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Journal of Chromatography A, 779 (1997) 307–313

JOURNAL OF CHROMATOGRAPHY A

Sulfur-selective chemiluminescence detection with packed column supercritical fluid chromatography

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Received 24 January 1997; received in revised form 16 April 1997; accepted 21 April 1997

Abstract

A new generation sulfur chemiluminescence detection (SCLD) system was interfaced and tested for supercritical fluid chromatography (SFC) with packed columns using 100% SF-CO_2 and methanol modified CO_2 as the mobile phase. The detection chemistry for the SCLD is based on ozone-induced chemiluminescence following a two-step combustion process of consecutive oxidation and reduction of sulfur-containing compounds. A seven-day evaluation study showed excellent sensitivity, selectivity and linearity, as well as day-to-day repeatability. The minimum detectable quantity was determined to be 3 pg sulfur (0.2 pg S/s) at the detector. Equimolar response of SCLD to sulfur compounds with different bonding environments was also observed. Unique applications and capabilities of the SFC–SCLD system for sulfur speciation and detection are presented for a petroleum product, thermally labile pesticides and herbicides which are difficult or impossible to analyze by GC techniques. © 1997 Elsevier Science B.V.

Keywords: Detection, GC; Sulfur-selective chemiluminescence detection; Chemiluminescence detection; Petroleum; Pesticides; Sulfonamides

1. Introduction

Detection techniques for sulfur-containing compounds have flourished over the years in both academic and industrial research resulting in numerous beneficial applications. Sulfur-selective detection based on ozone-induced chemiluminescence, developed by Parks in the early 1980s [1,2] is currently one of the best available techniques. Recently a new generation sulfur chemiluminescence detection (SCLD) system was introduced by Antek Instruments Inc.. The much larger furnace of the SCLD system is expected to provide better reproducibility, repeatability and long term detector stability for analyzing heavy as well as light sulfur species. When SCLD was interfaced with a GC, a long term stability (two months) study by two independent research facilities showed similar excellent day-to-day repeatability of the detector [3].

Development by Benner et al. [4] resulted in commercialization of a sulfur chemiluminescence detection (SCD) system by then Sievers Research Inc. for GC [5]. Some real world applications were also reported [6]. Sulfur chemiluminescence detection techniques were evaluated and reviewed along with other sulfur-selective detection methods such as flame photometric detection (FPD) and fluorine-induced sulfur chemiluminescence detection. Better

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0021-9673/97/$17.00
PII S0021-9673(97)00455-X

detection limits, linearity and detector response [7,8] were observed with sulfur chemiluminescence detection. In order to alleviate the problem of drastic changes in sensitivity and selectivity found in the early units, a modified SCD system utilizing a heated furnace assembly instead of the flame ionization detector flame was developed by Shearer and was referred to as flameless SCD [9]. The flameless SCD system was reported to exhibit better operability, precision and increased sensitivity by one order of magnitude. Recently Chen and Lo have reported the coupling of FID and flameless SCD in series after gas chromatography for dual-channel detection of sulfur compounds in three gasoline samples [10].

Determination of sulfur-containing compounds by SFC is of great interest since many sulfur-containing compounds are either thermally labile or non-volatile and therefore are not suitable for analysis by GC. Successful efforts have been made to interface both the flame and flameless SCD to SFC [11–13]. Chang et al. first reported the coupling of SCD to capillary SFC employing both 100% SF-CO_2 and 2% (w/w) methanol modified mobile phases [11]. A detection limit of 12 pg sulfur at the detector, selectivity of 10^7 and detector linearity of three orders of magnitude were achieved. However, 70% loss in signal was observed when a CO_2 gas flow-rate of 20 ml/min was used. Pekay et al. published a related article on capillary SFC–SCD where optimized flame (hydrogen rich) gas conditions were used to analyze organosulfur compounds [12]. A compromised flame gas composition was required to achieve the broadest linear dynamic range with the least variation in response to different types of sulfur compounds. Under these conditions however, optimum sensitivity was lost. With the advantages of the flameless SCD, Shearer and Skelton investigated coupling of this detector to a packed column SFC system using 100% CO_2 as the mobile phase via a post-column split [13]. About 2.5 ml/min of decompressed CO_2 was passed into the furnace of the flameless SCD via a frit restrictor. Minimum detection of 0.3 pg S/s, a sulfur to carbon (as in toluene) selectivity of 10^6 and a linearity of nearly 10^3 were reported along with approximate equimolar response of the detector. An upper limit of 10–12 ml/min of decompressed CO_2 gas flow-rate was, however, required to ensure successful chromatographic analysis.

To overcome the inequity of optimal detector and chromatographic flow-rates and to take full advantage of recent improvements in sulfur chemiluminescence detection technology, SCLD was interfaced to SFC. The evaluation with a packed column using 100% CO_2 as the mobile phase was accomplished and is described herein. Reproducibility and repeatability of the SFC–SCLD were also studied. Analyses of pesticides and herbicides, as well as a hydrotreated petroleum product are shown as applications using this new generation sulfur detector.

2. Experimental

2.1. Apparatus

A Model 704E sulfur chemiluminescence detector from Antek Instruments (Houston, TX, USA) was used for analyzing sulfur-containing compounds. The detector was interfaced with a Hewlett–Packard Model G1205A supercritical fluid chromatograph (Avondale, PA, USA) using a post-column split. The SFC system also includes a variable-wavelength UV detector. A 5-μl loop was used for sample injection. A tapered restrictor (50 μm I.D.) was used to control the 100% decompressed CO_2 flow-rate to the SCLD throughout this study, except where a higher flow-rate was needed and in this case a linear restrictor (25 μm I.D.) was used. For the methanol modified CO_2 mobile phase, a linear restrictor with 15 μm I.D. was used. Two columns from Keystone Scientific (Bellefonte, PA, USA) were used for the chromatographic separation. A Deltabond C_8 (250× 4.6 mm I.D., 5 μm particle size) column was used in the detector evaluation experiments, and a Deltabond phenyl (250×4.6 mm I.D., 5 μm particle size) column was used for the applications. The SCLD was operated under the general conditions suggested by the manufacturer. The furnace temperature was set at 950°C. The operating range for the hydrogen flow-rate was 85–160 ml/min, while the oxygen flow-rate was 3.5–6.5 ml/min. The oxygen flow-rate to the ozone generator was fixed at approximately 25 ml/min. The SCLD gain was set to High×50 with a 1 V full scale output voltage.

2.2. Reagents and standards

Dibenzothiophene, phenylsulfide, methylsulfide and octadecyl mercaptan were purchased from Aldrich (Milwaukee, WI, USA). Captan, disulfiram and folpet were purchased from Chem Service (West Chester, PA, USA). Sulfamerazine was purchased from Sigma (St. Louis, MO, USA) and dimethoate from Accu Standard (New Haven, CT, USA). Sulfamethazine, sulfaquinoxaline, sulfathiazole were provided by United States Department of Agriculture (Philadelphia, PA, USA). Sulfameturon methyl and 1,3-dibutyl-2-thiourea were provided by E.I. duPont de Nemours and Co. (Wilmington, DE, USA). The hydrotreated petroleum product was provided by a research facility. All chemicals and samples were used without further purification or clean-up. HPLC grade solvents from EM Science (Gibbstown, NJ, USA) were used for preparing standard solutions. Grade 4.3 oxygen from Airco (Murray Hill, NJ, USA) was used for both oxidation and ozone-generation. Hydrogen was also obtained from Airco. SFC-grade CO_2 was obtained from Air Products and Chemicals Inc. (Allentown, PA, USA).

2.3. Chromatographic conditions

Flow injection analysis was used to determine the relative response factors of the SCLD under a pressure of 200 atm at 50°C (1 atm=101 325 Pa). The remaining experiments were performed after elution of the analyte from a packed column under similar detector conditions. The split decompressed CO_2 flow-rate to the SCLD system was 36 ml/min. Additional chromatographic conditions are cited in the figure legends of each chromatogram. The SFC operating conditions with methanol modifier is provided in the legend of Fig. 5.

3. Results and discussion

3.1. Detection mechanism

Unlike the reaction mechanism proposed for SCD by Shearer et al. [5], the SCLD operation principle involves a post column two-step reaction process. Sulfur-containing analytes emerging from the chromatographic column are first oxidized to sulfur dioxide (SO_2), and subsequently the SO_2 is reduced to hydrogen sulfide (H_2S) and possibly other reduced species by a large excess of hydrogen. The H_2S, together with all other reduced products are then drawn into a reaction chamber where H_2S is oxidized with ozone to sulfur dioxide (SO_2^*) in the excited electronic state. The chemiluminescence of SO_2^* with a spectrum ranging approximately from 300 to 450 nm is then measured by a photomultiplier tube (PMT). The following equations summarize the SCLD detection mechanism:

$$R_1 - S - R_2 + O_2 \xrightarrow{950°C} SO_2 + \text{other oxides}$$

$$SO_2 + H_2 \rightarrow H_2S + \text{other reduced sulfur species}$$

$$H_2S + O_3 \rightarrow SO_2^*$$

$$SO_2^* \rightarrow SO_2 + h\nu$$

3.2. Detector configuration

SCLD was coupled with packed column SFC without any modification. Fig. 1 shows a schematic diagram of the dual SFC–SCLD/UV detection, and a more detailed representation of the actual interface is given in Fig. 2. The restrictor tip was threaded through the fitting until it reached the bottom of the

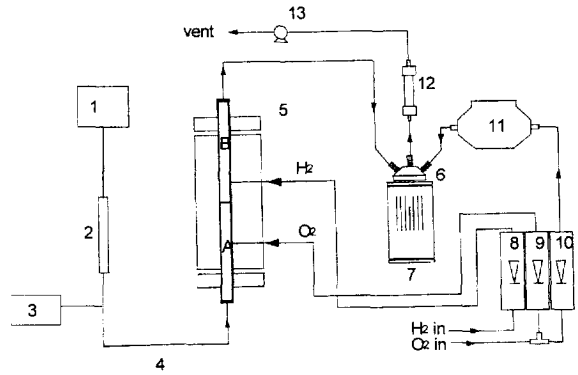


Fig. 1. Schematic flow diagram of the packed column SFC–SCLD/UV system. 1=SFC; 2=column; 3=UV detector; 4=restrictor; 5=furnace (A – oxidation zone, B – reduction zone); 6=reaction chamber; 7=PMT; 8=flow meter for pyro H_2; 9=flow meter for pyro O_2; 10=flow meter for ozone generator; 11=ozone generator; 12=scrubber; 13=vacuum pump.

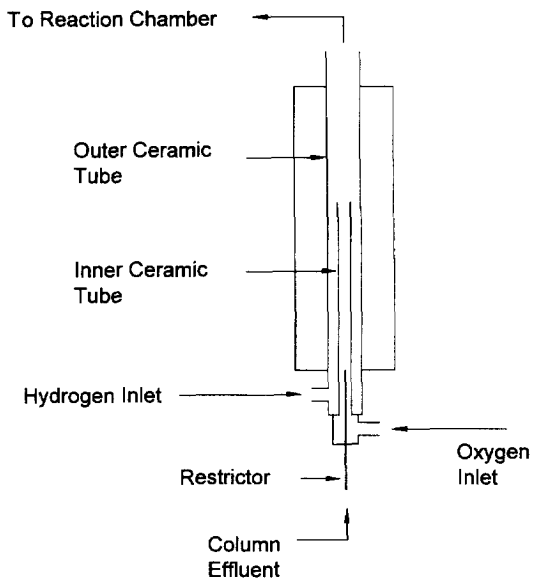


Fig. 2. Schematic of the SFC–SCLD interface.

furnace. In the evaluation of the detector performance under packed column SFC conditions, it was found that SCLD sensitivity was more dependent on oxygen flow than hydrogen flow-rate. More specifically the sensitivity increased with an increase of oxygen flow-rate. The oxygen flow-rate was higher for SFC–SCLD than for GC–SCLD.

3.3. Detector performance

Standard solutions were used to obtain minimum detectable quantity (MDQ), repeatability, linearity, selectivity and relative response factors (f_x) for packed column SFC–SCLD employing 100% CO_2 mobile phase. Injected MDQs were 85 pg sulfur and 80 pg sulfur for dibenzothiophene and phenylsulfide, respectively. Considering a split ratio of 30:1 (UV to SCLD), MDQs at the SCLD were 3 pg sulfur (0.2 pg S/s) for both dibenzothiophene and phenylsulfide with a signal-to-noise ratio of two. Since real world samples often contain coeluting non sulfur-containing compounds which interfere in the analysis, standard solutions of 1 ng/μl sulfur in toluene, as well as 0.2 ng/μl sulfur in hexane and methanol, were used to study selectivity of the detector. For all three solutions with operating conditions of the SCLD cited above, no solvent response was observed. Thus, sulfur to carbon selectivity was at least 10^6 to 10^7. Dibenzothiophene standards in methanol with concentrations ranging from 3 to 3000 ng S/μl were used to examine the linear dynamic range of the SCLD when interfaced with SFC. A linear detector response with a correlation coefficient of 0.9999 was obtained over a range of three orders of magnitude. In order to study the equimolar sulfur detection capability of the SFC–SCLD, different classes of sulfur-containing compounds were used. Table 1 shows the response factors relative to dibenzothiophene for several sulfur-containing compounds. These numbers were determined by flow injection analysis so as to avoid possible column discrimination. Each response factor was close to unity thus demonstrating the equimolar sulfur response of SCLD. Repeatability studies to assess the utility of the sulfur selective detector for routine use are very important. No report has been published in this area to our knowledge. A 7-day detector stability assessment of the SFC–SCLD system was accomplished. During this study, the SCLD system was set to run continuously around-the-clock. Only the PMT voltage was turned off when data were not being collected. The CO_2 mobile phase was pumped to the SFC instrument only during the chromatographic runs. Standard solutions of dibenzothiophene at two concentration levels (0.6 and 1.7 ng/μl sulfur) were used. Relative standard deviation (R.S.D.) values for the response afforded by the two standards during the 7-day period with three replications per day were 5.6 and 8.7%, respectively.

Table 1
Response factors (f_x) relative to dibenzothiophene by SCLD

Component	f_x
Captan	1.10
Dibenzothiophene	1.00
Dimethoate	1.04
Disulfiram	1.16
Methylsulfide	1.02
Octadecyl mercaptan	1.15
Phenylsulfide	1.02
Sulfamerazine	1.04
Average	1.07
R.S.D.	6%

3.4. Applications

A pesticide mixture containing octadecyl mercaptan, folpet and captan was chromatographed on a packed Deltabond phenyl column with 100% SF-CO_2 using a pressure program at constant temperature and a dual SFC–SCLD/UV detection system. These pesticides are normally analyzed by HPLC methods because of their thermally labile characteristics. Since a fixed restrictor was used, the decompressed CO_2 flow-rate increased during pressure programming. Fig. 3 shows the simultaneous UV and SCLD chromatograms of the three sulfur-containing pesticides mixture with a decompressed CO_2 flow-rate starting at 36 ml/min at the SCLD. A standard solution containing 15 ng/μl of octadecyl mercaptan (peak 1) and 12 ng/μl of both folpet (peak 2) and captan (peak 3) in methanol was injected to the SFC–SCLD/UV system via a 5-μl internal loop. The elution order of the pesticides in the mixture was confirmed by single injections of the three individual components. The advantage of SCLD is that, even with a split ratio of 30:1 (UV to SCLD), all three compounds are detected while only peak 2 was detected by the UV detector at both 219 and 254 nm due to the lack of a UV chromophore in the other two components. Because peak 2 had much better UV detector response at 219 nm than at 254 nm, only the chromatogram with UV detection at 219 nm was presented. The results of this study demonstrated near equimolar response of SCLD with excellent sulfur selectivity and baseline stability during CO_2 pressure programming. A retention time offset between UV detection and SCLD was observed with folpet (e.g., 0.2 min delay at the SCLD). When a higher starting decompressed CO_2 flow-rate (55 ml/min) was used, essentially zero-offset in retention time between the UV detector and SCLD peaks was achieved. At the higher decompressed CO_2 flow-rate, excellent signal-to-noise ratio was also achieved with the SFC–SCLD system. The flameless SCD on the other hand, has been reported to require a much lower decompressed CO_2 flow-rate (a threshold of 12 ml/min) to achieve successful chromatographic analysis. The SCLD consequently demonstrated better column effluent capacity and detector stability as compared to the flameless SCD. The higher decompressed CO_2 flow-rate can actually be advantageous in quantitative trace analysis since a larger fraction of column eluent can enter the detector thus providing more analyte at a fixed sample injection volume. As much as 10 μl of sample can be injected on the 4.6 mm I.D. packed column for SFC, thus maximizing the sample load to achieve lower MDQ. Smallbore (2 mm I.D.) packed column for example can tolerate up to 1 μl injection volume. Injection volumes are further reduced (up to 60 nl) when using fused silica (100 μm I.D.) open tubular columns for SFC separations.

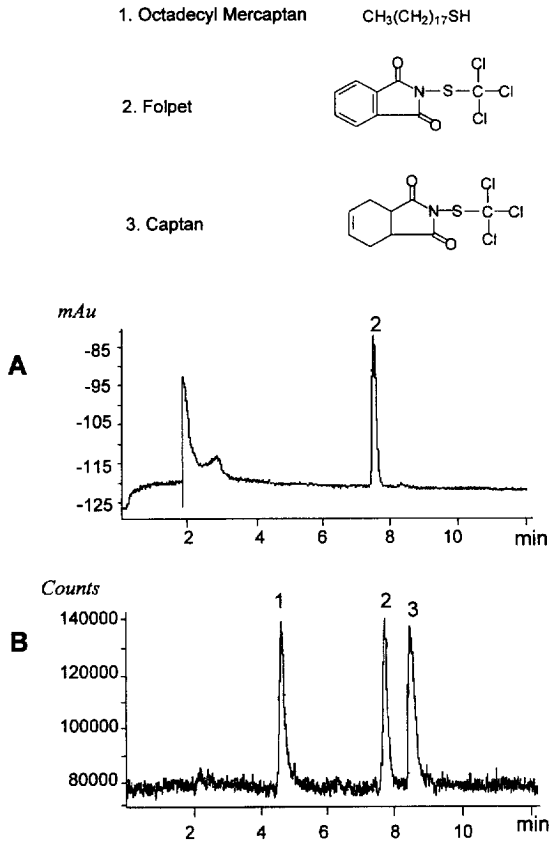


Fig. 3. Pesticide mixture profile with packed column dual SFC–SCLD/UV system. Mobile phase: 100% CO_2; Deltabond phenyl (250×4.6 mm I.D., 5 μm particle size) column. SFC conditions: 100 atm ramp to 220 atm at 10 atm/min; oven temperature held at 55°C; Decompressed CO_2 was 36 ml/min at 100 atm for the SCLD. Split ratio 30:1 (UV to SCLD). (A) UV detection at 219 nm. (B) SFC–SCLD.

Monitoring sulfur-containing compounds is very

important in petroleum and petrochemical industries since some of these compounds impede successful refining processes. A hydrotreated petroleum product was chromatographed on the SFC–SCLD/UV system using pressure programming from 100 to 260 atm. The simultaneous SCLD and UV profiles are shown in Fig. 4. Excellent selectivity was demonstrated by the SCLD. Although the components in the petroleum product eluted together in the UV profile, the two sulfur-containing components were easily resolved and detected by the SCLD. GC–SCLD analysis is possible for this sample, however, our interest was to obtain the aromatic hydrocarbon profile by UV detection at 254 nm, simultaneously with the SCLD. Since the UV detector can be readily interfaced to SFC, we chose to pursue the SF mode of separation for this particular application. If sulfur species need to be monitored quickly, a constant, moderately high SF-CO_2 pressure together with SCLD may be the solution to real world analytical problems.

The compatibility of this SFC–SCLD system with the use of methanol modified CO_2 was demonstrated by the analysis of a mixture of thermally labile sulfur compounds (Fig. 5). A mixture with concentration range from 5 to 7 ng/μl of 1,3-dibutyl-2-thiourea, sulfometuron methyl, sulfamethazine, sulfaquinoxaline and sulfathiazole was injected. Peak identification was achieved by injection of individual components. Methanol modifier was required to elute the polar and nonvolatile sulfonamides and sulfonylurea herbicides (Fig. 6). The efficient transport of these components from the restrictor to the furnace of the SCLD system is important. Best results were

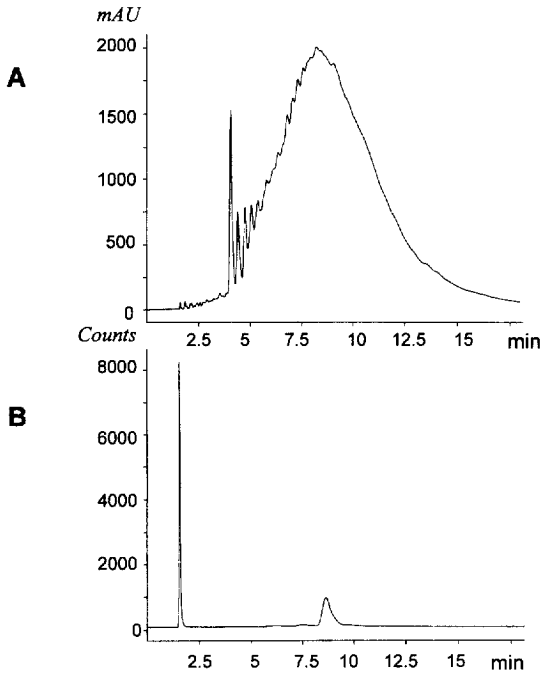


Fig. 4. Neat hydrotreated petroleum product profile with packed column dual SFC–SCLD/UV system. Mobile phase: 100% CO_2; Deltabond phenyl (250×4.6 mm I.D., 5 μm particle size) column. SFC conditions: 100 atm ramp to 260 atm at 10 atm/min; oven temperature held at 70°C; a 2 ft. long fused silica linear restrictor with 15 mm I.D. was used to the SCLD (1 ft. = 30.48 cm). Sample injection size was 5 ml. (A) UV detection at 254 nm. (B) SFC–SCLD.

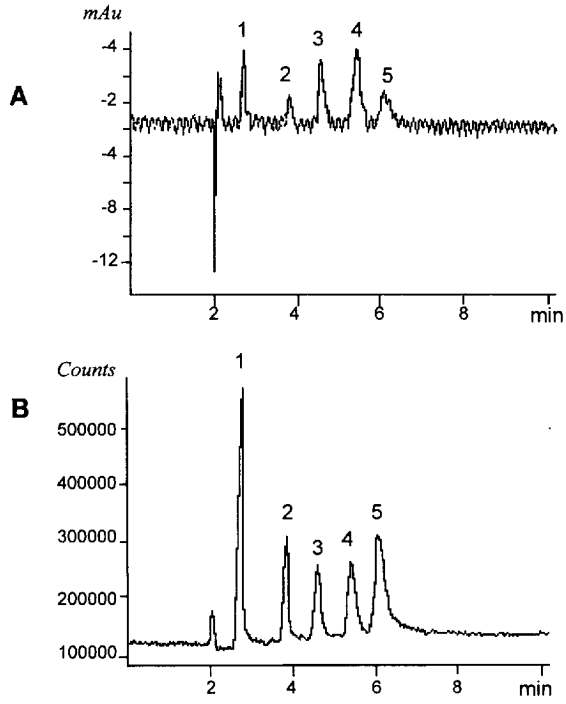
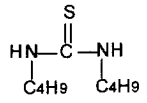
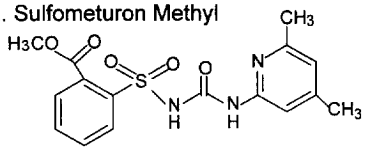


Fig. 5. Chromatographic separation of sulfonylurea herbicides and sulfonamides with packed column dual SFC–SCLD/UV system and methanol modified CO_2. Deltabond phenyl (250×4.6 mm I.D., 5 μm particle size) column. SFC conditions: methanol modifier starts at 8% (v/v), ramp to 10% at 0.2%/min; 150 atm pressure; liquid CO_2 flow-rate, 1.5 ml/min; oven temperature at 55°C; injection volume, 5 μl; split ratio 13.3:1 (UV to SCLD). (A) UV detection at 254 nm. (B) SFC–SCLD. Peaks: 1 = 1,3-dibutyl-2-thiourea, 2 = sulfometuron methyl, 3 = sulfamethazine, 4 = sulfaquinoxaline and 5 = sulfathiazole.

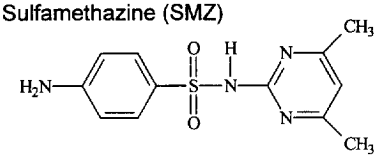
1. 1,3-Dibutyl-2-thiourea



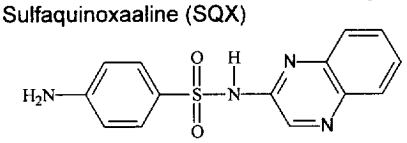
2. Sulfometuron Methyl



3. Sulfamethazine (SMZ)



4. Sulfaquinoxaaline (SQX)



5. Sulfathiazole (STZ)

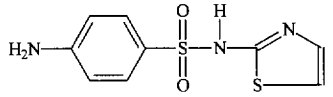


Fig. 6. Structures of sulfonylurea herbicides and sulfonamides of the SFC–SCLD/UV chromatogram in Fig. 5.

achieved with a 15-μm I.D. linear restrictor. As can be seen in Fig. 5 that even at a split ratio of 13.3 to 1 (UV to SCLD), SCLD has a much higher signal-to-noise ratio than UV detection. Therefore, much lower minimum detectable quantities can be achieved by SCLD than by UV.

4. Conclusion

SCLD was interfaced with packed column SFC using 100% CO_2 as mobile phase and tested simultaneously with UV detection. SCLD demonstrated high sensitivity, selectivity, a wide linear dynamic range, as well as equimolar responses to sulfur. The day-to-day repeatability over a 7-day period was excellent with less than 10% R.S.D.. The main advantage of SCLD (important for trace analysis) is the detector stability and compatibility with higher decompressed CO_2 flow-rates (for increased sensitivity) than other sulfur selective detectors presently available. When SFC separation is required, SCLD offers an excellent method for sensitive and selective detection of sulfur species in complex sample matrices. Analysis of sulfonamides and sulfonylurea herbicides by packed column SFC–SCLD using methanol modified CO_2 as mobile phase is also demonstrated.

Acknowledgments

Special thanks to Antek Instruments Inc. for the loan of 704E sulfur chemiluminescence detector. Additionally, we would like to thank Air Products and Chemicals Inc., for the donation of carbon dioxide, and Hewlett–Packard Company for the loan of the SFC system. Financial assistance provided by the USDA Northern Regional Laboratory, Philadelphia, PA, is appreciated (Cooperative Agreement # 58-1935-4-012).

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