" ion source ion volume for the "EI probe" volume. Electron energy and emission current were held at 70 eV and 200 μA, respectively. The electron multiplier was operated at -1500 V with an electrometer gain of 10^{-7} A/V.

All the Neodol samples but Neodol 23-3 were commercial samples obtained from the Shell Chemical Co. (Houston, TX, U.S.A.). Neodol 23-3 was obtained courtesy of W. R. West (Shell Development, Houston, TX, U.S.A.). Approximately 2% (w/v) solutions of the Neodol samples were prepared in dichloromethane (American Burdick & Jackson, Muskegon, MI, U.S.A.) and injected without further preparation. The 1-pentaethoxy tetradecanol was synthesized and purified in house. Retention standards were obtained from PolyScience Corp. (Niles, IL, U.S.A.).

The Neodol samples were also analyzed by SFC–flame ionization detection (FID) using a Lee Scientific Model 622 SFC–GC system. The capillary column was a 10 m $\times$ 50 μm I.D. DB-1 (J&W Scientific, Folsom, CA, U.S.A.) with a 0.2-μm film thickness. The flow restrictor was a 50-μm I.D. ceramic frit (Lee Scientific). Injection was performed using a 0.2-μl internal loop (Valco, Houston, TX, U.S.A.) and flow splitting with a rougly 10:1 split ratio. The oven temperature was 120°C while the detector was held at 300°C. The mobile phase in all the work described in this publication was unmodified, SFC-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, U.S.A.). In the SFC–FID runs of the Neodol samples, the mobile phase pressure was held at 10.1 MPa (100 atm) for 5 min and then ramped to 35.5 MPa (350 atm) over 50 min. In the SFC–MS runs the SFC oven and interface probe stem were held at 100°C. The mobile phase pressure was held at 9.1 MPa (90 atm) for 8 min after the direct injection and then ramped at 0.5 MPa/min (5 atm/min) to 34.5 MPa (340 atm) during the methane CI runs. A slight modification of this pressure program was used in later ammonia CI work. The injection was performed at 7.1 MPa (70 atm). After 5 min at 7.1 MPa the pressure was stepped to 10.1 MPa (100 atm). The pressure was then ramped at 0.5 MPa/min (5 atm/min) to 35.5 MPa (350 atm). The mass spectrometer was scanned from m/z 100 to 1200 every second for Neodol 91-6, from m/z 100 to 1000 every 1.5 s for Neodol 45-7T, and from m/z 100 to 900 every 1.5 s for Neodol 23-3.

Inositol triphosphate was obtained from W. R. Sherman (Washington University School of Medicine, St. Louis, MO, U.S.A.). The sample was silylated as described in ref. 19. The solution injected had a concentration of 4.1 mg/ml of the trimethylsilyl ester/ether of inositol trophosphate. The SFC oven temperature was held at 150°C while the mobile phase pressure was held at 12.2 MPa (120 atm) for 4 min and then ramped to 32.4 MPa (320 atm) at 1.0 MPa/min (10 atm/min). The mass spectrometer was scanned from m/z 300 to 1200 in the CI mode and from m/z 100 to 1200 in the EI-like mode, both with a cycle time of 2 s. During EI-like ionization, at a mobile phase pressure of 27.4 MPa (270 atm) [approximately 2.0 MPa (20 atm) above the elution pressure of the analyte], the "ion source" pressure (as previously described) was 26 mTorr. Under these same EI-like conditions the analyzer pressure was $1.1 \cdot 10^{-5}$ Torr, as measured with an uncalibrated Bayert–Alpert ion gauge.

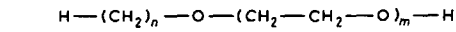
Benzoyl peroxide was obtained from Aldrich (Milwaukee, WI, U.S.A.). Terpineol succinate peroxide was synthesized in-house. The former was dissolved in

dichloromethane (American Burdick & Jackson) to a concentration of 0.5 μg/μl, while the latter was dissolved in acetone (American Burdick & Jackson) at the same concentration. The mass spectrometer was scanned from *m/z* 100 to 300 with a cycle time of 1 s for benzoyl peroxide. The scan range for terpineol succinate peroxide was *m/z* 100 to 550, also with a 1-s cycle time. A variety of component temperatures were used during the benzoyl peroxide runs, but the spectrum presented in the following section was obtained with an SFC oven and interface probe stem temperature of 80°C, a probe-tip temperature of roughly 120°C, and an ion source temperature of 150°C. Temperatures for the terpineal succinate peroxide run presented in the following section were: oven and probe-stem at 40°C, probe-tip at 100°C, and source at 85°C. The mobile phase pressure was held at 7.1 MPa (70 atm) for 2 min and then ramped to 27.4 MPa (270 atm) at 1.0 MPa/min (10 atm/min) for both peroxides.

RESULTS AND DISCUSSION

Ethoxylated surfactant

Neodol (Scheme 1) is a trade name for ethoxylated alcohols, an important class of non-ionic surfactants. They are generally named Neodol xx-z, where "xx" indicates a range of *n* values and "z" is an average *m* value. For example, Neodol 45-7 indicates that the starting alcohols were 14 and 15 carbon alcohols and that the average ethoxylate value is 7. The average length and range of the alkyl and ethoxylate chains determine the properties of each mixture. Neodols are used in a wide variety of industrial and household products. Yet they are difficult to analyze using traditional chromatographic methods. Many Neodols contain components which are too low in volatility for traditional GC analysis. Ethoxylated alcohols lack a good chromophore or other functionality for HPLC detection, and are thus difficult to analyze with good sensitivity by HPLC. In addition, they are often the product of the ethoxylation of a mixture of alcohols. It is difficult to resolve the various components of such a complicated mixture using traditional HPLC, especially if the chosen detector requires isocratic elution. We have found that SFC using carbon dioxide and FID is an excellent tool for analyzing Neodol samples. SFC–MS has been used to identify unknown components of the ethoxylated mixtures and to confirm postulated peak identities.



Scheme 1. Structure of neodol.

Fig. 1 shows an SFC–FID trace of Neodol 45-7T ("T" stands for "topped", meaning that the concentration of the lower oligomers in the mixture has been reduced) to which 1-tetradecanol and 1-pentadecanol have been added. The two straight-chain alcohols co-elute with the first members of each ethoxylated series allowing one to postulate identities for the other members of the series by simply counting from the unethoxylated peak (E_0). Postulating peak identities in this manner was straightforward for the chromatograms of most Neodol mixtures. Fig. 2 shows the chromatogram of Neodol 45-8NRE, where NRE stands for "narrow

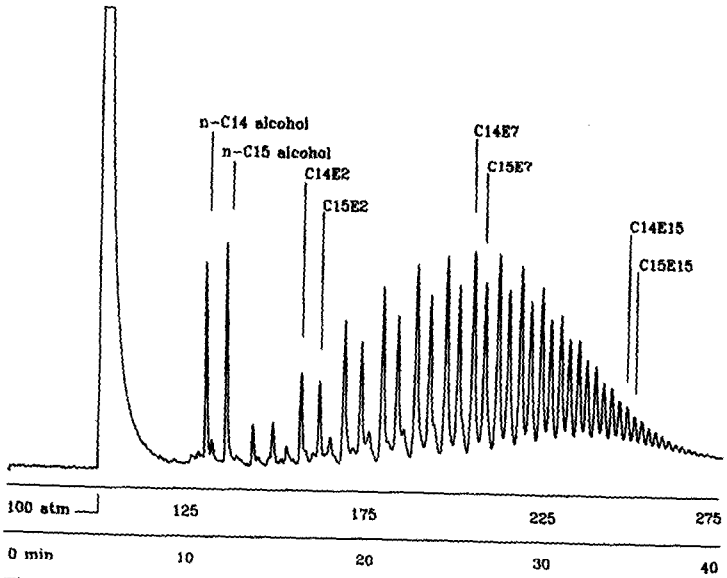


Fig. 1. SFC–FID chromatogram of Neodol 45-7T with straight-chain C_{14} and C_{15} alcohols "spiked" as retention standards.

range ethoxylate". It is obvious from the chromatograms that the second sample does indeed have a tighter ethoxylate distribution than does the Neodol 45-7T, Table I summarizes the results of the analysis of a number of Neodol samples. These results assume that the FID response factor is constant during the chromatographic run, in spite of the increase in carbon dioxide flow into the flame due to pressure programming. Our results agree well with the expected composition of the mixtures.

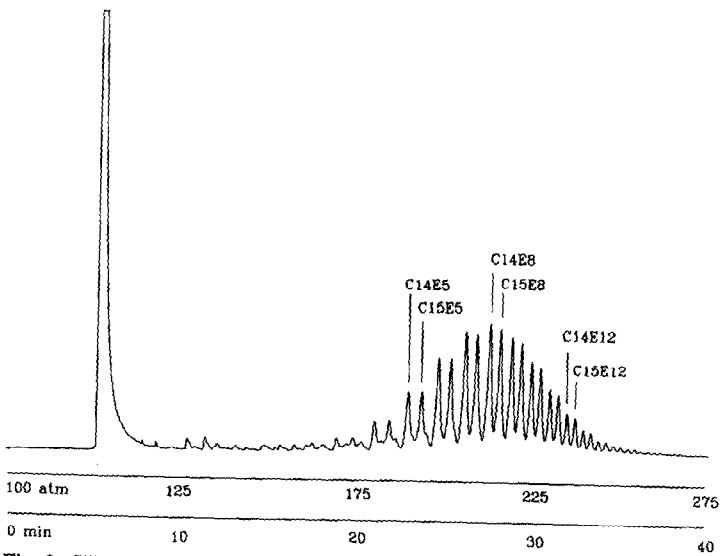


Fig. 2. SFC–FID chromatogram of Neodol 45-8NRE.

TABLE I

SUMMARY OF SFC–FID ANALYSIS OF NEODOL SAMPLES

Neodol sample	*Average ethoxylate number*	*Detectable ethoxylate range*	*Ethoxylate range carrying 68.3% mass*	*Average alkyl chain length*
23.3	3.1	0–15	0–3	12.5
45-7T	7.4	0–21	3–9	14.5
45-8NRE	8.1	1–18	6–10	14.5
91-6	6.8	0–22	3–10	10.2
91-6NRE	6.7	3–19	5–8	10.2

The postulated peak identities of the Neodol samples were confirmed by SFC–MS. Some controversy exists in the literature concerning the CI of ethoxylated alcohols. Stephanou[20] discusses spectra exhibiting the ammonium adduct ion as the base peak, a protonated molecule with a relative abundance of 3–17% depending on the length of the ethoxylate chain, and many structurally significant fragment ions of relative abundance generally 3–18%. On the other hand, spectra discussed in the work of Rudewicz and Munson[21] are much simpler, showing primarily the ammonium adduct ion, a protonated molecule with less than 2% relative abundance, and even lower levels of fragment ions. These results were obtained with either pure ammonia or 1% ammonia in methane as reagent gas. Fig. 3 shows the ammonia CI SFC–MS spectrum of an ethoxylated alcohol standard, 1-pentaethoxy tetradecanol. The base peak of the spectrum in Fig. 3 corresponds in mass to the ammonium adduct ion. The next most abundant ion corresponds to the protonated molecule. The other major peaks in the spectrum correspond in mass to logical fragments of the ethoxylated standard that would be expected based on Stephanou's work[20]. Ions at $[M + 32]^+$ and at $[M + 46]^+$ may be due to the addition of $[CH_3–NH_3]^+$ and of $[C_2H_5–NH_3]^+$, produced in the 1% ammonia in methane CI plasma, to the ethoxylated standard.

Mass chromatograms of the ammonium adduct ions of a number of components of a typical Neodol sample, Neodol 23-3, are shown in Fig. 4. Under the conditions of

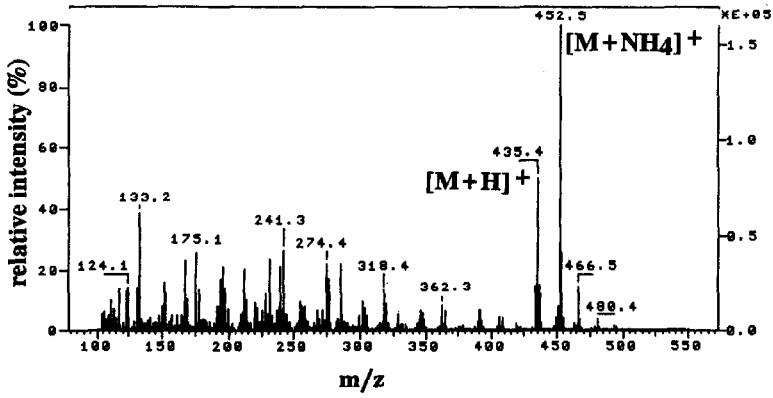


Fig. 3. Ammonia CI SFC–MS spectrum of 1-pentaethoxy tetradecanol.

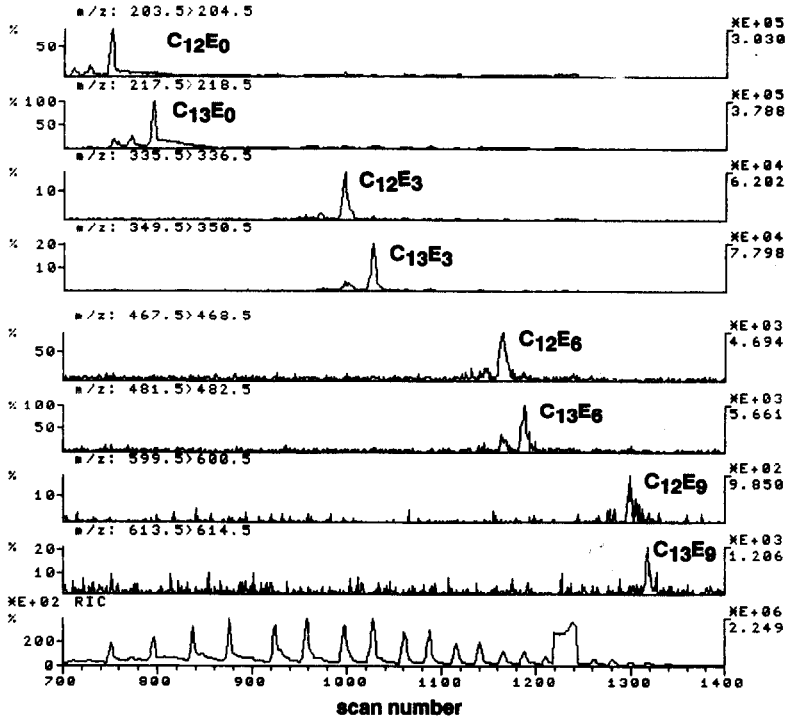


Fig. 4. Ammonia CI SFC–MS run of Neodol 23-3 showing a portion of the reconstructed total ion current chromatogram (bottom) and representative ammonium adduct mass chromatograms.

this run ethoxylated, branched-chain alcohols would elute before their straight-chain isomers[22]. Small amounts of branched-chain alcohols are known to be present in the starting alcohols used in this type of ethoxylation reaction[23]. Thus small peaks eluting before the major peak in each mass chromatogram are probably due to branched-chain isomers of the starting alcohols. The shift in baseline in the reconstructed total ion current chromatogram is due to rezeroing the electrometer during the run.

Fig. 5 shows the SFC–FID chromatogram of Neodol 91-6. We could not unambiguously assign structures to the peak series using coinjection of retention standards due to the complexity of the mixture. Fig. 6 illustrates mass chromatograms and the reconstructed total ion current chromatogram from a methane CI SFC–MS run of Neodol 91-6. Using the data from this run we were able to quickly and unambiguously assign the peak identities shown in Fig. 5. The chromatographic resolution is lower in the SFC–MS runs than in the SFC–FID runs. This has been observed and discussed in previous work[17]. It is most likely due to the use of direct injection in SFC–MS instead of flow-splitting injection, as well as column overload.

The analysis of non-ionic, ethoxylated surfactants such as Neodols by SFC–FID and SFC–MS is straightforward. These data illustrate why we feel that SFC is the most appropriate separation technique for dealing with problems which involve surfactants such as these. Some problems involving Neodol mixtures may be solved more rapidly using Rudewicz and Munson's probe distillation/CI MS method[21]. This method,

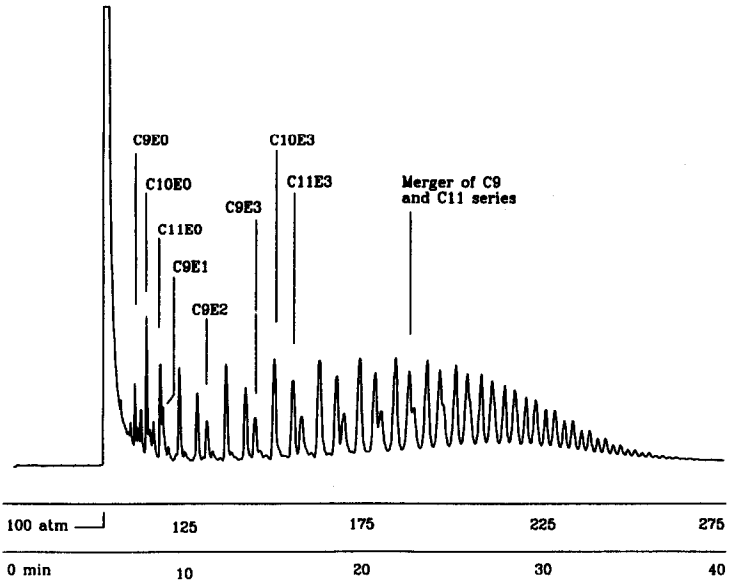


Fig. 5. SFC–FID chromatogram of Neodol 91-6.

however, would not be suitable for very complex Neodol mixtures, especially those where information about isomers was desired, or for Neodol mixtures containing components of interest too low in volatility to be desorbed from the distillation probe. Finally, the method would be difficult if ionization conditions producing the very simple spectra reported by Rudewicz and Munson[21] were not achieved (as in our work).

Thermospray LC–MS has recently been shown to be a viable alternative for the analysis of non-ionic ethoxylated surfactants[24]. Thermospray LC–MS does suffer from at least two disadvantages when compared to SFC–MS in this type of analytical

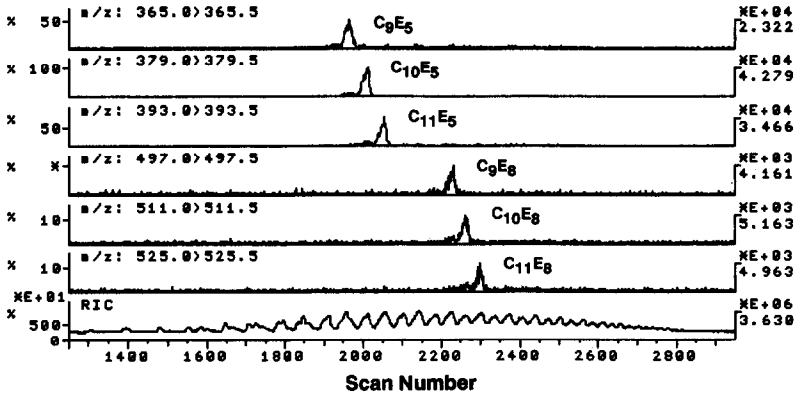


Fig. 6. Methane CI SFC–MS run of Neodol 91-6 showing a portion of the reconstructed total ion current chromatogram (bottom) and mass chromatograms of the protonated molecules of representative components.

challenge, however. Chromatographic efficiency per unit time on a given column is inherently lower with a liquid mobile phase than with a supercritical mobile phase[1]. Thermospray LC–MS is often performed with packed columns, while our results were obtained using capillary columns. Thus the results reported in ref. 24 cannot be directly compared with our results. However, individual oligomers and their isomers in each ethoxylated series are better resolved by capillary SFC–MS than by thermospray LC–MS[24]. Secondly, and perhaps more importantly, most traditional CI and even EI (see below) ionization methods are available in capillary SFC–MS, which is not the case with thermospray LC–MS.

Inositol triphosphate

The phosphorylated inositols are important in many biological systems (for a review see refs. 25 and 26). Certain isomers are involved in cellular stimulus-response coupling via calcium mobilization[25–27], and it's been postulated that lithium treatment of manic illness acts on an enzymatic pathway involving phosphorylated inositols[28]. The more highly phosphorylated inositols (*i.e.*, pentakis- and hexakis-phosphates) are commonly found in grains, seeds, soils, and sediments.

The chromatographic analysis of the members of this series with higher degrees of phosphorylation is difficult using conventional chromatographic methods. Significant losses due to adsorption during GC analysis are apparent with the TMS and methyl esters of the di- and triphosphates[29,30]. The lack of a sensitive method of detection and adsorption of the more highly phosphorylated inositols are the obstacles in HPLC analysis[30,31]. Capillary SFC has relatively inert instrumental components and is able to elute low volatility solutes at comparatively low temperatures. We have found that capillary SFC is well suited for analyzing the TMS derivatives of inositol phosphates[19]. Isobutane CI SFC–MS was used to confirm the identity of the TMS derivative of inositol triphosphate[19]. The base peak of the spectrum is at *m/z* 1069, corresponding in mass to the protonated molecule.

We have extended this work by running TMS-inositol triphosphate derivative by EI or, more appropriately, "EI-like" ionization. At least a portion of the ionization is probably due to carbon dioxide charge exchange[32,33]. The spectrum obtained from this EI run is shown in Fig. 7. The spectrum is indeed EI-like. The base peak at *m/z* 299,

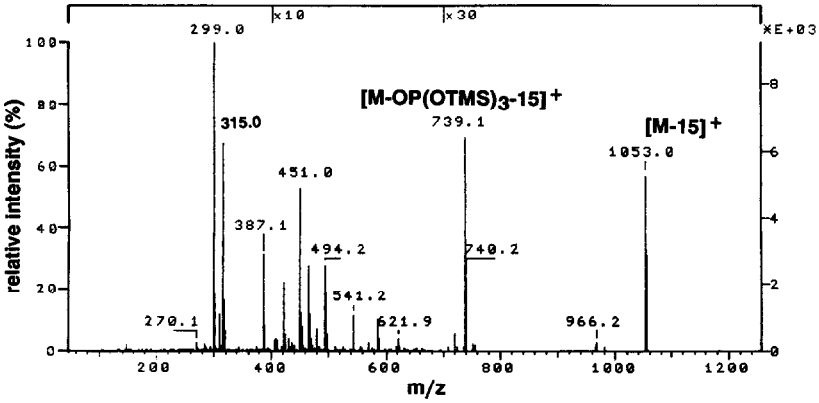


Fig. 7. EI-like SFC–MS mass spectrum of the TMS derivative of inositol triphosphate.

$[OP(OSi(CH_3)_2)\ (OTMS)_2]^+$, is a common base peak in the EI spectra of TMS derivatives of phosphate-bearing molecules. The ion of highest mass in the spectrum is the $[M - CH_3]^+$ peak at m/z 1053. The ion at m/z 739 corresponds in mass to $[M - OP(OTMS)_3 - 15]^+$. Most of the other major ions in the spectrum correspond in mass to fragments containing portions of the phosphate group. For example, m/z 315 corresponds to $[HO\text{–}P(OTMS)_3]^+$, m/z 387 to $[P(OTMS)_4]^+$, and m/z 451 to $[H(OP(OTMS)_2)_2]^+$.

A library search of the EI spectrum of the TMS derivative of inositol triphosphate against the NBS/EPA library of EI spectra as supplied with the TSQ-70 yields the spectrum of the TMS derivative of inositol diphosphate as the highest match (the spectrum of the TMS derivative of inositol triphosphate is not in the library). Ions at m/z 299, 315 and 387 are the most abundant in both spectra and have similar relative intensities. This is not surprising since these are low mass ions related to the TMS derivative of the phosphate group.

Peroxides

The use of organic peroxides as polymerization initiators, bleaching agents and oxidizing agents is widespread. They are also involved in many biochemical processes[34]. Success in the separation of mixtures of peroxides by HPLC has been reported[35–38], though evidence of decomposition was observed under certain conditions[37]. All but the smallest peroxides are difficult to analyze by traditional GC methods due to their lability[39]. Since the critical temperature of carbon dioxide is just 31°C, SFC with carbon dioxide can be performed at temperatures just above room temperature if necessary. In addition, the components of a capillary SFC system are relatively inert. SFC with FID, nitrogen–phosphorus, and ultraviolet detection has been used to analyze thermally labile materials such as peroxides[40] and azo-compounds[5,6]. We wanted to further investigate the use of SFC in the analysis of peroxides. As part of this investigation we decided to use SFC–MS to confirm that the "peroxide" peak seen by SFC–FID was indeed the intact peroxide and not a degradation product. Earlier work with a different SFC–MS system[16] yielded

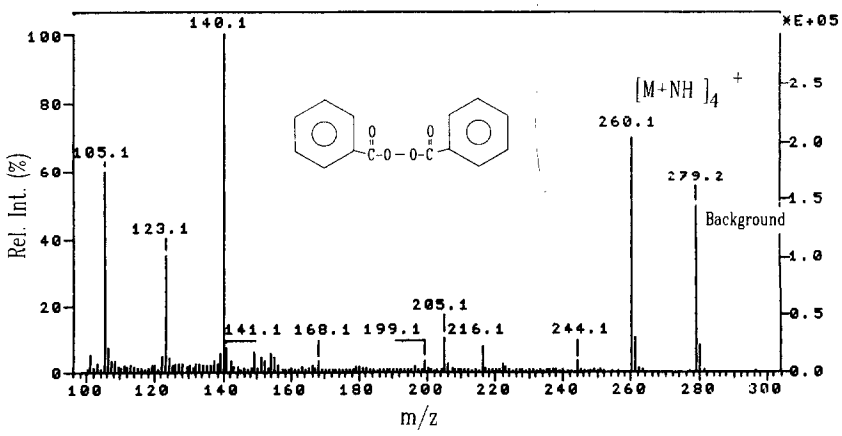


Fig. 8. Ammonia CI SFC–MS spectrum of benzoyl peroxide. Ions at m/z 205 and m/z 279 are background ions.

a single, sharp chromatographic peak for benzoyl peroxide. The major ions in the isobutane and ammonia CI spectra of this peak were related to decompensation products of benzoyl peroxide.

We recently undertook a study of the effects of the temperatures of various components of the SFC–MS system on the spectrum of benzoyl peroxide. We varied the temperatures of the mass spectrometer ion source, the SFC–MS interface probe-tip, the SFC column oven and the interface probe-stem. We also investigated the use of ammonia (1% in methane) and isobutane CI gases. Fig. 8 illustrates one result of our investigations, an ammonia CI SFC–MS spectrum of benzoyl peroxide with a large ion at *m/z* 260 corresponding to the ammonium adduct of intact benzoyl peroxide. Other major ions correspond in mass to expected thermal degradation products of benzoyl peroxide. The ammonium adduct of benzoic acid anhydride has a *m/z* of 244. Ions at *m/z* 216, 199, 140 and 123 correspond to the ammonium adducts and the protonated molecules of the phenyl ester of benzoic acid and of benzoic itself. Fig. 9 shows mass chromatograms of the ammonium adducts of intact benzoyl peroxide, benzoic acid anhydride, and benzoic acid, as well as the reconstructed total ion current chromatogram. The decomposition products co-elute with the intact peroxide in sharp, clean peaks. These results and our SFC–FID work strengthen our previous belief[16] that this peroxide is migrating through the SFC system with minimal degradation until it reaches the interface probe-tip and ion source where some degradation does occur.

We found that the temperature of the SFC–MS interface probe-tip was crucial in determining the degree of peroxide decomposition observed. The lowest probe-tip temperature where noise from sputtering and spiking of the restrictor was not a problem (100–120°C) gave the most abundant ammonium adduct of the intact peroxide. A change in the column oven temperature from 40 to 80°C and a change in the ion source temperature from 80 to 150°C did not have a significant effect on the spectrum. We did not determine the probe-tip temperature at which the adduct of the intact peroxide disappears, but it is two orders of magnitude less intense at our

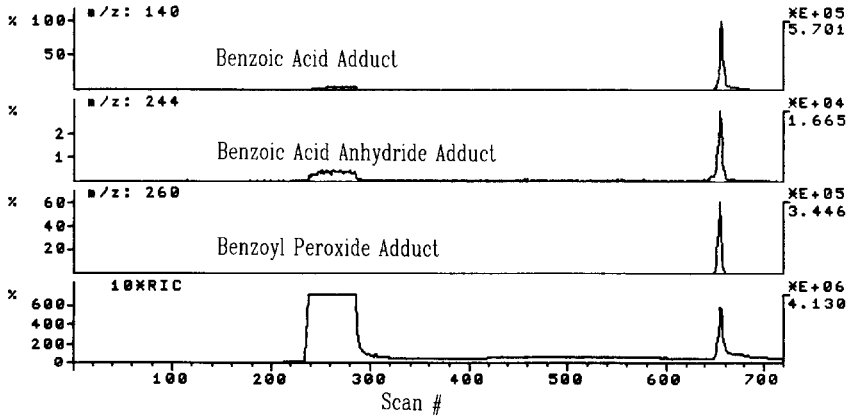
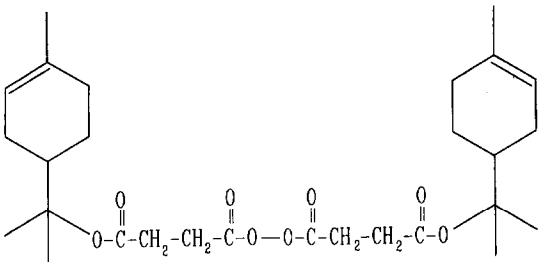


Fig. 9. Reconstructed total ion current chromatogram (bottom) and mass chromatograms of the ammonium adducts of benzoyl peroxide and of some of its degradation products from the ammonia CI SFC–MS run of benzoyl peroxide.

customary operating temperature of 300 than at 100°C. Isobutane CI did not yield the protonated molecule of the peroxide. Only two major ions were observed in the isobutane CI spectra from duplicate SFC–MS runs of benzoyl peroxide: the base peak at *m/z* 123, corresponding to protonated benzoic acid, and an ion of 20% relative abundance at *m/z* 105, corresponding to $[C_7H_5O]^+$. These isobutane CI results are consistent with the previously described behaviour of peroxides under isobutane CI conditions[41].

Armed with the knowledge gained during our work with benzoyl peroxide, we decided to attempt to confirm the molecular weight of the terpineol succinate peroxide whose postulated structure is shown in Scheme 2. MS analysis using direct-probe introduction revealed no ions related to the expected intact peroxide.



Scheme 2. Postulated structure of terpineol succinate peroxide.

Fig. 10 shows the ammonia CI SFC–MS spectrum of the terpineol succinate peroxide. The ion at *m/z* 524 corresponds in mass to the ammonium adduct of the expected peroxide, thus confirming its molecular weight of 506. As with benzoyl peroxide, the interface probe-tip temperature was crucial. The adduct ion was most intense near the lowest possible interface probe-tip temperature, in this case 100°C.

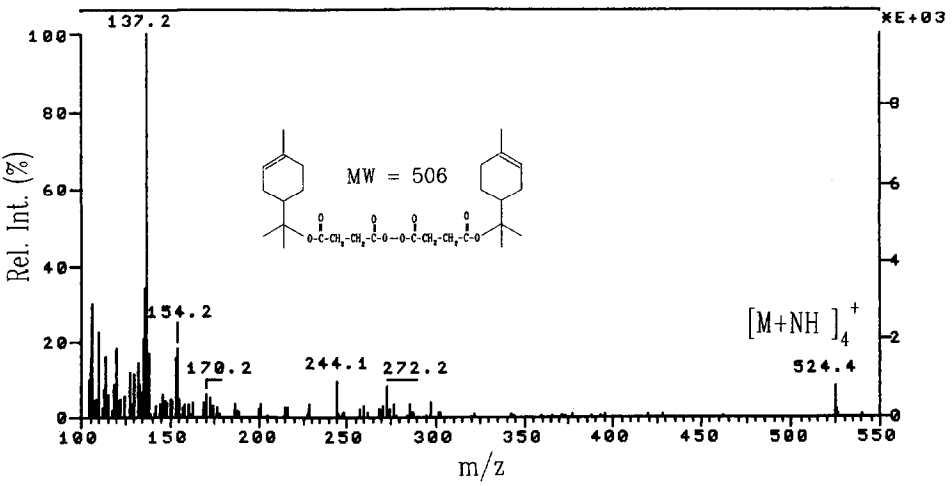


Fig. 10. Ammonia CI SFC–MS spectrum of terpineol succinate peroxide.

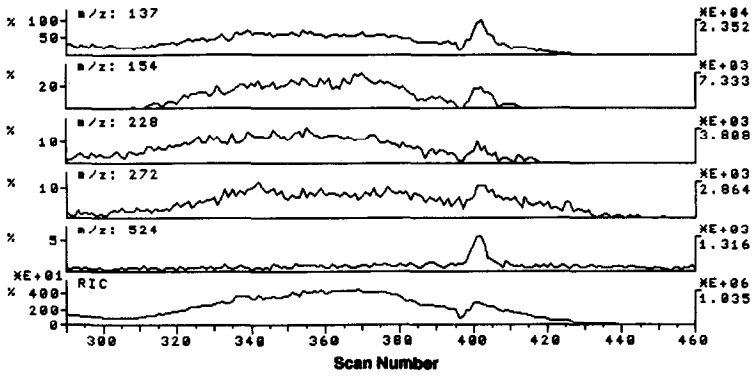


Fig. 11. Reconstructed total ion current chromatogram (bottom) and mass chromatograms of the protonated molecules and/or of the ammonium adducts of terpineol succinate peroxide and of some of its degradation products from the ammonia CI SFC–MS run of terpineol succinate peroxide.

Other temperatures were kept as low as possible and the pressure program was relatively fast, as listed in the Experimental section. Certain ions in the spectrum correspond to probable thermal degradation products of the peroxide. The ion at *m/z* 272 corresponds to the ammonium adduct of terpineol succinate monoester/free acid. The ammonium adduct of terpineol propionate has an *m/z* ratio of 228. The base peak at *m/z* 137 and the ion at *m/z* 154 correspond to the protonated molecule and the ammonium adduct of limonene. Mass chromatograms of these ions are shown in Fig. 11. While the intact peroxide (Scheme 2) elutes in a single peak less than 10 s wide, the ions related to the peroxide's thermal degradation products elute in broad "humps". This probably indictes that a significant portion of this peroxide decomposes as it travels through the SFC column. In spite of this degradation, we were able to quickly and easily confirm the postulated molecular weight of this peroxide by SFC–MS. As with benzoyl peroxide, no ions related to the intact peroxide were observed when using isobutane CI.

CONCLUSION

These three recent applications illustrate the use of SFC and of SFC–MS in the analysis of relatively non-volatile or thermally labile materials. In all three cases the analysis would have been difficult by traditional GC or HPLC. Elution and detection by SFC–FID and SFC–MS were straightforward. Inositol triphosphate did require derivatization, but this derivative was easily prepared and appeared stable under the SFC conditions used. SFC–MS was invaluable in quickly confirming the structures of the eluted species.

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