Packed-Column Supercritical Fluid Chromatography with Chemiluminescent Nitrogen Detection at High Carbon Dioxide Flow Rates

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Abstract

The use of chemiluminescence nitrogen detection (CLND) coupled with packed-column supercritical fluid chromatography is investigated. The pyrolysis tube design, position of the restrictor, and reaction chamber pressure are shown to affect the response of the detector. By modifying the pyrolysis chamber and controlling the pressure at the reaction chamber, the response of the detector remains constant when different concentrations of methanol modifier are used. This detector design also tolerates high flow rates of decompressed CO_2. As a result, no post-column split is required and the total column effluent is delivered to the CLND. Sensitive detection (limit of detection = 1 ng nitrogen) is achieved at the high flow rates of decompressed CO_2 with 8% methanol-modified CO_2.

Introduction

Chromatographic detectors based on chemiluminescence are becoming popular because they are very selective and sensitive. Because these detectors are selective for specific classes of analytes (i.e., sulfur-containing or nitrogen-containing), the desired analytes can be quantitated in complex matrices without complete chromatographic resolution. This type of selective detection can simplify or eliminate the need for sample preparation and provide qualitative and quantitative information for individual components.

Detectors based on chemiluminescence have been interfaced with gas chromatography (GC) (1–5) and high-pressure liquid chromatography (6–10). More recently, the chemiluminescence nitrogen detector (CLND) (11) has been successfully interfaced with supercritical fluid chromatographs (SFC) (12–15). The CLND selectively detects nitrogen-containing compounds by monitoring the chemiluminescence produced by the following series of reactions (16):

$$R\text{–}N + O_2 \rightarrow NO + \text{other compounds}$$
$$NO + O_3 \rightarrow NO_2^* + O_2$$
$$NO_2^* \rightarrow NO_2 + h\nu$$

where $h\nu$ is the energy emitted as light.

SFC–CLND was first reported by Shi et al. (13) using an open tubular capillary column. These authors found that the response of the detector was affected by the restrictor position and oxygen gas flow rate, and the response of the detector was not affected by pressure programming (increased CO_2 flow rate). A linear dynamic range of three orders of magnitude and a detection limit of 60 pg nitrogen were reported. In a later study, Shi et al. (15) described a packed-column SFC–CLND system. They found that the addition of methanol decreased the response of the detector, and their detector design was limited to a decompressed CO_2 flow rate of 10–150 mL/min at the detector. To achieve these flow rates, a post-column split was performed to reduce the amount of mobile phase being delivered to the CLND. This also reduced the amount of sample that reached the detector. Though low detection limits were reported (213 pg nitrogen as indole at the detector), post column splits of 10:1 to 15:1 were needed for optimum sensitivity. To expand the utility of the CLND as an SFC detector, it must be capable of handling higher flow rates of decompressed CO_2.

This paper reports on an SFC–CLND system using a modified pyrolysis tube that can tolerate high flow rates of decompressed CO_2 (100–600 mL/min). The effects of methanol composition and oxygen gas flow rate were also studied. In addition, the analysis of nitrogen-containing steroids by SFC–CLND is presented.

Experimental

Instrumentation

The instrumentation used for this study consisted of a Hewlett Packard (Little Falls, DE) HP SFC 5890A in the down-

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stream mode equipped with an HP GC SFC 7673 injector with a 20-µL injection loop, an HP G1205A pumping module, and an HP 1050 photodiode array detector. The data acquisition was performed using an HP 486/66XM Vectra computer along with the HP Chemstation software (revision A.01.02). Chromatography was performed using a Zorbax RX-C_8 column (25 × 0.46-cm i.d.) purchased from MAC-MOD Analytical (Chadds Ford, PA). An unmodified CLND model 7000B from Antek Instruments (Houston, TX) was used for nitrogen selective detection. The pyrolsis tube of the CLND was heated to 1100°C. The detector photomultiplier was chilled to 5°C and set to a voltage of 785 V. The outlet of the photodiode array detector was connected to the CLND via an integral restrictor (75-µm fused silica, manufactured in-house) (Figure 1). The flow rate of the system was held constant by the pump control software. Because the system pressure is maintained by the integral restrictor, pressure programming cannot be used with this system without changing the flow rate. All pressures related to the chromatographic system reported here are the inlet pressures at the pump.

Chemicals

High-performance liquid chromatography-grade methanol was purchased from Fisher Scientific (Springfield, NJ). Pyridine solutions were provided by Antek Instruments. All the solvents were used without further purification. The compounds used in this study were synthesized by Process Research of Merck Research Laboratories (Rahway, NJ).

Results and Discussion

Detector optimization

In the initial investigation, we used the pyrolysis tube depicted in Figure 1B with the restrictor placed inside the inner quartz tube. The depth of the restrictor in the small quartz tube was found to be important. The restrictor should be far enough inside the furnace to allow the analytes to be vaporized but not so far that the restrictor melts. With this pyrolysis tube, we found that the restrictor had a tendency to rest against the quartz tube. When this occurred, the response of the detector decreased. This was caused by the restrictor spraying the effluent and analytes onto the side of the small quartz tube, resulting in a loss of the analyte. Therefore, a new pyrolsis tube was used (Figure 1C).

The tip of the restrictor was positioned just inside the inner

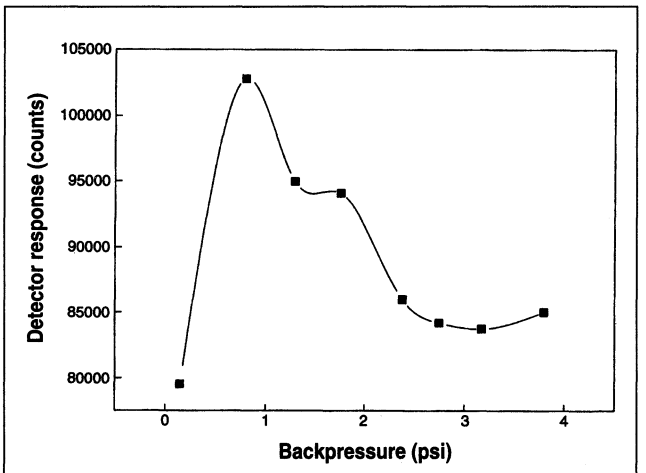

Figure 2. Effect of backpressure on the response of the CLND. SFC conditions: pressure, 250 bar; flow rate, 1.0 mL/min (liquid CO_2); oven temperature, 35°C; 8% (v/v) methanol-modified CO_2; injection loop, 20 µL; integral restrictor, 75-µm i.d. fused silica. CLND conditions: pyrolysis tube temperature, 1100°C; oxygen flow rate, 50 mL/min; ozone flow rate, 10 mL/min. The response of a 100-µg/mL solution of MK-0386 dissolved in methanol was measured.

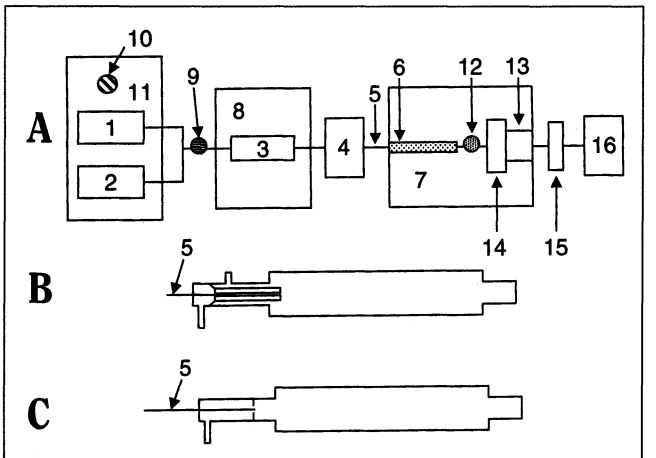

Figure 1. SFC–CLND system (A), the pyrolysis tube initially used (B), and the modified pyrolysis tube (C). Equipment identification: 1, CO_2 pump; 2, modifier pump; 3, column; 4, photodiode array detector; 5, integral restrictor; 6, pyrolysis tube; 7, CLND; 8, SFC oven; 9, injector; 10, variable restrictor; 11, SFC pump module, 12, backpressure control valve; 13, photomultiplier tube; 14, reaction chamber; 15, A/D converter; 16, computer for data storage and processing.

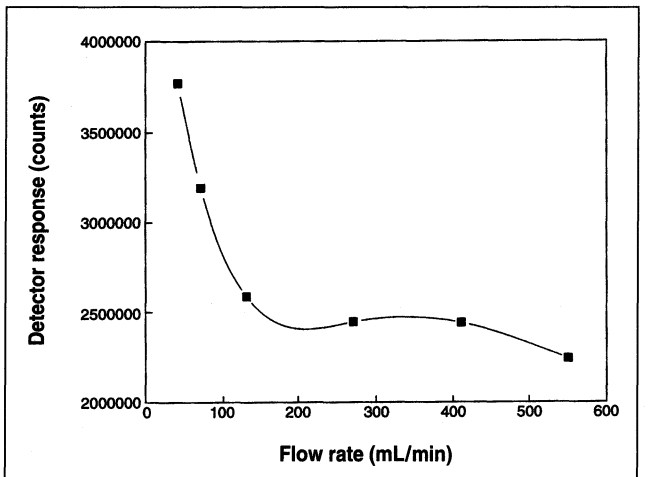

Figure 3. Effect of oxygen gas flow rate on the response of the CLND. SFC conditions: pressure, 250 bar; flow rate, 1.0 mL/min (liquid CO_2); oven temperature, 35°C; 8% (v/v) methanol-modified CO_2; injection loop, 20 µL; integral restrictor, 75-µm i.d. fused silica. CLND conditions: pyrolysis tube temperature, 1100°C; backpressure, 1.3 psi; ozone flow rate, 10 mL/min. The response of a 100-µg/mL solution of MK-386 dissolved in methanol was measured.

orifice of the quartz tube. In this position, the restrictor sprayed the effluent and analytes directly into the pyrolysis tube where the effluents and analytes were oxidized. The pyrolysis tube and restrictor were positioned in the furnace so that the restrictor tip was heated but did not melt. This pyrolysis tube was utilized in the remaining experiments.

Shi et al. (15) found that the introduction of methanol into the CLND reduced the response of the detector. One explanation for this is that the chemiluminescence reaction for nitrogen is a pressure-sensitive reaction. Therefore, the pressure at the reaction cell should be controlled, especially when modifiers are used. When the modifier is vaporized, the pressure of the overall system will increase, which can reduce the performance of the detector. One method of stabilizing the pressure at the detector is to restrict the flow entering the detector. This was accomplished by using a gas backpressure valve (Figure 1). The valve controls the flow of gas entering the reaction cell. By closing the valve, the pressure of the reaction cell decreases while the pressure of the pyrolysis tube increases. The backpressure of the system is monitored at the pyrolysis tube. The effect of backpressure was studied using a 100-µg/mL solution of MK386, an azasteroid, dissolved in methanol at an oxygen gas flow rate of 400 mL/min. A maximum response was observed with a backpressure of 0.8 psi (Figure 2). The response slowly declined with pressures above 1.3 psi. A backpressure of 0.8–1.3 psi was used for all remaining experiments.

The effect of oxygen gas flow rate in the pyrolsis tube was also studied by varying the oxygen flow rate from 50 to 550 mL/min at a constant backpressure of 1.3 psi. The peak area obtained for a 100-µg/mL solution of MK386 in methanol was measured at the varying oxygen flow rates. The lower oxygen flow rates (< 100 mL/min) yielded higher detector responses (Figure 3). These results were similar to the open tubular SFC–CLND work of Shi et al. (13). The optimum setting for the oxygen flow rate was 50 mL/min. This flow rate was used for the remaining experiments.

Once the oxygen gas flow rate and backpressure were optimized, the effect of methanol concentration was investigated (Figure 4). A 50-µg/mL solution of pyridine in methanol was used. The response of the detector was found to remain constant within experimental error over the methanol concentration of 2–10% (v/v) methanol-modified CO_2. This result contradicts the previous work of Shi et al. (15). It is likely that the backpressure valve helps reduce the effect of methanol on the system by maintaining a lower, more stable pressure at the reaction cell.

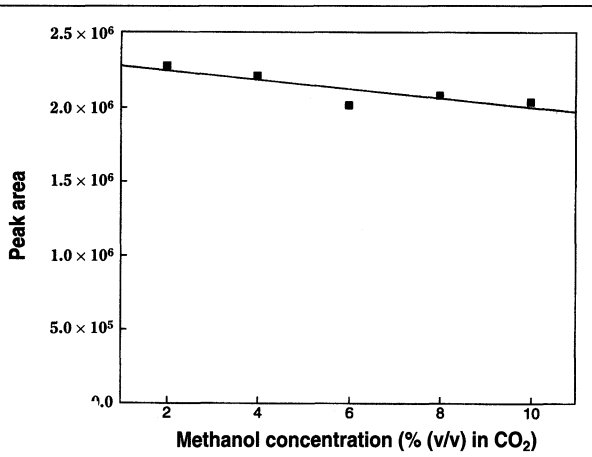

Figure 4. Effect of methanol concentration on the response of the CLND. SFC conditions: pressure, 250 bar; flow rate, 1.0 mL/min (liquid CO_2); oven temperature, 35°C; injection loop, 20 µL; integral restrictor, 75-µm i.d. fused silica. CLND conditions: pyrolysis tube temperature, 1100°C; backpressure, 1.0 psi; oxygen flow rate, 50 mL/min; ozone flow rate, 10 mL/min. The response of a 50-µg/mL solution of pyridine dissolved in methanol was measured.

Detector performance

The SFC–CLND system was evaluated by determining the linear dynamic range (LDR), limit of detection (LOD), and injection precision. A calibration curve for MK386 was

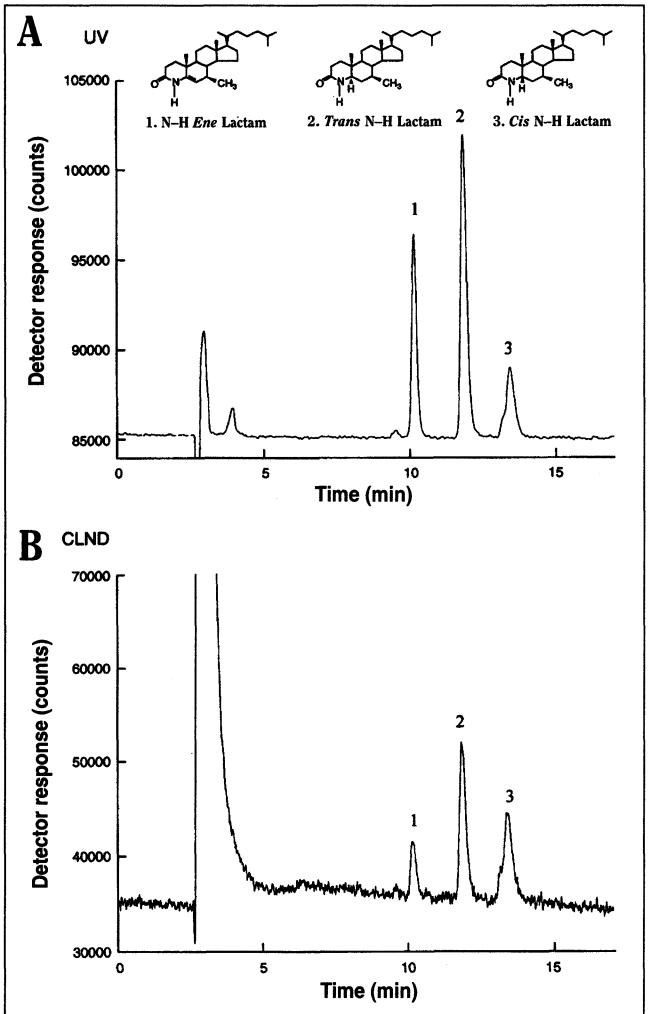

Figure 5. Separation of *cis, trans,* and *ene* MK386 derivatives. Peak identification: 1, N–H-*ene* Lactam (13.9 µg/mL; 0.49 µg/mL nitrogen); 2, *trans*-N–H Lactam (22.3 µg/mL; 0.78 µg/mL nitrogen); 3, *cis*-N–H Lactam (8.3 µg/mL; 0.29 µg/mL nitrogen). SFC conditions: pressure, 250 bar; flow rate, 1.0 mL/min (liquid CO_2); oven temperature, 35°C; 8% (v/v) methanol-modified CO_2; injection loop, 20 µL; integral restrictor, 75-µm i.d. fused silica; ultraviolet detection at 215 nm. CLND conditions: pyrolysis tube temperature, 1100°C; backpressure, 1.2 psi; oxygen flow rate, 50 mL/min; ozone flow rate, 10 mL/min.

constructed from standards ranging from 3.7 to 370 µg/mL nitrogen. Regression of the curve resulted in a correlation coefficient of 0.9997 with an LDR of three orders of magnitude. The injection precision was found to be < 3% relative standard deviation for the calibration standards. An LOD (signal-to-noise ratio = 3) was calculated to be 1 ng nitrogen for MK386 (600 mL/min decompressed CO_2 or 1 mL/min liquid at 250 bar and 8% methanol-modified CO_2; integral restrictor, 75-µm i.d. fused silica). The CLND pyrolysis tube temperature was 1100°C. The oxygen flow rate was 50 mL/min with a back-pressure of 1.5 psi and an ozone flow rate of 10 mL/min.

Applications

The analysis of MK386 and L-751,788 derivatives (Figures 5 and 6) was undertaken using the SFC–CLND system. An ultraviolet photodiode array detector (PDA) was incorporated into the system between the column and CLND. The PDA was used to evaluate the effect that the CLND had on peak shape. The peak shapes observed with the PDA and the CLND were similar, indicating that no additional band broadening was occurring in the CLND. The separation of *cis*, *trans*, and *ene* lactams of MK386 was accomplished using a Zorbax RX-C_8 column with 8% methanol-modified CO_2 at 600 mL/min decompressed CO_2 (Figure 5). The solvent peak was observed in the CLND, which indicated a nitrogen contamination in the solvent or injector. Using the same chromatographic conditions, the separation of L-751,788 and its derivatives (N–H, 7-desmethyl, and *p*-Br) was accomplished (Figure 6). All four peaks were seen by both detectors, but the N–H derivative was close to the detection limit of the CLND.

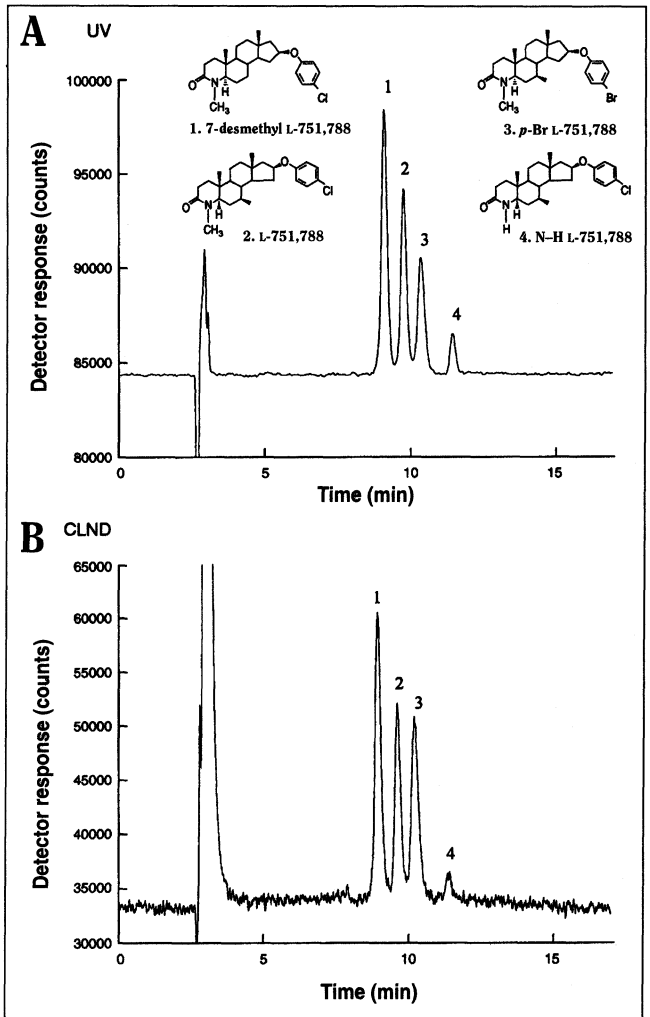

Figure 6. Separation of L-751,788 and derivatives. Peak identification: 1, 7-desmethyl (15 µg/mL); 2, L-751,788 (14 µg/mL); 3, *p*-Br (11 µg/mL); 4, N–H (2 µg/mL). SFC-CLND conditions listed in Figure 5.

Conclusion

High flow rates of decompressed CO_2 can be used with CLND. As a result, no post-column split is required and the total column effluent is delivered to the CLND. The pyrolysis tube design and position of the restrictor were found to affect the response of the detector. By controlling the pressure at the reaction chamber, the response of the detector remained constant when different concentrations of methanol modifier were used. Sensitive detection (LOD = 1 ng) was achieved at the high flow rates of decompressed CO_2 with 8% methanol-modified CO_2. SFC–CLND is an alternative technique for detecting nitrogen-containing compounds with little or no chromophore, such as azasteroids.

Acknowledgments

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