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Chemiluminescence nitrogen detection for packed-column supercritical fluid chromatography with methanol modified carbon dioxide

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Abstract

The novel interface of a chemiluminescent nitrogen detector (CLND) with a packed column chromatographic system, utilizing supercritical methanol modified carbon dioxide, has been achieved. Both pressure gradients and mobile phase gradients have been successfully demonstrated. Detector optimization in terms of sensitivity and performance is described. Polymeric materials such as nitrogen containing cyclic oligomers and triazine herbicides were successfully analyzed. Pharmaceutical applications are also demonstrated using a combination of SFC–UV and SFC–CLND.

Keywords: Chemiluminescence nitrogen detection; Optimization; Detectors, SFC; Methanol; Carbon dioxide; Oligomers, nitrogen containing; Triazine herbicides

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 and coworkers uses low temperature catalytic decomposition of N-nitroso- and nitrocompounds followed by chemiluminescence detection with ozone [1–3]. The TEA has been coupled to both GC [4–7] 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 nitrocompounds 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 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 at least 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 en-

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vironmental and petroleum samples have been reported by Britten [13] and Fujinari and co-workers [14–17]. Taylor et al. recently coupled open tubular SFC with CLND and reported the detection of nitrogen-containing compounds found in foods and flavors. A minimum detectable quantity of 60 pg of nitrogen with a dynamic range of at least 3 orders of magnitude was found [18]. A simultaneous detection system using the CLND and flame ionization detector (FID) for open-tubular SFC was also accomplished [19]. In this paper, a new interface between the CLND and a packed-column SFC system will be described for the first time and evaluated, showing its utility for especially pharmaceutical applications with methanol modified CO_2 as the mobile phase.

2. Experimental

2.1. Apparatus

A chemiluminescent nitrogen detector Model 705D with a Model 771 pyrolysis system from Antek Instruments (Houston, TX, USA) was interfaced to a Model G1205 supercritical fluid chromatographic system with a multiple wavelength UV detector from Hewlett–Packard (Little Falls, DE, USA). An auxiliary 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. (Apple Valley, MN, USA). 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°C; photomultipiler (PMT) voltage, 720 volts; range, ×50; detector output, 1 mV. 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.

2.2. 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 (Milwaukee, WI, USA). Caffeine was purchased from Sigma (St. Louis, MO, USA). Drug Standard Mixture 2 and the corresponding single components (glutethimide, meprobamate, pentobarbital, phenobarbital and secobarbital) were purchased from Supelco (Bellefonte, PA, USA). The sample of cyclic oligomers was obtained from a polymer research group at Virginia Tech. (Blacksburg, VA, USA). All chemicals were used without further purification. HPLC grade solvents from EM Science (Gibbstown, NJ, USA) were used for preparing standard solutions. Grade 4.3 oxygen from Airco (Murray Hill, NJ, USA) was used for the CLND pyro-furnace and ozone-generator gas. SFC-grade CO_2 (without helium head space) was obtained from Air Products and Chemical (Allentown, PA, USA).

3. Results and discussion

3.1. Detector sensitivity optimization

An increasing number of industrial applications utilizing supercritical fluid chromatography have been reported. 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. 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 the interface between the chemiluminescent nitrogen detector and the packed-column SFC system (Fig. 1). Supercritical methanol modified CO_2 was used as the mobile phase. A linear restrictor (25 mm 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°C in order to oxidize the eluting nitrogen-containing compounds to NO. The chemiluminescence resulting from the interaction of NO and O_3 in the reduced pressure reaction chamber

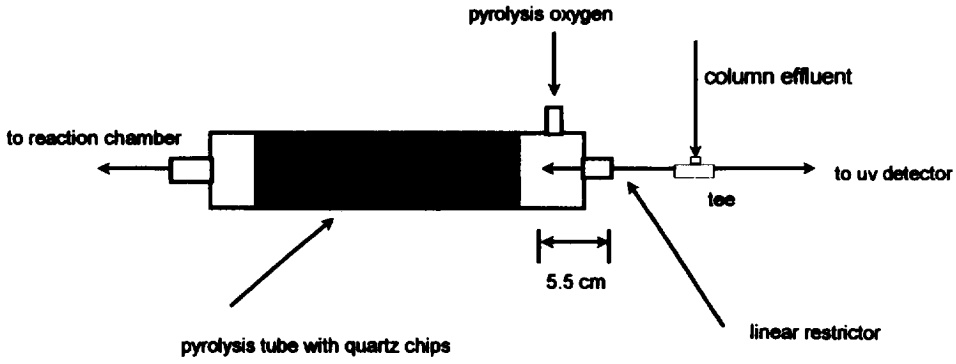

Fig. 1. Schematic diagram of the packed-column SFC–CLND interface.

was detected by the photomultiplier tube (PMT). The detection mechanism is believed to be as follows:

$$R\text{–}N + O_2 \rightarrow NO + \text{other products}$$

$$NO + O_3 \rightarrow NO_2^* + O_2$$

$$NO_2^* \rightarrow NO_2 + h\nu.$$

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 how efficient the column effluent was being mixed with oxygen to achieve the necessary oxidation in the furnace. Plots of the CLND responses (area counts) of the indole standard vs. restrictor position, with respect to the start point of the fitting (see Fig. 1) for 100% CO_2 and 10% methanol modified CO_2 mobile phases, are presented in Fig. 2. Maximum CLND response was found to occur 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 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 a similar decompressed CO_2 flow-rate. Results (Fig. 3) 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 pyrolysis oxygen and decompressed CO_2 flow-rates, the next phase in our study was to test the

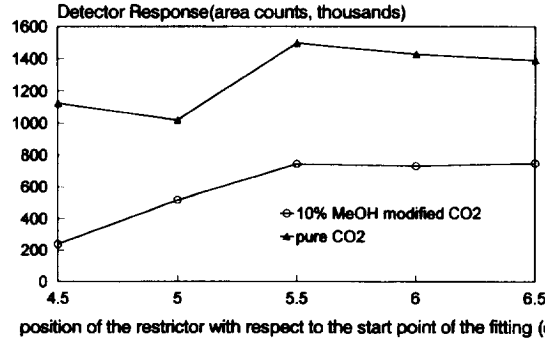

Fig. 2. CLND optimization of restrictor position at the SFC interface. Conditions: 60°C, flow injection analysis (FIA); pressure at 320 atm; decompressed CO_2 split ratio of 8 to 1 (UV/CLND); 25 mm I.D. linear restrictor; sample: indole in methanol.

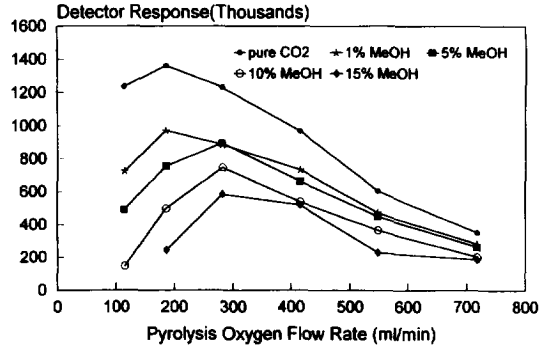

Fig. 3. CLND optimization of pyrolysis oxygen flow-rate. Conditions: 60°C, FIA; pressure at 320 atm; decompressed CO_2 split ratio of 8 to 1 (UV/CLND); 25 mm I.D. linear restrictor; sample: indole in methanol.

effects of methanol modified decompressed CO_2 flow-rates on the CLND response (Fig. 4). The decompressed CO_2 with methanol flow-rate can affect the detector response in several ways. Some variables to consider are: (1) residence time of analyte(s) will be decreased in the pyro-furnace (decrease in CLND response) by 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. At high decompressed CO_2 flow-rates, a short residence time for analyte(s) and methanol (as well as sample matrix) in the furnace

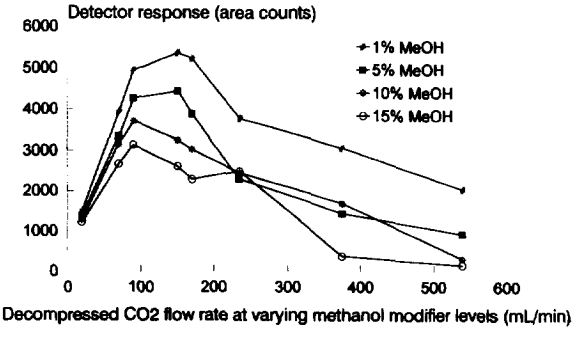

Fig. 4. Profiles of methanol modified decompressed carbon dioxide flow-rate vs. CLND response for packed-column SFC. Conditions: 50°C FIA; pressure at 280 atm; Deltabond CN (250×4.6 mm I.D., 5-μm particle size) column; indole sample with 23.9 ppm of nitrogen in methanol.

was anticipated. In turn, the uncombusted methanol and other organic compounds entered the reaction chamber causing a significant decrease in detector response. Optimum decompressed CO_2 flow-rates for 1–5% and 10–15% (v/v) methanol modifier were found to be at 150 and 90 ml/min, respectively. It should be noted here that no attempt was made to obtain a global optimum, but just to examine the effects of the variables in turn.

3.2. 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 herein. Detector selectivity of 10^7 (N/C ratio) was observed by injecting a solution containing 0.67 ppm indole in to the 5% methanol modified mobile phase at 250 atm. No solvent peak was found under these conditions. Minimum detectable quantities of nine nitrogen-containing compounds at a signal-to-noise ratio equal/greater than two are provided in Table 1. Different experimental 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

Table 1
Minimum detectable quantities by packed-column SFC–CLND

Analyte	MDQ at CLND[a] (pg N)	Conditions[b]
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

[a] Signal-to-noise ratio of 2–3.

[b] A: Deltabond cyano (250×4.6 mm I.D., 5-μm particle size) column, 50°C oven, 250 atm pressure, 5% (v/v) methanol modifier, split ratio of 15.7 to 1 (UV/CLND). B: Deltabond cyano (250×4.6 mm I.D., 5-μm particle size) column [except Amino 1 (150×4.6 mm I.D., 5-μm particle size)] was used for diphenhydramine, 50°C oven. 280 atm pressure, 12% (v/v) methanol modifier, split ratio of 11.0 to 1 (UV/CLND).

Table 2
Relative response factors (RRF)[a] for CLND from flow injection analysis as a function of methanol modifier percentage

Analyte	Methanol percentage			
	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°C, CO_2 pressure at 320 atm, decompressed CO_2 flow split ratio of 8 to 1 (UV/CLND).
[a] RRF = area counts per ppm N of compound/area counts per ppm N of indole.

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 three orders of magnitude. Calibration curves using sulfanilamide and acetaminophen (1–1000 ppm of nitrogen) standards in methanol-modified CO_2 eluent, showed linear correlation coefficients greater than 0.9999. Response factors for

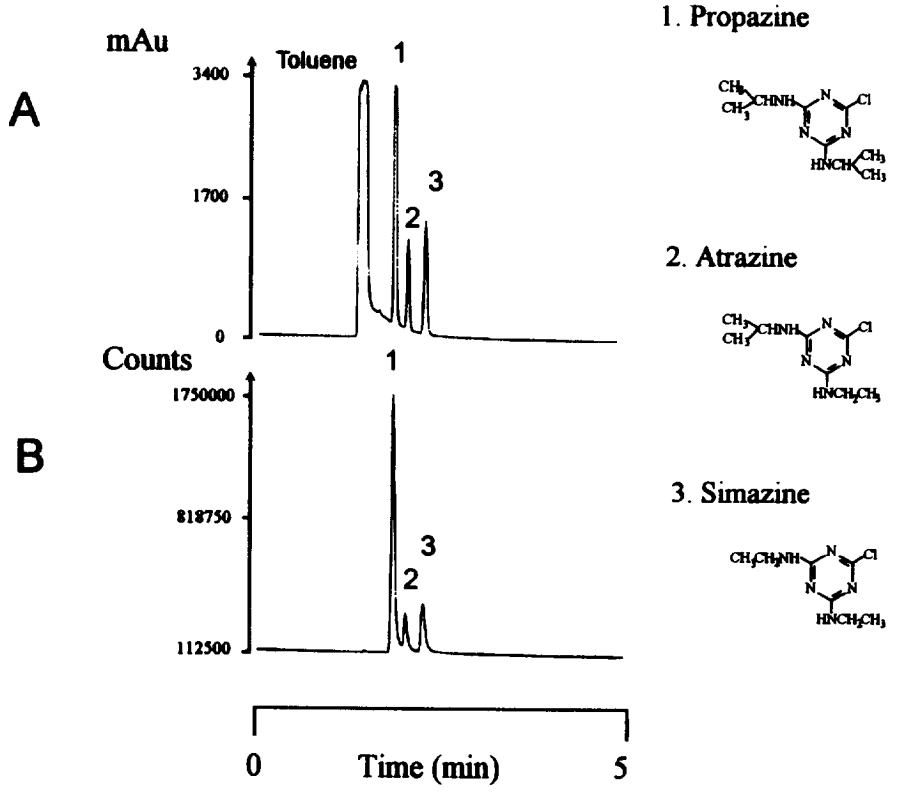

Fig. 5. Packed-column SFC–CLND/UV profile of triazine herbicides. Conditions: 70°C oven; pressure program: 250–280 atm at 10 atm/min; methanol modifier: 10% (v/v); Hypersil silica (250×4.6 mm I.D., 3-μm particle size) column; 5-μl injection loop; liquid CO_2 flow-rate of 2.5 ml/min; decompressed CO_2: 1460 ml/min at UV and 160 ml/min at CLND; toluene is sample solvent. (A) UV detection at 219 nm. (B) SFC–CLND.

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 2). Equimolar nitrogen response was observed by SFC–CLND at each of the methanol modifier concentrations, just as had been observed previously with 100% CO_2. A mean response factor value of 1.02 (R.S.D. = ±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.

3.3. Applications

Triazine herbicides by capillary SFC using 100% CO_2 are immobile because of their polarity and can not be chromatographed. However, packed-column SFC using 10% methanol modified CO_2 and pressure programming can readily elute these compounds

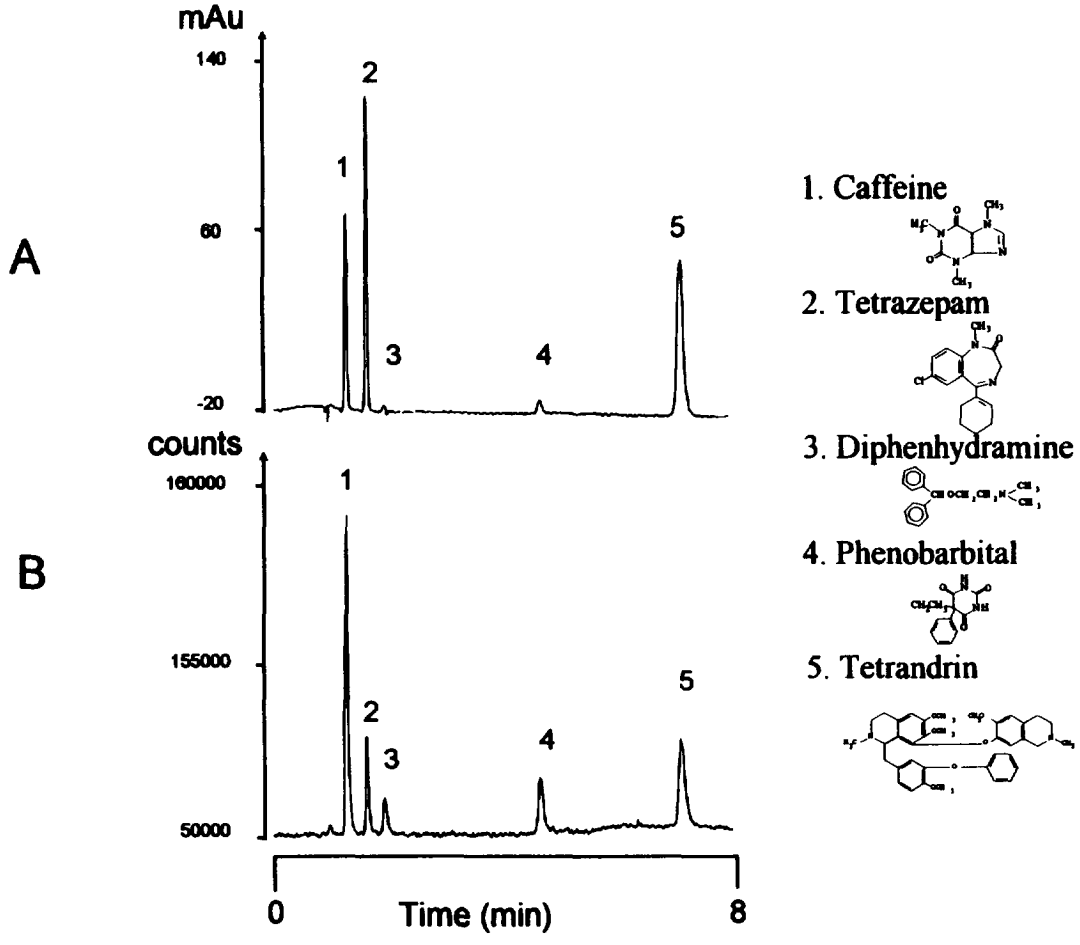

Fig. 6. Packed-column SFC–CLND/UV profile of a pharmaceutical mixture. Conditions: 50°C oven; pressure program: 250–300 atm at 10 atm/min, and hold; methanol modifier program: 8% (v/v) hold 2 min, ramp to 15% at 1.5%/min, and hold; Valuepak amino (150×4.6 mm I.D., 5-μm particle size) column; 5-μl injection loop; liquid CO_2 flow-rate of 2.0 ml/min; decompressed CO_2 of 1200 ml/min at UV and 160 ml/min at CLND (25 mm I.D. integral 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.

[20]. Using this approach with simultaneous CLND (nitrogen-specific mode) and UV (219 nm), detection of propazine, atrazine and simazine is shown in Fig. 5.

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. Fig. 6 demonstrates the usefulness of this technique as a confirmatory method, since caffeine, tetrazepam, diphenhydramine, phenobarbital and tetrandrin each contain both aromatic UV chromophores and nitrogen functional groups. Both pressure and modifier programs are used to elute these polar compounds. The retention times in minutes (UV/CLND) for these 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 Fig. 7. Because of the high polarity of these compounds, a constant modifier concentration as high as 15% (v/v) was used to elute

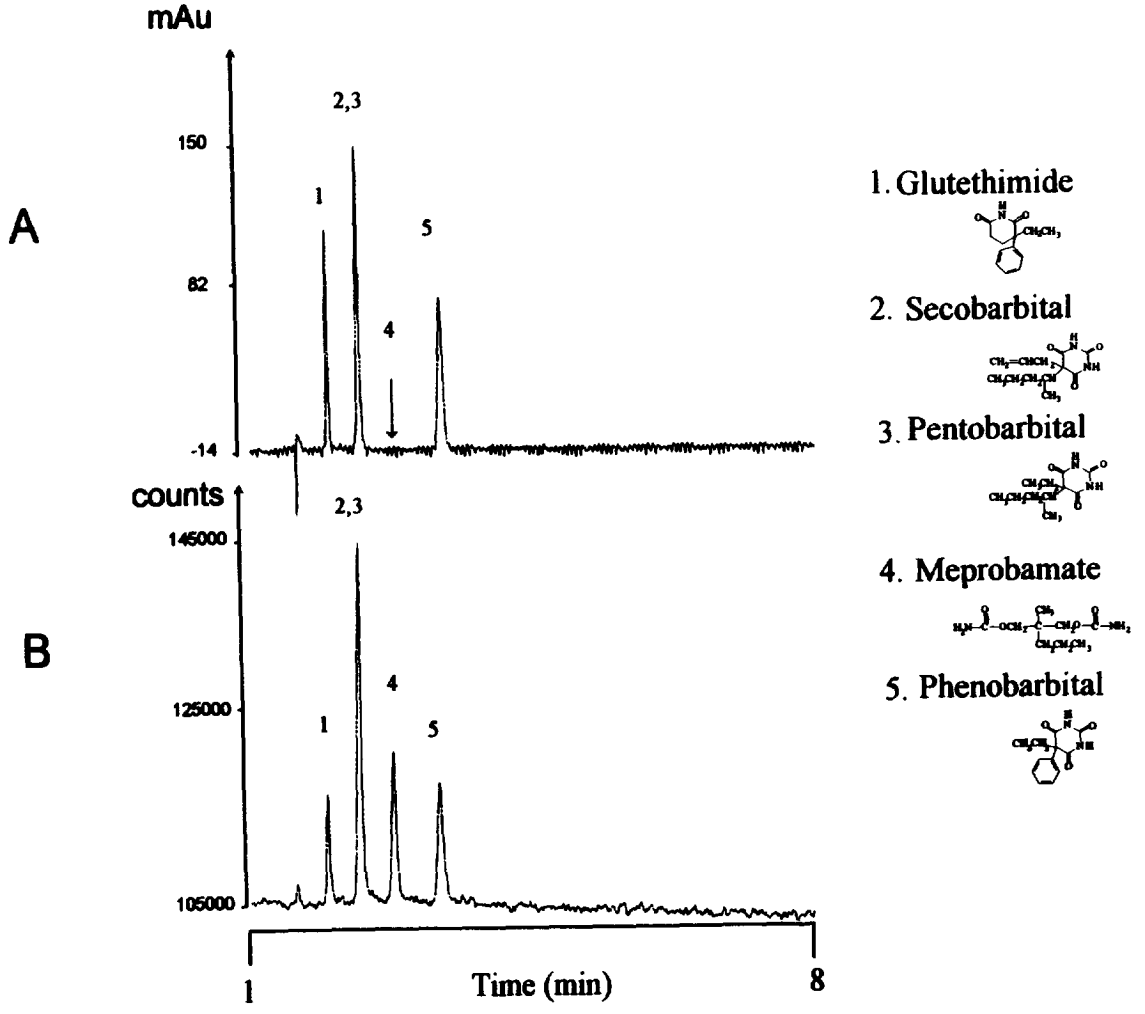

Fig. 7. Packed-column SFC–CLND/UV separation of sedatives. Conditions: 50°C oven; pressure 250 atm; methanol modifier: 15% (v/v); Amino 1 (150×4.6 mm I.D., 5-μm particle size) column; 5-μl injection loop; liquid CO_2 flow-rate of 2.5 ml/min; decompressed CO_2 of 1300 ml/min at UV and 120 ml/min at CLND; methanol as sample solvent; sample concentration of 50 ppm of each component. (A) UV detection at 219 nm. (B) SFC–CLND.

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.

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 (Fig. 8). These oligomers are not volatile and consequently can not be separated by GC. Although these compounds can be chromatographed by HPLC, detection is a problem because they are void of UV chromophores (Fig. 8A). The CLND profile of the cyclic nitrogen-containing oligomer conformational mixture is shown in Fig. 8B. Peak identification and characterization of these oligomers were elucidated by Si^{29} NMR spectroscopy.

4. Conclusion

The 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 wide linear dynamic range. Packed-column SFC–CLND provides another alternative for chromatographic separation, and at the same time can unleash

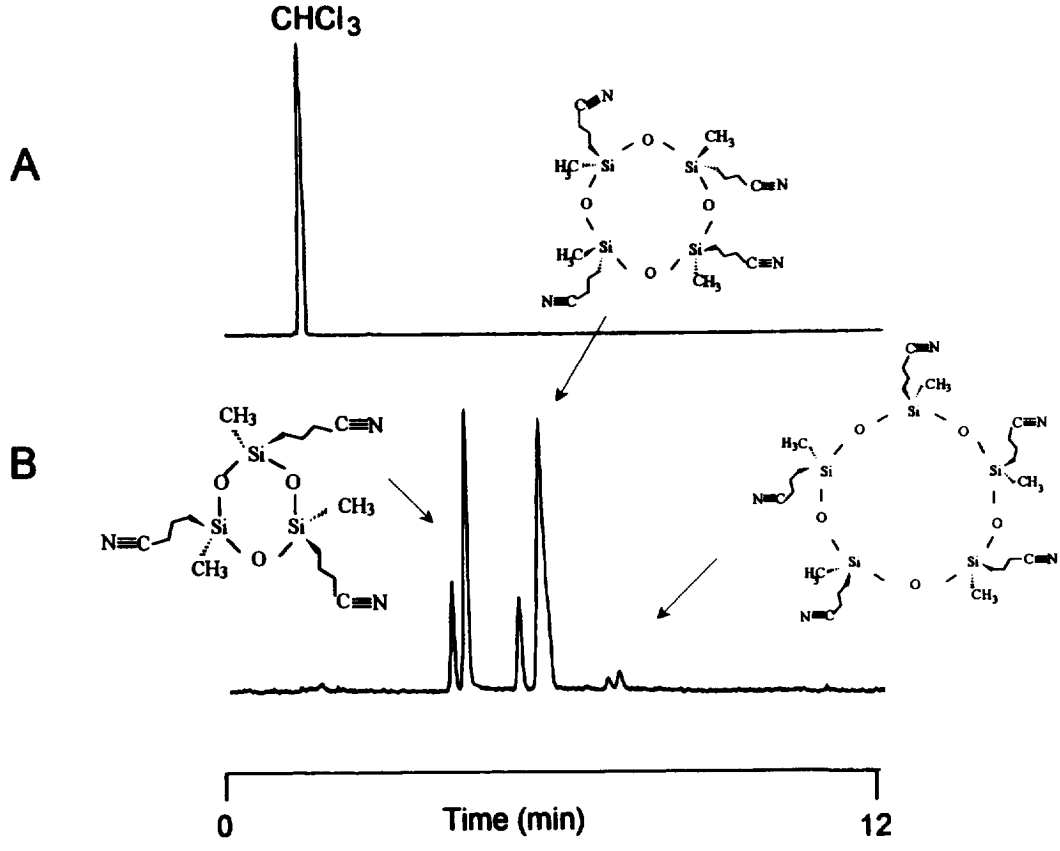

Fig. 8. Packed-column SFC–CLND/UV profile of cyclic oligomers. Conditions: 60°C oven; pressure program: hold 2 min, 150–250 atm at 15 atm/min and hold; methanol modifier program: 5% (v/v) hold 1 min, to 14% at 1% min, and hold; Hypersil silica (250×4.6 mm I.D., 3-μm particle size) column; 5-μl injection loop; liquid CO_2 flow-rate of 2.0 ml/min; decompressed CO_2 of 980 ml/min at UV and 120 ml/min at CLND (25 mm I.D. integral restrictor); chloroform as sample solvent; total sample concentration of 7 mg/ml. (A) UV detection at 219 nm. (B) SFC–CLND.

the power of nitrogen detection by simplifying analysis of complex samples.

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