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Use of sulfur chemiluminescence detection after supercritical fluid chromatography

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ABSTRACT

A newly developed sulfur chemiluminescence detector is evaluated for supercritical fluid chromatography (SFC). The detection chemistry for sulfur chemiluminescence detection (SCD) is based on the chemiluminescent reaction between sulfur monoxide, which is produced from the decomposition of sulfur-containing analytes in a H_2/O_2-reducing flame and ozone. Detection limits are determined to be 12 pg sulfur via capillary-column SFC–SCD. Detector linearity is three orders of magnitude, and a selectivity of at least 10^7 was obtained. Quenching of SCD signals resulting from both the supercritical-fluid mobile phase and co-eluting hydrocarbons is discussed. Separation of thermally labile pesticides, and the determination of the ethylene oxide distribution for a thioethoxylate surfactant are demonstrated.

INTRODUCTION

Specific detection of sulfur-containing compounds, such as polycyclic aromatic compounds and pesticides, is important because of their natural toxicity or mutagenicity. The separation and detection of sulfur-containing compounds has been most often accomplished with gas chromatography (GC) coupled with different sulfur-selective detectors[1,2]. In these studies, excellent separation and detection of sulfur compounds was achieved. However, many sulfur-containing compounds are difficult to analyze by GC due to either their thermal instability or their non-volatility.

Supercritical fluid chromatography (SFC) offers some advantages over GC for analytical problem solving. For example, analysis of thermally unstable and relatively non-volatile compounds which cannot be achieved in GC has been performed by SFC[3–5]. Furthermore, because SFC employs supercritical mobile phases like CO_2, which is a gas under ambient conditions, it is relatively easy to interface SFC to GC-like detectors[6]. A variety of sulfur-selective detections have been coupled to SFC, such as flame photometric detection (FPD)[7–9], surface-wave-sustained microwave-induced plasma detection (surfatron MIP)[10], radio-frequency plasma detection (RPD)[11] and fluorine-induced sulfur chemiluminescence detection (SCD)[12,13]. Good

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performance with certain limitations has been shown from each coupling.

In spite of its non-linear response to sulfur compounds, FPD is the most popular sulfur-selective detector used in GC. Detailed characterization and optimization of FPD for use after capillary SFC has been recently studied[8]. A detectivity of 8.2 pg S/s for dibenzothiophene was reported. In another study, analysis of thermally labile and relatively high-molecular-weight compounds was demonstrated using capillary SFC–FPD[9]. A detection limit of 150 pg/s (or 26 pg S/s) of dibenzothiophene was reported via a capillary SFC–surfatron MIP system[10]. Detector linearity was reported to be only two orders of magnitude and signal quenching from CO_2 was severe. However, it was reported that improved performance could be obtained if the SFC–surfatron MIP system was optimized. The element-selective RPD when operated in the sulfur mode provided detection limits ranging from 50 to 300 pg S/s depending on the mass flow of CO_2 into the detector[11]. The advantage for the surfatron MIP and RPD is that both can provide selective detection to other elements, such as chlorine, bromine and nitrogen.

Fluorine-induced SCD in which the detection chemistry is based on the gas-phase reaction of F_2 with organic sulfur compounds is probably the most sensitive sulfur-selective detector that has been reported for SFC[12,13]. A 0.72 pg S/s detection limit for dodecanethiol[13] was obtained, and a selectivity of 10^4 was observed. The fluorine-induced SCD is, however, somewhat limited in that it shows strong response only for those organic sulfur compounds that have certain structural configurations[12]. For example, a sulfur compound whose structure is represented as R_1–S–R_2, yields a very strong response if the R-groups bonded to the sulfur are hydrogen or have an alkyl group which contains hydrogen.

Recently, a new sulfur chemiluminescence detector was developed for analyzing sulfur-containing compounds[14]. Its detection principle is based on the chemiluminescence reaction of ozone gas with sulfur monoxide (SO) which is produced in a H_2/O_2-reducing flame that contains the sulfur-containing analyte. This reaction mechanism can be summarized as follows:

$$\text{S-compounds} + H_2/O_2 \rightarrow SO + \text{other products} \quad (1)$$

$$SO + O_3 \rightarrow SO_2^{\ddagger} + O_2 \quad (2)$$

$$SO_2^{\ddagger} \rightarrow SO_2 + h\nu \quad (3)$$

Reaction (2) is highly exothermic, and the energy produced is large enough to excite SO_2 at the same time. The wavelength of the emitted light ranges from 260 to 480 nm with a peak maximum at 350 nm. Unlike the fluorine-induced SCD, this ozone-induced SCD responds to all sulfur-containing compounds. Nearly equimolar response to various sulfur compounds and a selectivity (g S/g C) greater than 10^7 was observed from the GC–SCD studies[14–16]. A detection limit of 400 fg/s of sulfur was reported by Shearer *et al.*[15] In addition, it was found that the SCD sensitivity was not affected significantly by environmental CO_2 and water vapor, which are the two major interferences in FPD.

This report describes the direct interfacing of this newly developed SCD to open tubular and packed capillary column SFC employing both CO_2 and 2% (w/w) meth-

anol-modified CO_2 as the mobile phase. Signal quenching from the mobile phase is addressed. Applications of the SFC–SCD to the analyses of thermally labile pesticides and a sulfur-containing surfactant are given.

EXPERIMENTAL

Chromatography

The flame sampling probe of the sulfur chemiluminescence detector (SCD 350, Sievers Research, Boulder, CO, U.S.A.) was mounted directly on top of the flame ionization detector of the Lee Scientific 501 supercritical-fluid chromatograph [Lee Scientific (LSI), Salt Lake City, UT, U.S.A.]. The FID temperature was maintained at 375°C. Grade 4.3 oxygen (Airco, Murray Hill, NJ, U.S.A.) was used as the ozone-generator gas. SFC-grade CO_2 and 2% (w/w) methanol-modified CO_2 were obtained from Scott Specialty Gases (Plumsteadville, PA, U.S.A.).

Both 50-μm and 100-μm I.D. frit restrictors (LSI) were used to control the capillary column and packed-capillary-column flow, respectively. Direct injection with a helium actuated Valco (Houston, TX, U.S.A.) injector (60-nl rotor) was employed as the sample introduction method[17]. Exact chromatographic conditions are cited in the figure legends. All data were recorded by a Spectra-Physics 4290 integrator.

Columns

Both capillary columns (50 μm I.D. coated with a 0.25-μm film thickness; LSI) and packed-capillary columns (250 μm I.D. packed with 5 μm Deltabond-CN; Keystone, Bellefonte, PA, U.S.A.) were used to achieve chromatographic separations. The packed-capillary columns were slurry-packed in-house. A 3–5% (w/w) slurry of stationary phase in methanol was used as the packing medium. A Haskel air pump was used to deliver the methanol. A pressure of 6000 p.s.i. was employed for packing the column. The pressure was increased to 7000 p.s.i. for 4–5 h after packing in order to condition the column.

Reagents

Dimethyl sulfide (49.9 ppm in nitrogen; Scott Specialty Gases) was used for the signal-quenching study. Sulfur-containing pesticides were purchased from Chemical Service (West Chester, PA, U.S.A.). Benzo[*b*]thiophene and dibenzothiophene were obtained from Aldrich (Milwaukee, WI, U.S.A.). These pesticides and chemicals were used as-received without further purification. The surfactant (ethoxylated thiols) was obtained from Shell (Houston, TX, U.S.A.). Garlic oil (Nature Made Nutritional Product, Los Angeles, CA, U.S.A.) was purchased from a local store. HPLC-grade solvents (Fisher, Raleigh, NC, U.S.A.) were used for preparing standard solutions.

RESULTS AND DISCUSSION

Without any modification, the SCD system was used after capillary SFC employing CO_2 or 2% (w/w) methanol-modified CO_2 as the mobile phase. A schematic diagram of this SFC–SCD is given in Fig. 1. All the flame-decomposed products were drawn from FID to SCD via a ceramic sampling probe and a transfer line by a

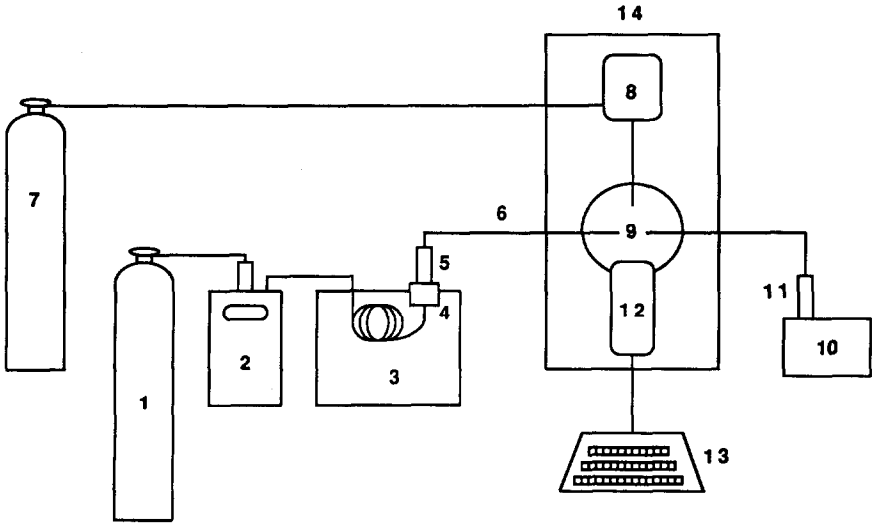

Fig. 1. Schematic diagram of SFC–sulfur chemiluminescence detector. Key: 1 = SFC grade CO_2 or 2% (w/w) methanol-modified CO_2; 2 = syring pump; 3 = chromatographic oven; 14 = flame ionization detector; 5 = flame sampling probe; 6 = transfer line; 7 = oxygen; 8 = ozone generator; 9 = reaction cell; 10 = vacuum pump; 11 = chemical trap; 12 = photomultiplier tube; 13 = integrator; 14 = SCD 350 main body.

vacuum pump. In order to efficiently transfer the short-lived sulfur monoxide to the reaction cell, pressure inside the SCD system was maintained at 15 Torr. This low-pressure system also prevented water condensation from occurring inside the transfer line and reaction chamber and reduced the collisions of other molecular species with sulfur monoxide.

The flame chemistry involved in SCD is expected to be as complex as that in FPD. It has been reported[18] that the major combustion products from sulfur analytes are SO, SH, SO_2, S_2, H_2S and some non-sulfur-containing products when a hydrogen-rich reducing flame is employed. The relative abundances of these species are strongly dependent on the flame gas composition (ratio of the H_2/O_2) and the position in the flame. It was also reported[13] that in a hydrogen-rich flame, SO_2, was the dominant sulfur species and SO accounted for at least 20% of the total products while the amount of S_2, was less than 1%.

The suggested hydrogen and air flows for obtaining maximum selectivity in GC–SCD are arround 200 and 380 ml min^{-1}, respectively[15]. It was found that SCD sensitivity was more dependent on the air flow-rate than the hydrogen flow-rate. While decreasing air flows increased sensitivity, it was accompanied by a loss of selectivity. This phenomenon was also observed in the SFC–SCD system. At a fixed hydrogen flow (205 ml min^{-1}), the optimum air flow was found to be lower in SFC–SCD than that in GC–SCD. An air flow of 330 ml min^{-1} and a hydrogen flow of 205 ml min^{-1} were used throughout this SFC–SCD study. Further investigation of the optimized flame gas composition for SCF–SCD may be necessary.

Signal quenching

It is believed that quenching resulting from collisions between the primary monitored species, S_2^*, and other molecular species produced in the flame are the main factors that cause signal quenching in FPD[16]. Quenching of SO_2^* emission is probably a considerable problem in SCD because the reaction mechanism can be considered similar for both FPD and SCD. However, it is important to note that the chemiluminescence is occurring in the hot flame at atmospheric pressure in FPD, and it occurs downstream at reduced pressure and room temperature in SCD. Consequently, collisional quenching in SCD would be expected to be less than in FPD. Indeed, this reaction in SCD quenching has been observed[14,15].

A significant signal quenching in FPD results from co-eluting hydrocarbons. Co-eluting hydrocarbons can serve as fuel gas in the flame, hence, they change the flame temperature which consequently perturbs the flame chemistry and therefore, changes the distribution of sulfur species. In addition, if the amount of hydrocarbons is large enough, the probability of collisions between the monitored sulfur species, *e.g.* S_2^* in FPD and SO_2^* in SCD, and the flame-decomposed products (such as CH_4, CO_2 or H_2O) is increased. In order to examine the quenching from co-eluting hydrocarbons in SFC–SCD, an experiment was designed such that benzol[*b*]thiophene (1 ng injected) co-eluted with 20 nl of either *n*-hexane, methanol, or toluene ($\approx$ 20 μg) using a capillary column (1 m $\times$ 50 μm I.D.; SB-Methyl-100). A decompressed CO_2 gas flow of less than 1 ml min^{-1} was controlled by frit restrictor. It was found that quenching resulting from co-elution of the sulfur-containing analytes with *n*-hexane or methanol was not significant. However a 15% decrease in signal was observed if the component co-eluted with toluene. This quenching from toluene has also been observed with SFC–FPD[8].

Another quencher which is thought to be important in SFC–SCD is the mobile phase employed in SFC. Most of the mobile phases used in SFC are polyatomic, *e.g.* CO_2, N_2O or modified CO_2. These mobile phases are more effective quenchers than most of the mobile phases used in GC such as monoatomic He or diatomic H_2. Furthermore, the decompressed mobile phase gas flow-rates (at their high densities) at the outlet of the column are usually higher in SFC than those from GC using a column with the same dimensions. It has been reported[19] that a 15% signal decrease was observed from a redox chemiluminescence detector by comparing the signal generated from decompressed CO_2 at a flow-rate of 0.75 ml min^{-1} with that from helium.

Quenching of the SCD signal resulting from the SFC mobile phase was examined for CO_2, which is the most widely used mobile phase. Curve a in Fig. 2 is the SCD background signal at different decompressed CO_2 gas flow-rates. The reason why the SCD signal increases with increasing CO_2 flows is not clear. It may be due to the CH* emission (at 390 nm) resulting from hydrocarbon contaminants (less than 5 ppm) in CO_2 (ref. 18). Curve c in Fig. 2 is the hypothetical SCD response for low nanogram level (49.9 ppm) of dimethyl sulfide. This curve is plotted under the assumption that no quenching was obtained from CO_2. However, the experimental SCD response of the dimethyl sulfide (49.9 ppm) is shown in curve b which indicates that CO_2 does quench the signal at a decompressed flow-rate above 5 ml min^{-1}. From the difference between curves b and c, this quenching becomes more and more severe with increasing CO_2 gas flow above 15 ml min^{-1}. About 70% of the signal is

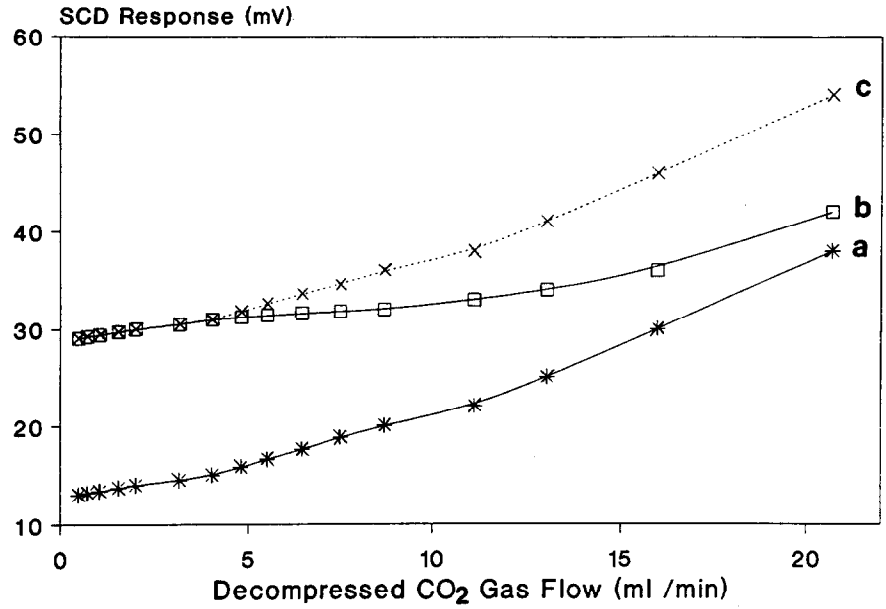

Fig. 2. Quenching of SCD signals resulting from different decompressed CO_2 gas flow-rates. a = CO_2; b = dimethyl sulfide (49.9 ppm) in CO_2 (experimental data); c = dimethyl sulfide (49.9 ppm) in CO_2 (hypothetical data obtained by adding 15 mV, SCD response of 49.9 ppm dimethyl sulfide, to curve a.

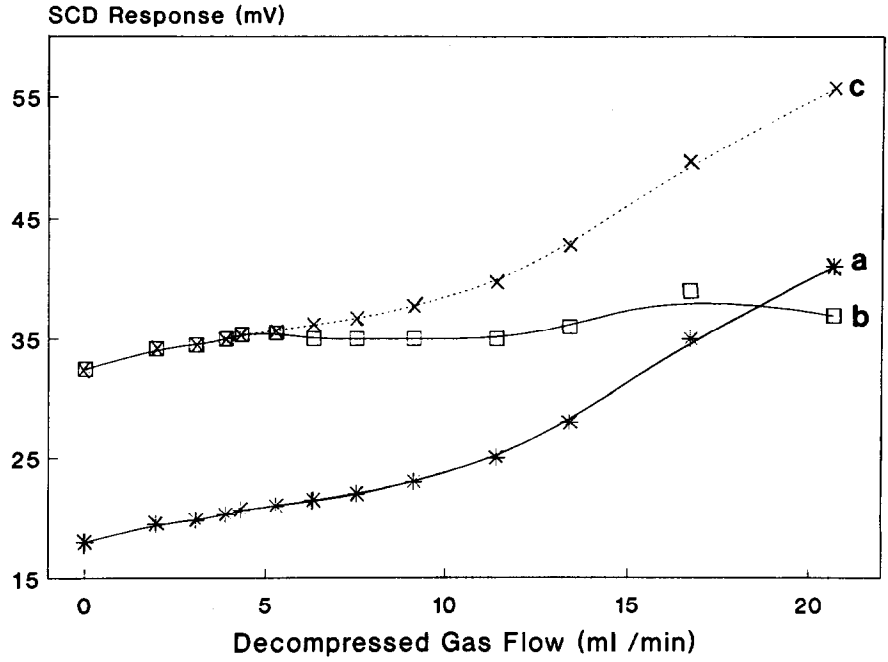

Fig. 3. Quenching of SCD signals resulting from different decompressed 2% (w/w) methanol-modified CO_2 gas flow-rates. (a) 2% (w/w) methanol-modified CO_2; (b) dimethyl sulfide (49.9 ppm) in 2% (w/w) methanol-modified CO_2 (experimental data); (c) dimethyl sulfide (49.9 ppm) in 2% (w/w) methanol-modified CO_2 (hypothetical data obtained by adding 15 mV, SCD response of 49.9 ppm dimethyl sulfide, to curve a.

lost at a CO_2 gas flow of 20 ml min^{-1}. This suggests that a packed column (I.D. $\geqslant$ 1 mm), which produces gas flow higher than 20 ml min^{-1}, is not compatible with the current SFC–SCD configuration.

In contrast to the high gas flow, the quenching in SCD is not significant when the gas flow is less than 5 ml min^{-1}. For typical capillary column (*i.e.* 50 μm I.D.) SFC operating conditions, the measured decompressed CO_2 gas flow was less than 3 ml min^{-1} (which corresponds to 390 atm CO_2 pressure). Hence, the signal quenching due to the presence of CO_2 should not be a significant problem when a capillary column is employed for SCF–SCD analysis.

The quenching phenomenon resulting from the SFC mobile phase was also studied for 2% methanol-modified CO_2. The flame gas composition and chromatographic conditions were the same as those used in the study of quenching from 100% CO_2. The results are shown in Fig. 3. For low decompressed flow-rates (< 4 ml min^{-1}), quenching does not appear to be a problem. However, the rate of SCD signal decrease was faster in this case than that observed with 100% CO_2 at flow-rates higher than 10 ml min^{-1}. It was also noticed that quenching from either CO_2 or 2% methanol-modified CO_2 was even more severe when a higher concentration of dimethyl sulfide was used.

Although quenching is significant at high gas flows for both CO_2 and modified CO_2, a packed capillary column (250 μm I.D.) can be used in SFC–SCD because its highest decompressed CO_2 flow is typically less than 10 ml min^{-1}. One advantage of using a packed capillary column is that a higher sample capacity can be obtained than with an open tubular capillary column, although some loss of the SCD sensitivity and detector linearity are observed.

Detector performance

A detection limit was found to be 12 pg S (signal-to-noise ratio 3) or 2 pg S/s with dibenzothiophene in capillary SFC–SCD. Detector linearity was at least three orders of magnitude (0.07–70 ng of dibenzothiophene) using a packed capillary column. Almost equimolar response was observed when injecting benzo[*b*thiophene, dibenzothiophene, and tri-allate (a thiocarbamate) using identical chromatographic conditions. Selectivity of seven orders of magnitude was determined by comparing methanol response and benzo[*b*thiophene response. It is interesting to note that hexane did not give a response, and toluene gave a negative response under these conditions.

Fig. 4 demonstrates the superior selectivity afforded by SCD. It is known that garlic oil contains many sulfur-containing compounds, many of which are thermally labile. The garlic oil analyzed here was sealed in a gel tablet in which the garlic oil had been premixed with vegetable oil. The garlic oil was then dissolved in *n*-hexane after being removed from the tablet. One advantage of using this SCD system is that the signals from both FID and SCD could be obtained simultaneously when employing 100% CO_2 as the SFC mobile phase. The FID and SCD chromatograms are shown in Fig. 4. These volatile sulfur compounds were detected by SCD (Fig. 4a). However, they were not detected using FID either because they had low response in FID or co-eluted with solvent (Fig. 4b). Non-sulfur triglycerides (micrograms were injected) contained in the vegetable oil were detected by FID; however, they were not detected by SCD.

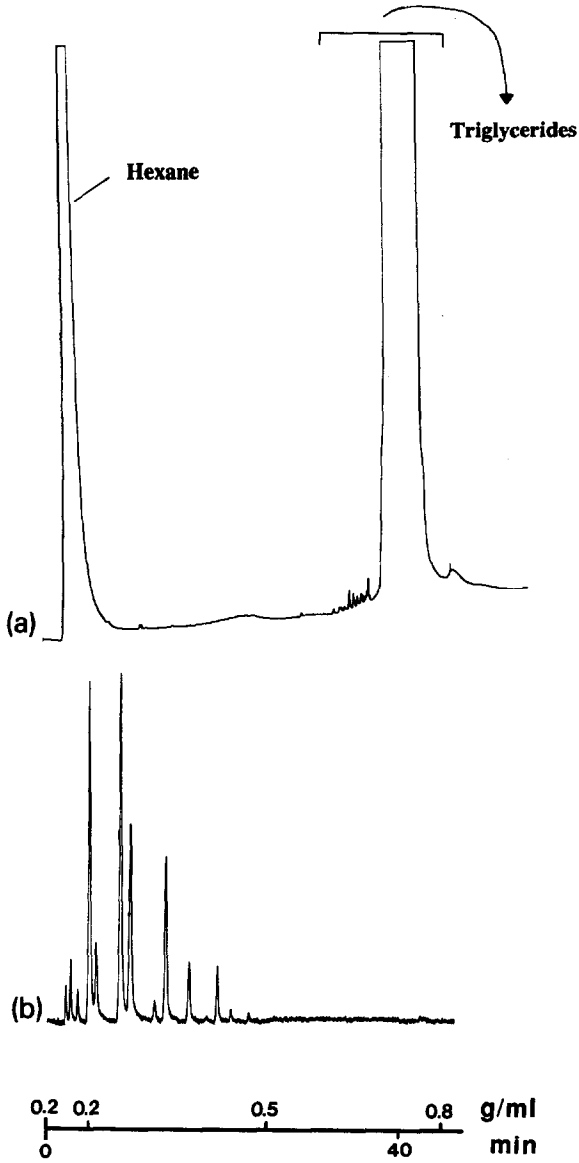

Fig. 4. Detection of sulfur-compounds from garlic oil by (a) FID and (b) SCD. Chromatographic conditions: CO_2 density programmed from 0.20 to 0.8 g ml^{-1} at 0.015 g ml^{-1} min^{-1} after a 3-min isoconfertic period; oven temperature at 80°C; packed capillary column was used.

Applications

The separation of C_{12}-ethoxylated thiols by capillary SFC–SCD is demonstrated in Fig. 5. Ethoxylated alcohols or thiols are widely used as non-ionic surfactants. Determination of the distribution of oligomers and average number of ethylene

$$C_{12}—S—(-CH_2CH_2O-)_n-H$$

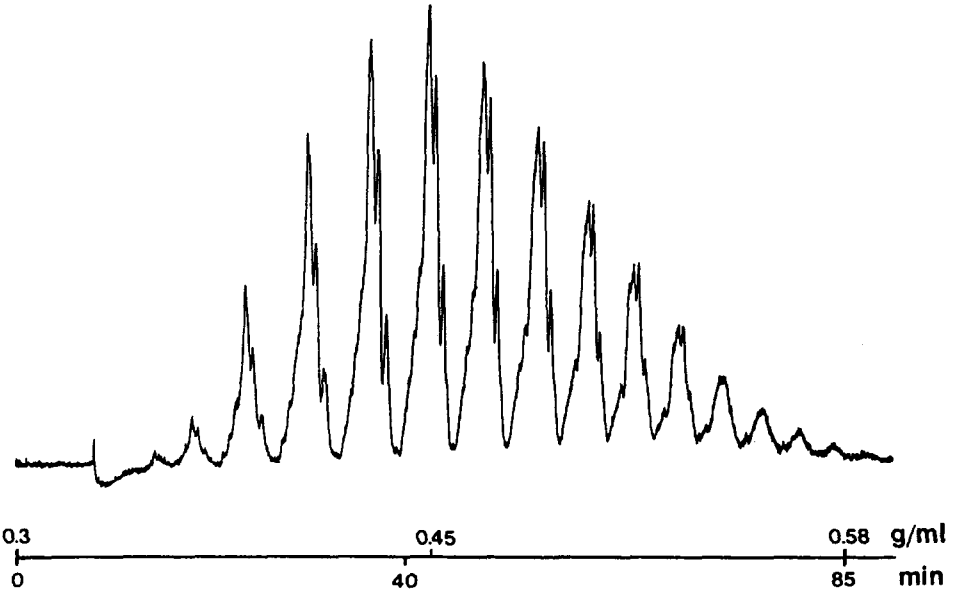

Fig. 5. Capillary supercritical fluid chromatogram of C_{12}-ethoxylated thiols. Chromatographic conditions: CO_2 density programmed from 0.30 to 0.45 g ml^{-1} at 0.004 g ml^{-1} min^{-} after a 5-min isoconfertic period, then to 0.58 g ml^{-1} at 0.003 g ml^{-1} min^{-}; oven temperature held at 100°C; column was SB-Phenyl-5.

oxide (EO) units is important because they affect the chemical properties and performance of the surfactant. SFC has been shown to be an effective method for separating the oligomers in ethoxylated alcohols using FID[20]. Because SCD responds only to the amount of the sulfur contained in each oligomer (*i.e.* one sulfur atom per molecule), the average number of EO units per mole of ethoxylate can be determined directly employing SCD. Since the response factors vary in the separated oligomers such a direct analysis via FID is not possible. By integrating each broad peak (each peak was comprised of many branched isomers) which represents one oligomer (Fig. 5), the calculated average EO unit was 6.8, which was in good agreement with the number provided by a combination of other techniques.

Analysis of thermally labile and polar sulfur-containing pesticides with 2% methanol-modified CO_2 is demonstrated in Fig. 6. A low nanogram amount of each pesticide was injected. It was found that these pesticides started to decompose when the analysis temperature was increased above 70°C; therefore, their separation and detection using GC was precluded. It was also difficult to chromatograph these compounds from the same column using 100% CO_2 because of the irreversible adsorption between these polar pesticides and the stationary phase[21]. Methanol, however, changes the surface activity of the packing material, thus enabling the polar compounds to be eluted.

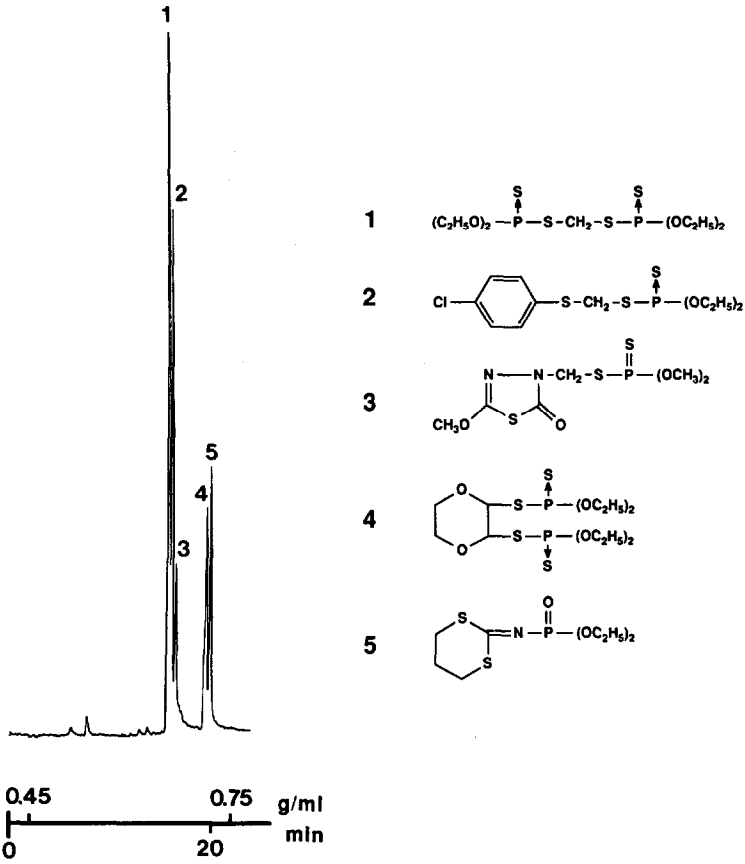

Fig. 6. Separation of five thermally labile sulfur-containing pesticides. Chromatographic conditions: density programmed from 0.45 to 0.75 g ml^{-1} at 0.015 g ml^{-1} min^{-1} after a 3-min isoconfertic period; oven temperature at 50°C; 2% (w/w) methanol-modified CO_2 was the mobile phase; packed capillary column was used. Peaks: 1 = ethion; 2 = carbophenothion; 3 = methidathion; 4 = dioxathion; 5 = phosfolan.

CONCLUSIONS

The ozone-based sulfur chemiluminescence detector, which was originally designed for GC, has been found to be suitable for use in capillary SFC without any modification. It affords the most selective detection for sulfur that has been reported for capillary SFC. In addition, this detector has been demonstrated to be useful for SFC employing 2% methanol-modified CO_2 as the mobile phase, thus increasing the SFC–SCD capability for analyzing polar compounds and relatively non-volatile compounds. Although suffering some loss of sensitivity, SCD also has been shown to be compatible for use after packed-capillary-column (250 μm I.D.) SFC. A study of the complex flame chemistry involved in this ozone-based SCD is necessary in order to more efficiently utilize this detector in SFC employing modified CO_2 as the mobile phase.

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REFERENCES

1 M. Dressler, *Selective Gas Chromatographic Detectors*, Elsevier, Amsterdam, 1986, Ch. 7–9.
2 R. J. Skelton, Jr., H.-C. K. Chang, P. B. Farnsworth, K. E. Markides and M. L. Lee, *Anal. Chem.*, 61 (1989) 2292.
3 M. Novotny, S. R. Springston, P. A. Peaden, J. C. Fjeldsted and M. L. Lee, *Anal. Chem.*, 53 (1981) 407A.
4 K. D. Bartle, in R. M. Smith (Editor), *Supercritical Fluid Chromatography*, St. Edmundsbury Press, England, 1988, pp. 1–28.
5 R. D. Smith, B. W. Wright and C. R. Younker, *Anal. Chem.*, 60 (1988) 1323A.
6 B. E. Richter, D. J. Bornhop, J. T. Swanson, J. G. Wangsgard and M. A. Andersen, *J. Chromatogr. Sci.*, 27 (1989) 303.
7 K. E. Markides, E. D. Lee, R. Bolick and M. L. Lee, *Anal. Chem.*, 58 (1986) 740.
8 S. V. Olesik, L. A. Pekay and E. A. Paliwoda, *Anal. Chem.*, 61 (1989) 58.
9 L. A. Pekay and S. V. Olesik, *Anal. Chem.*, 61 (1989) 2616.
10 D. R. Luffer, L. J. Galante, P. A. David, M. Novotny and G. M. Hieftje, *Anal. Chem.*, 60 (1988) 1365.
11 R. J. Skelton, Jr., P. B. Farnsworth, K. E. Markides and M. L. Lee, *Anal. Chem.*, 61 (1989) 1815.
12 W. T. Foreman, C. L. Shellum, J. W. Birks and R. E. Sievers, *J. Chromatogr.*, 465 (1989) 23.
13 D. J. Bornhop, B. J. Murphy and L. Krieger-Jones, *Anal. Chem.*, 61 (1989) 797.
14 R. L. Benner aznd D. H. Stedman, *Anal. Chem.*, 61 (1989) 1268.
15 R. L. Shearer, D. L. O'Neal, R. Rios and M. D. Baker, *J. Chromatogr. Sci.*, 28 (1990) 24.
16 M. Legier, Sievers Research, Inc., personal communication.
17 M. L. Lee, B. Xu, E. C. Huang, N. M. Djordjevic, H-C. K. Chang and K. E. Markides, *J. Microcol. Sep.*, 1 (1989) 7.
18 S. O. Farwell and C. J. Barinaga, *J. Chromatogr. Sci.*, 24 (1986) 483.
19 W. T. Foreman, R. E. Sievers and B. W. Wenclawiak, *Fresenius Z. Anal. Chem.*, 330 (1988) 231.
20 P. R. Geissler, *J. Am. Oil Chem. Soc.*, 66 (1989) 685.
21 M. Ashraf-Khorassani and L. T. Taylor, in C. M. White (Editor), *Modern Supercritical Fluid Chromatography*, Hüthig, Heidelberg, 1988, pp. 115.