SUPERCRITICAL FLUID CHROMATOGRAPHY WITH CHEMILUMINESCENT NITROGEN AND SULFUR DETECTION

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(ABSTRACT)

The need for sensitive and selective detectors in supercritical fluid chromatography (SFC) is particularly evident since SFC can be used to analyze classes of compounds that are not readily amenable to either gas chromatography (GC) or liquid chromatography (LC). These compounds include species that are nonvolatile or thermally labile and , in addition, contain no chromophore that can be used for spectrophoto detection. The objective of this research is therefore to interface selective chemilumninescent detectors with SFC in the sensitive detection of nitrogen- and/or sulfur containing compounds.

The chemiluminecent nitrogen detector (CLND), a gas-phase detector which is specific for nitrogen-containing compounds, was first evaluated as a detector for use with capillary SFC. The potential use of the CLND for food flavor and petroleum samples was demonstrated. In addition to equimolar nitrogen response, the CLND showed good sensitivity and large linear dynamic range. Minimum detectable quantity

(MDQ) was 60 pg of nitrogen with a linear range of over 3 orders of magnitude. Nitrogen to carbon selectivity of 10^5 was obtained. Capillary SFC with simultaneous flame ionization and chemiluminescent detection was also demonstrated.

The second portion of the research investigated the CLND for packed column SFC with methanol modified CO_2. The only modification made in the CLND for packed column SFC is the pyrolysis furnace. The CLND and UV were used to interface with SFC via a post-column split. Methanol-modified CO_2 was also demonstrated to be compatible with the CLND even with a high mobile phase flow rate. The use of pressure and modifier programs appears to be feasible as is evidenced by the baseline studies which have been performed, as well as by the applications demonstrated.

The last portion of the research focused on the evaluation of a new generation sulfur chemiluminescent detector (SCLD), which is also a gas-phase detector, with packed column SFC using both pure and methanol modified CO_2. The minimum detectable quantities were determined to be 2.6 pg or 14 pg sulfur for mobile phase employing pure CO_2 or 8% methanol modified CO_2 respectively. The evaluation study also showed excellent selectivity and linearity, as well as day-to-day repeatability. The capabilities of the SFC-SCLD system for sulfur speciation and detection of thermally labile pesticides and polar sulfonamides, as well as petrochemical samples were presented.

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Chapter I

Introduction

1.1 Chemiluminescence Detection in Chromatography

When chemical reactions are sufficiently exoergic, they usually produce vibrationally or electronically excited species. Relaxation of the excited species resulting from those exoergic chemical reactions by the emission of photons is referred to as chemiluminescence. This thesis describes the use of chemiluminescent reactions for the detection of chromatorgraphed species.

Chemiluminescent reactions can occur in both gas and liquid phases and have been applied in the chemiluminescent detection of compounds after gas and liquid chromatography. In the cases where the phenomenon of chemiluminescence has been used for chemical analysis, the method has generally proven to be extremely sensitive, because the light is detected against a dark background. The method is also very selective, since the selectivity of chemiluminescent detection can be easily achieved by using specific chemiluminescent reagents. The use of an optical filter can provide additional selectivity, because only light from a particular emitting species is allowed to reach the detector under this circumstance.

Several highly efficient chemiluminescent reactions in solutions have been used for detection in liquid chromatography. The general configuration for liquid phase chemiluminescent detection in liquid chromatography is given in Figure 1.1 As shown in

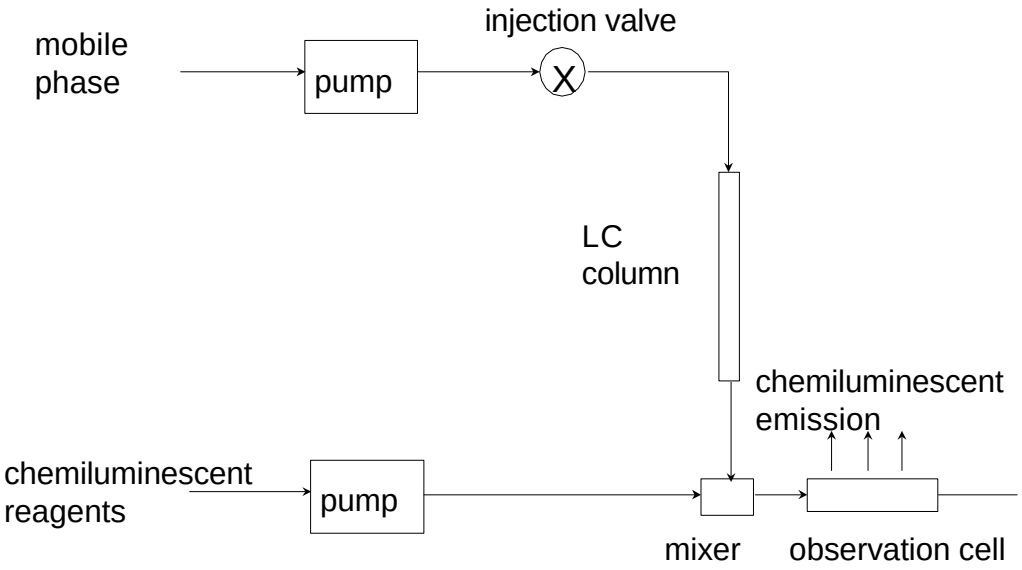
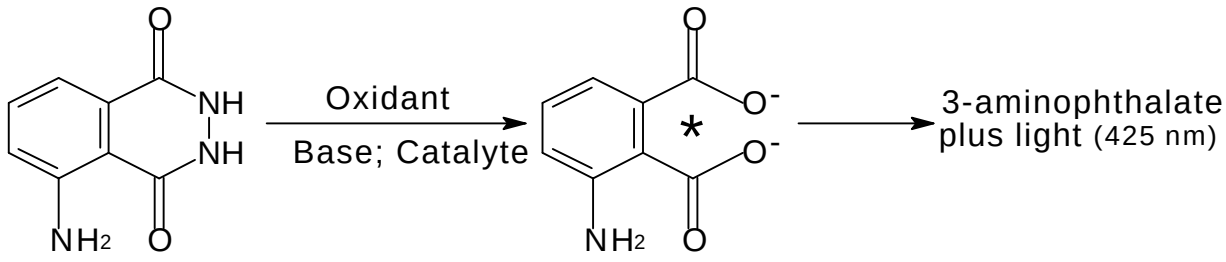


Figure 1.1. General arrangement for liquid phase chemiluminescent detection in liquid chromatography.

Figure 1.1, chemiluminescent reagents are mixed with column effluent, and detection of chemiluminescence emission is measured. Because the intensity of light emission from a chemiluminescent reaction is transient, the time from mixing to detection is critical and must be appropriately chosen in order to achieve optimum detection. This time can be controlled by both the volume between mixer and observation cell and the mobile phase flow rates.

A typical example (Figure 1.2) for liquid phase chemiluminescent detection is the metal catalyzed reaction of oxidizing agents with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). In basic solution, luminol can be oxidized to 3-aminophthalate in an excited state, and the light emitted is measured as the detector signal.[1]



Luminol 3-aminophthatale

Figure 1.2. The chemiluminescent reaction of oxidizing luminol.

[1] E.H. White; D.F. Roswell; In J. Burr, ed, "*Chemi- and Bioluminescence*", Dekker, New York, 1985, chap 4, pp215-244.

This chemiluminescent reaction has been used to quantitate transition metal ions such as Fe^{2+}, Cr^{3+}, Cu^{2+} etc. after ion chromatography, because the chemiluminescent intensity is directly proportional to the concentration of these ions.[2]

However, the majority of chemiluminescent reactions take place in the gas phase. The chemiluminescent reactions between ozone and certain molecules such as NO and SO are commonly used in detectors for gas chromatography.[3] The chemiluminescence induced by the reaction of ozone with these small molecules can be attributed to the weak bonds in ozone (104 KJ/mol) which can be easily broken to yield the stronger bond in molecular oxygen (497 KJ/mol). For example, the standard enthalpy change for the NO + O_2 reaction is -836 KJ/mol which is sufficiently exoergic to produce NO_2 in an electronically excited state (equation 1.1):

$$NO + O_3 \longrightarrow NO_2^* + O_2 \qquad \Delta H^O = -836 \text{ KJ/mol} \qquad (1.1)$$

The reaction of SO + O_3 also has a high enthalpy change of -443 KJ/mol which provides the necessary energy for electronic excitation of SO_2 (equation 1.2):

$$SO + O_3 \longrightarrow SO_2^* + O_2 \qquad \Delta H^O = -443 \text{ KJ/mol} \qquad (1.2)$$

---

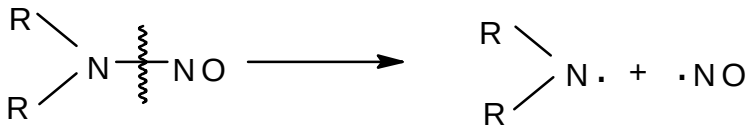
[2] W.R. Seitz; *CRC Crit. Rev. Anal.Chem*., 13 (1981) 1

[3] J.W. Birks, " *Chemiluminescence and Photochemical Reaction Detection in Chromatography*", VCH Publishers, Inc. New York, 1989, pp46

Probably the most widely used chemiluminescent detectors for gas chromatography are based on the NO + O_3 reaction[4] and the general arrangement for these chemiluminescent detectors is shown in Figure 1.3.

The column effluent is mixed with a post column reagent gas in a thermostatted reactor which may simply consist of a pyrolyzer, with/without catalyst. The reaction of ozone with the post column reaction product (NO) is the source of the signal in the chemiluminescent detector for gas chromatography. Because the intensity of light from the chemiluminescent reaction can be increased at reduced pressure, a vacuum pump is always used. The post column reaction is critical to the detector performance. The detection can be optimized by choice of appropriate post column reaction parameters such as temperature, residence time and reagent gas flow rate. The utilization of the NO + O_3 reaction in detection schemes is very attractive owing to its high sensitivity and large range of linearity. Selectivity for the detector can by controlled by varying the post column reaction temperature, or the catalyst type used in the reactor.

Nitrosamines are frequently detected using a NO + O_3 based chemiluminescent detector called the Thermal Energy Analyzer (TEA). The N-NO bond in N-nitroso compounds can be easily cleaved by pyrolysis to form nitric oxide (equation 1.3):



(1.3)

[4] A. Fontijn; A.J. Sabadell and R.J. Ronco; *Anal. Chem*. 42 (1970) 575

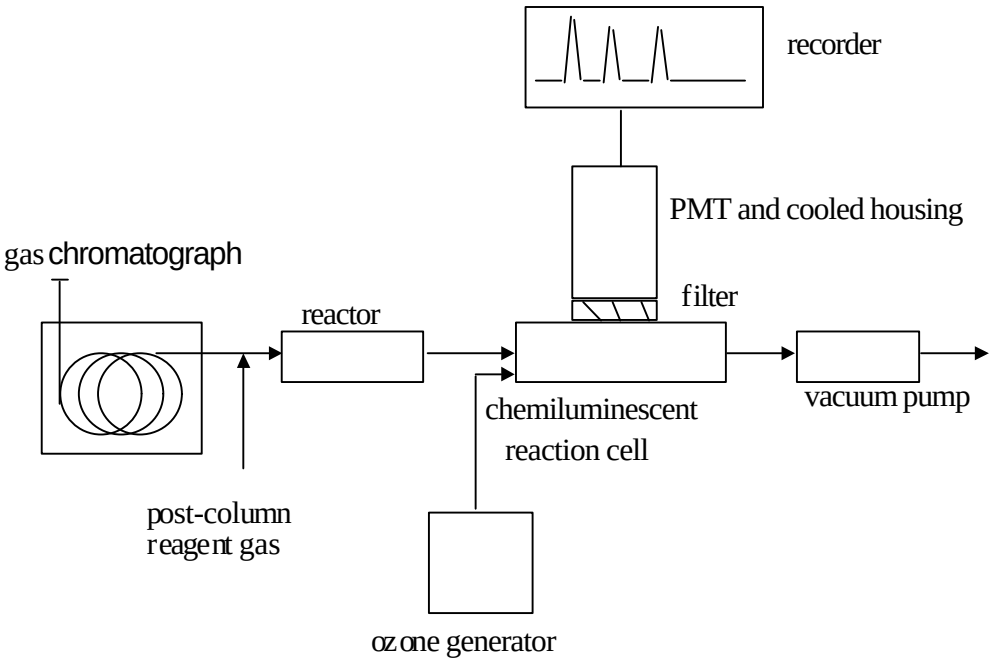


Figure 1.3. General arrangement for NO + O_3 chemiluminescent detector

The nitric oxide is then detected by its chemiluminescent reaction with ozone. The TEA has been coupled to gas chromatography[5,6,7] and liquid chromatography,[8] as well as to supercritical fluid chromatography.[9] Another variation on the nitric oxide and ozone detector is a Redox Chemiluminescent Detector (RCD).[10] In the RCD, a nitrogen-containing reagent such as NO_2 is reduced to nitric oxide by the analytes (equation 1.4) which are then detected on a catalyst surface at elevated temperature and thereby provide a surrogate NO signal which is proportional to the concentration of the analytes:

$$\text{analyte} + NO_2 \xrightarrow[\text{heat}]{\text{Au}} NO + \text{oxidized analyte} \qquad (1.4)$$

The NO is subsequently detected by its chemiluminescent reaction with ozone. The RCD has been used in gas chromatography for the analysis of a wide array of compounds such as alcohols, olefins and phenols.[11] Later, the feasibility of interfacing the RCD to supercritical fluid chromatography was demonstrated by the separation of antioxidant isomers and some compounds of biological relevance.[12]

Recently a new gas chromatography detector which is also based on the NO + O_3 chemiluminescent reaction has been described. Unlike TEA and RCD, this new

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5 D.H. Fine; F. Rufeh; B. Gunther, *Anal. Lett*., 6 (1973) 731
6 D.H. Fine; F. Rufeh; D. Lieb; D.P. Roundbehier, *Anal. Chem*., 47 (1975) 1188
7 J.J. Hansen; M.C. Archer; S.R. Tannenbaum, *Anal. Chem*., 51 (1979) 1526
8 J.H. Phillips; R.J. Coraor; S.R. Prescott, *Anal. Chem*., 55 (1983) 889
9 E.S. Francis; D.J. Eatough; M.L.Lee, *J. Microcol. Sep*., 6 (1994) 2074
10 S.A. Nyarady; R.M. Barkley; R.E. Sievers, *Anal. Chem*., 57 (1985) 2074
11 R.S. Hutte; R.E. Sievers; J.W.Birks, *J. Chromatogr. Sci*., 24 (1986) 499

detector is very specific and quite sensitive to nitrogen-containing compounds.[13,14] The commercial detector of this type was manufactured by Antek Instruments Inc. (Houston, TX) and was named Chemiluminescent Nitrogen Detector (CLND). In the CLND, the formation of nitric oxide is based on post column oxidization of nitrogen-containing species at temperatures above 1000°C. At this high temperature, the oxidization of nitrogen-containing compounds results in the exclusive formation of nitric oxide (equation 1.5):

$$R_1\text{–}\underset{\displaystyle R_3}{\overset{\displaystyle R_2}{\underset{|}{\overset{|}{N}}}} + O_2 \xrightarrow{>1000^{o}C} NO + \text{other products} \qquad (1.5)$$

The chemiluminescent reaction of nitric oxide and ozone is the same as that in the TEA and RCD detections. This novel chemiluminescent detector may be used for either gas chromatography[15,16] or liquid chromatography[17] in the quantitation of nitrogen containing compounds in complex sample matrices.

The chemiluminescence produced by the reaction of small sulfur-containing compounds with ozone has also been used for chemical analysis. When sulfur-

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[12] W.T. Foreman; R.E. Sievers; B.W. Wenclawiak, *Fresenius Z Anal. Chem*., 330 (1988) 231
[13] A.J. Britten, *Res. Dev*. 31 (1989) 76
[14] L.O. Courthanden; E.M. Fujinari, *LC-GC*, 9 (1991) 732
[15] E.M. Fujinari; L.O. Courthaudon; H. Hernandez, presented at the 1992 Pittsburgh Conference, New Orleans, LA, March, 9-12, 1992
[16] S.M.Benn; K. Nyung; E.M. Fujinari, "*Food Flavors, Ingredients and Composition"*, G.Charlambous Ed, Elsevier Science Publishers, Amsterdam, 1993, pp65

containing compounds are combusted in a hydrogen/air flame, the major products are SO, SO_2, H_2S and S_2.[18] As mentioned earlier, ozone reacts with SO to produce electronically excited sulfur dioxide (SO_2*). It is logical to make use of this chemiluminescent reaction for selective detection of sulfur-containing compounds. A commercial instrument to do this is Model 350 Sulfur Chemiluminescent Detector (SCD) manufactured by Sievers Research Inc. (Boulder, CO). In the original version of the SCD, sulfur compounds that emerged from the chromatographic column were first burned in a hydrogen-rich (reducing) flame (FID) and were converted to SO. The SO and the other products are collected by means of a ceramic probe positioned in the flame and then transferred to a reduced pressure reaction cell to react with ozone. The resulting chemiluminescence at 350 nm from excited SO_2* species is detected. The following equations illustrate the principle of the SCD:

$$\text{S-compounds} \xrightarrow{H_2\text{-rich flame}} SO + \text{other products}$$

$$SO + O_3 \longrightarrow SO_2^* + O_2$$

$$SO_2^* \longrightarrow SO_2 + h\nu \; (\lambda_{max}=350 \text{ nm})$$

The performance of the SCD has been found to surpass that obtained with other sulfur selective detectors[19, 20] and the SCD has become the most widely used sulfur

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[17] E.M. Fujinari; L.O. Courthaudon, *J. Chromatogr*., 592 (1992) 209
[18] C.F. Cullis; M.F.R. Mulcahy, *Combustion and Flame*, 18 (1972) 225
[19] A.L. Howard; L.T. Taylor, *J. High Resolut. Chromatogr*., 14 (1991), 785
[20] M. Dyson, *Anal. Proceedings*, 30 (1993) 79

selective detector by its coupling to gas chromatography,[21, 22, 23] supercritical fluid chromatography,[24, 25, 26] and liquid chromatography.[27, 28] The main weakness of the detector is, however, the operational difficulties related to conditioning and positioning of the ceramic probe interface in the FID which is critical to the detector performance. Thus, a newer version of the combustion module for SCD has been developed for gas chromatography called flameless SCD. Instead of using a FID flame. the flameless SCD utilize a furnace assembly heated at 780^{o}C in order to generate SO species. The flame SCD is considered to be one of the most sensitive detectors for sulfur compounds. The coupling of flameless SCD to supercritical fluid chromatography has been reported by Shearer et al.[29] A maximum of 12 mL/min of decompressed CO_2 was the limit to ensure successful detection. This limitation is probably owing to the small size of the heated furnace which may cause problems in generating SO species. Recently, a new generation sulfur chemiluminescent detector (SCLD) for gas chromatography was described by Antek Instruments, Inc..[30] A furnace assembly is also used in the SCLD, but the furnace size is much larger compared to the one used in flameless SCD. Therefore, the SCLD is expected to have better performance than

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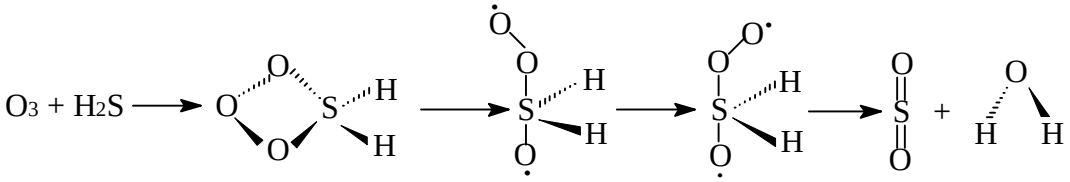
[21] R.L. Benner; D.H. Stedman, *Anal. Chem*., 61 (1989) 1268
[22] R.L. Shearer; D.L. O'Neal;R. Rios; M.D. Baker, *J. Chromatogr. Sci*., 28 (1990) 24
[23] N.G. Johansen; J.W. Birks, *Amer. Lab*., Feb. (1991) 112
[24] H.-C.K. Chang; L.T. Taylor, *J. Chromatogr*., 517 (1990) 491
[25] L.A. Pekay; S.V. Olesik, *J. Microcol. Sep*., 2 (1990) 270
[26] A.L.Howard; L.T. Taylor, *Anal. Chem*., 61 (1993) 724
[27] H.-C.K. Chang; L.T. Taylor, *Anal.Chem*., 63 (1991) 486
[28] A.L.Howard; C.L.B. Thomas; L.T. Taylor, *Anan. Chem*. 66 (1994) 1432
[29] R.L. Shearer; R.J. Skelton, *J. High Resolut. Chromatogr*., 17 (1994) 251

[30] X.Yan; E.M. Fujinari, presented at Pittsburgh Conference, Chicago, IL., March 3-8, 1996, paper # 434

flameless SCD. Besides being structurally different from flameless SCD, the chemiluminescent precursor for the SCLD is reportedly H_2S rather than SO for the excited sulfur dioxide species based on a single collision reaction (equation 1.6).[31]

$$H_2S + O_3 \longrightarrow SO_2^* \qquad (1.6)$$

The mechanism for the single-collision reaction between H_2S and ozone was envisioned as follows:



The enthalpy change of this reaction is -660 KJ/mol, so that it is sufficiently exoergic to form SO_2 in an excited state. In addition, the chemiluminescent spectrum recorded from $H_2S + O_3$ reaction was found to be essentially identical with that of the $SO + O_3$ reaction. A dispute exists as to whether the chemiluminescent precursor is either H_2S or SO. However, the result of either H_2S or SO with ozone is the same production of excited SO_2 species.

1.2 The Importance of Nitrogen and Sulfur Detection

Both sulfur and nitrogen are very important elements. Nitrogen and/or sulfur are

[31] R.J. Glinski; J.A. Sedarski; D.A. Dixon, *J. Am. Chem. Soc*., 104 (1982) 1126

responsible for many of the more exciting flavors and tastes in food and drink. Horse-radish sauce derives its strong taste from allyl sulfide and hot mustard oils contain allyl isothiocyanide. Water and soil samples may be contaminated by trace level of harmful pesticides and herbicides. The pharmaceutical industry makes nitrogen- and/or sulfur-containing drugs which are widely used in everything from ointments to medication and dietary tablets. Most biological samples contain nitrogen, and certain amino acids also contain sulfur. Since nitrogen and/or sulfur are the major heteroelements in coal and petroleum components, diesel fuel and gasoline contain nitrogen and/or sulfur. Nitro-compounds found in diesel exhaust particulate samples may lead to an increased probability of cancer. Nitrogen compounds adversely affect catalytic refining processes, thus resulting in the instability of stored fuels.[32, 33] The presence of sulfur components in feed stock reactions also causes a detrimental effect on modern metallic catalysts.[34]

Often the nitrogen and/or sulfur compounds are present at trace levels, with detection and quantification being complicated by high levels of interfering components. Notwithstanding the continuing development of various high resolution stationary phases for chromatographic columns, the use of highly sensitive chemiluminescent detectors such as CLND, SCLD for selective detection of nitrogen or sulfur provides unmatched advantages over universal detectors. The need for extensive sample cleanup runs the risk of either contamination or loss of analytes. These steps can be

[32] H.V. Drushel; A.L. Sommers, *Anal. Chem*., 38 (1966) 19
[33] J.H. Worstell; S.R. Paniel; G. Frauenhoff , *Fuel*, 60 (1981) 485
[34] H.V. Drushel, *J. Chromatogr. Sci*., 21 (1983) 375

minimized ated by using selective detectors, since the detection of analytes of interest can be achieved in unresolved mixtures.

1.3 Advantages of Supercritical Fluid Chromatography

Many nitrogen- and/or sulfur-containing compounds are either thermally unstable or non- volatile which limits their analyses by gas chromatography(GC). High performance liquid chromatography(HPLC) is applicable to analyze compounds of classes, but it generates a large amount of organic solvents which need to be ultimately disposed. The disposal cost for organic solvents typically range from $5 to $10 per gallon and are constantly rising due to stricter environmental regulations.[35] Supercritical fluid chromatography(SFC) is emerging as a separation technique that is superior to both gas chromatography and liquid chromatography for analysis of thermally labile or non volatile compounds. The information in Table 1 indicates that physical properties

Table 1. Comparision of Properties of Supercritical Fluids, Liquids and Gases

	Gas (STP)	Supercritical Fluid	Liquid
Density g/cm^3	(0.6-2) x 10^{-3}	0.2-0.5	0.6-1.6
Diffusion coefficient, cm^2/s	(1-4) x 10^{-1}	10^{-3}-10^{-4}	(0.2-2) x 10^{-5}
Viscosity, g/cm.s	(1-3) x 10^{-4}	(1-3) x 10^{-4}	(0.2-3) x 10^{-2}

[35] B.D. Jones, USACHPPM Information Paper, Aug. 1992

of supercritical fluids are intermediate between those of gases and liquids. As a consequence, SFC combines some of the best features of both GC and HPLC. Like GC, SFC is inherently faster than HPLC, because of its lower viscosity and higher diffusion rates compared to HPLC. It is well documented that SFC provides a combination of a 3-5 times increase in speed of analysis, and a decrease in analysis cost through savings in organic solvent. [36, 37] The "liquid-like" density of supercritical fluids makes it possible for SFC to extend the molecular range of GC to larger non-volatile molecules. Low critical temperatures for such supercritical fluids as carbon dioxide(31^{o}C) and nitrous oxide(37^{o}C) allows SFC to analyze thermally unstable compounds that are not amenable to GC.

Unlike GC or HPLC, where the mobile phase dominates the type of detector to be used, SFC utilizes mobile phases which can be either liquid-like, or gas-like. Therefore both GC and HPLC detectors are applicable to SFC. This multidetector compatibility makes SFC a technique of unparalleled success in the analysis of thermally labile species and/or relatively high molecular weight compounds. Conventional gas-phase detectors such as flame ionization detector(FID) and flame photometric detector(FPD) coupled to SFC have been described in various publications.[38, 39, 40] Liquid phase detectors such as ultraviolet-visible(UV-Vis)

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[36] J.T.B. Strode III; L.T. Taylor; A.L. Howard; M.A. Brooks, *J. Pharm & Bio. Anal*., 12 (1994) 1003

[37] T.L.Chester; J.D. Pinkston, The Procter & Gamble Company, User-Manufacturer Interchange Session, Atlanta, GA, March, 1997

[38] C.H. Burnett; D.F. Adams;S.O. Fauell, J. *Chromatogr. Sci*., 16 (1978) 68

[39] B.E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun*., 8 (1985) 297

spectrophotometric detector,[41] light scattering detector,[42] and refractive index detector[43] have been employed for SFC. Mass spectrometry and Fourier transform infrared spectrometry (FTIR) can also be used effectively with SFC.[44]

1.4 Objective of the Research

The objective of this research is to extend the utility of a new generation of chemiluminescent detectors such as CLND and SCLD to SFC. The optimization and evaluation, as well as application of SFC/CLND and SFC/SCLD for both packed and open tubular columns using 100% or methanol modified supercritical fluid carbon dioxide as mobile phase have been studied. Applications of dual-channel detection of samples by FID/CLND or FID/SCLD are also presented, thus providing information on both carbon and nitrogen or sulfur by a single injection.

In Chapter II we describe the feasibility of SFC/CLND with open tubular columns using 100% CO_2 as the mobile phase. It was reasonable to first interface the CLND to capillary SFC, since the CLND was originally designed for GC. Flow injection analysis was used in all the optimization and evaluation studies. Indole, which is a very stable nitrogen-containing compound was used as a target analyte. The separation of several mixtures of nitrogen-containing compounds was undertaken to show the application of

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[40] J.C. Fjeldsted; R.C. Kong; M.L. Lee, *J. Chromatogr*., 279 (1983) 449
[41] S.M. Fields; K.E. Markides; M.L. Lee, *Anal. Chem*., 60 (1988) 802
[42] P. Carroud; D. Thiebaut; M. Caude; R. Rosset, *J. Chromatogr. Sci*., 25 (1987) 395

the capillary column SFC/CLND system.

Chapter III describes the use of open-tubular column supercritical fluid chromatography for the separation of aniline isomers, alkyldimethylamines, and dialkylmethylamine mixtures, as well as a food flavor extract with simultaneous flame ionization detection(FID) and selective nitrogen detection(CLND).

In Chapter IV we describe the evaluation and optimization of a packed column SFC/CLND system using methanol modified CO_2. The use of packed columns in SFC has several advantages due to their larger inner diameter and shorter length. Packed columns have higher sample capacities and offer faster analysis time than capillary columns. More important, a packed column SFC system makes it possible to utilize methanol modified CO_2 as the mobile phase, thus, high polarity analytes such as most pharmaceuticals which usually require HPLC methods can be analyzed by SFC. In order to accommodate high mobile phase flow rates from packed column SFC, the original furnace of the CLND was substituted by a larger pyrolysis furnace. The packed column SFC/CLND system was optimized and evaluated using several polar, as well as non-polar nitrogen-containing compounds. The analyses mainly focused on pharmaceuticals.

Chapter V describes the interface of the new generation sulfur

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[43] W. Asche,Chromtographia, 11(1978) 411

chemiluminescence detector (SCLD) to SFC using pure and methanol modified SF-CO_2 as the mobile phases. The SCLD was evaluated for packed column SFC using dibenzothiophene and sulfonamides as target analytes. The separation of a thermally labile pesticide mixture, polar pharmaceuticals, as well as petroleum products by packed column SFC-SCLD/UV was studied. The advantages of using SCLD were demonstrated by comparing chromatograms obtained by a sulfur selective detector and a UV detector. Open tubular columns are to be preferred when high efficiency is required. Therefore, the analysis of several heavy diesel samples by open tubular column SFC with dual channel sulfur selective detection and flame ionization detection was studied.

[44] D.W. Later; D.J. Bornhop; E.D. Lee; J.D. Henion; R.C. Weibolt, LC-GC, 5 (1987) 804

Chapter II

Studies of Interfacing a Nitrogen Chemiluminescent Detector after Open Tubular Column Supercritical Fluid Chromatography

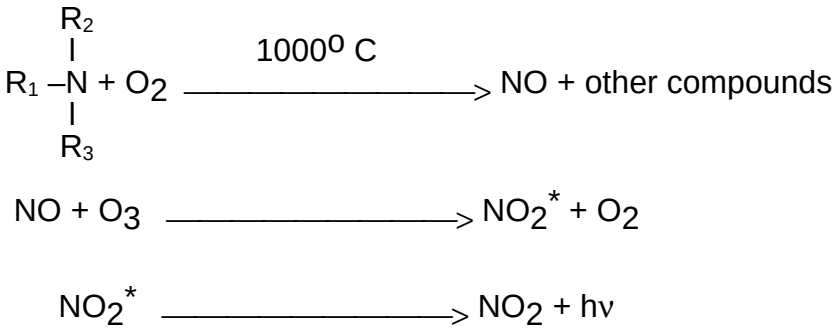
2.1 Introduction

In general, chemiluminescence detection is gaining in popularity because it offers advantages over other optical techniques [1]. The chemiluminescence response, together with the low background current, places these detectors among the most sensitive of analytical instruments. Because of the unique chemistry of each chemiluminescent reaction, the detectors are inherently very selective. These characteristics make it possible to detect very small amounts of desired analytes in complex matrices. The need for extensive sample clean-up and preparation prior to chromatographic analysis is thus eliminated. In addition, good repeatability and accuracy of the chemiluminescent technique makes it a viable tool for routine analysis.

As stated previously, the analysis of nitrogen containing compounds is greatly valued in biological, industrial, and environmental samples, such as body fluids, food flavors, and pesticides. The detection of nitrogen by chemiluminescence became possible after Fontjin and coworkers [2] demonstrated the usefulness of chemiluminescent light resulting from the gas phase reaction between nitric oxide and ozone. Several years later, a chemiluminescent nitrogen detector for GC (GC-CLND)

[1] R.S. Hutte; R.E. Sievers; J.W. Birks, *J.Chromatogr. Sci*., 24 (1986) 499
[2] A. Fontijn; A.J. Sabadell; R.J. Ronco, *Anal. Chem*., 42 (1970) 575

was developed by Parks et al..[3] The detection mechanism is illustrated in the following series of reaction:



The light emitted in the process occurs in the wavelength region of 600-900 nm and is detected by a photomultiplier tube.

The first GC detector utilizing nitric oxide/ozone chemiluminescence was the thermal energy analyzer (TEA) developed by Fine et al.[4, 5, 6] for nitrosamine determinations. N-Nitroso compounds were analyzed based on a catalytic low-temperature pyrolysis reaction, followed by chemiluminescence detection with ozone. Kashihira et al. used a chemiluminescent nitrogen oxide analyzer in conjunction with formation of nitrogen monoxide from nitrogen-containing compounds by pyrolysis on a hot platinum catalyst. This system was also applied to the GC measurement of

[3] R.E. Parks; R.L. Marietta, U.S. Patent 4,018,562,24 October 1975
[4] D.H. Fine; F. Rufeh; D. Lieb; D.P. Roundbehler, *Anal. Chem*., 47 (1975) 1188
[5] D.H. Fine; D.P. Roundbehler, *J. Chromatogr*., 109 (1975) 271
[6] D.H. Fine; D. Lieb; Frufeh, *J. Chromatogr*., 107 (1975) 351

atmospheric ammonia and amines [7]. More recently, an improved nitrogen specific GC-CLND system was reported by Fujinari et al..[8] A wide range of GC-CLND applications was presented, which included pesticide residue, food flavor, pharmaceutical and petroleum light cycle oil samples. The GC-CLND method has proven to be very sensitive and highly selective, enabling detection of as little as 12 pg nitrogen.

The CLND has also been successfully interfaced with high performance liquid chromatography. Fujinari et al. [9] described a novel HPLC-CLND system in the quantitation of ammonium hydroxide in waste water. The limit of detection of the system was found to be 5 ng nitrogen.

Supercritical fluid chromatography (SFC) has now become a widely accepted analytical method and is used in various industries. The multidetector compatibility is a major advantage of SFC over both GC and HPLC. A variety of liquid-like and gas-like detectors have been successfully coupled to SFC, such as ultraviolet-visible [10], flame ionization [11], mass spectrometry, Fourier transform infrared spectrometry [12]and sulfur chemiluminescence detection [13, 14, 15]. This chapter describes the direct interfacing of the CLND for the first time to open tubular SFC employing 100% CO_2 as the mobile

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[7] N. Kashihira; K. Kirita; Y. Watanabe, *J. Chromatogr*., 239 (1982) 617
[8] L.O. Courthaudon; E.M. Fujinari, *LC-GC*, 9 (1991) 732

[9] E.M. Fujinari; L.O. Courthaudon, *J. Chromatogr*., 592 (1992) 209
[10] S.M. Fields; K.E. Markides; M.L. Lee, *Anal. Chem*., 60 (1988) 802
[11] B.E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun*., 8 (1985) 297
[12] D.W. Later; D.J. Bornhop; E.D. Lee; J.D. Henion; R.C. Weibolt, LC-GC, 5 (1987) 804
[13] D.J. Burnhop; B.J. Murphy, *Anal. Chem*., 61 (1989) 797
[14] H.-C.K. Chang; L.T. Taylor, *J. Chromatogr*., 517 (1990) 491

phase. Results from the performance evaluation of the SFC-CLND system are discussed, and the utility of the system is illustrated by the analysis of several food-related matrices.

2.2 Experimental

Instrumentation

A model 705D CLND nitrogen specific detector from Antek Instruments Inc. (Houston, TX) was interfaced to either a Hewlett-Packard supercritical fluid chromatograph (for flow injection analysis) or a Dionex Lee Scientific (Salt Lake City) series 600 supercritical fluid chromatograph (for chromatographic separation) via a 50 μm i.d. frit restrictor. A SB-cyanopropyl capillary column (20 m x 100 μm i.d., 0.25 μm film thickness) obtained from Dionex was used to achieve chromatographic separation. Time split injection with a helium actuated Valco (Houston, TX) injector (500 nL rotor) was employed for sample introduction. Data were recorded by a Hewlett-Packard 3310 integrator (Avondale, PA).

Reagents

Allyl cyanide, allylisothiocyanate, 2,3-dimethylindole, 2,6-dinitrotoluene, diphenylamine, indole, 4-nitrotoluene, 2-phenylindole, phenyl ethyl isothiocyanate, and

[15] A.L.Howard; L.T. Taylor, *Anal. Chem*. 61 (1993) 724

pyridine were purchased from Aldrich Chemical Co.(Milwaukee, WI). 2-Butyl-isothiocyanate was purchased from Lancaster Synthesis Inc. (Windham, NH). 2,5-Lutidine was purchased from Chem Service Inc. (West Chester, PA). Caffeine was purchased from Sigma Chemical (St. Louis, MO). p-Nitroaniline was purchased from Fisher Scientific Co.(Pittsburgh, PA). Dimethoate was purchased from Accu Standard Inc. (New Haven, CT). All chemicals were used without further purification. Horseradish oil standard and hot mustard extract were received from commercial sources. HPLC grade solvents (EM Science, Gibbstown, NJ) were used for preparing standard solutions. Grade 4.3 oxygen (Airco, Murray Hill, NJ) was used as both pyrolysis and ozone-generator gas. SFC-grade CO_2 was obtained from Air Products and Chemical Inc. (Allentown, PA).

Chromatographic Conditions

All analyses were performed with pressure programming. In all cases, the oven temperature was held constant throughout the experiment. Integrator conditions: 1 volt input, attenuation 7. CLND conditions: pyrolysis temperature 1050^{o}C, PMT voltage 750 volts, range x 50, detector output 1 volt. Additional chromatographic conditions are cited in the Figure legends.

2.3 Results and Discussion

Detector Sensitivity Optimization

The CLND was used with capillary SFC employing 100% CO_2 as the mobile phase without modifier. A simple interface between the SFC and the CLND was accomplished by using a conventional length (25 cm) frit restrictor which was threaded through the bottom of the pyrolysis furnace. A schematic diagram of the SFC-CLND is given in Figure 2.1. When a sample was chromatographed on a column, the eluting compounds under supercritical fluid conditions were decompressed through the restrictor and then introduced into the pyrolysis furnace where they mixed with oxygen and underwent high-temperature oxidation. All compounds containing nitrogen were converted into nitric oxide. The nitric oxide is then drawn into the reaction chamber and reacted with ozone. This resulted in the formation of nitrogen dioxide in the excited state (NO_2^*) and the chemiluminescence from the NO_2^* was detected by a photomultiplier tube (PMT).

There are two operating variables which are critical to the detector response. They are (a) restrictor position, and (b) pyrolysis oxygen flow rate. First, we optimized the CLND sensitivity by proper insertion of the restrictor to the detector interface. The restrictor positions and the corresponding indole signal responses of the CLND are shown in Figure 2.2. The result shows that the optimum position for the restrictor is 15 cm into the tube adjoining the detector base of the pyrolysis furnace and is described in Figure 2.3.

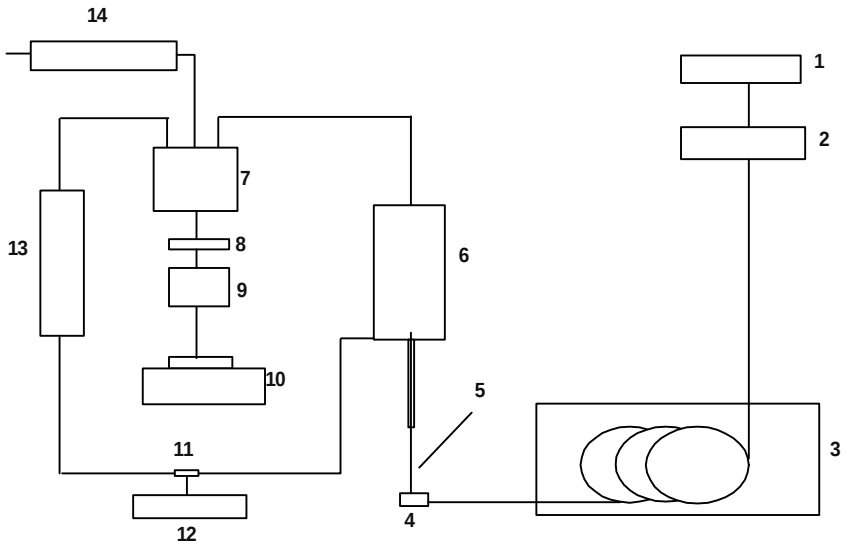

Figure 2.1. Schematic drawing of the SFC-CLND system. 1. CO_2 tank, 2. CO_2 pump, 3. SFC oven, 4. butt connector, 5. frit restrictor, 6. pyrolysis furnace, 7. reaction chamber, 8. filter, 9. PMT, 10. integrator, 11. tee splitter, 12. oxygen tank, 13. ozone generator, 14. scrubber.

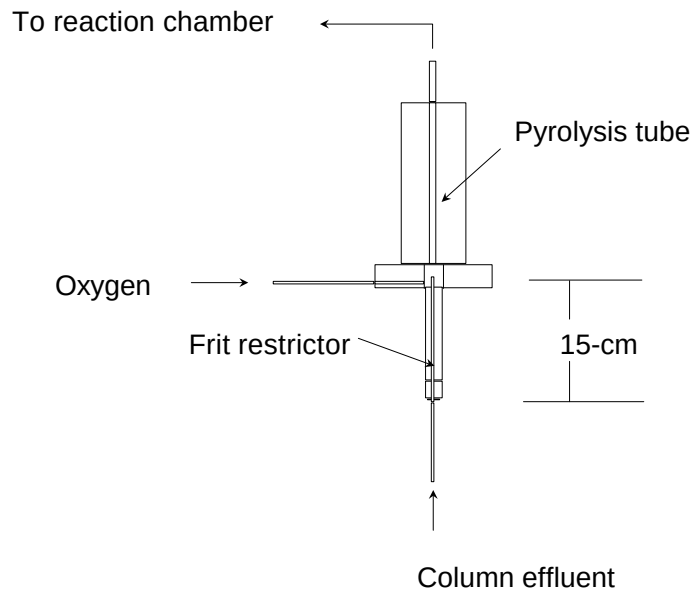

Figure 2.2. Schematic interface of the SFC/CLND system.

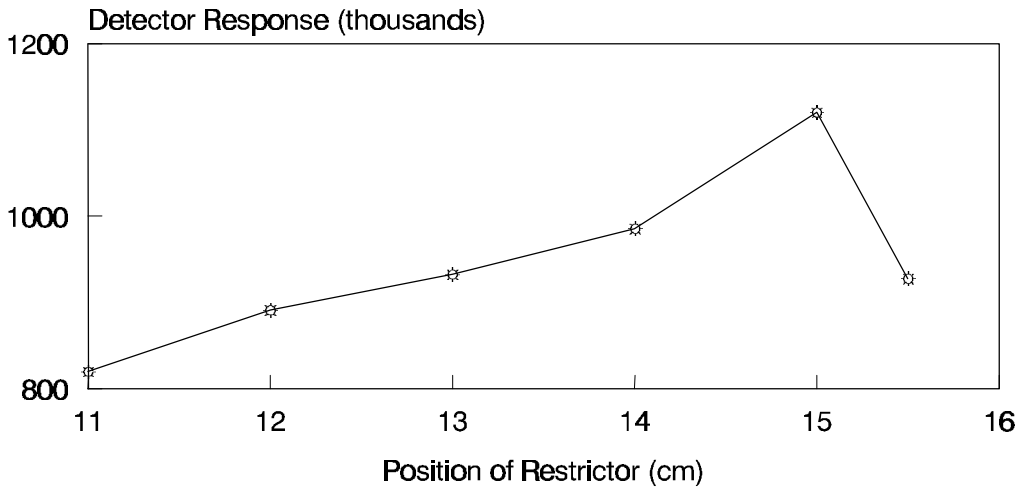

Figure 2.3 Effect of restrictor position on CLND response. Conditions: pressure program 100 atm (hold 1 min), ramp to 180 atm at 20 atm/min, hold 3 min; Biphenyl column (50 μm x 3 m, 0.25 μm); time split injection 0.3 sec; sample: indole in methanol.

At the optimum position, the restrictor tip is placed above the oxygen inlet to the pyrolysis chamber. Therefore, the column effluent is mixed with the pyrolysis oxygen and effectively swept into the furnace. The efficient pyrolysis of the sample and the resulting nitric oxide gas provide for maximum sensitivity.

The CLND was further optimized by maintaining a constant oxygen flow rate through the ozone generator at 8 mL/min and varying the pyrolysis oxygen flow from 20 to 65 mL/min. The detector response (peak area) in this case was obtained by injecting 2,6-dinitrotoluene in methanol. As shown in Figure 2.4, a significant drop in the detector response was observed when the pyrolysis oxygen flow rate is either below 30 mL/min or above 55 mL/min. A possible explanation for this is that (a) low pyro-oxygen flow rate into the furnace results in incomplete oxidation of nitrogen to nitric oxide and (b) incomplete oxidation is also obtained from short residence time of the analyte in the pyrolysis chamber when the pyro-oxygen flow rate is set too high . The best CLND response was achieved at the flow rate range from 35 to 50 mL/min. The pyrolysis oxygen flow rate of 40 mL/min was selected and used in subseqent experiments.

Detector Performance

The SFC-CLND system was evaluated by determining linear dynamic range (LDR), response factors, selectivity and detection limit. Calibration curves were

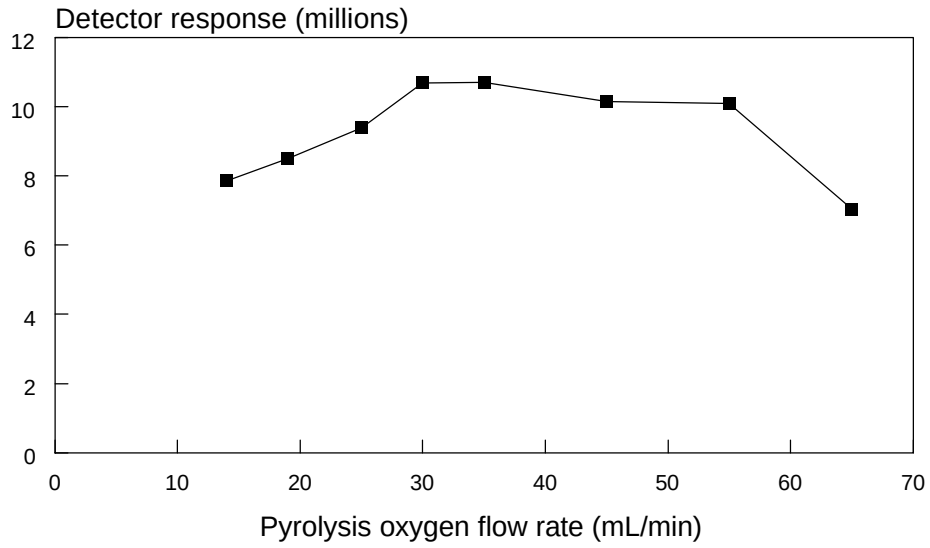

Figure 2.4. Effect of pyrolysis oxygen flow rate on CLND response. Conditions: (flow injection analysis) 60^{o}C transfer line; CO_2 pressure: 300 ate; Sample: indole in methanol.

constructed from standard solutions of indole in methanol ranging form 2.96 to 850 ppm of nitrogen. Linear least square analysis resulted in correlation coefficients greater than 0.999. The LDR of 3 orders of magnitude was obtained. Minimum detectable quantity

(MDQ) was determined by a signal (S/N=3) (Figure 2.5) resulting from a flow injection of 1.4 ppm nitrogen of indole solution via a 5-μL sample loop with a split ratio of 1/116 (CLND/UV), and was found to be 60 pg nitrogen at the detector. The CLND's minimum detectable quantity for SFC are compared to that of GC and HPLC (Table 2.1).

As reported previously, the chemiluminescent nitrogen detector is molar responsive [16, 17], and responds to the nitrogen content of each nitrogenous compound. This is because all nitrogen species are converted to NO and the nitrogen is detected by means of the oxidation of NO to NO_2*, so that the first reaction strips away inter-molecular differences, the second reaction is thus similar for all species and so their molar responses are similar. Peak areas per unit mass of nitrogen for some selected compounds were used to calculate relative response factors. Table 2.2 lists the response factors for those selected nitrogen compounds relative to the response form indole (arbitrary units of the detector response per unit mass of nitrogen). As expected the result showed equimolar nitrogen response for the detector. The advantage of this detector for quantifying nitrogen-containing compounds in a complex sample is that a single calibration curve may be used, provided that the proper chromatographic

[16] A.J. Robbat; P.N. Corso; J.P. Doherty; H.M. Wolf, *Anal. Chem*., 58 (1986) 2078

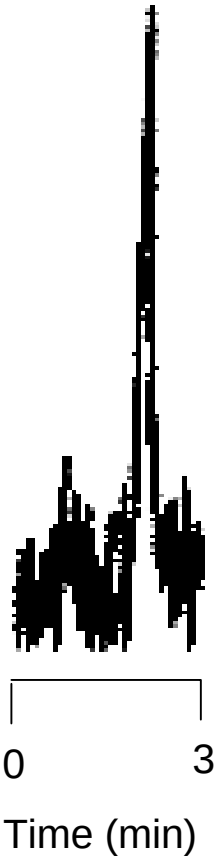

Figure 2.5. Peak resulting from flow injection of 1.4 ppm nitrogen of indole via a 5-μL sample loop with a split ratio of 1/116.5 (CLND/UV). Minimum detectable quantity is 60 pg nitrogen at S/N = 3

[17] E.M. Fujinari; J.D. Manes, "*Food Flavors: Generation, Analysis and Process Influence"*, G.Charlambous Ed, Elsevier Science Publishers, Amsterdam, 37A, 1995, pp929

Table 2.1. CLND Minimum Detectable Quantity (MDQ) Comparison for SFC, GC, and HPLC

Chromatographic System	MDQ (pg of N)	Analyte	Reference
GC	12	nitric oxide	8
SFC	60 (FIA)	indole	This work
HPLC	50×10^2	ammonium ion	9

Table 2.2. Relative Response Factors in CLND

Component	Relative Response
4-nitrotoluene	1.01
2,6-dinitrotoluene	1.05
2,3-dimethylindole	1.04
pyridine	1.01
caffeine	1.07
diphenylamine	1.06
indole	1.0 (reference standard)
2-phenylindole	1.01

conditions are chosen. Selectivity (N/C) of 5 orders of magnitude using SFC-CLND was achieved by comparing the indole to methanol response.

In most cases, pressure programming was needed to achieve the necessary SFC separations. Therefore, preliminary linear pressure programming experiments (without sample injection) from 100-400 atm were conducted at various ramp rates (10, 15, 20, 25, 30, 35, and 40 atm/min) in order to study the effects of pressure changes on the detector. The CLND showed a stable baseline for every ramp as depicted in Figure 2.6, indicating the feasibility for using pressure programming to obtain SFC separations.

Applications

Capillary SFC-CLND chromatography of a mixture of 2,5-lutidine, 4-nitrotoluene, 2,6-dinitrotoluene, caffeine, indole, dimethoate and p-nitroaniline prepared in methanol has been accomplished (Figure 2.7). No significant base line shift was observed by the detector when pressure programming from 100 to 340 atm was used. Equimolarity of the CLND was not observed from Figure 2.7 because the roughly estimated concentration range of the mixture plus possible column discrimination. Analysis of a hot mustard methanol extract by the capillary SFC-CLND system is demonstrated in Figure 2.8. Peaks A and B are allylisothiocyanate and 2-butylisothiocyanate, respectively. These nitrogen-containing compounds are also the 'hot' components

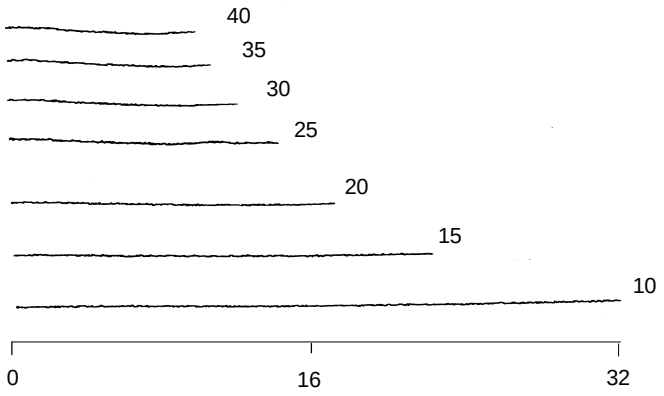

Figure 2.6. The CLND baseline stability is shown during linear pressure programming with SF-CO_2. Conditions: pressure program 100 atm (hold 1 min), ramp to 400 atm (hold 1 min) at different ramps (10, 15, 20, 25, 30, 35, 40 atm/min respectively); Column SB-cyano-25 (10 m x 100 μm, 0.25 μm d_f); without sample injection.

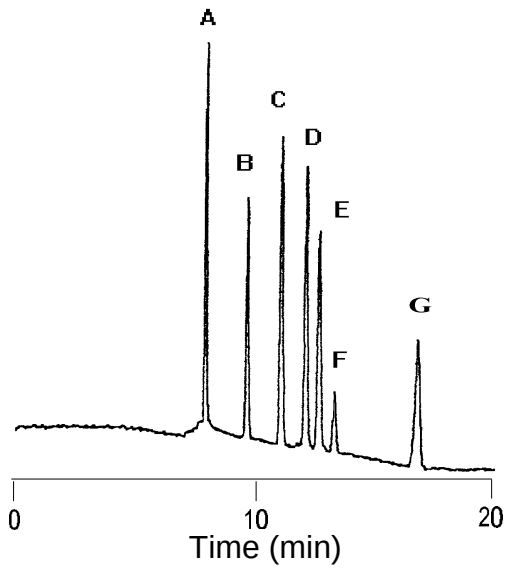

Figure 2.7. SFC-CLND chromatogram of a nitrogen-containing mixture. A = 2,5-lutidine, B = 4-nitrotoluene, C = 2,6-dinitrotoluene, D = caffeine, E = indole, F = dimethoate, and G = p-nitronailine. The concentration of each component in the mixture varies from about 50 ppm to 80 ppm. Chromatographic conditions: pressure program from 100 atm (hold 4 min), ramp to 250 atm at 15 atm/min, then ramp to 340 atm at 30 atm/min (hold 3 min); Column SB-cyano-25(20 m x 100 μm i.d., 0.25 μm d_f); time split injection 0.2 sec; sample in methanol.

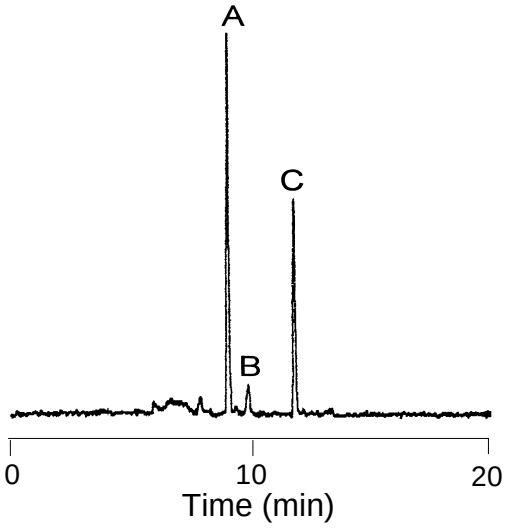

Figure 2.8. SFC-CLND separation of a hot mustard extract { 0.1 gram of hot mustard powder extracted with a 1-mL water/methanol (30/70 v%) mixture}. A = allylisothiocyanate, B = 2-butylisothiocyanate, and C = an unknown nitrogen-containing compound. Chromatographic conditions: pressure program from 80 atm (hold 5 min) ramp to 150 atm at 10 atm/min, then ramp to 200 atm at 15 atm/min; Column SB-cyano-25 (20 m x 100 μm, 0.25 μm d_f); time split injection 0.2 sec.

present in horseradish oil. Peak C is an unknown component in the hot mustard sample.

Horseradish oil, a high impact flavor, is used in low amounts to enhance flavors. The separation of a horseradish oil standard by capillary SFC-CLND is demonstrated in Figure 2.9. The peaks A, C, D, and E were identified by injecting single component standards. A standard for identifying peak B was unavailable, therefore assignment (B = allylthiocyanate) was made by comparing the SFC-CLND trace to a GC-CLND chromatogram [18] of known a horseradish oil standard. The molecular structures of these compounds are shown in Figure 2.10.

2.4 Conclusion

The chemiluminescent nitrogen detector has been successfully interfaced to capillary SFC. In addition to equimolar nitrogen responses, the detector demonstrated high nitrogen selectivity, sensitivity, and linearity. Pressure programming used in this study showed no perturbation of the detector base line. The SFC-CLND technique provides an alternative approach to current GC and HPLC methods.

[18] S.M.Benn; K. Nyung; E.M. Fujinari, "*Food Flavors, Ingredients and Composition"*, G.Charlambous Ed, Elsevier Science Publishers, Amsterdam, 1993, pp65

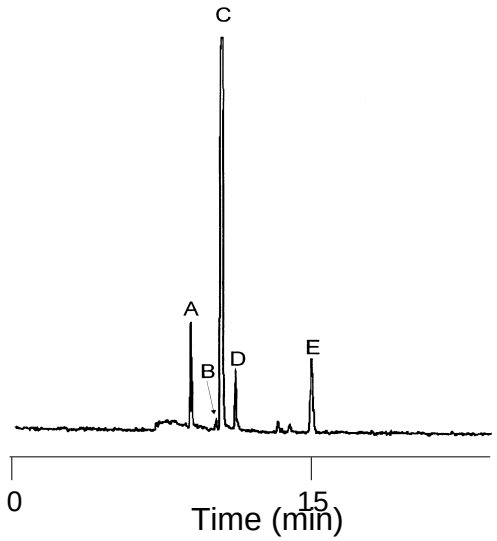

Figure 2.9. SFC-CLND separation of a 0.03% (w/w) horseradish oil standard. A = but-3-enonitrile, B = allylthiocyanate, C = allylisothiocyanate, D =2-butylisothiocyanate, E = phenylethylisothiocyanate. Chromatographic conditions: pressure program from 80 atm (hold 5 min) ramp to 150 atm at 10 atm/min, then ramp to 200 atm at 15 atm/min; Column SB-cyano-25 (20 m x 100 μm, 0.25 μm d_f); time split injection 0.2 sec; sample in ethanol.

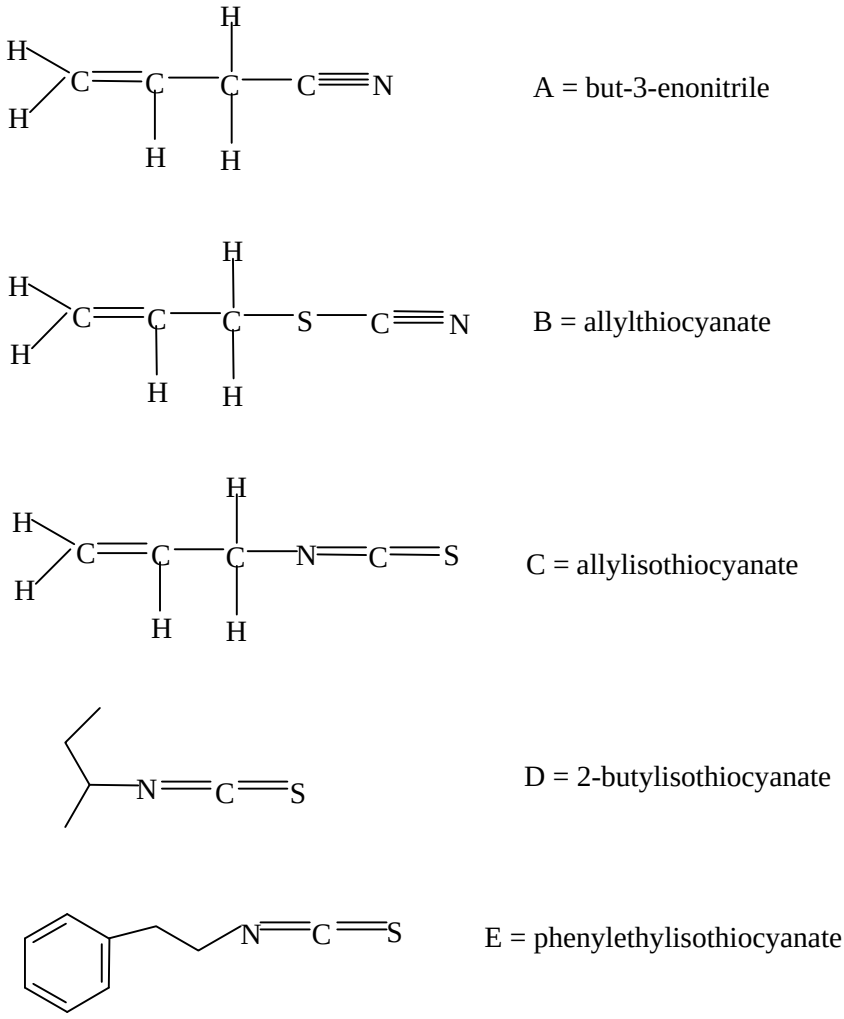

Figure 2.10. Structure of the nitrogen-containing components in the horseradish oil of the SFC/CLDN chromatogram in Figure 2.9.

Chapter III

Open-Tubular Column Supercritical Fluid Chromatography with Simultanious Flame Ionization and Chemiluminescent Nitrogen Detection

3.1 Introduction

A growing interest in using element selective detectors in chromatography is evident by the increasing number of published applications. One reason for using such detectors is to accurately achieve simplified chromatograms with shorter analysis time. Element selective detectors have also been shown to have greater sensitivities than universal detectors [1]. In addition, the total analytical method can be shortened by the elimination of unnecessary sample clean-up steps, thereby preventing loss of analyte(s) and possible sample contamination.[2]

Supercritical fluids possess viscosities that are much lower than liquids, while their diffusivities are much greater than those of liquids. These physical properties in particular, provide higher separation efficiencies for supercritical fluid chromatography (SFC) than for high performance liquid chromatography (HPLC) per unit time. Since high molecular weight sample components as well as thermally labile compounds may be analyzed, SFC can also provide an advantage over gas chromatography (GC).

Significant uses of element selective detectors with open-tubular SFC have been reported. Examples include the use of a flame photometric detector (FPD) where parathion, a pesticide containing both phosphorus (P) and sulfur (S), was

[1] R.L. Shearer; D.L. O'Neal;R. Rios; M.D. Baker, *J. Chromtogr. Sci*., 28 (1990) 24

simultaneously analyzed on the P and S channels [3]. An ozone based sulfur chemiluminescence detector (SCD) for determination of sulfur containing sulfonylurea herbicides has also been employed [2]. A nitrogen-phosphorus based chemiluminescence detector (NPD) used for the determination of nitro- and nitroso-compounds in such materials as tobacco and explosives has been used for monitoring several nitrogen containing carbamate pesticides in parsley [4]. The thermal energy analyzer (TEA), has been evaluated for open-tubular SFC [5]. There is a need for a sensitive detector for analyzing a large variety of functionalized nitrogen containing compounds from a host of industrial and academic research endeavors. Unlike conventional flame detectors such as FID, FPD and NPD, the CLND does not lose detector response due to solvent flame-out problems at the interface. The CLND has detected nitrogen levels as low as 60 pg when pure CO_2 was used as the mobile phase. CLND also demonstrated a nitrogen to hydrocarbon selectivity on the order of 10^7, and detector linearity of 10^3 was observed. The detector is also reported to be easy to use and demonstrated very stable response in continuous day to day operation.

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[2] A.L.Howard; L.T. Taylor, *Anal. Chem*. 61 (1993) 724

[3] K.E. Markides; E.D. Lee; R. Bolick; M.L. Lee, Anal. Chem., 58 (1986) 740

[4] B. E. Richter, M.R. Anderson, D. E. Knowles, E. R. Campbell, N. L. Porter, L. Nixon, and D.W. Later, "Supercritical Fluid Extraction and Chromatography: Techniques and Applications", ACS Symp. Ser. 366; B. A. Charperter and M. R. Sevenants, Eds, p197, Washington, DC

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In this chapter, a chemiluminescent nitrogen detector (CLND) and a flame ionization detector (FID) were configured at the end of the open tubular column supercritical fluid chromatography via a zero- to dead-volume tee. The dual detection was achieved with a post-column split at a 1 : 2.5 ratio for the CLND and FID, respectively. Pure CO_2 was used as mobile phase to achieve the chromatographic separations. The chromatograms employing the simultaneous detectors are compared.

3.2 Experimental

A chemiluminescent nitrogen detector (CLND) Model 705D from Antek Instruments, Inc. (Houston, TX) and a Dionex Lee Scientific (Salt Lake City, UT) series 600 supercritical fluid chromatograph equipped with a flame ionization detector and a helium actuated Valco (Houston, TX) injector (500 nL) were used in this study. Each sample mixture was introduced onto the open-tubular column using a time split injection. The split times varied from 0.05-0.3 seconds depending on sample concentration as described in the figure legends. Chromatographic separations were performed on a J & W Scientific (Folsom,CA) DB1701 bonded fused silica open-tubular column (10 m x 100 μm I.D.) with a 0.4 μm film thickness. Simultaneous chemiluminescent nitrogen and flame ionization detection were achieved via a post-column split using a zero- to dead-volume tee from Chrom Tech, Inc. (Apple Valley, MN). Two home-made 25 μm I.D. integral restrictors were used as the interface

between the chromatographic column and the two detectors. A schematic flow diagram of the SFC-CLND/FID is shown in Figure 3.1. Flow of the decompressed carbon dioxide to FID and CLND was 7.5 mL/min and 3.0 mL/min, respectively under 100 atm at room temperature.

CLND conditions were: pyrolysis temperature 1070 ^{o}C, PMT voltage 700 volts, pyrolysis oxygen flow rate 48 mL/min, and oxygen flow rate for ozone generator 10 mL/min. For data acquisition, the detector output was set at 1 volt with a range x 50. FID conditions were detector temperature at 350 ^{o}C, air flow rate at 360 mL/min, hydrogen flow rate at 65 mL/min, and nitrogen (make up gas) flow rate at 33 mL/min. For data acquisition, the detector range was set at zero which corresponds to a signal output value of 0-1000 picoamp and offers the highest resolution. Only 0- 500 picoamp was plotted vertically for all the FID chromatograms.

SFC grade carbon dioxide was obtained from Air Products and Chemical Inc. (Allentown, PA). N,N-Dimethylaniline was obtained from Fisher Scientific (Pittsburgh, PA) while 2-nitroaniline, 3-nitroaniline, and 4-nitroaniline were purchased from Aldrich Chemical Co. (Milwaukee, WI). An octyldimethylamine, decyldimethylamine, dodecyldimethylamine, tetradecyldimethylamine, hexadecyldimethylamine, octadecyldimethylamine mixture and a di-octylmethylamine, octyldecylmethylamine, di-decylmethylamine mixture were obtained from Albemarle Corp.(Baton Rouge, LA).

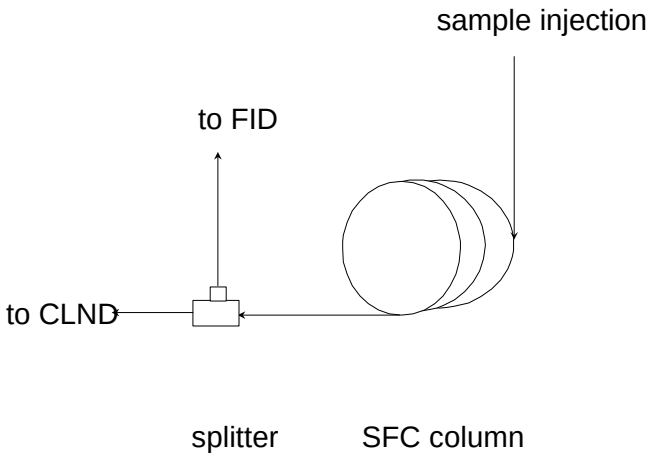

Figure 3.1 Flow scheme for simultaneous SFC-CLND/FID. Split ratio of FID to CLND is 2.5 : 1.

HPLC grade solvents from EM Science (Gibbstown, NJ) were used for sample preparation.

Wasabi, a Japanese horseradish powder, was purchased from a local food store. A sample extract was prepared by mixing 1 gram Wasabi with 3 mL aqueous methanolic (1:3) solution. The mixture was shaken for several minutes and left to stand overnight. The supernatant was concentrated two fold after filtration and analyzed by open-tubular SFC-CLND/FID.

3.3 Results and Discussion

Several applications using the simultaneous dual detection (CLND/FID) system to monitor open-tubular supercritical fluid chromatographic separations with 100% carbon dioxide mobile phase are presented. Peak identification was achieved by comparing component retention times (r.t.) with those times obtained by injection of single analytical standards. In Figure 3.2, the FID chromatogram shows a very large peak for the benzene solvent and four very small peaks for the (aniline isomers) analytes. The N,N-Dimethylaniline (peak 1, r.t. = 6.20 min) is not resolved from the solvent peak and 4-nitroaniline (peak 4, r.t. = 12.07 min) is nearly undetected by the FID. The CLND chromatogram on the other hand shows only the four nitrogen containing

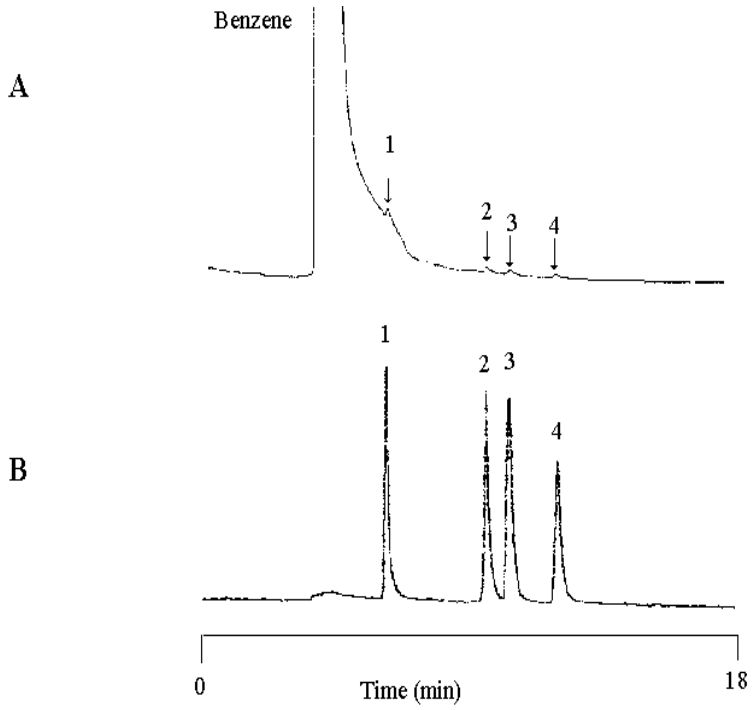

Figure 3.2. Separation of a nitrated aniline mixture using 100% CO_2 as mobile phase on a DB-1701 column (10 m x 100 μm, i.d., film thickness 0.4 μm) with flame ionization detection (A) and chemiluminescent nitrogen detection (B). SFC conditions: 100 atm for 4 min, ramp to 380 atm at 20 atm/min; oven temperature 70^{O}C; time split injection 0.3 sec. The concentration of each sample component ranged from 0.52-0.85 μg/μL. Sample solvent : benzene. 1. *N,N*-dimethylaniline; 2. 2-nitroaniline; 3. 3-nitroaniline; 4. 4-nitroaniline.

compounds with baseline resolution and excellent peak shapes, while benzene remained virtually transparent to the CLND detector. In comparing the two detectors, the CLND has significant advantages in high signal to noise ratio and low background signals and the average r.t. offset between FID and CLND was only 0.02 min.

SFC separation of a series of relatively large alkyldimethylamine and dialkylmethylamine mixtures are presented in Figures 3.3 and 3.4, respectively. The FID chromatogram in Figure 3.3, shows octyldimethylamine (peak 1, r.t. = 2.97 min) partially masked by the solvent front which could not be quantitated due to the toluene interference. The quantity of octyldimethylamine (peak 1, r.t. = 3.00 min) was easily determined in the corresponding CLND trace since it is free from the hydrocarbon interference and no additional sample clean-up was needed. The average r.t. offset between the FID and CLND was 0.02 min and 0.01 min, for alkyldimethylamine (Figure 3.3) and dialkylmethylamine (Figure 3.4) separations, respectively.

For a food application using SFC-CLND/FID, two hot ingredients in Wasabi have been separated (Figure 3.5). Peak assignments (1 = allylisothiocyanate and 2 = phenylethylisothiocyanate) were accomplished by the standard addition method. Phenylethylisothiocyanate concentration is below the detection limit of the FID and consequently undetected by FID. However, the two hot components (r.t. = 5.32 and 12.34 min) in the Wasabi extract were determined in the same analysis by the CLND.

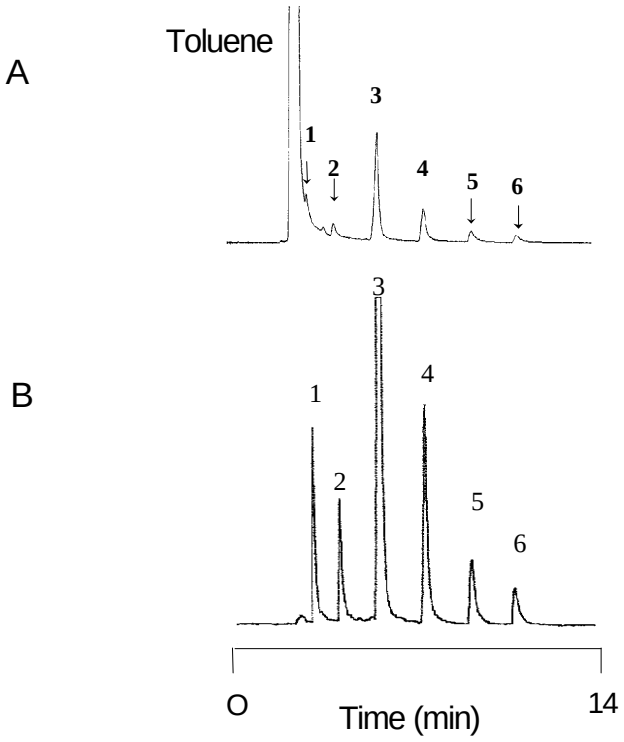

Figure 3.3. Separation of an alkyldimethylamine mixture using 100% CO_2 as mobile phase on a DB-1701 column (10 m x 100 μm, i.d., film thickness 0.4 μm) with flame ionization detection (A) and chemiluminescent nitrogen detection (B). SFC conditions: 100 atm hold for 3 min, ramp to 200 atm at 10 atm/min; oven temperature 125^{o}C; time split injection 0.05 sec. About 0.2 gram of the alkyldimethylamine mixture was dissolved in 6 mL toluene.
1. octyldimethylamine; 2. decyldimethylamine; 3.dodecyldimethylamine;
4. tetradecyldimethylamine; 5. hexadecyldimethylamine; 6. octadecyldimethylamine.

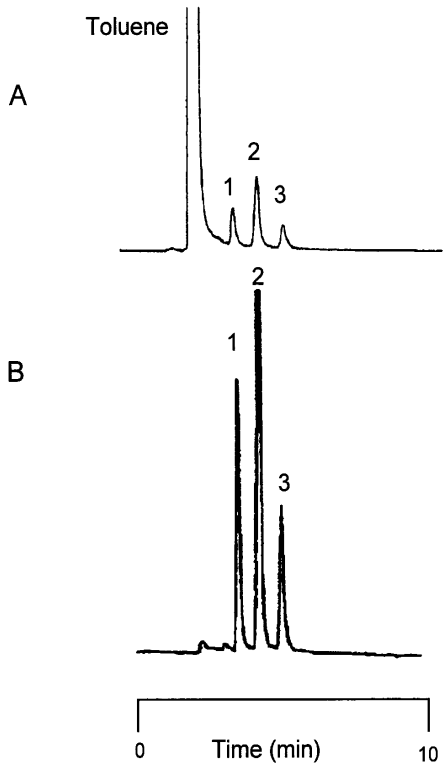

Figure 3.4. Separation of a dialkylmethylamine mixture using 100% CO_2 as mobile phase on a DB-1701 column (10 m x 100 μm, i.d., film thickness 0.4 μm) with flame ionization detection (A) and chemiluminescent nitrogen detection (B). SFC conditions: 100 atm hold for 3 min, ramp to 200 atm at 10 atm/min; oven temperature 125^{o}C; time split injection 0.05 sec. About 0.2 gram of the dialkylmethylamine mixture was dissolved in 2 mL toluene. 1, dioctyl-methylamine; 2, octyl-decyl-methylamine; 3, didecyl-methylamine.

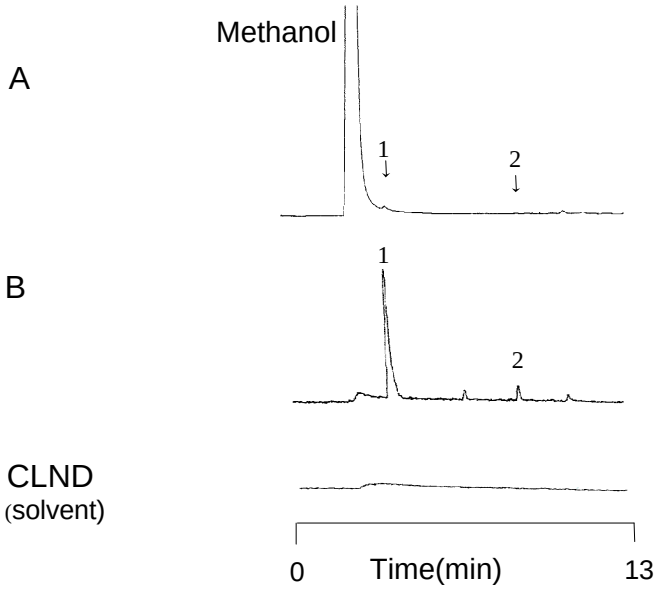

Figure 3.5. Separation of a Wasabi extract using 100% CO_2 as mobile phase on a DB-1701 column (10 m x 100 μm, i.d., film thickness 0.4 μm) with flame ionization detection (A) and chemiluminescent nitrogen detection (B). SFC conditions: 100 atm hold for 8 min, ramp to 180 atm at 15 atm/min, then ramp to 250 atm at 20 atm/min and hold 1 min; oven temperature 60^{o}C; time split injection 0.3 sec. 1 gram Wasabi was extracted with 3 mL water/methanol mixture at a water to methanol ratio of 1: 3 and the extract was concentrated two fold before injection. 1. allylisothiocyanate; 2. phenylethylisothiocyanate.

3.4 Conclusion

The use of a simultaneous detection system for open-tubular supercritical fluid chromatographic separation of aniline isomers, alkyl(C-8 to C-18) dimethylamines, a dialkylmethylamine mixture, and an extract of a Japanese horseradish powder " Wasabi" was demonstrated. Nitrogen containing compounds are easily detected by the chemiluminescent nitrogen detector. It has significant advantages over the FID. The CLND is well suited for trace-level analysis of nitrogen bearing compounds since there are 1) no hydrocarbon interferences, 2) high signal to noise ratio, 3) no additional sample clean-up, and 4) the nitrogen-specific detection mode is easily attainable. Since FID is the most widely used detector for SFC,[6] a dual (SFC-CLND/FID) detection system is a distinct advantage over the use of the stand alone SFC-FID system when analyzing nitrogen containing compounds.

[6] M. L. Lee and K. E. Markides, "Analytical Supercritical Fluid Chromatography and Extraction" Chromatography Conferences, Inc., 1990, p194, Provo, VT

Chapter IV

Chemiluminescent Nitrogen Detection for Packed Column Supercritical Fluid Chromatography with Methanol Modified CO_2

4.1. Introduction

Nitrogen detection based on nitric oxide (NO) and ozone (O_3) chemiluminescence is a powerful tool for chromatographic analyses. Currently two types of nitrogen-selective detectors using the NO + O_3 reaction are commercially available. The thermal energy analyzer (TEA) developed by Fine et al. uses low temperature catalytic decomposition of N-nitroso- and nitro-compounds followed by chemiluminescence detection with ozone. [1, 2, 3] The TEA has been coupled to both GC [4, 5, 6, 7] and HPLC. [8] Grolimund et al. first reported the use of the TEA with open tubular SFC for the detection of volatile nitrosamines. [9] Separation and detection of explosives containing nitro-compounds by this SFC technique was reported by Douse et al.. [10] Recently, Lee et al. used open tubular SFC/TEA for the analysis of tobacco specific nitrosamines and explosives. [11] In their evaluation, minimum detectable

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[9] K. Grolimund; W.P. Jackson; M. Joppich; W. Nussbaum; K. Anton; H. M. Widmer, *Proc. Seventh Int. Symp. Cap. Chromatogr.*, P. Sandra (Ed.), Huethig, Heidelberg, 1986, p. 625

[10] J.M.F. Douse; *J. Chromatogr.*, 445 (1988) 244

[11] S.E. Francis; J. D. Eatough; M.L. Lee, *J. Microcol. Sep.*, 6 (1994) 395

quantities (MDQ) of some selected compounds were reported in the 16 to 171 pg level and a linear dynamic range for the TEA under SFC conditions was found to be four orders of magnitude. A second chemiluminescent nitrogen detector (CLND) was developed originally for GC by Parks et al.. [12] The advantage of the CLND over TEA is that the total nitrogen content, regardless of the chemical state of nitrogen can be assayed except for diatomic nitrogen (N_2). CLND applications coupled with GC and HPLC for the analysis of pesticide residues, foods, flavors, pharmaceuticals and environmental and petroleum samples have been reported by Britten [13] and Fujinari et al.. [14, 15, 16, 17]

In this Chaper, results are given for the chemiluminescent nitrogen detector (CLND) interfaced with a packed column chromatographic system utilizing supercritical methanol modified carbon dioxide. Detector optimization in terms of sensitivity and performance is described. Polymeric materials such as nitrogen containing cyclic oligomers were successfully analyzed. Pharmaceutical applications were also demonstrated using SFC-CLND with shorter analysis time than by typical HPLC

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[12] R.E. Parks; R. L. Marietta, U. S. Patent 4,018,562, October 1975

[13] A. J. Britten; *R&D* magazine, 31 (1989) 76

[14] L.O. Courthaudon; E. M. Fujinari, *LC-GC*, 9 (1991) 732

[15] E.M. Fujinari;L.O. Courthaudon, *J. Chromatogr.*, 592 (1992) 209

[16] S.M. Benn; K. Myung; E. M. Fujinari, "*Food Flavors, Ingredients and Composition*," G. Charalambous (Ed.), Elsevier Science Publishers, Amsterdam, 1993, p. 65

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methods. In addition, significant savings for SFC were evident in cost of organic solvents and their waste disposal than for HPLC.

4.2. Experimental

Apparatus

A chemiluminescent nitrogen detector Model 705D with a Model 771 pyrolysis system from Antek Instruments Inc. (Houston, TX) was interfaced to a Model G1205 Supercritical Fluid Chromatographic system with a multiple wavelength UV detector from Hewlett-Packard (Little Falls, DE). An auxillary reciprocating pump in the HP SFC system allowed for methanol modifier to be added on-line to the carbon dioxide mobile phase. Samples were injected via Model 7673 auto injector configured to an air-actuated Rheodyne valve with a 5 µL sample loop. Simultaneous CLND and UV detection was achieved with a post-column split using a zero dead-volume tee from Chrom Tech, Inc. (Apple Valley, MN). Data from both detectors were stored on the HP ChemStation and later plotted separately. The post-column split ratio, fixed restrictor type, and column used in each chromatographic separation are cited in the Figure legends.

CLND conditions: pyrolysis temperature 1070^{o}C, photomultiplier(PMT) voltage 720 volts, range x50, detector output 1 mVolt. Pyrolysis oxygen flow rates were 185-280 mL/min and oxygen flow rates for ozone generator were 17-24 mL/min for detector evaluation experiments and subsequent applications.

Reagents and Standards

Acetaminophen, atrazine, 2,3-dimethylindole, 2,6-dinitrotoluene, diphenhydramine, diphenylamine, indole, 4-nitrotoluene, propazine, simazine, sulfadiazine, sulfanilamide, tetrandrine, tetrazepam and pyridine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Caffeine was purchased from Sigma Chemical (St. Louis, MO). Drug Standard Mixture 2 and the corresponding single components (glutethimide, meprobamate, pentobarbital, phenobarbital and secobarbital) were purchased from Supelco (Bellefonte, PA). Sample of cyclic oligomers was obtained from a polymer research group at Virginia Tech (Blacksburg, VA). All chemicals were used without further purification. HPLC grade solvents from EM Science (Gibbstown, NJ) were used for preparing standard solutions. Grade 4.3 oxygen from Airco (Murray Hill, NJ) was used for the CLND pyro-furnace and ozone-generator gas. SFC-grade CO_2 (without helium head space) was obtained from Air Products and Chemical Inc. (Allentown, PA).

4.3. Results and discussion

Detector Sensitivity Optimization

An increasing number of industrial applications utilizing supercritical fluid chromatography have been reported. Packed column SFC has been found to be more suitable for separating polar compounds with faster analysis time than with open

tubular (capillary) columns. With methanol modified CO_2 as the mobile phase, traditional HPLC columns can be routinely used for SFC of highly polar material such as pharmaceuticals. Packed columns also provide higher sample capacity. It is therefore desirable to develop a packed column SFC-CLND system for analysis of nitrogen-containing analytes. The key for such a tool is coupling the chemiluminescent nitrogen detector to packed column SFC system (Figure 4.1), and the detailed interface is shown in Figure 4.2. Supercritical methanol modified CO_2 was used as the mobile phase. A linear restrictor (25 µm i.d., 16" in length) was introduced into the inlet of the CLND furnace. Oxygen was mixed with the column effluent in the pyrolysis tube which was heated at 1070^{o}C in order to oxidize the eluting nitrogen-containing compounds to NO. The resulting NO and O_3 chemiluminescence in the reduced pressure reaction chamber was detected by the photomultiplier tube (PMT).

Efficient oxidation is an important step for maximizing the CLND sensitivity. The restrictor position at the SFC-CLND interface was first investigated using an indole (23.9 ppm N) standard in methanol. Varying the position of the restrictor showed the mixing efficiency of the column effluent with oxygen to achieve the necesary oxidation in the furnace. Plots of the CLND responses (area counts) of the indole standard versus. restrictor position for 100% CO_2 and 10% methanol modified CO_2 mobile phases are presented in Figure 4.3. The maximum CLND response was found to be when the restrictor was positioned at or just beyond the junction of the pyro-oxygen inlet. Another important factor for efficient oxidation of nitrogen-containing compounds

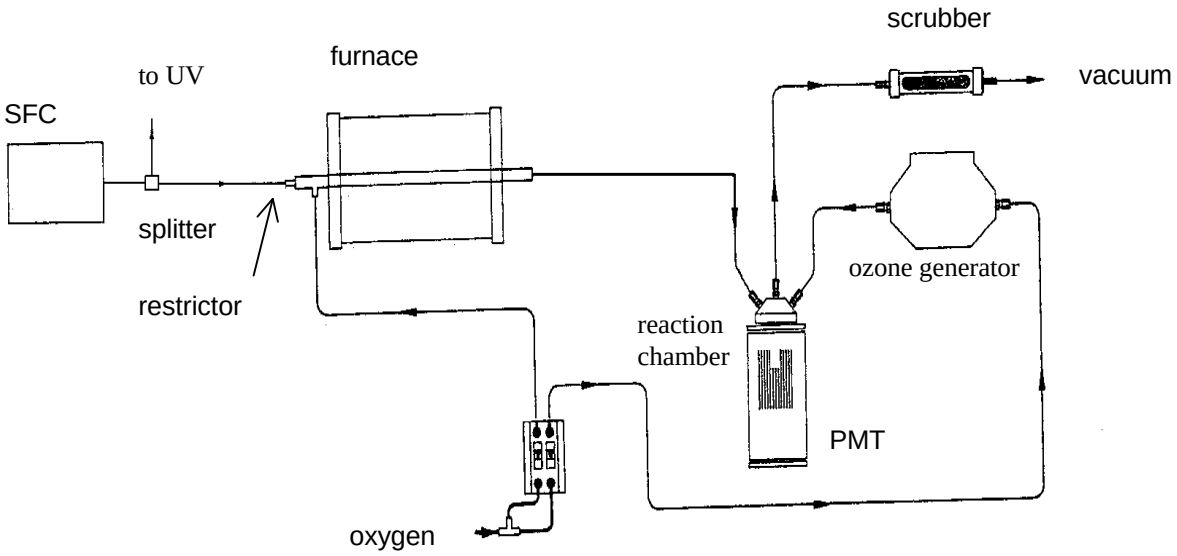


Figure 4.1 Schematic of the packed column SFC-CLND

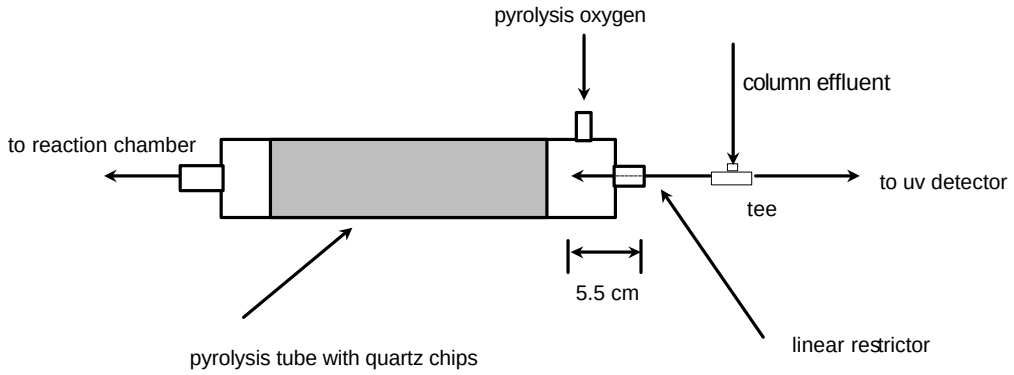


Figure. 4.2 Detailed diagram of the packed-column SFC/CLND interface.

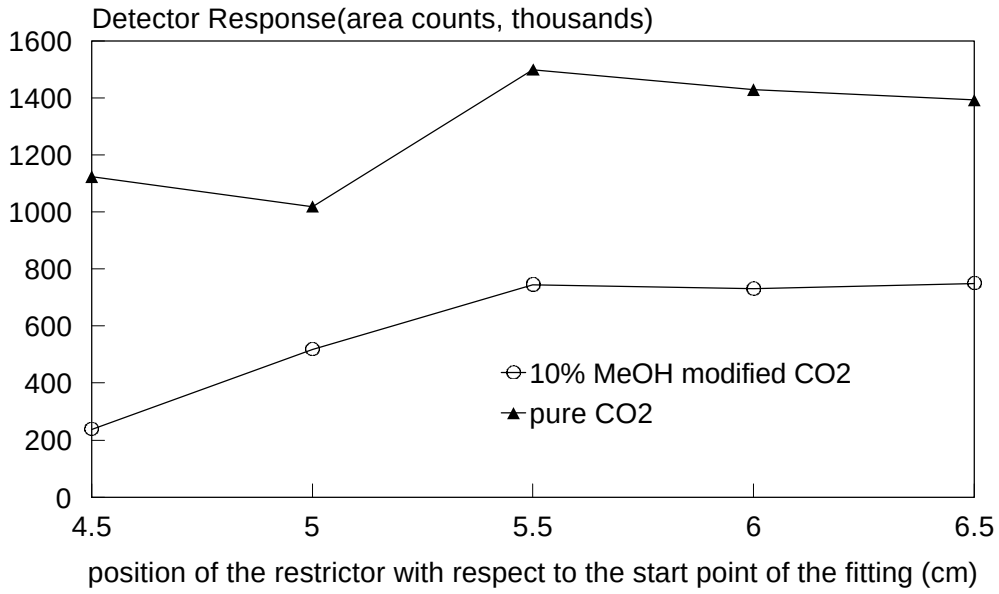


Figure 4.3 CLND optimization of restrictor position at the SFC interface. Conditions: 60^{o}C, flow injection analysis(FIA); pressure at 320 atm; decompressed CO_2 split ratio of 8 to 1 (UV/CLND); 25 m i.d. linear restrictor; sample: indole in methanol.

to NO is the pyrolysis O_2 flow rate. A balance must be struck between providing sufficient pyro-oxygen for achieving efficient oxidation and at the same time maintaining sufficient residence time of analytes in the furnace. In order to study this balance, the restrictor position was fixed at 5.5 cm and CLND responses to indole were measured between 100-730 mL/min of pyro-oxygen using supercritical 100% CO_2 as well as 1%, 5%, 10% and 15% methanol modified mobile phases while maintaining similar decompressed CO_2 flow rate. Results (Figure 4.4) under these conditions indicated that optimum pyrolysis oxygen flow rates were related to supercritical mobile phase compositions. For 100% CO_2 and 1% methanol modified CO_2, the detector response reached maxima at approximately 180 mL/min of oxygen. Mobile phases in this study containing greater than 5% methanol content in CO_2 needed a higher oxygen flow rate (290 mL/min) in order to achieve a maximum detector response. In this case, extra oxygen was apparently needed for the oxidation of methanol in the mobile phase albeit at the cost of reduced residence time of indole in the furnace.

Since the total flow rate is actually a combination of pyro-oxygen and decompressed CO_2 flow rates, the next phase in the study was to test the effects of methanol modified decompressed CO_2 flow rates on the CLND response (Figure 4.5). The decompressed CO_2 flow rate can affect the detector response in several ways. Some variables to consider at the start of method development are: 1) residence time of analyte(s) will be decreased in the pyro-furnace (decrease in CLND response) by

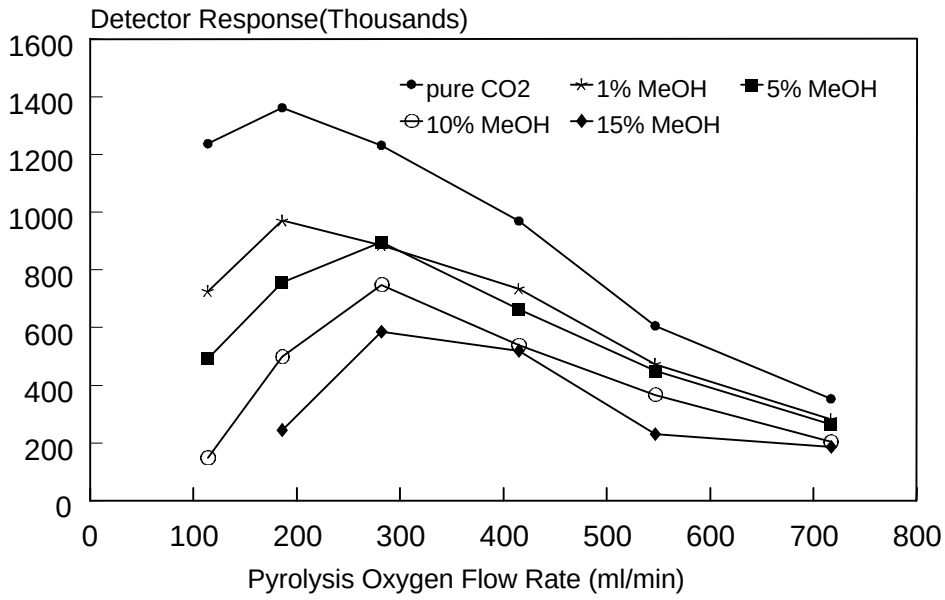


Figure 4.4 CLND optimization of pyrolysis oxygen flow rate. Conditions: 60^{o}C, FIA; pressure at 320 atm; decompressed CO_2 split ratio of 8 to 1 (UV/CLND); 25 µm i.d. linear restrictor; sample: indole in methanol.

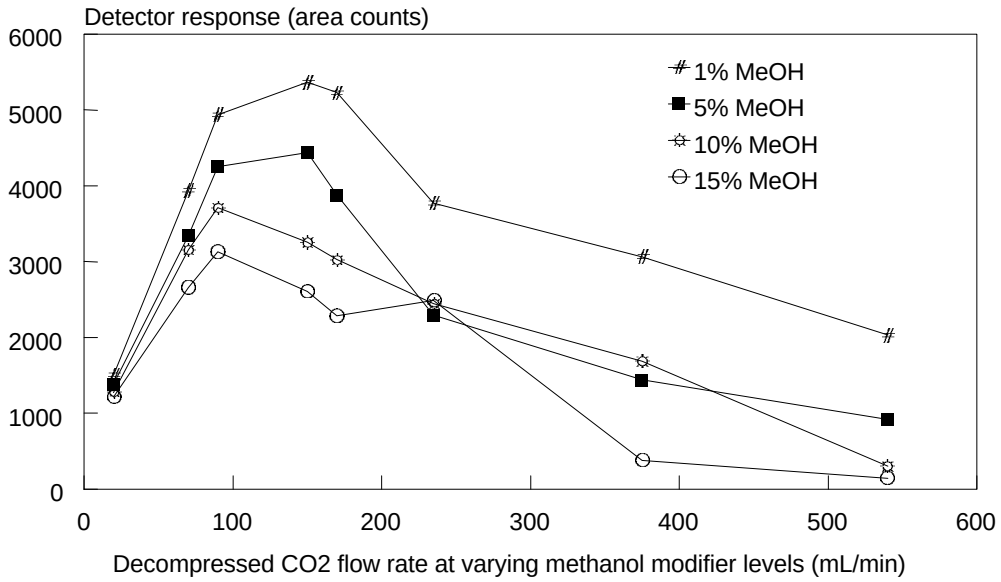


Figure 4.5 Profiles of methanol modified decompressed carbon dioxide flow rate vs. CLND response for packed-column SFC. Conditions: 50^{o}C FIA; pressure at 280 atm; Deltabond CN (250x4.6mm i.d., d_p=5-µm) column; Indole sample with 23.9 ppm of nitrogen in methanol.

increasing flow rate, 2) inefficient oxidation of nitrogen-containing analytes to NO with increasing methanol content in the mobile phase may lower the detector response, and 3) quenching of the chemiluminescence signal by uncombusted organic species may occur in the reaction chamber. High methanol content in the mobile phase at high decompressed CO_2 flow rates resulted in a short residence time for analyte(s) and methanol (as well as sample matrix) in the furnace. In turn, the uncombusted methanol and other organic compounds entered the reaction chamber causing a significant decrease in detector response. As shown in Figure 4.5, optimum decompressed CO_2 flow rates for 1-5% and 10-15% (v/v) methanol modifier were found to be at 150 mL/min and 90 mL/min, respectively.

Detector Performance

Results for nitrogen selectivity, minimum detectable quantities (MDQ), linear dynamic range (LDR), and response factors (f_X) relative to indole for the packed-column SFC-CLND system are presented here. Detector selectivity of 10^7 (Nitrogen/Carbon ratio) was observed by injecting a solution containing 0.67 ppm indole into the 5% methanol modified mobile phase at 250 atm. No solvent peak was found under these conditions. The selectivity was also tested with mobile phases of different methanol concentrations in CO_2 and the results are showed in Figure 4.6. Minimum detectable quantities of nine nitrogen-containing compounds at a signal-to-noise ratio equal/greater than two are provided in Table 4.1. Different experimental

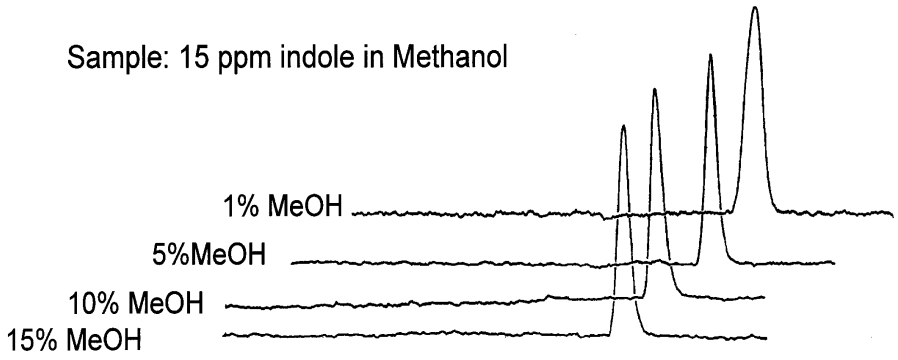


Figure 4.6 The selectivity of packed column SFC-CLND with mobile phases of different methanol concentration in CO_2. Conditions: Hypersil silica column (250 x 4.6 mm, d_p=5-µm); Oven: 60 ^oC; Pressure: 250 atm.

Table 4.1

Minimum detectable quantities by packed-column SFC-CLND .

Analyte	MDQ at CLND *(pg N)	**Conditions
4-nitrotoluene	276	A
indole	213	A
caffeine	276	A
tetrazepam	351	A
diphenylamine	316	A
acetaminophen	453	B
sulfanilamide	453	B
sulfadiazine	453	B
diphenhydramine	448	B

*Signal to noise ratio of 2-3.

**A : Deltabond cyano (250x4.6mm i.d., d_p=5-μm) column, 50^{O}C oven, 250 atm pressure, 5% (v/v) methanol modifier, split ratio of 15.7 to 1 (UV/CLND).

**B : Deltabond cyano (250x4.6mm i.d., d_p=5-μm) column {except Amino 1 (150x4.6mm i.d., 5 μm particle size) was used for diphenhydramine}, 50 ^{O}C oven. 280 atm pressure, 12% (v/v) methanol modifier, split ratio of 11.0 to 1 (UV/CLND).

conditions were used to elute compounds of different polarities. Since the CLND response is lower at higher modifier concentration, some variation in the MDQ was observed as a result of different mobile phase compositions. Lower MDQs were obtained for compounds (e.g. 213 pg N of indole) using 5% (v/v) methanol modified CO_2 mobile phase than for compounds (e.g. 448 pg N of diphenhydramine) eluting at 12% (v/v) and higher methanol level. The CLND provided a linear dynamic range of 3 orders of magnitude. Calibration curves using sulfanilamide and acetaminophen (1-1000 ppm of nitrogen) standards in methanol showed linear correlation coefficients greater than 0.9999. Response factors for six compounds were measured by flow injection analysis (FIA) in order to eliminate any possible bias from the column such as peak tailing, which makes accurate peak integration measurements impossible. Response factors relative to indole were measured at different methanol modifier (1%, 5%, 10%, and 15% v/v) concentrations in CO_2 (Table 4.2). Equimolar nitrogen response was observed by SFC-CLND at each of the methanol modifier concentrations. A mean response factor value of 1.02 (RSD = $\pm$ 0.05) was obtained. The significance of this particular study is that only one calibration curve (using a stable nitrogen-containing compound) is needed to quantitate the nitrogen content of any (unknown) peak in the chromatogram. Baseline stability for the packed column SFC-CLND was also investigated. The baselines during both pressure and modifier programs are given in Figure 4.7 and the result showed that the detector baseline was fairly stable considering both pressue and modifier concentration changes within wide ranges.

Table 4.2

Relative Response Factors (RRF*) in CLND using packed column parameters

* RRF = area counts per ppm N of compound / area counts per ppmN of indole

Analyte	1% (v/v)	5% (v/v)	10% (v/v)	15% (v/v)
4-nitrotoluene	1.10	1.04	1.27	1.20
2,6-dinitrotoluene	1.15	1.06	1.24	1.10
2,3-dimethylindole	1.01	0.95	0.97	0.96
pyridine	1.09	1.09	1.08	1.01
caffeine	0.96	0.96	1.01	0.98
diphenylamine	1.14	1.09	1.20	0.99

Conditions: 60^{o}C FIA, pressure at 320 atm, decompressed CO_2 split ratio of 8 to 1 (UV/CLND).

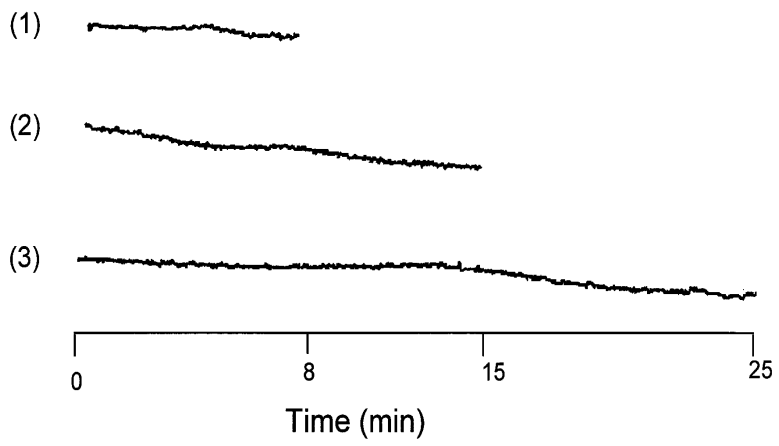


Figure 4.7 Packed column SFC-CLND baseline stability during both pressure and modifier program.

Conditions: (Oven: 50 ^{o}C; Phenyl column (250 X 4.6 mm, d_p=5-μm)

(1). Pressure: 150 atm ramp to 350 atm at 25 atm/min; Modifier: 5% (v/v) ramp to 26% at 3%/min, then hold for 1 min.

(2). Pressure: 100 atm ramp to 360 atm at 20 atm/min, then hold for 2 min; Modifier: 1% (v/v) ramp to 25% at 2%/min, then hold for 3 min.

(3). Pressure: 100 atm ramp to 350 atm at 10 atm/min; Modifier: 1% (v/v) ramp to 20% at 1%/min, then hold for 6 min.

Applications

Triazine herbicides using 100% CO_2 are immobile because of their polarity and cannot be chromatographed by capillary SFC. However, packed-column SFC using 10% methanol modified CO_2 and pressure programming can readily elute these compounds. Using this approach with simultaneous CLND (nitrogen-specific mode) and UV (219 nm), detection of propazine, atrazine and simazine is shown in Figure 4.8. Dual SFC-CLND/UV detection using packed-column and a CO_2 mobile phase with methanol modifiers is particularly amenable for analyses of pharmaceuticals since most are polar and contain nitrogen moieties. Figure 4.9 demonstrates the usefulness of this technique as a confirmatory method since caffeine, tetrazepam, diphenhydramine, phenobarbital, and tetrandrin (structures see Figure 4.10) each contain both aromatic UV chromophores and nitrogen functional groups. Both pressure and modifier programming are used to elute these polar compounts. The retention times(UV/CLND) for those compounds are 1.289/1.292, 1.641/1.644, 1.940/1.950, 4.644/4.638 and 7.121/7.114 respectively. The confirmation of peaks 3 and 4 by the CLND is quite evident. Sedatives can also be analyzed as shown in Figure 4.11. The structures of the sedatives are given in Figure 4.12. Because of the high polarity of these compounds, a constant modifier concentration as high as 15%(v/v) was used to elute them. Meprobamate (peak 4) does not have an aromatic group and was not observed by UV detection at 219 nm, however, it is clearly visible by the CLND.

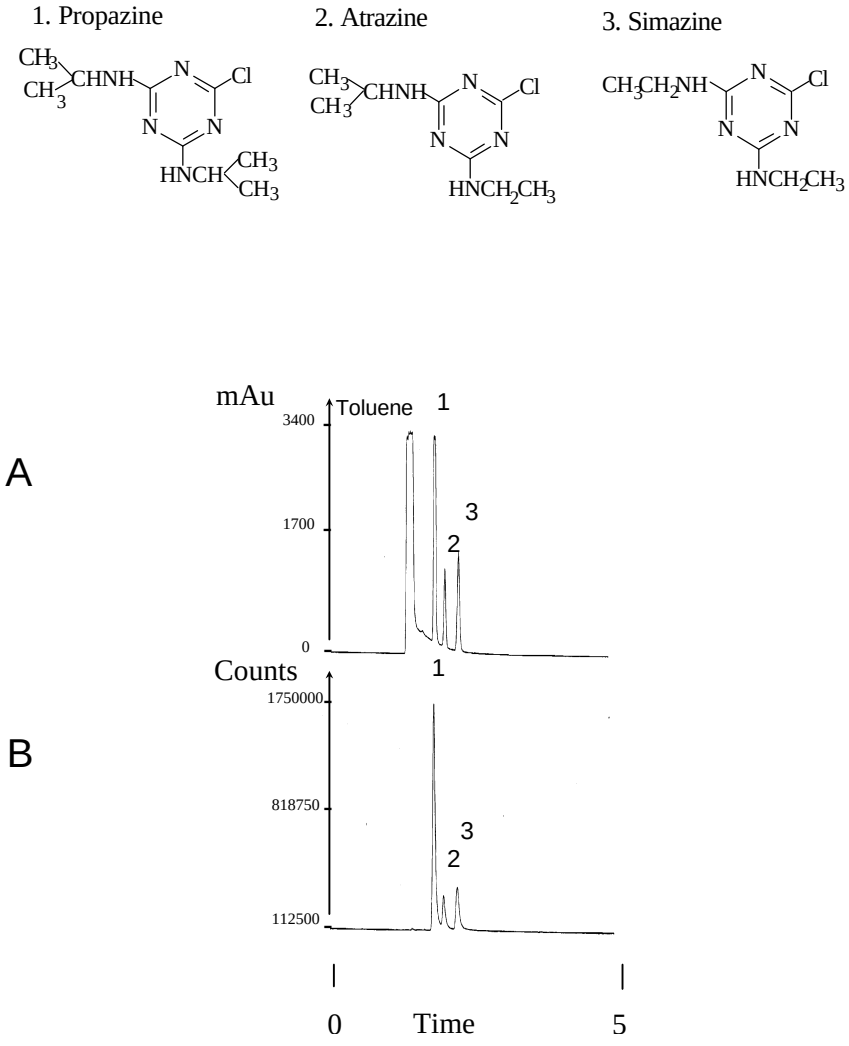


Figure 4.8 Packed-column SFC-CLND/UV profile of triazine herbicides. Conditions: 70^{o}C oven; Pressure program: 250-280 atm at 10 atm/min; methanol modifier: 10% (v/v); Hypersil silica (250x4.6mm i.d., d_p=3-μm) column; 5 μL injection loop; Liquid CO2 flow rate of 2.5 mL/min; Decompressed CO2: 1460 mL/min at UV and 160 mL/min at CLND; Toluene is sample solvent. (A) UV detection at 219 nm. (B) SFC-CLND.

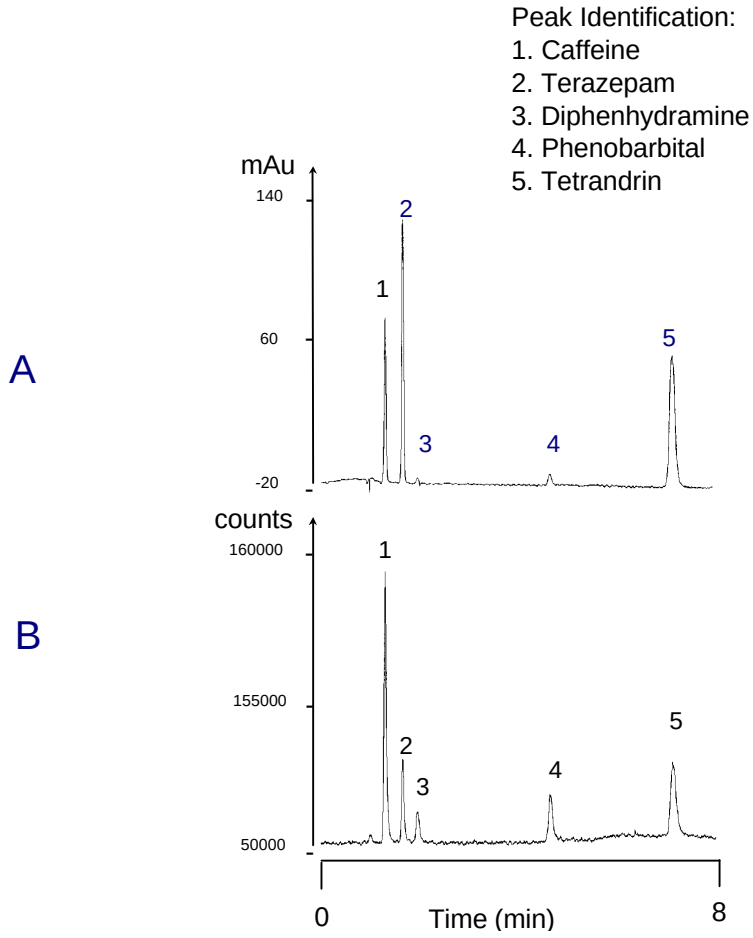
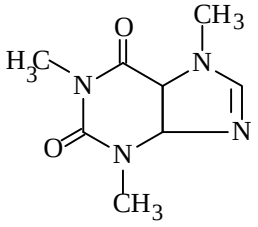
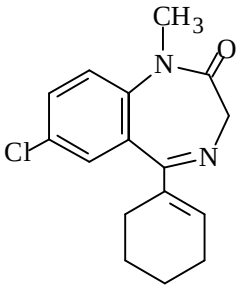


Figure 4.9 Packed-column SFC-CLND/UV profile of a pharmaceutical mixture. Conditions: 50^{o}C oven; Pressure program: 250-300 atm at 10 atm/min, and hold; Methanol modifier program: 8% (v/v) hold 2 min, ramp to 15% at 1.5%/min, and hold; Valuepak amino (150x4.6mm i.d., d_p=5-µm) column; 5 µL injection loop; Liquid CO2 flow rate of 2.0 mL/min; Decompressed CO2 of 1200 mL/min at UV and 160 mL/min at CLND (25 µm i.d. intergral restrictor); Methanol as sample solvent; Sample concentration of 167 ppm of each compound except tetrandrin at approx. 250 ppm. (A) UV detection at 254 nm. (B) SFC-CLND.

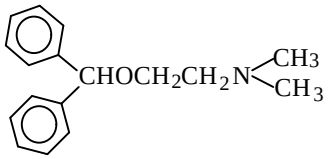
1. Caffeine



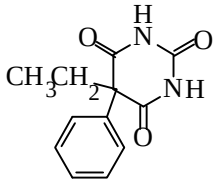
2. Tetrazepam



3. Diphenhydramine



4. Phenobarbital



5. Tetrandrin

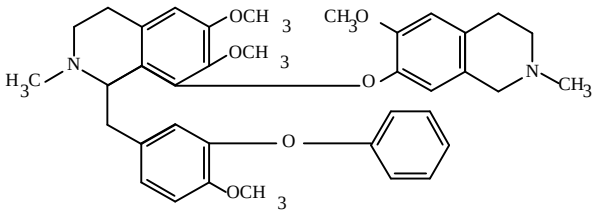


Figure 4.10 Structures of pharmaceuticals in Figure 4.9

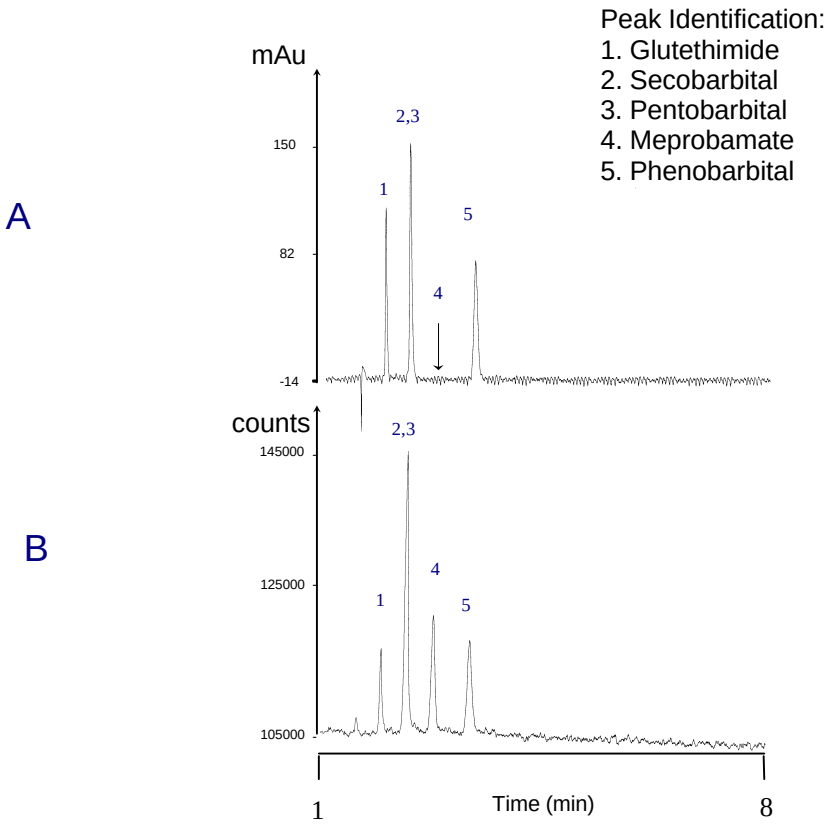
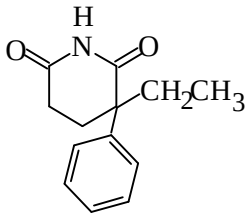
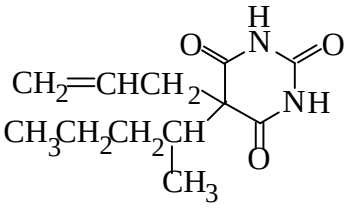


Figure 4.11 Packed-column SFC-CLND/UV separation of sedatives. Conditions: 50^{o}C oven; Pressure 250 atm; Methanol modifier: 15% (v/v); Amino 1 (150x4.6mm i.d., d_p=5-µm) column; 5 µL injection loop; Liquid CO2 flow rate of 2.5 mL/min; decompressed CO_2 of 1300 mL/min at UV and 120 mL/min at CLND; methanol as sample solvent; Sample concentration of 50 ppm of each component. (A) UV detection at 219 nm. (B) SFC/ CLND.

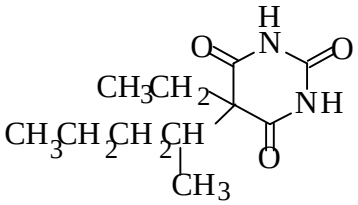
1. Glutethimide



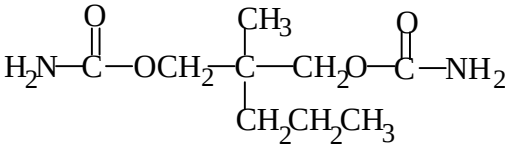
2. Secobarbital



3. Pentobarbital



4. Meprobamate



5. Phenobarbital

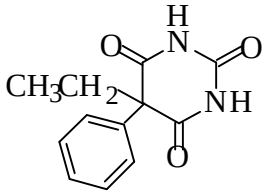


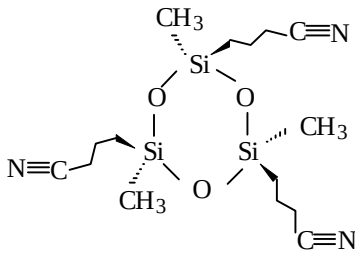
Figure 4.12 Structures of sedatives in Figure 4.11.

The SFC-CLND/UV system was used to analyze a mixture of cyclic oligomers containing trimer, tetramer, and pentamer of methylcyclosiloxanes with pendant 3-cyanopropyl groups and their conformational isomers. These oligomers (structures see Figure 4.13) are not volatile and consequently can not be separated by GC. Although these compounds can be chromatographed by HPLC, UV detection is a problem because they are void of UV chromophores. Packed-column SFC with methanol modified CO_2 was the choice of separation method. The CLND profile of the cyclic nitrogen-containing oligomer mixture is shown in Figure 4.14.

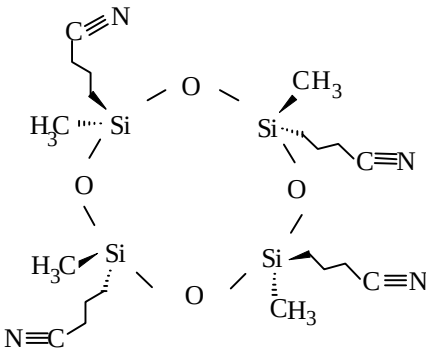
4.4 Conclusion

Chemiluminescent nitrogen detector is compatible with methanol modified CO_2 mobile phase for packed-column SFC. The detector provides equimolar nitrogen response to nitrogen containing compounds with high sensitivity, selectivity, and a wide linear dynamic range. Packed-column SFC-CLND provides another alternative for chromatographic separation and at the same time shows the value of nitrogen detection allowing simple analysis of complex samples.

Trimer



Tetramer



Pentamer

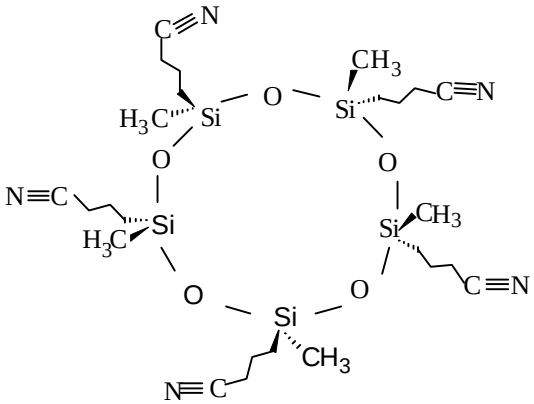


Figure 4.13 Structures of cyclic oligoers in Figure 4.14

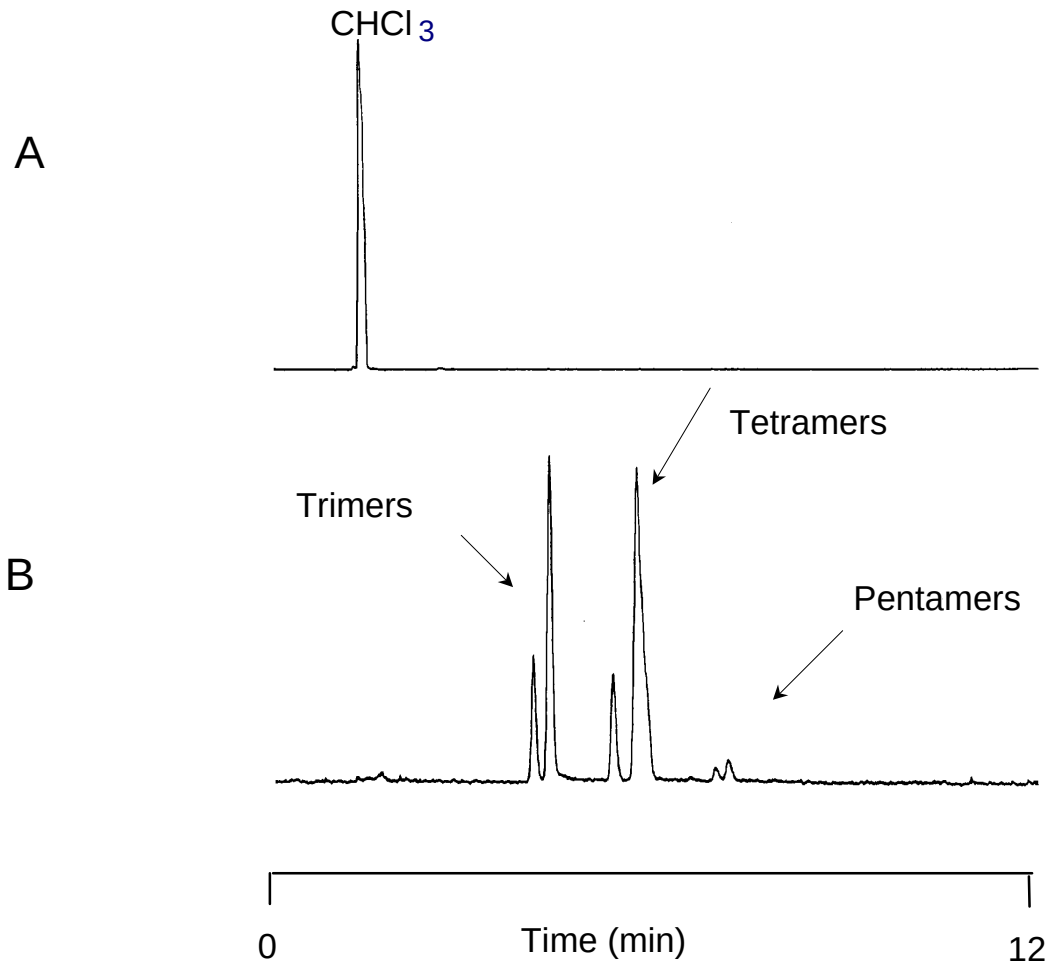


Figure 4.14 Packed-column SFC-CLND/UV profile of cyclic oligomers. Conditions: 60^{o}C oven; Pressure program: hold 2 min, 150-250 atm at 15 atm/min and hold; Methanol modifier program: 5% (v/v) hold 1 min, to 14% at 1%/min, and hold; Hypersil silica (250x4.6mm i.d., d_p=5-µm) column; 5 µL injection loop; Liquid CO2 flow rate of 2.0 mL/min; Decompressed CO2 of 980 mL/min at UV and 120 mL/min at CLND (25 µm i.d. intergral restrictor); Chloroform as sample solvent; Total sample concentration of 7 mg/ mL. (A) UV detection at 219 nm. (B) SFC-CLND.

Chapter V

Sulfur-Selective Chemiluminescence Detection after Supercritical Fluid Chromatography

5.1 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, pioneered by Antek Instruments, Inc. in the early 1980s [1, 2] is currently one of the best available techniques. Recently a new generation sulfur chemiluminescence detector (SCLD) was introduced by Antek Instruments, Inc.. The much larger furnace of the SCLD is expected to provide better reproducibility, repeatability, and long term detector stability than other commercially available sulfur detectors for analyzing heavy as well as light sulfur species. When the 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]

Developments by Benner et al. [4] resulted in commercialization of another sulfur chemiluminescence detector (SCD) by then Sievers Research, Inc. for GC. [5] Additional real world applications were reported. [6] Good detection limits, linearity, and detector response [7, 8] were observed with this sulfur chemiluminescence detector. In order to

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[1] R. E. Parks, *U.S. Pat*., 4,352,779 (1982)
[2] R. E. Parks, *U.S. Pat*., 4,678,756 (1987)
[3] X. Yan; E. M. Fujinari, presented in *Gas Chromatography: Detectors,* Pittsburgh Conference, Chicago, IL., March 3-8, 1996, paper #434
[4] R. L. Benner; D. H. Stedman, *Anal. Chem.*, 61, (1989) 1268
[5] R. L. Shearer; D. L. O'Neal; R. Rios; M.D. Baker, *J. Chromatogr.*, 28 (1990) 24
[6] N. G. Johansen; J. W. Birks, *Amer. Lab.*, February (1991) 112
[7] A. L. Howard; L. T. Taylor, *J. High Resolut. Chromatogr.,* 14 (1990) 785

alleviate the problem of drastic changes in sensitivity and selectivity found in the early units, a modified SCD utilizing a heated furnace assembly was developed by Shearer and was referred to as the flameless SCD. [9] The flameless SCD 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]

Separation 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, 12, 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]

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[8] K. K. Gaines; W. H. Chatham; S. O. Farwell, *J. High Resolut. Chromatogr.*, 13 (1990) 489-493.

[9] R. L. Shearer, *Anal. Chem.*, 64 (1992) 2192
[10] Y.C. Chen; J.G. Lo, *Chromatographia*, 43 (1996) 522
[11] H. -C. K. Chang, L. T. Taylor, *J. Chromatogr.*, 517 (1990) 491
[12] L. A. Pekay; S. V. Olesik, *J. Microcol. sep.*, 2 (1990) 270
[13] R. L. Shearer; R. J. Skelton, *J. High Resolut. Chromatogr.*, 17 (1994) 251

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. The flameless SCD was reported to be more sensitive and selective than any other sulfur selective detector for SFC. Minimum detection of 0.3 pg S/sec, a sulfur to carbon (as in toluene) selectivity of 10^6 and a linearity of nearly 10^3 were obtained 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 take full advantage of recent improvements in sulfur chemiluminescence detection technology, a new generation of sulfur chemiluminescent detector was interfaced to SFC. The evaluation with packed columns employing both pure and methanol modified CO_2 as mobile phases was accomplished and is described in this chapter. Reproducibility and repeatability of the new SFC-SCLD were also studied. Analysis of pesticides, sulfonamides and petroleum products by open tubular and

packed column SFC are shown as applications using this new generation sulfur detector.

5.2 Experimental

Instrumentation

A model 704E sulfur chemiluminescence detector from Antek Instruments, Inc. (Houston, TX) was used for analyzing sulfur-containing compounds. The detector was interfaced with either a Hewlett-Packard Model G1205A supercritical fluid chromatograph (Avondale, PA) for packed columns, or a Dionex Lee Scientific series 600 supercritical fluid chromatograph (Salt Lake City, UT) for open tubular columns. A 5-μL loop was used for sample injection in the packed column SFC system. While time split injection with a helium actuated Valco (Houston, TX) injector (500 nL rotor) was employed for the open tubular SFC system. Tapered restrictors (50-μm i.d.) or linear restrictors (25-μm i.d.) were used depending on the mobile phase composition or decompressed CO_2 flow rates. The chromatographic columns used in this study included a SB-methyl-100 (10-m x 100-μm i.d., 0.25-μm film thickness) column obtained from Dionex (Salt Lake City, UT), a Deltabond C_8 (250 mm x 4.6 mm i.d., d_p=5-μ) column, and a Deltabond phenyl (250 mm x 4.6 mm i.d., 5 μm particle size) column obtained from Keystone Scientific (Bellefonte, PA). 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 84-133 mL/min, while the oxygen flow rate was 3.4-6.3 mL/min. The oxygen flow rate to the ozone generator was fixed at 27.1 mL/min. The SCLD gain was set to High x 50, or High x 10 with a 1 volt full scale output voltage.

Reagents

Dibenzothiophene, phenylsulfide, methylsulfide, octadecyl mercaptan and piperine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Captan, disulfiram, and folpet were purchased from Chem Service, Inc. (West Chester, PA). Sulfamerazine was purchased from Sigma Chemical (St. Louis, MO), and dimethoate from Accu Standard, Inc. (New Haven, CT). The hydrotreated petroleum product, diesel A, heavy diesel and vacuum gas oil were provided by a research facility. 1,3-Dibutyl-2-thiourea and sulfometuron methyl were obtained from Dupont (Wilmington, DE). Sulfamethazine, sulfadimethoxine, sulfaquinoxaline, sulfathioazole were obtained from United States Department of Agriculture (Philadelphia, PA). All chemicals and samples were used without further purification or clean-up. HPLC grade solvents (EM Science, Gibbstown, NJ) were used for preparing standard solutions. Grade 4.3 oxygen (Airco, Murray Hill, NJ) was used for both oxidation and ozone-generation.

Hydrogen was also obtained from Airco. SFC-grade CO_2 was obtained from Air Products and Chemical, Inc. (Allentown, PA).

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. The remaining experiments were performed with either a packed column or capillary column. Detailed chromatographic conditions are cited in the Figure legends of each chromatogram.

5.4 Results and Discussion

Detection Mechanism

Unlike the reaction mechanism proposed for SCD by Sievers Instruments, 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 (SO2*) in the excited state. The chemiluminescence of SO2* with a spectrum ranging approximately from 300 to 450 nm is then measured by a photomultiplier tube. The following equations summarize the SCLD detection mechanism:

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

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

$$H_2S + O_3 \longrightarrow SO_2^{*}$$

$$SO_2^{*} \longrightarrow SO_2 + h\nu$$

Detector Configuration for Packed Column

The SCLD was coupled with packed column SFC without any modification. Figure 5.1 shows a schematic diagram of the SFC-SCLD, and a more detailed representation of the actual interface is given in Figure 5.2. The restrictor tip was threaded through the fitting until it reached the bottom of the 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.

Detector Performance

The detector performance was first evaluated for packed column SFC using 100% CO_2 mobile phase. Standard solutions were used to obtain a minimum detectable quantity (MDQ), repeatability, linearity, selectivity, and relative response factors (f_x). MDQs at the SCLD were 3 pg sulfur (0.2 pg S/sec) for both for dibenzothiophene and phenylsulfide, respectively. These values at the detector were calculated based on a split ratio of 30 : 1 (UV to SCLD) and 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 ppm sulfur in toluene, as well as 0.2 ppm sulfur in both hexane and methanol were used to study selectivity of the detector. For all three solutions operating under the SCLD conditions 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 ppm 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 observed 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

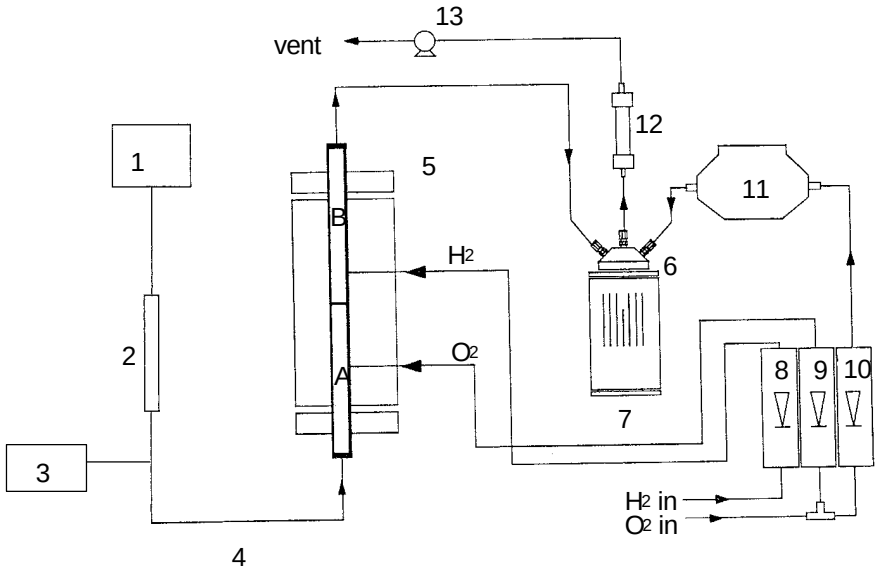


Figure 5.1 Schematic flow diagram of packed column SFC-SCLD system. 1. SFC; 2. Column; 3. UV detector; 4. Restrictor; 5. Furnace (A-oxidation zone, B-reduction zone); 6. Reaction chamber; 7. PMT; 8. Flow meter for pyro H_2; 9. Flow meter for pyro O_2; 10. Flow meter for ozone generator; 11. Ozone generator; 12. Scrubber; 13. Vacuum pump.

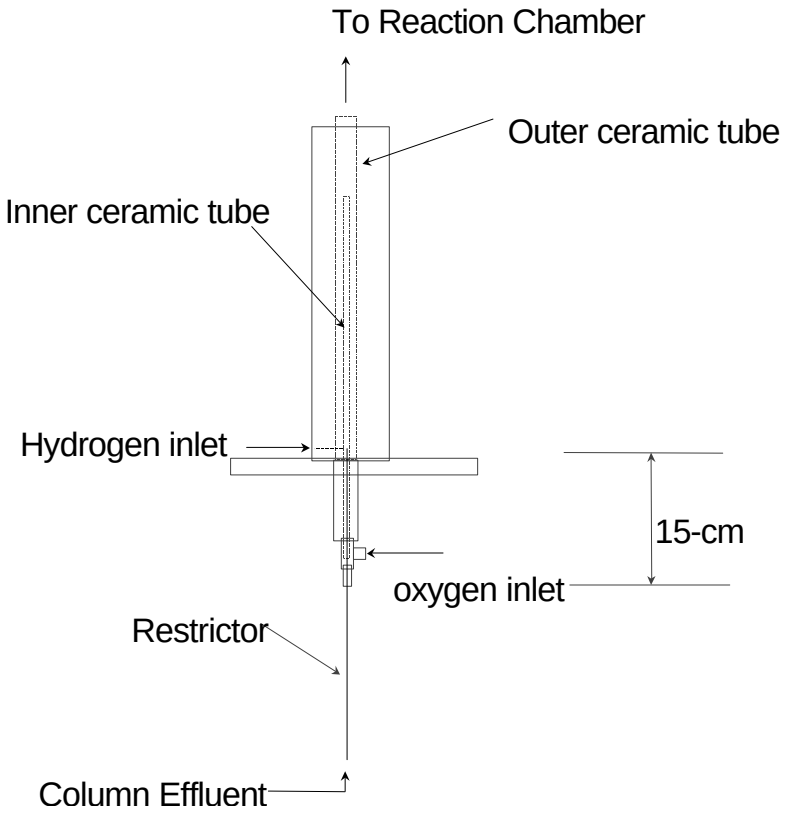


Figure 5.2 Detailed schematic of the SFC-SCLD interface.

compounds were used. Table 5.1 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 the 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 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 ppm sulfur) were used. Relative standard deviations (RSDs) 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. Figure 5.3 shows a profile of three replicates of the two standards on day five used in the repeatability studies.

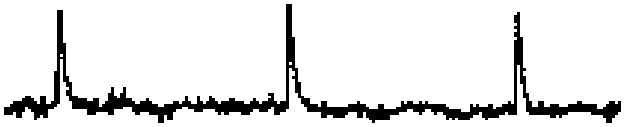
When methanol modified CO_2 was used as the mobile phase, the evaluation studies for the packed column SFC-SCLD system were mainly focused on obtaining

Table 5.1

Response Factors (f_x) relative to dibenzothiophene by SCLD

Component	f_x
captan	1.10
dibenzothiopene	1.00
dimethoate	1.04
disulfiram	1.16
methylsulfide	1.02
octadecyl mercaptan	1.15
phenylsulfide	1.02
sulfamerazine	1.04

A



B

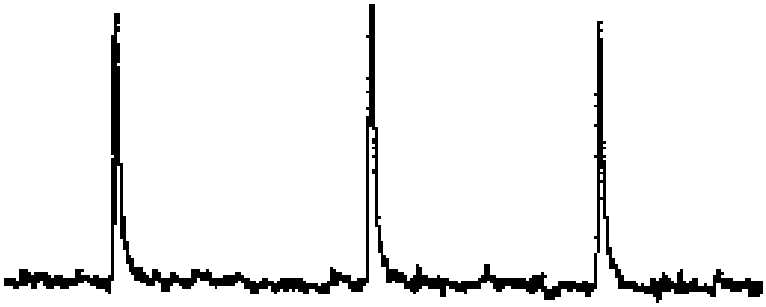


Figure 5.3 SCLD profile of three replicates of the two standard solutions (0.56 and 1.7 ppm sulfur) on day five. Mobile phase: 100% CO_2; Deltabond C_8 (250 x 4.6 mm i.d., d_p=5-μm). SFC conditions: 200 atm, oven temperature held at 50°C; Decompressed CO_2 was 36 mL/min at 100 atm for the SCLD. Split ratio 30 :1 (UV to SCLD). (A) SCLD for 0.56 ppm solution. (B) SCLD for 1.7 ppm solution.

data on linearity, reproducibility in the low nanogram range, as well as selectivity and minimum detectable quantity using sulfonamides. Because methanol modifier was required to elute polar and nonvolatile sulfonamides the efficient sample transport from restrictor to the furnace of SCLD is very critical.

As shown in Figure 5.2, the restrictor tip was located at the low portion of the heated furnace of the SCLD. The precipitation of polar and nonvolatile compounds at the interface may be encountered due to inefficient sample transfer in the heated interface where the methanol modifier evaporates. This will happen very often with a tapered restrictor, because of the extremely small inner diameter at the exit. For our SFC-SCLD system, precipitation of polar and nonvolatile compounds was indicated by observation of a non-sulfur-containing solvent peak and/or analyte peak splitting. The size of the 'solvent peak is related to the sample concentration, as well as the chromatographic conditions. For example, a small 'solvent peak' was observed for mobile phases with a higher modifier percentage. The use of higher flow rates may help reduce the probability of sample precipitation. A linear rather than a tapered restrictor (25-μm i.d.) was used at the interface for the SFC-SCLD employing methanol modified CO_2 as the mobile phase. It was necessary, however, to flush the system frequently with mobile phase of high methanol concentration and elevated CO_2 pressure to keep the interface free of precipitates.

Figure 5.4 shows the SCLD signal (S/N>2) resulting from the injection of 400 ppb sulfamethazine solution via a 5-μL sample loop after the SFC-SCLD system was flushed with 20% methanol modified CO_2 at 300 atmospheres with a liquid CO_2 pumping rate at 2.5 mL/min. For a post column split ratio of 15.4 to 1 (UV to SCLD), the minimum detectable quantity was found to be 15 pg of sulfur at the SCLD. No response was observed from the methanol injection solvent which indicates that the sulfur to carbon selectivity is at least 10^7.

As shown in Figure 5.5, piperine and sulfamethazine both at low nanogram level can be separated on a packed phenyl column with good resolution. It is desireable to use piperine to examine carbon to sulfur selectivity of the detector because piperine contains nitrogen. Since the formation of a nitrogen-containing chemiluminescent species is in much the same way as the SO_2* species is formed, piperine could possibly result in a sulfur selective detector response. Thus, a standard solution containing both sulfamethazine and piperine at a concentration ratio 1:10,638 (sulfamethazine : piperine) was prepared and injected into the SFC-SCLD/UV system. Excellent sulfur to carbon selectivity was demonstrated in the chromatogram of the sulfamethazine and piperine mixture. As illustrated in Figure 5.6, the UV detector is saturated by the extremely high concentration of piperine, while the SCLD is totally transparent to piperine at this high level. On the other hand picogram levels of sulfamethazine can be well detected at the same time.

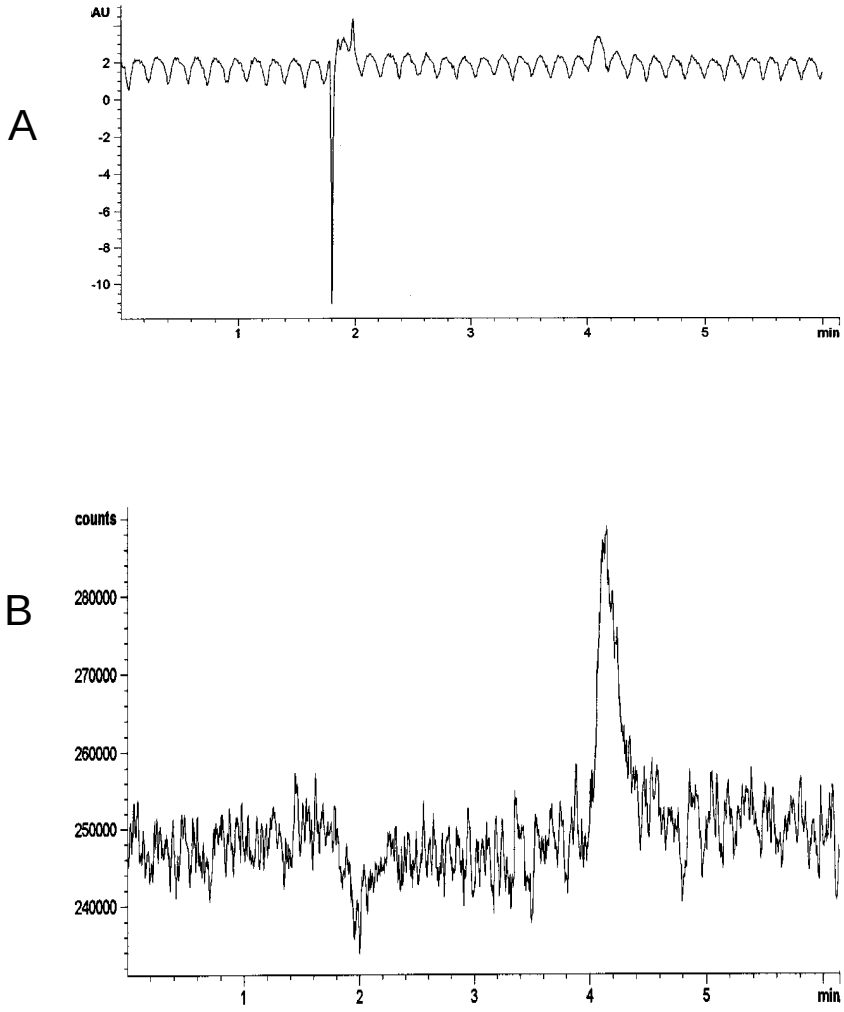


Figure 5.4 Chromatograms of 400 ppb sulfamethazine in methanol with SFC-UV/SCLD system. Conditions: postcolumn split ratio 15.4 to 1(UV to SCLD); oven 55 ^{o}C; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p=5-μm); liquid CO2 flow rate 1.5 mL/min; injection solvent methanol; injection volume 5-μL. (A). UV detection at 266-nm; (B). SCLD.

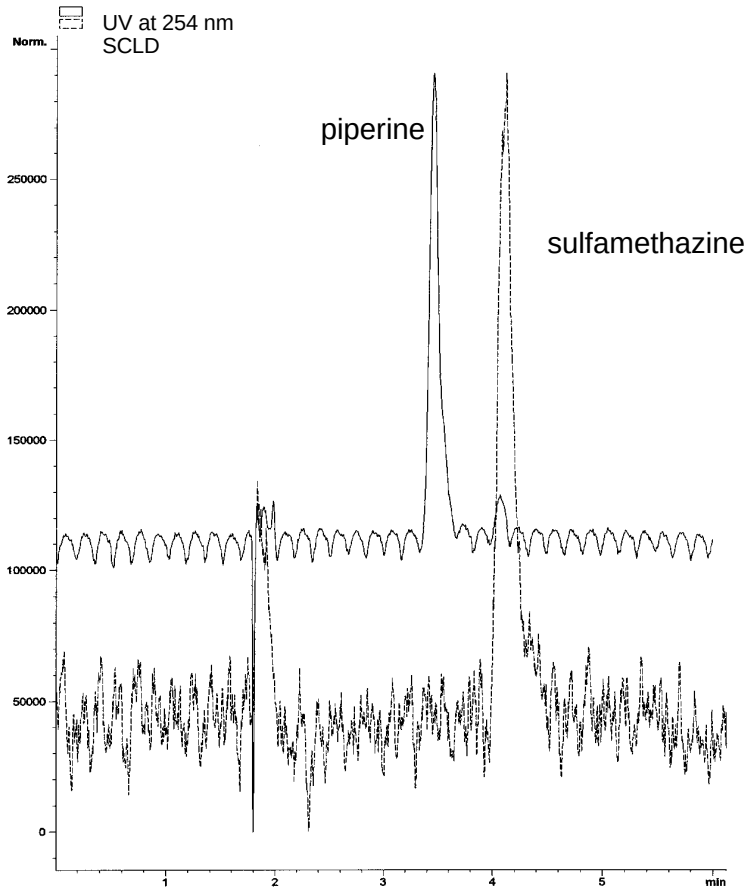


Figure 5.5 Chromatograms of a sulfamethazine and piperine mixture at low nanogram level in methanol with SFC-UV/SCLD system. Conditions: postcolumn split ratio 15.4 to 1(UV to SCLD); oven, 55 ^{o}C; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p= 5-μm); liquid CO2 flow rate, 1.5 mL/min; injection volume, 5-μL. UV-Solid line; SCLD-dashed line.

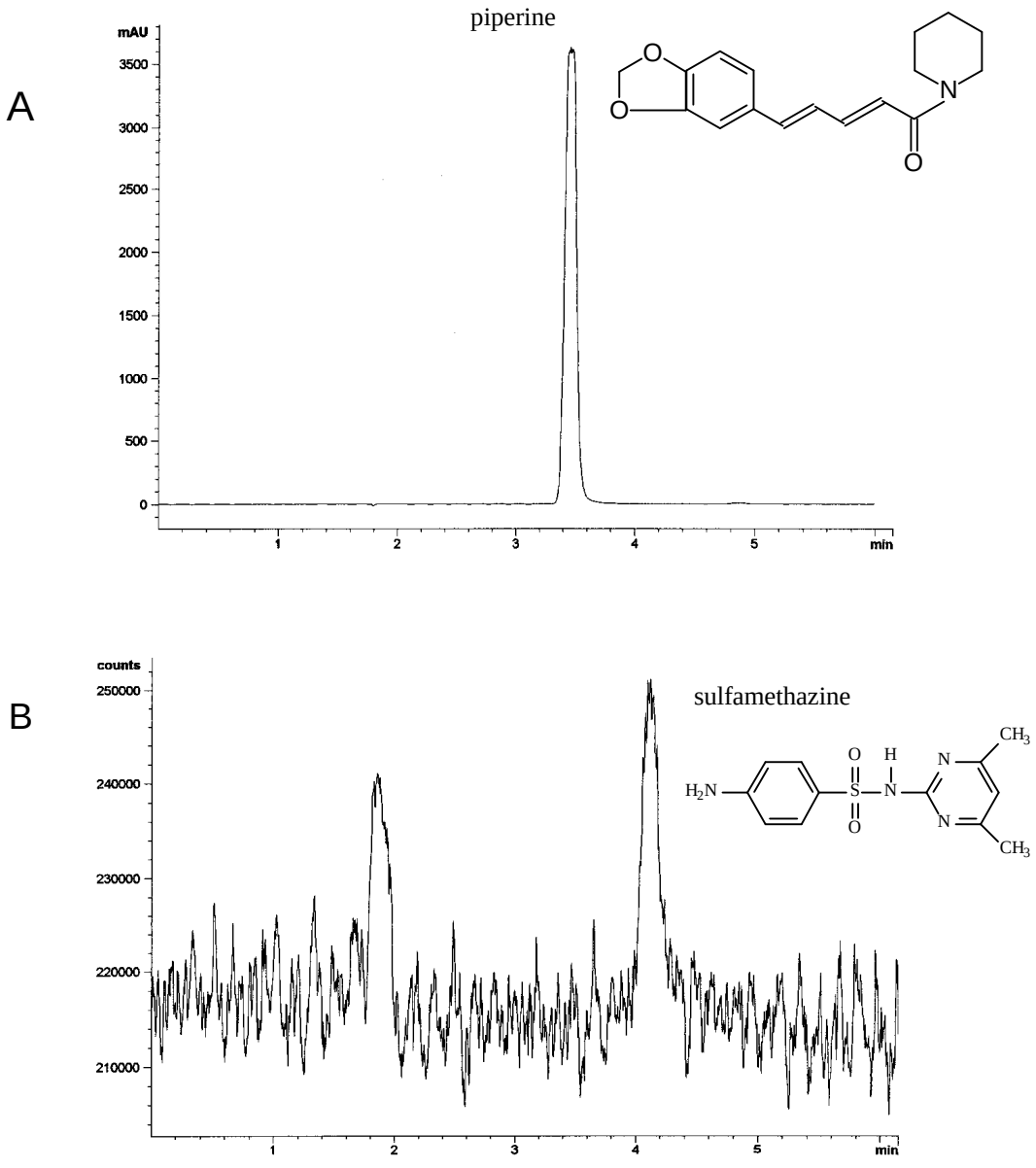


Figure 5.6 Chromatograms of a sulfamethazine and piperine ($1:10^4$) mixture in methanol with SFC-UV/SCLD system in order to show carbon to sulfur selectivity of the SCLD. Conditions: postcolumn split ratio 15.4 to 1(UV to SCLD); oven, 55 ^oC; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p= 5-µm); liquid CO2 flow rate, 1.5 mL/min; injection volume, 5-µL. (A). UV detection at 254-nm; (B). SCLD.

The SCLD showed a good linear dynamic range covering three orders of magnitude when used in SFC. Since most pesticides and drug residues are present at a low concentration level in a complex matrix, it is important to evaluate the detector linearity within the low concentration ranged. Therefore, a series of standard solutions containing 0.40, 0.50, 1.0, 2.5, and 5.0 ppm sulfamethazine in methanol was prepared to investigate the detector linearity. Indeed, the SCLD was linear as illustrated in Figure 5.7.

The reproducibility and day-to-day repeatability were also investigated for the SCLD when methanol modified CO_2 was used as the mobile phase in packed column SFC. Relative standard deviation (RSD) of three injections from the system was generally around 5%. Three injections of a 0.40 ppm and a 1.0 ppm of sulfamethazine (SMZ) standard solution in methanol are illustrated in Figure 5.8 and Figure 5.9 respectively. Two standards freshly prepared everyday from an original solution were used in a 6-day detector repeatability study. The RSDs for the two standards (0.40 and 1.0 ppm SMZ) during the 6-day period were 7.9% and 4.1%, respectively.

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

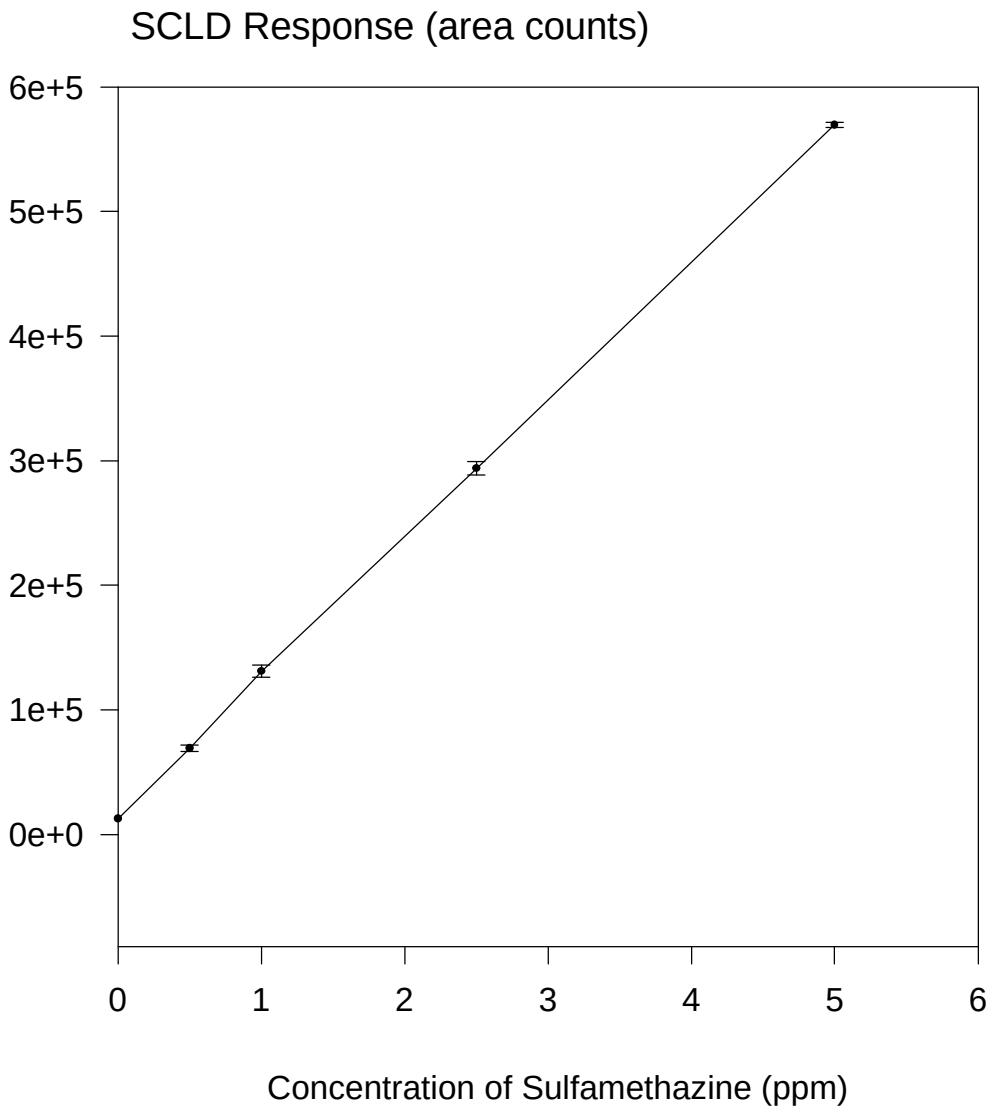


Figure 5.7 Linearity of detector response at low concentration range using sulfamethazine as a probe. Data points represent the means of three injections. The correlation coefficient is larger than 0.999. Conditions: post-column split ratio 15.4 to 1(UV to SCLD); oven, 55 ^{o}C; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p= 5-μm); liquid CO2 flow rate, 1.5 mL/min; injection volume, 5-μL.

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. However, the SF mobile phase used in this study is a simple single component carrier much like the single component carrier used in GC. A single component carrier ultimately simplifies the overall application in the SFC mode rather than in the HPLC mode where the chromatography is more dependent on multicomponent mobile phase mixtures. Since a fixed restrictor was used, the decompressed CO_2 flow rate increased during pressure programming. Therefore, the decompressed CO_2 flow rate reported is the one measured at the starting point of the program.

The simultaneous UV and SCLD chromatograms of the three sulfur-containing pesticides (Figure 5.10) mixture with a decompressed CO_2 flow rate starting at 36 mL/min at SCLD is presented in Figure 5.11. A standard mixture containing 15 ppm of octadecyl mercaptan (peak 1) and 12 ppm of both folpet (peak 2) and captan (peak 3) 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 the 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

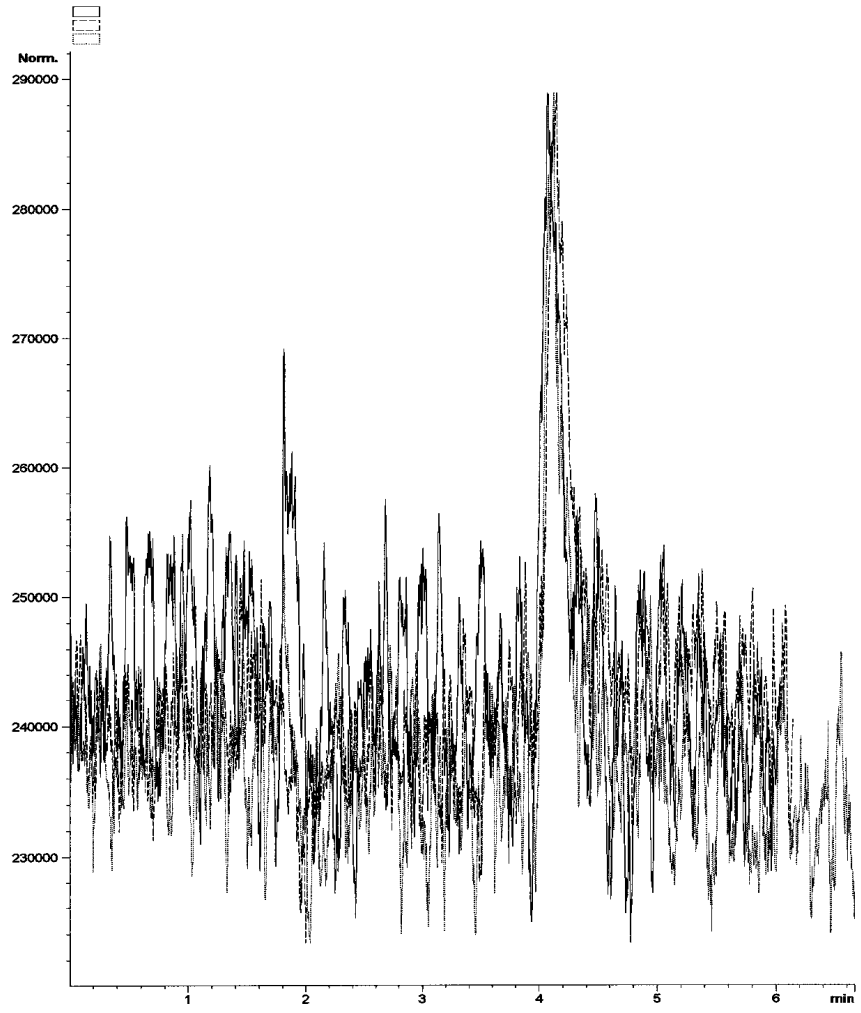


Figure 5.8 Three replicate injections of 400 ppb sulfamethazine in methanol to the SFC--SCLD system. Conditions: postcolumn split ratio 15.4 to 1(UV to SCLD); oven, 55 ^{o}C; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p= 5-μm); liquid CO2 flow rate, 1.5 mL/min; injection volume, 5-μL.

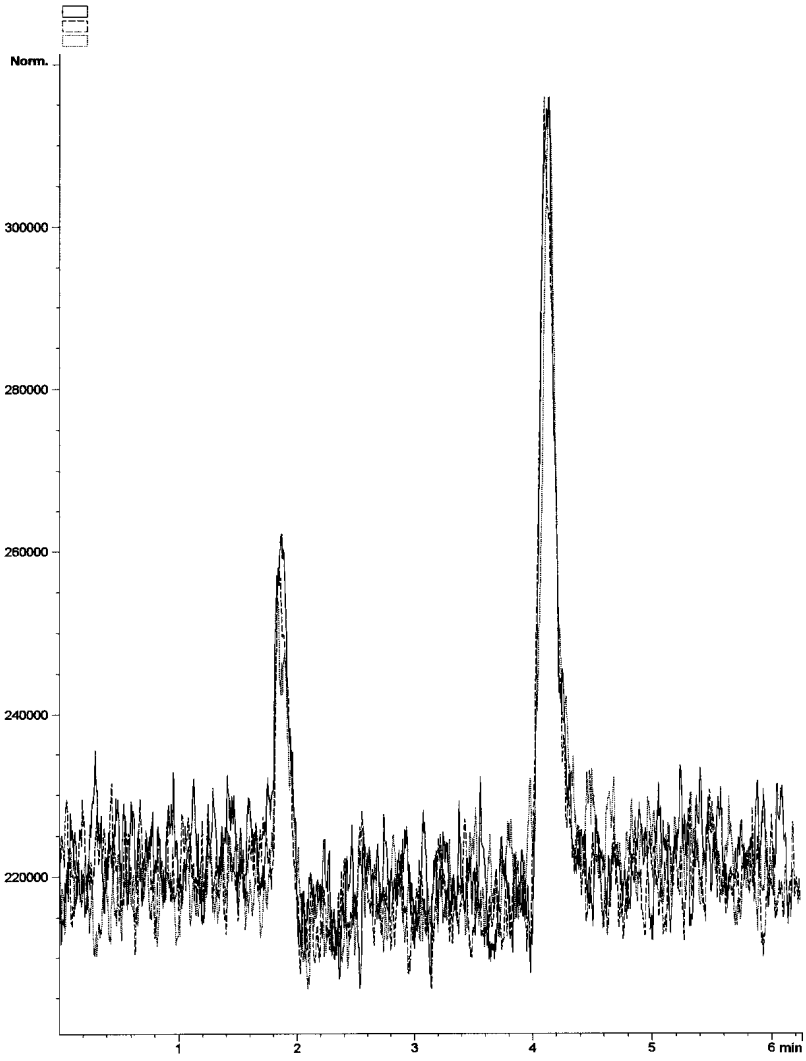
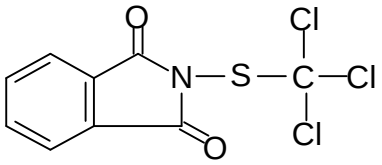


Figure 5.9 Three replicate injections of 1000 ppb sulfamethazine in methanol to the SFC-SCLD system. Conditions: postcolumn split ratio 15.4 to 1(UV to SCLD); oven, 55 ^{o}C; 8% methanol modified CO_2; 150 atm; Deltabond phenyl column (250 x 4.6-mm i.d., d_p=5-µm); liquid CO2 flow rate, 1.5 mL/min; injection volume 5-µL.

1. Octadecyl Mercaptan $CH_3(CH_2)_{17}SH$

2. Folpet



3. Captan

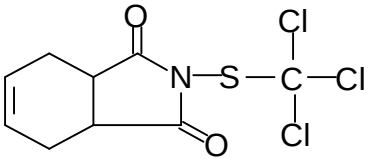


Figure 5.10 Chemical structures of sulfur-containing compounds in Figure 5.11.

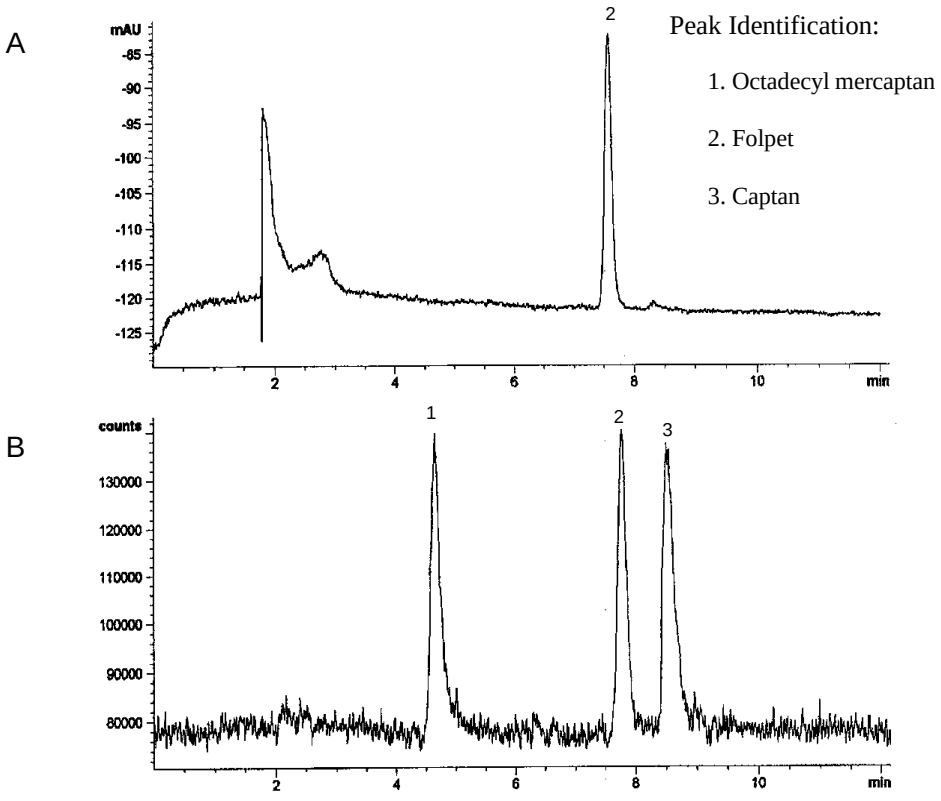


Figure 5.11 Chromatograms of a sulfur-containing compound mixture in methanol with packed column SFC-SCLD/UV system. Mobile phase: 100% CO_2; Deltabond phenyl (250 x 4.6-mm i.d., d_p=5-µm) with SCLD and UV detection at 219 nm. SFC conditions: 100 atm ramp to 220 atm at 10 atm/min; oven temperature held at 55°C; Decompressed CO_2 was 36 mL/min at 100 atm for the SCLD. Split ratio 30 :1 (UV to SCLD). Injection volume: 5 µL; 75 ng of octadecyl mercaptan, 60 ng of both folpet and captan injected. (A) UV detection at 219-nm. (B) SCLD.

at 254 nm, only the chromatogram from UV detector at 219 nm is presented. The results of this study demonstrated near equimolar response of the SCLD with excellent sulfur selectivity and baseline stability during CO_2 pressure programming.

A retention time offset between the UV detector and SCLD was observed with folpet (e.g. 0.2 minute 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 and SCLD peaks was achieved. As shown in Figure 5.12, 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.

The separation of a mixture containing polar sulfonamides and thermally labile sulfonylurea herbicides is demonstrated in Figure 5.13. A low nanogram level of each component (Figure 5.14) was injected. Peak identification was achieved by injection of single standard components under the same chromatographic conditions as used in the separation. The SCLD has significant advantages in high signal-to-noise ratio compared to UV detector even with a split ratio of 13.3 to 1 (UV to SCLD).

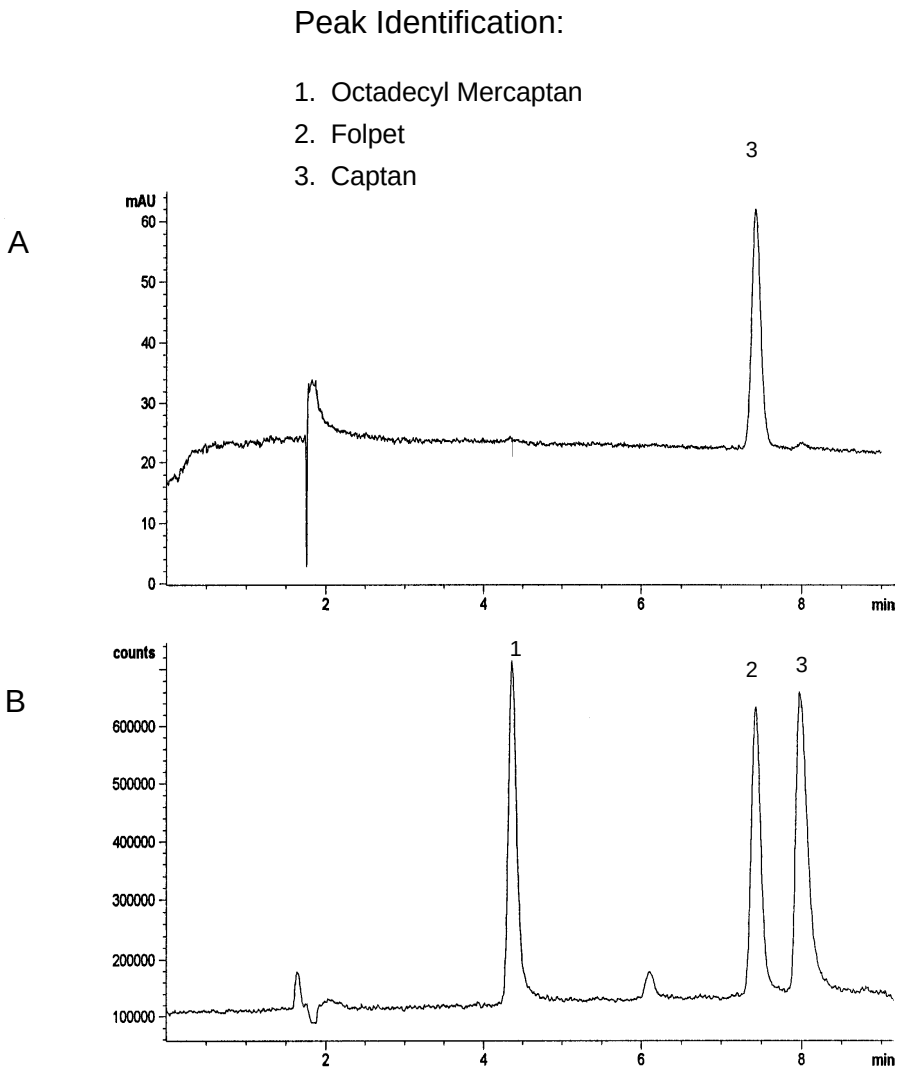


Figure 5.12 Chromatograms of a sulfur-containing compound mixture in methanol with packed column SFC-SCLD/UV system. Same operating conditions as in Figure 5.11, except decompressed CO_2 flow rate was 55 mL/min at 100 atm for the SCLD. (A) UV detection at 219-nm. (B) SCLD.

Peak Identification:

1. 1,3-Dibutyl-2-thiourea
2. Sulfometuron Methyl
3. Sulfamethazine
4. Sulfaquinoxaline
5. Sulfathiazole

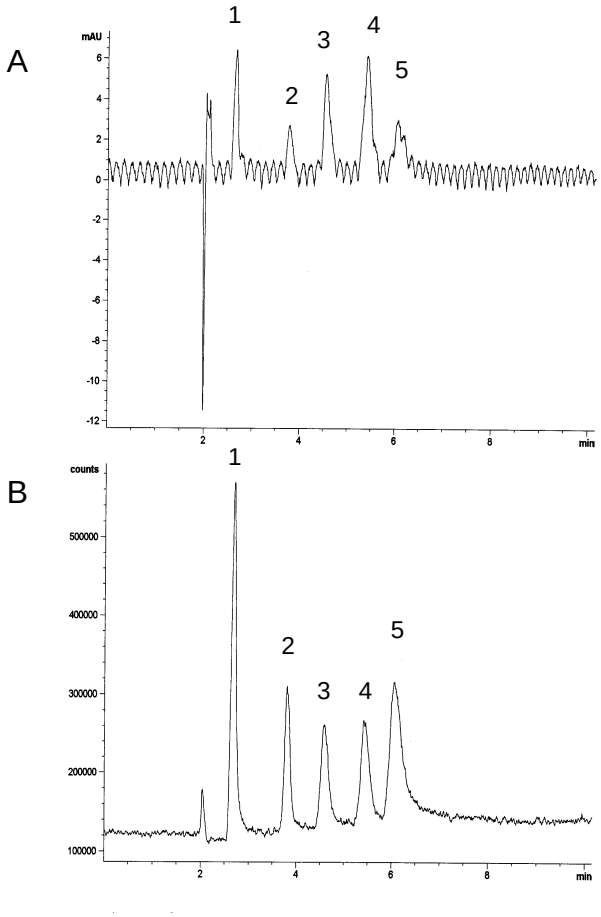
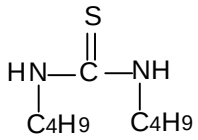
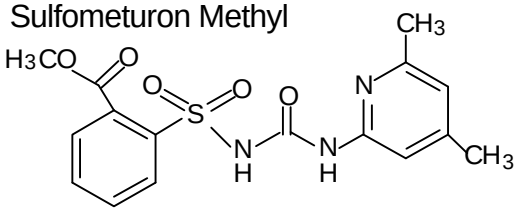


Figure 5.13 Chromatograms of sulfonylurea herbicides and sulfonamides mixture in methanol with packed column SFC-SCLD/UV system. Conditions: Deltabond phenyl column (250 x 4.6-mm i.d., d_p=5-µm); oven, 55°C; mobile phase, methanol modified CO_2, methanol starts at 8% (v/v), ramp to 10% at 0.2%/min; pressure,150 atm; liquid CO_2 flow rate, 1.5 mL/min; injection volume, 5-µL; Split ratio 13.3 :1 (UV to SCLD). (A) UV detection at 254-nm. (B) SCLD.

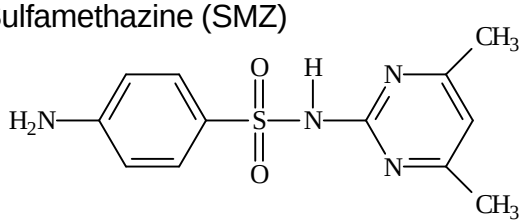
1. 1,3-Dibutyl-2-thiourea



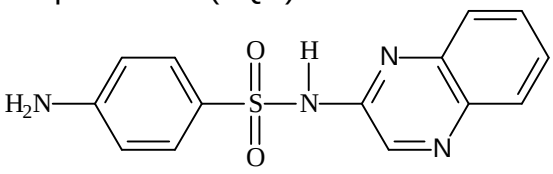
2. Sulfometuron Methyl



3. Sulfamethazine (SMZ)



4. Sulfaquinoxaline (SQX)



5. Sulfathiazole (STZ)

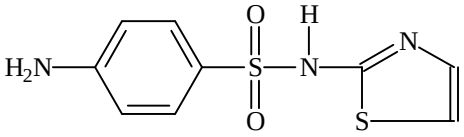


Figure 5.14 Chemical structures of the components in Figure 5.13.

Because sulfa drugs are widely used as an animal medicine, the drug residue in animal products may cause health problems to human beings. It was reported that some people exhibit hypersensitivity to drug residues and/or the drug residue may resist the drug therapy that people are taking.[14] A recent study also indicated that sulfamethazine, one of the most widely used sulfa drugs, may be a thyroid carcinogen.[15] Since the SCLD has demonstrated high sensitivity and selectivity for sulfur-containing compounds, the packed column SFC-SCLD system was used in the separation of a sulfa drug extract from poultry tissue provided by USDA employing methanol modified CO_2 as the mobile phase. The extract of sulfa drug which contains sulfamethazine (SMZ), sulfadimethoxine (SDM) and sulfaquinoxaline (SQX) was obtained after spiking 0.5-mg each of the drug onto 1.0-kg of chicken liver. After supercritical fluid extraction at a pressure of 10,000-psi by pure CO_2, the spiked sulfonamides were collected in a water/methanol mixture (65/35 v/v%) which resulted in a concentration of 0.5 ng/µL of each component. The extract as received was injected onto the system. As shown in Figure 5.15, although the sulfonamides were detected by SCLD, peak splitting and poor peak shapes were observed. This can be explained by the immiscibility of water and CO_2 and is most likely a chromatographic problem since it happened with both UV and SCLD. This was not pursued any further.

[14] B.M. Kagen, " Antimicrobial Therapy", W.B. Saunders, Philadelphia, 1974
[15] N. Littlefield, Technical Report, National Center for Toxicological Research, Jeffersons, AR.,

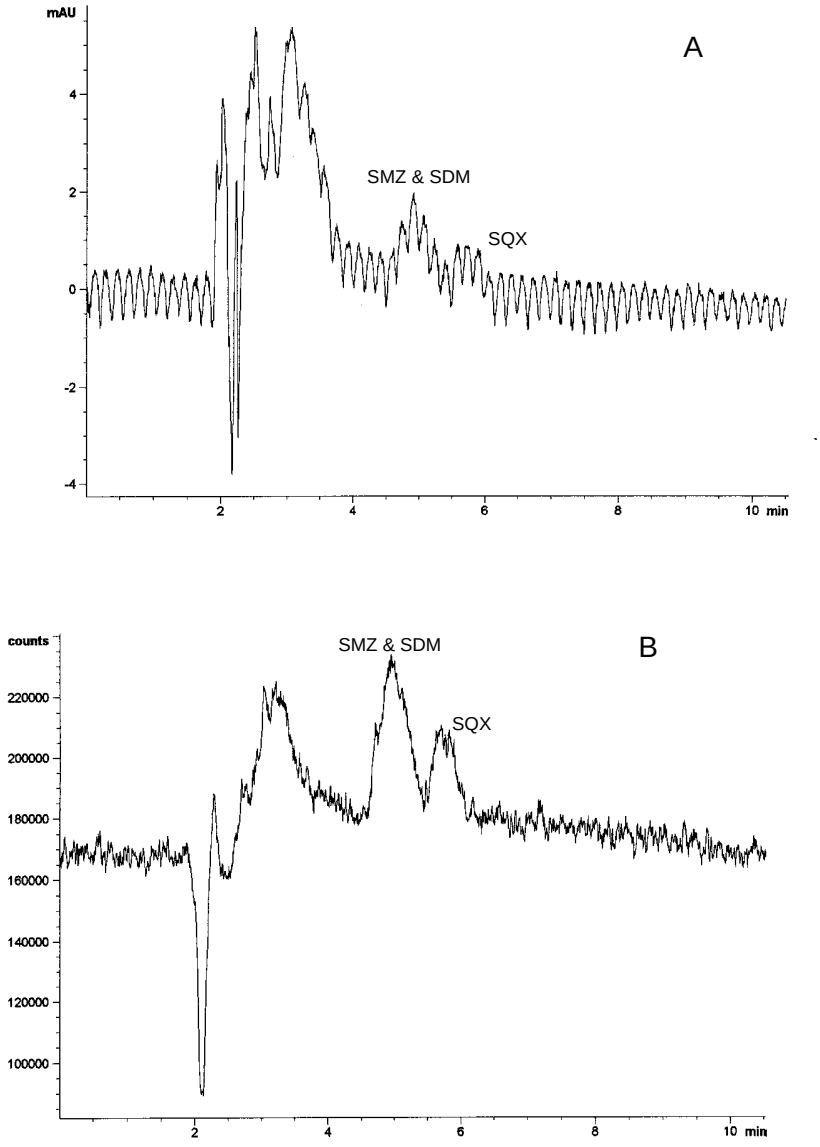


Figure 5.15 Chromatograms of an extract of spiked sulfonamides (0.5-mg/kg) on chicken liver with packed column SFC-SCLD/UV system. Conditions: Deltabond phenyl column (250 x 4.6-mm i.d., d_p=5-µm); oven, 55°C; mobile phase, methanol modified CO_2, methanol starts at 8% (v/v), ramp to 10% at 0.2%/min; pressure program starts at 150 atm, then ramp to 180 atm at 3 atm/min ; liquid CO_2 flow rate, 1.5 mL/min; injection volume, 5-µL; Split ratio 13.3:1 (UV to SCLD). (A) UV detection at 266-nm. (B) SCLD.

1988

Monitoring sulfur-containing compounds is very important in the petroleum and petrochemical industries, since some of these compounds impede successful 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 Figure 5.16. Excellent selectivity was demonstrated by the SCLD. This sample was further analyzed under a constant pressure of 200 atm, as shown in Figure 5.17. 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. 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.

Diesel samples are generally analyzed by gas chromatography. When selective detection of sulfur-containing compounds in diesel becomes necessary, chemiluminescence detectors are, of course, the first choice. However, heavier components, or components with high boiling point may pose a problem when they are analyzed by GC because of the temperature limit set by the chromatograph. Figure 5.18 presents a chromatogram of a sample ‘ diesel A’. As can be seen from Figure 5.18, there is a broad hump together with a baseline rise at the end of the GC-SCLD profile. The hump possibly resulted from column bleeding or heavier sulfur compounds. However, a chromatogram obtained from the injection of only thiophene under the same chromatographic conditions negates our column bleeding suspicion,

since there was no base line rise in the profile with thiophene. Therefore, a heavy sulfur-containing component is mostly likely present in this diesel sample. Since SFC can extend the molecular weight range of GC and the SCLD has demonstrated feasibility to be used after SFC, the same sample was injected onto a capillary SFC and detected by both SCLD and FID. As shown in Figure 5.19, the patterns of the sulfur-containing compounds in ‘diesel A ‘ obtained by GC-SCLD is quite similar to those obtained by capillary SFC-SCLD, the hump in the GC chromatogram appeared in the SFC as a normal peak and the baseline was normal after all the sulfur-components eluted. The capillary SFC was also used in the analysis of other diesel samples such as ‘heavy diesel’ and ‘ vacuum gas oil’. The SFC chromatograms of these two samples with dual FID and SCLD detection are given in Figure 5.20 and 5.21 respectively. The SCLD demonstrated the capability of dealing with heavy sulfur components, while exhibiting high selectivity.

5.4. Conclusion

The SCLD was interfaced with packed column SFC using both pure and methanol modified CO_2 as mobile phase and tested simultaneously with UV detection. Capillary SFC with dual FID and SCLD detection of heavy diesel samples was also

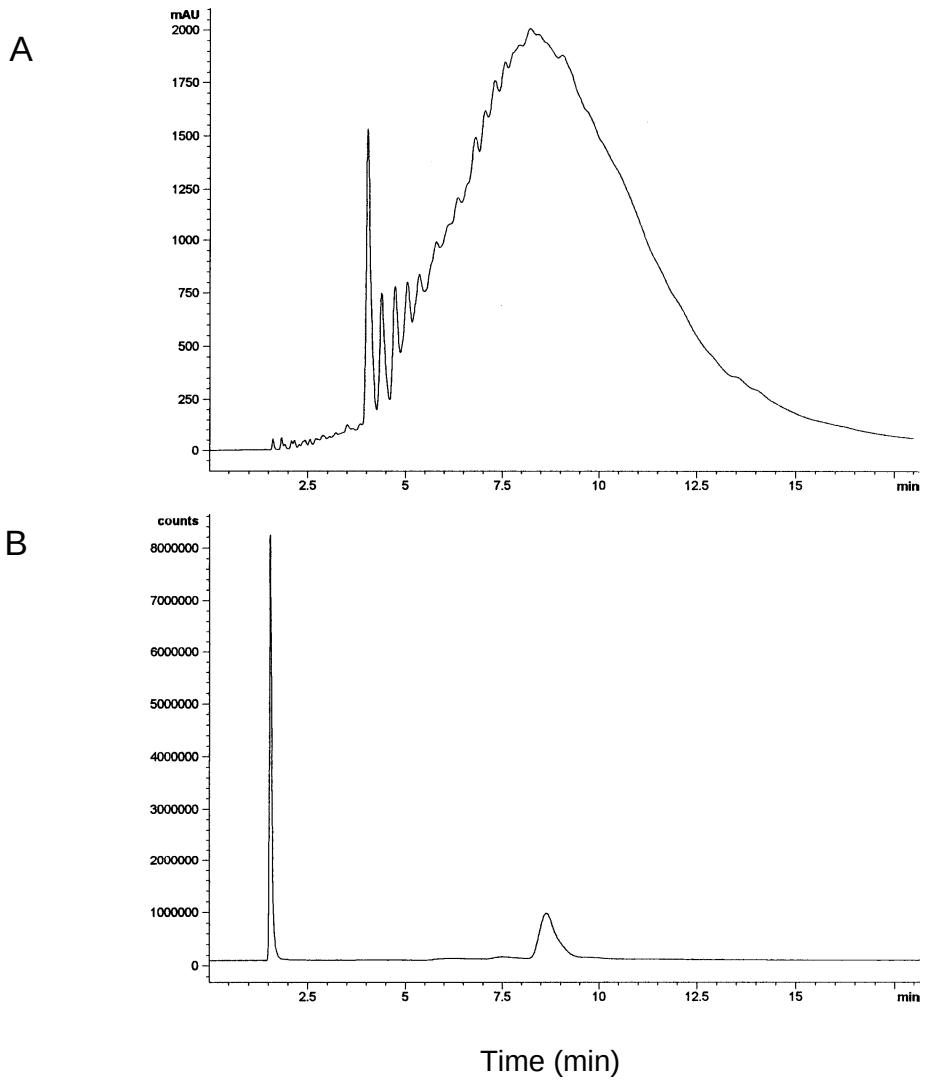


Figure 5.16 Neat hydrotreated petroleum product profile with packed column SFC-SCLD/UV system. Mobile phase: 100% CO_2; Deltabond phenyl (250 x 4.6 mm i.d., d_p=5-μm) with SCLD and UV detection at 254 nm. SFC conditions: 100 atm ramp to 260 atm at 10 atm/min; oven temperature held at 70°C; A 2 feet long fused silica linear restrictor with 15 μm i.d. was used to the SCLD. Sample injection size was 5 μL. (A) UV detection at 254 nm. (B) SCLD.

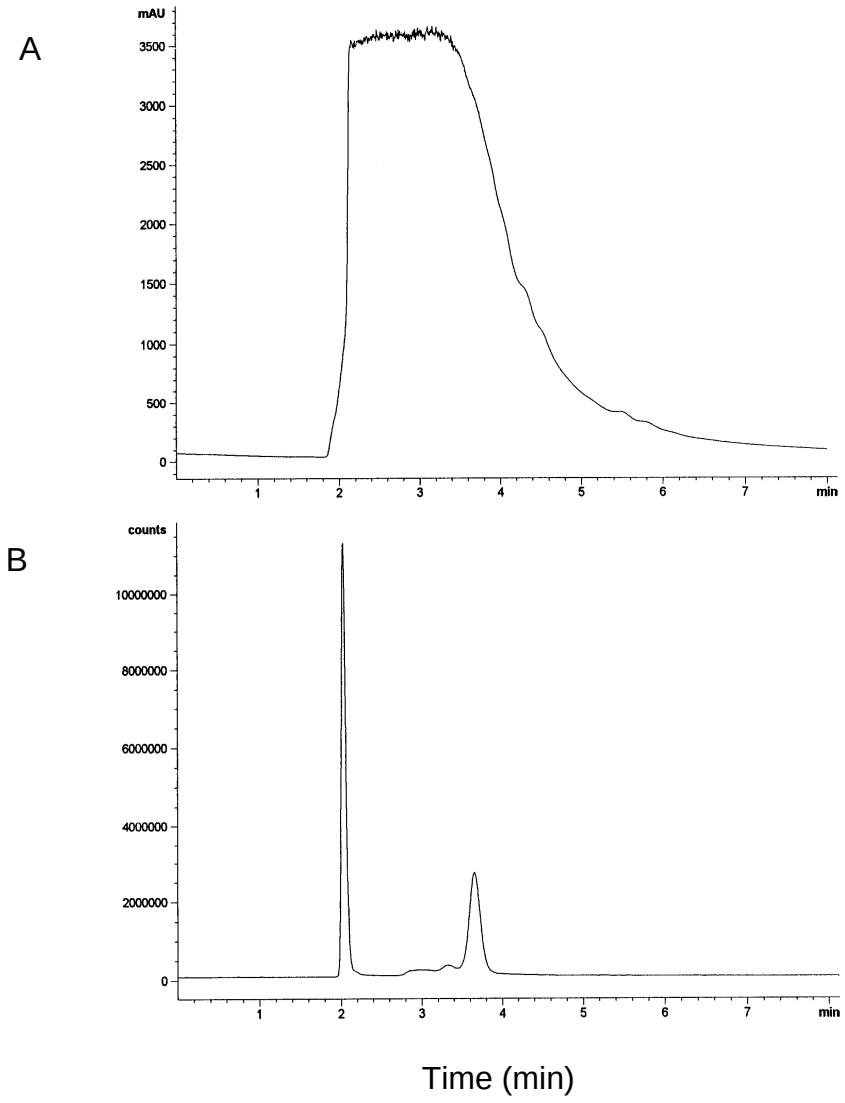


Figure 5.17 Hydrotreated petroleum product profile with packed column SFC-SCLD/UV system. Same operating conditions as in Figure 5.15, except that a constant 200 atm SF-CO_2 pressure was used. (A) UV detection at 254 nm. (B) SCLD.

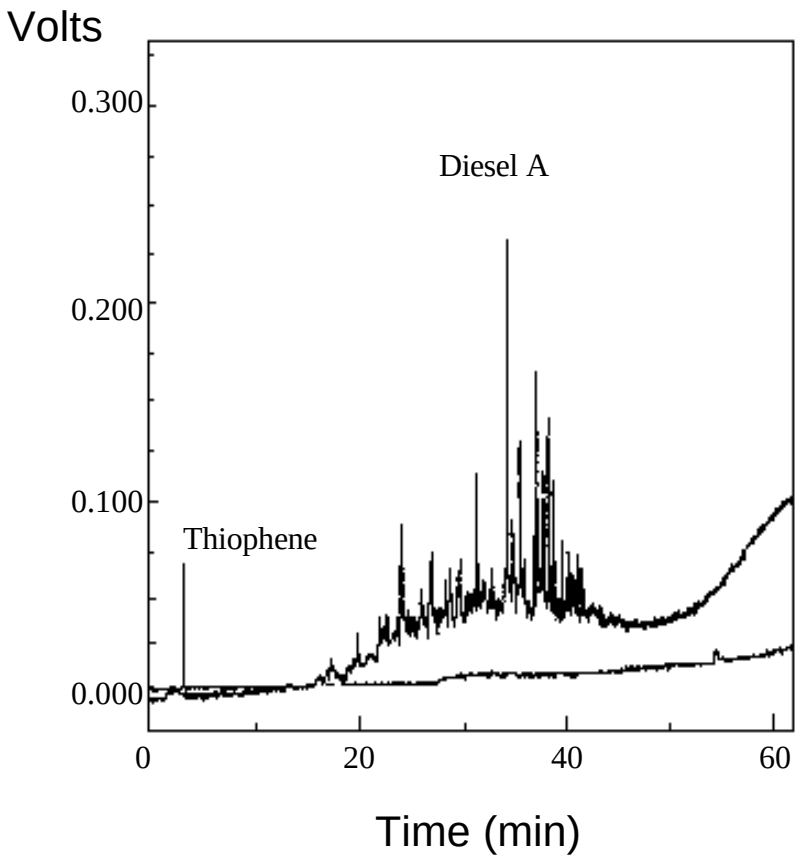


Figure 5.18 Chromatograms of diesel A and thiophene by GC-SCLD. Conditions: oven, 50 ^{o}C - 200 ^{o}C at 25 ^{o}C/min, then 200 ^{o}C - 320 ^{o}C at 3 ^{o}C/min and hold at 320 ^{o}C for 14 min; column, Rtx-1 (30-m x 0.32-mm i.d., 1.0 µm film thickness); carrier, helium at 1 mL/min; injector, on-column at 50 ^{o}C, septum purge at 0.75 mL/min, and cooling gas at 0.11 mL/min.

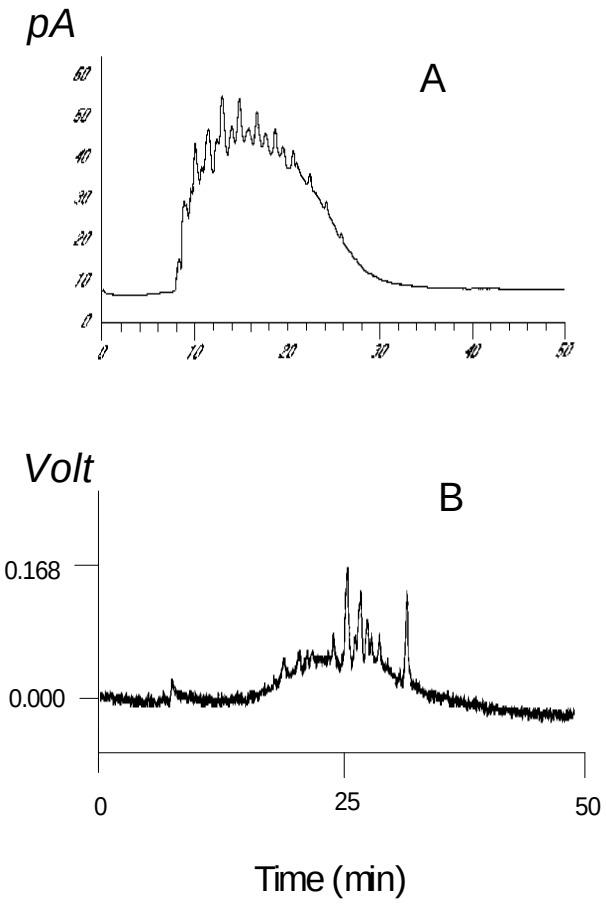


Figure 5.19 Chromatograms of diesel A by SFC-FID/SCLD. Conditions: SB-Methyl-100 column (10 m x 100 μm i.d., 0.25 μm film thickness); Oven temperature at 100 ^{o}C; Pressure Program from 100 atm to 250 atm CO_2 at a ramp of 3 atm/min; 0.05 sec time split injection. Tapered restrictors at both SCLD and FID. Post column split ratio 3:1 (SCLD to FID). A. FID, B. SCLD.

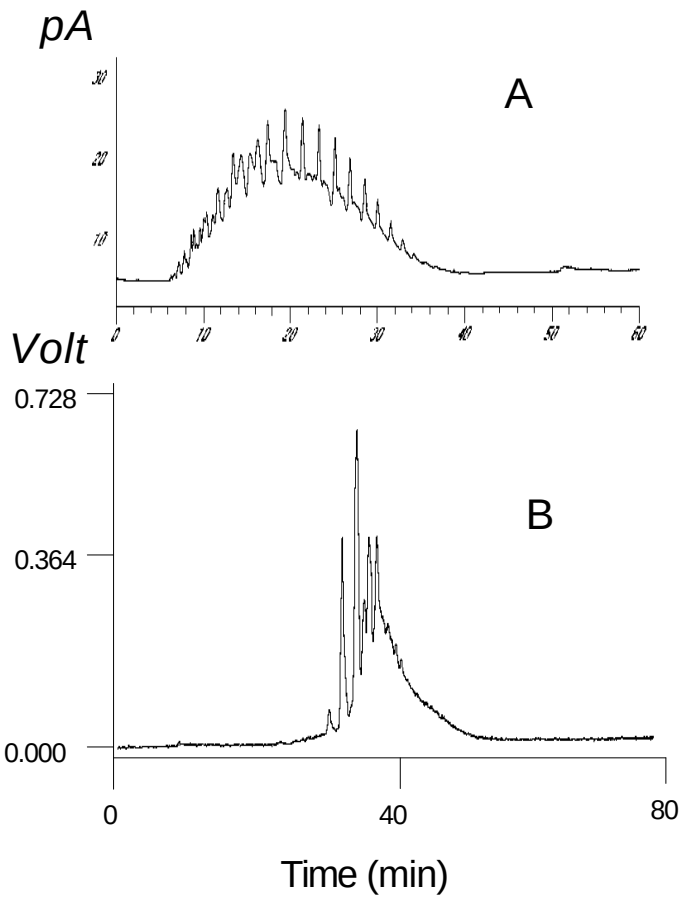


Figure 5.20 Chromatogram of heavy diesel by SFC-FID/SCLD. Conditions: SB-Methyl-100 column (10 m x 100 μm i.d., 0.25 μm film thickness); Oven temperature at 100 ^{o}C; Pressure Program from 100 atm to 280 atm CO_2 at a ramp of 3 atm/min; 0.05 sec time split injection. Tapered restrictors at both SCLD and FID. Post column split ratio 3:1 (SCLD to FID). A. FID, B. SCLD.

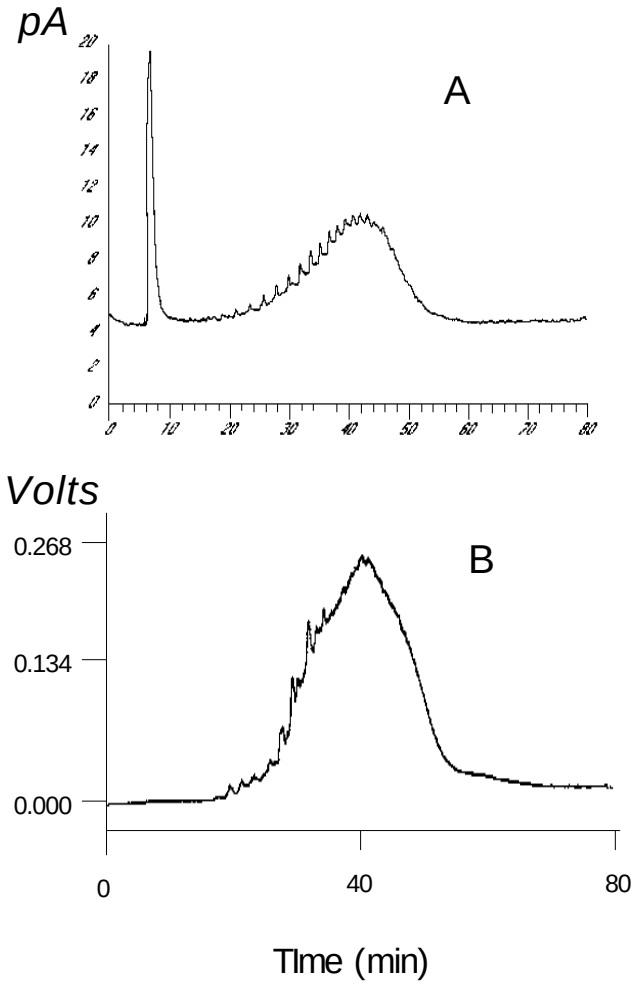


Figure 5.21 Chromatograms of vacuum gas oil by SFC-FID/SCLD. Conditions: SB-Methyl-100 column (10 m x 100 μm i.d., 0.25 μm film thickness); Oven temperature at 100 ^{o}C; Pressure Program from 100 atm to 340 atm CO_2 at a ramp of 3 atm/min; 0.05 sec time split injection. Tapered restrictors at both SCLD and FID. Post column split ratio 3:1 (SCLD to FID). A. FID, B. SCLD.

achieved. The SCLD demonstrated high sensitivity, good selectivity, a wide linear dynamic range, as well as equimolar responses to sulfur. The day-to-day repeatability was excellent with less than 10%RSD. The main advantage of the SCLD (important for trace analysis) is the detector stability and compatibility with higher decompressed CO_2 flow rates than other sulfur selective detectors presently available. The capability of dealing with heavy sulfur components also made this detector superior to other sulfur selective detectors. When SFC separation is required, the SCLD offers an excellent method for sensitive and selective detection of not only light , but also heavy sulfur species in complex sample matrices. An added advantage is that the use of methanol modified CO_2 mobile phases made it possible to analyze of polar drugs and pesticides by packed column SFC-SCLD.

Chapter VI

Summary

The objective of this research has been to extend the use of gas phase chemiluminescent nitrogen and sulfur selective detectors to supercritical fluid chromatography. Due to inherent advantages of SFC high diffusivity, low viscosity and relatively mild analysis temperature, it is believed that SFC with chemiluminescent detection provides an alternative technique for GC and HPLC for the selective detection of nitrogen- and sulfur-containing compounds. Many high molecular weight or thermally labile nitrogen- and sulfur-containing compounds are not amenable to analysis by GC, or they can be separated by HPLC but detection may pose a problem for those analytes with no chromophore. The CLND or SCLD, therefore, can be utilized as a detector for SFC in order to address these problems. The SFC- CLND and SFC-SCLD are both capable of quantitatively detecting picogram levels of nitrogen (Table 6.1) and sulfur-containing compounds. In addition to equimolar response, both detectors showed large linear range, high selectivity and compatability with gradients of both pressure and mobile phase modifier elution and have already proven themselves quite useful in SFC.

The advantages of using selective detectors are well demonstrated by the applications of SFC-CLND/FID for the separation of nitrogen-containing compound via open tubular columns. The CLND selectively detected nitrogen-containing compounds which is contained in a solvent front or at the tail of the solvent peak. Since about 80% of drugs contain nitrogen, pharmaceutical testing will especially benefit from the feasibility of packed column SFC-CLND employing methanol modified CO_2 considering fast analysis time for SFC with added advantage of savings from organic solvent, as

Table 6.1 CLND Minimum Detectable Quantity (MDQ) for GC, SFC and HPLC

System	MDQ (pg of N)	Analyte	Correlation Coefficient	References
Capillary column SFC	60	indole	0.999	
Packed column SFC	4.5×10^{2}	acetaminophen	0.999	
GC	12	nitric oxide	0.999	[1]
HPLC	5000	ammonium hydroxide	0.998	[2]

[1] L.O. Courthandon; E.M. Fujinari, *LC-GC*, 9 (1991) 732
[2] E.M. Fujinari; L.O. Courthandon, *J. Chromatogr*., 592 (1992) 209

well as from organic waste disposal.

Owing to modifications such as the arrangement of the inner and outer ceramic tubes, and the size of the furnace made in the sulfur chemiluminescent detector (SCLD), the SCLD appears to be have advantages compared to the flameless SCD as is evidenced by its compatability with higher decompress CO_2 flow rate up to 100 mL/min. The packed column SFC using the new generation of sulfur chemiluminescent detector provides many advantages such as fast analysis time and larger sample size for the analysis of trace sulfur-containing compounds in extremely complex matrices, such as those found in petroleum products. As is expected that excellent selectivity was achieved comparing the UV and SCLD chromatograms obtained from SFC for the separation of petrochemical samples. The promise of using the SCLD with packed columns with methanol modifier is also encouraging. Low nanogram levels of sulfonamides and pesticides were found to be easily detected by packed column SFC-SCLD with methanol modified mobile phase. In addition, the SCLD's high sensitivity was demonstrated by comparing the SFC-UV and SFC-SCLD chromatograms for the separation of sulfur-containing compounds that contain UV chromophore. However, difficulties were encountered for the SFC-SCLD system when water was used as a sample solvent which caused high detector noise level, as well as broadened peaks, or a decrease in chromatographic efficiency. Precipitation of nonvolatile and polar analytes poses another problem to the packed column SFC-SCLD system when modified CO_2 was used as mobile phases. The main reason for the precipitation can

be explained as solubility decrease of the nonvolatile and polar analyte in the mobile phase since modifier evaporates at the hot interface. Frequently flushing the system with mobile phase of high modifier concentration and elevated CO_2 pressure is necessary to avoid the accumulation of the precipitattion which may cause restrictor clogging. The reduction in sensitivity is inevitable when analytes deposit at the SFC-SCLD interface. Therefore, optimization of restrictor position and the temperature at the interface according to the boiling point of modifier needs to be done in the future. In this research, the restrictor tip was positioned above the furnace entrance by 15 cm. One suggestion is lowering the restrictor tip to where the tip just passes the oxygen inlet. Since the oxygen inlet is far from heated furnace plus the cooling effect from the incoming oxygen, the precipitation of nonvolatile and polar analytes may be reduced. A appropriate oven temperature could be chosen to avoid possible icing resulted from supercritical fluid CO_2.

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