Evaporative Light Scattering Detection for Supercritical Fluid Chromatography

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Abstract

A high-performance liquid chromatograph-evaporative light scattering detector is modified and interfaced with a supercritical fluid chromatograph. The detector performance is evaluated by monitoring the response of several steroids. Specifically the effects of nitrogen makeup gas flow rate, carbon dioxide modifier type, modifier concentration, evaporative light scattering detector orifice size, and detector temperature are determined. As the nitrogen gas flow rate increases, the response of the analyte decreases, but the increased flow improves peak shape to mimic that obtained with ultraviolet detection. Furthermore, increasing the detector temperature causes the response of the analytes to decrease. A detection limit of 10 ng or less is determined for progesterone and testosterone with 2% and 20% (v/v) methanol-modified carbon dioxide on a Deltabond cyano column (4.6 mm × 15 cm, 5 µm) at 150 mL/min and 1000 mL/min decompressed carbon dioxide. The separations of polyethylene glycols and *ginko biloba* leaf extract with the optimized conditions are reported.

Introduction

In packed-column supercritical fluid chromatography (SFC), modifiers and additives (i.e., organic solvents, acids, and bases) are frequently required to efficiently elute polar analytes off the column (1). The modifiers and additives perform this function by either increasing the solubility of the analyte in the supercritical fluid or reducing the activity of the column. Unfortunately, the introduction of modifiers and additives can interfere with the use of some detectors including flame-ionization detectors (FID) or ultraviolet (UV) detectors (1). Therefore, a detector is needed in which the modifier does not interfere and detection is possible. One such detector is the evaporative light scattering detector (ELSD), which responds to the light scattered by nonvolatile analytes after the mobile phase has been evaporated (2–5).

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The first reports of packed-column SFC–ELSD were by Carraud and co-workers (6) and Nizery and co-workers (7). The interface was a modified version of that used in high-pressure liquid chromatography (HPLC). The nebulizer that atomizes the mobile phase in HPLC applications was thought to be unnecessary in SFC applications since the restrictor that controls pressure and flow rate in SFC was believed to perform like a nebulizer. At first, a tapered stainless steel tube was used as the restrictor, but it was difficult to reproducibly control the pressure and flow rate (6). It was replaced with a linear restrictor, which was a short length of fused silica (60–200 mm × 75-µm i.d.). The response of the detector was found to be highly dependent on the flow rate of the decompressed CO_2. A maximum response at 2.7 L/min decompressed flow was observed (6), but this high flow rate was not practical for most analytical-scale packed-column (4.6-mm i.d.) SFC analyses. At the higher flow rates, the chromatographic resolution of nonpolar to moderately polar analytes would suffer since the resulting liquid flow rate of 4.5 mL/min was much higher than the widely used value of 2.0 mL/min (1).

Furthermore, in these early studies, the Joule-Thomson cooling effect at the high decompressed CO_2 flow rates caused methanol ice to form, which increased the background noise and decreased the performance of the detector. To alleviate this problem, the tip of the restrictor was placed inside a heated brass ring that heated the restrictor and prevented the methanol ice formation (7). However, the formation of methanol ice could not be completely eliminated when the methanol content was increased from 2.8% (w/w) to 10% (w/w). Methanol ice formation caused by the additional methanol was reduced by increasing the drift tube temperature, but the detector's performance was also reduced. Nizery and co-workers (7) found that the formation of methanol ice and resulting noise could be minimized without affecting the performance of the detector by heating a small section of tubing after the tip of the restrictor but before the drift tube (7). This reduced the noise when both 100% CO_2 and low concentrations of methanol-modified CO_2 (3% w/w) were used as the mobile phase; however, at a methanol concentration of 4.8% (w/w), a makeup gas was necessary to aid in the evapo-

ration of the mobile phase at the high decompressed CO_2 flow rates (>3 L/min). Detection limits of 12 ng were reported for docosanol and octadecanol on a LiChrospher Sil column, but the mobile phase composition was not reported (7). The analysis of carbohydrates (6), opium alkaloids (6), triglycerides (6), fatty alcohols (7), and fatty acids (7) were given as applications.

Later, using the same interface, Herbreteau and co-workers (8) discussed the separation of sugars by packed-column SFC–ELSD with a cyanopropyl derivatized-silica column, a methanol-modified CO_2 gradient from 2.4% (w/w) to 11.1% (w/w), 2.1 L/min decompressed CO_2, and a pressure of 265 bar. Brossard and co-workers (9) then described the separation of ethoxylated alcohols by packed-column SFC and HPLC. They used a silica column, 2.5 L/min decompressed CO_2, methanol-modified CO_2, and a pressure of 268 bar. They also found that the retention time decreased when either methanol–water–CO_2 or methanol–water–triethylamine–CO_2 was used as the mobile phase rather than methanol-modified CO_2 alone. More importantly, these complex mobile phases did not interfere in the detection of ethoxylated alcohols by ELSD. In a later study, Brossard and co-workers (10) published a paper about the use of packed-column SFC–ELSD for the analysis of synthetic waxes (1). They used a diol derivatized-silica column with 2-propanol–formic acid–carbon dioxide as the mobile phase. Lafosse and co-workers (11) reported the separation of pharmaceuticals, sugars, phospholipids, and surfactants by packed-column SFC–ELSD. Furthermore, Cocks and Smith (12) discussed the separation of fatty acid methyl-esters (FAME) by packed-column SFC–ELSD with 100% CO_2 and methanol-modified CO_2 at a mobile phase flow rate of 2.4 L/min decompressed CO_2. Like Carraud and co-workers (6), they found that CO_2 could form ice (water) from nondried nitrogen and increase the background noise. To eliminate this noise, they suggested placing glass wool inside the heated drift tube to improve the heat transfer and the evaporation process. However, it was suggested that the glass wool interferes in the detection if the analytes adsorb onto it.

All of these papers discuss the use of 4.6-mm i.d. packed-column SFC–ELSD at high mobile phase flow rates (3 L/min decompressed CO_2). Takeuchi (13) coupled SFC to a conventional HPLC–ELSD to permit the use of smaller columns and slower flow rates. This was accomplished by connecting the outlet of a variable restrictor to a mixing tee, which was connected to the inlet of a conventional HPLC nebulizer on the ELSD. A particle-forming solvent via the mixing tee was found to be necessary for making a proper aerosol. This approach allowed the use of slower mobile phase flow rates (300 µL/min) and smaller diameter columns (250 × 1.7 mm). No detection limit was reported. The separa-

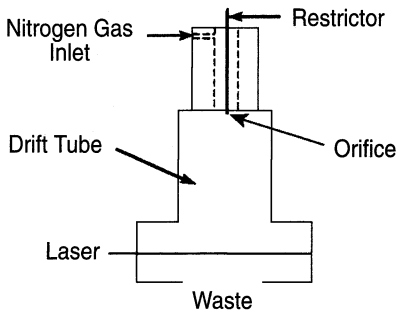

Figure 1. ELSD interface.

Table I. Conditions for 2% and 20% (v/v) Methanol-Modified CO_2 Factorial Experiments

Parameter	CO_2 flow rate (mL/min)	Level –1	Level 0	Level 1
Nitrogen gas flow rate	150	0.5	1	1.5
(SLPM)	1000	0.25	0.75	1.25
Drift tube temperature	150	30	50	70
(°C)	1000	50	70	90
Orifice size	150	0.0185	0.0193	0.0213
(in.)	1000	0.0185	0.0193	0.0213

Table II. Calculated *F* Values from ANOVA Using 2% and 20% Methanol-Modified CO_2 at Low Decompressed CO_2 Flow Rate*

Parameter	Methanol-Modified CO_2	Progesterone	Testosterone	17-α-hydroxyprogesterone
Nitrogen flow rate	0.02	4300	4600	1200
	0.2	2200	3900	2700
Drift tube temperature	0.02	770	660	4200
	0.2	1800	2900	1200
Orifice size	0.02	90	50	500
	0.2	170	15	20

* If $F_{calc} > F_{table}$ then the parameter had a statistical effect. F_{table} for all experiments was 3.23.

Table III. ANOVA Results Using 2% and 20% Methanol-Modified CO_2 at High Decompressed CO_2 Flow Rate*

Parameter	Methanol-Modified CO_2 (%)	Progesterone	Testosterone	17-α-hydroxyprogesterone
Nitrogen flow rate	2	1900	2300	220
	20	1400	1200	900
Drift tube temperature	2	1700	1500	2400
	20	2100	1700	850
Orifice size	2	300	310	300
	20	330	200	370

* If $F_{calc} > F_{table}$ then the parameter had a statistical effect. F_{table} for all experiments was 3.23.

tions of Triton X-100, polyoxypropylene glycol, and alcohol-polyethoxylate were reported.

Hoffmann and Greibrokk (14) modified a conventional HPLC–ELSD for use with packed capillary SFC with typical flow rates of 10 mL/min decompressed CO_2 (20 µL/min liquid). They removed the nebulizer and the drift tube. The drift tube heating block was used as the column oven. The restrictor was placed at the entrance of the detection cell. They observed a detection limit of less than 5 ng for Irgafos 168 and trimyristin with 5.6% (mol) *n*-propanol-modified CO_2 and a Nova-Pak-4 ODS column (100 mm × 0.32-mm i.d.). The separations of glyceryl monosterate and ethylene bisstearamide were discussed. Similarly, Demirbüker and co-workers (15) described a packed-capillary SFC system in which a miniaturized ELSD was used. The restrictor (130 mm × 10 µm fused silica) was connected to a miniaturized drift tube ($^1/_{16}$- in. o.d. stainless steel tube). The drift tube was then connected to a small detection cell where the analyte particles could scatter light and be detected. They found that the lower volume ELSD did not suffer the mobile phase flow rate dependence that was observed by Carraud. For example, the response of the detector remained constant over a range of 8–16 mL/min decompressed CO_2 (13–26 µL/min liquid). They also discussed the argentation chromatography of triacylglycerols with modified CO_2 mobile phases. A detection limit of 6 ng was obtained for triolein with a packed capillary column; this result compares well with previously reported packed-column SFC–ELSD work that involved a higher flow rate (4 mL/min liquid) (15).

The main focus of our study was to develop an ELSD for SFC that could detect 5 ng of analyte at mobile phase flow rates between 150 mL/min and 1.0 L/min decompressed CO_2 and could handle methanol-modified CO_2 ranging from 2% to 20% modifier. This work did not suffer the sample loadability or chromatographic resolution problems that the previously reported work did. Furthermore, a detailed study of several detector parameters (i.e., orifice size, restrictor position, drift tube temperature) that affect the response of the detector was performed. Detection limits for several steroids have been determined. The separation of polyethylene glycol (PEG) and ginkgolides are given as applications.

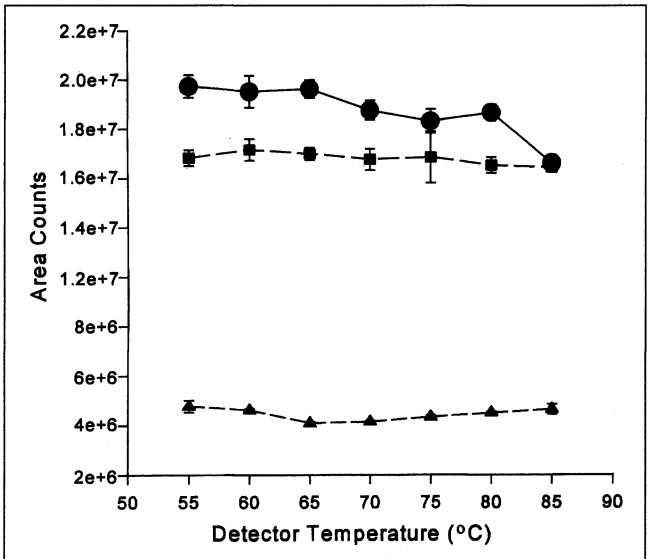

Figure 2. Effect of detector temperature on the response of progesterone. Conditions: 800 mL/min decompressed CO_2; 5% modified CO_2; column, Deltabond cyanoproyl derivatized silica (250 × 4.6 mm; particle size, 5 µm); pressure, 200 bar; oven temperature, 50°C; liquid flow rate measured at the pump, 2 mL/min CO_2; makeup gas, 0.4 SLPM N_2; and orifice size, 0.0165 in. Key: ●, 5% (v/v) methanol-modified CO_2; ■, 5% (v/v) ethanol-modified CO_2; and ▲, 5% (v/v) isopropanol-modified CO_2.

Experimental

Instrumentation

A prototype of the Hewlett-Packard Model G1205 SFC system (Little Falls, DE) was used for detector evaluation and subsequent separations. System pressure was maintained electronically by a computer-controlled, back pressure regulator, which allowed the flow rate and pressure to be independently controlled. The mobile phase flow rate was measured as a liquid at the pump. Organic modifier was added via an auxiliary pump. A postcolumn split via a Valco zero dead volume three-way tee (Supelco; Bellefonte, PA) was introduced to divert a percentage of column effluent through a restrictor to the ELSD with the remaining effluent directed towards a standard HP 1050 multiwavelength detector that used a 13-µL high-pressure flow cell. For the detector study, a 250- × 4.6-mm Deltabond cyano-derivatized silica (5 µm) column (Keystone Scientific; Bellefonte, PA) was used. A 150- × 4.6-mm Deltabond amino-derivatized silica (5 µm) column (Keystone Scientific) was used to separate the ginkgolides. A 250- × 4.6-mm Hypersil silica (3 µm) column was used for the analysis of the FAMEs and PEG.

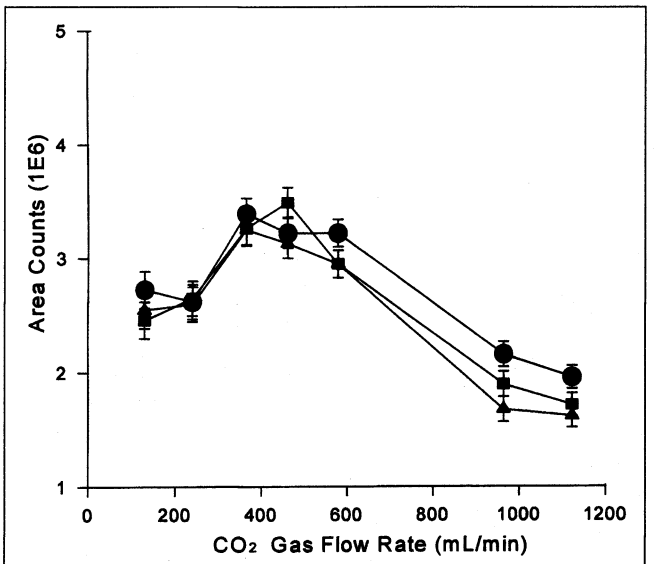

Figure 3. Effect of CO_2 gas flow rate on the response of steroids. Conditions: 5% methanol-modified CO_2; column, Deltabond cyanoproyl derivatized silica (250 × 4.6 mm; particle size, 5 µm); pressure, 200 bar; oven temperature, 50°C; liquid flow rate measured at the pump, 2 mL/min CO_2; ELSD, 60°C; and orifice size, 0.0165 in. Key: ●, progesterone; ■, testosterone; and ▲, 17-α-hydroxyprgesterone.

A commercially available Mark III ELSD (Alltech Associates; Deerfield, IL) was used. The nebulizer was removed and replaced with either a linear restrictor (30 cm × 50-µm i.d. fused silica) or an integral restrictor (50 or 100 µm i.d.), which was made in-house to deliver flow rates between 100 and 1200 mL/min decompressed flow. An integral restrictor was chosen over a crimped stainless steel tube because the integral restrictor gave better control over the flow rate. All decompressed flow rates were measured by a soap flow meter. An interface was positioned on top of the drift tube to hold the restrictor (Figure 1). The interface allowed nitrogen makeup gas to be introduced into the drift tube through an orifice. The

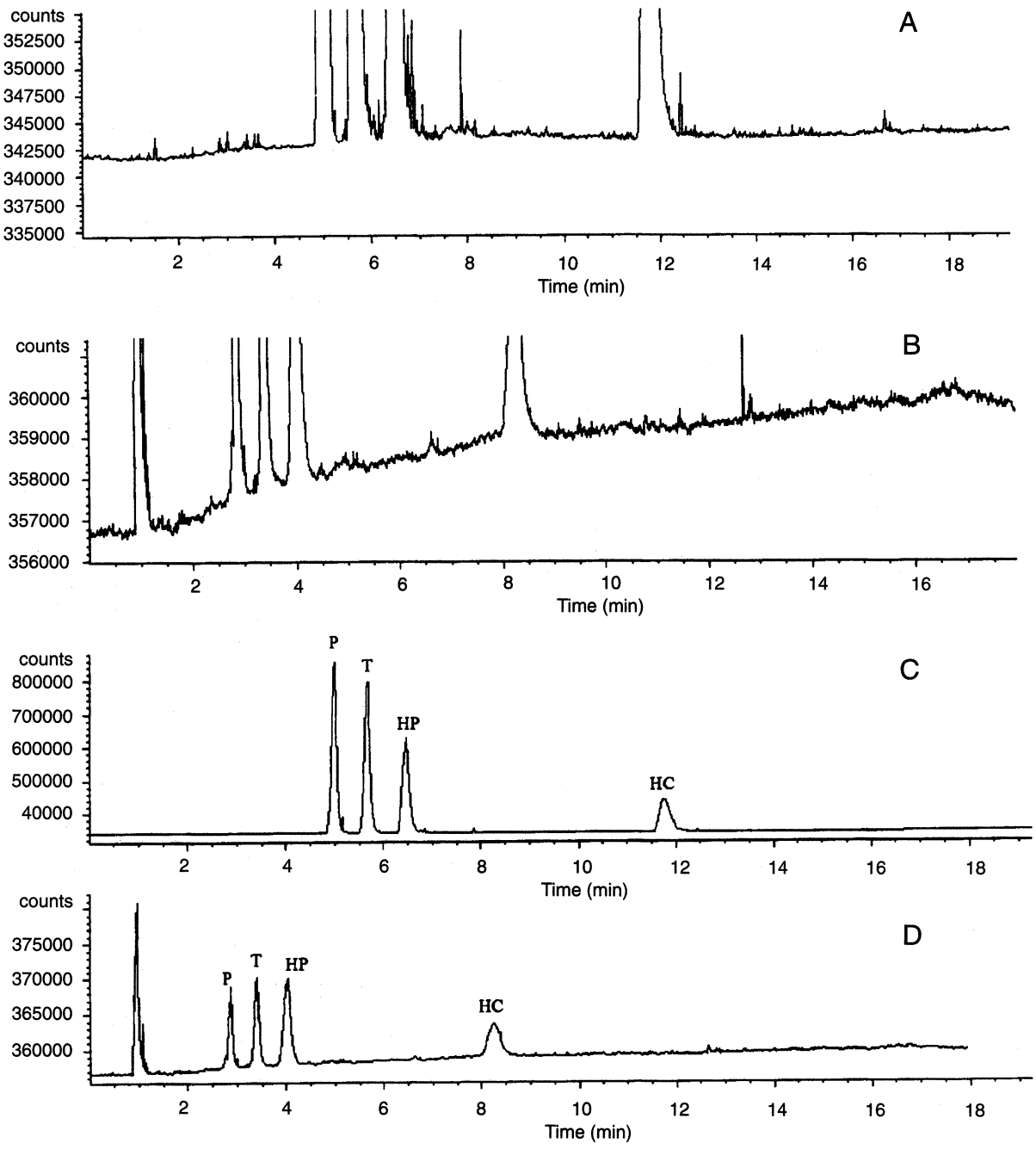

Figure 4. Effect of pressure gradient programming on the performance of the ELSD. Conditions for the low decompressed CO_2 flow rates (A and C): 5% methanol-modified CO_2; liquid CO_2 measured at the pump, 2.0 mL/min; pressure program, 100 bar CO_2 (1.5-min hold) to 350 bar CO_2 (1-min hold) at 15 bar/min; column, Deltabond cyanopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 138 mL/min; oven temperature, 60°C; makeup gas flow rate, 0.89 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in. Conditions for the high decompressed CO_2 flow rates (B and D): 5% methanol-modified CO_2; liquid CO_2 measured at the pump, 2.0 mL/min; pressure program, 100 bar CO_2 (1.5-min hold) to 350 bar CO_2 (1-min hold) at 15 bar/min; column, Deltabond cyanopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 858 mL/min; oven temperature, 60°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in. Peak identity as follows: P, progesterone; T, testosterone; HP, 17-α-hydroxyprogesterone; HC, hydrocortisone.

diameter of the orifice was varied from 0.0165 to 0.0213 in. The restrictor tip was placed 1–2 mm outside the orifice. Microsoft Excel (Version 5.0) was used to calculate the three-way analysis of variance (ANOVA) results.

Chemicals

SFE/SFC-grade CO_2 (no helium padding) was obtained from Air Products and Chemicals (Allentown, PA). The methanol and isopropanol were obtained from Fisher Scientific

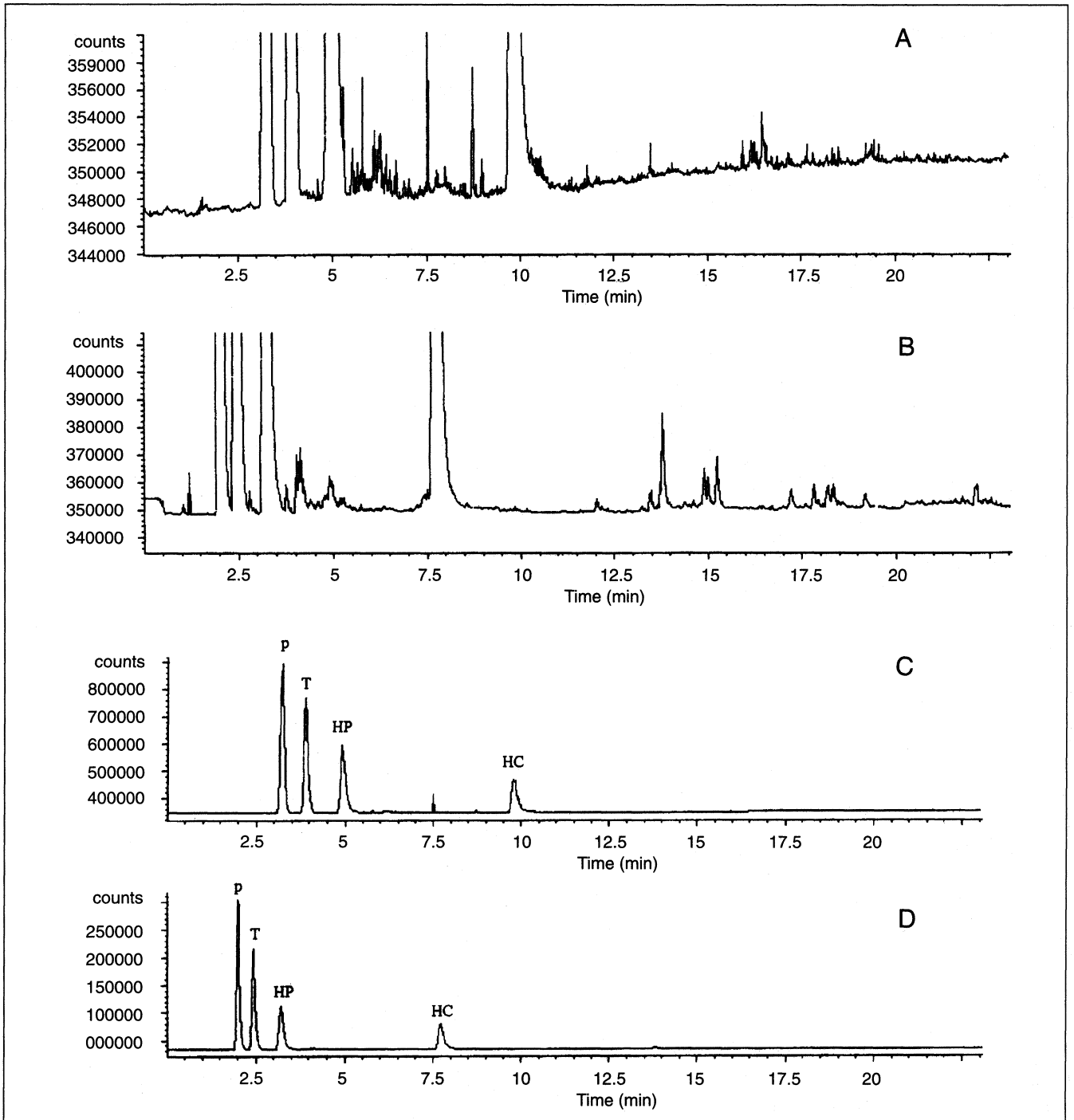

Figure 5. Effect of modifier gradient programming on the performance of the ELSD. Conditions for the low decompressed CO_2 flow rate (A and C): 1% methanol-modified CO_2 (0.5-min hold) to 20% methanol-modified CO_2 (3-min hold) at 1%/min; pressure, 280 bar CO_2; column, Deltabond cyanopropyl derivatized silica; liquid CO_2 measured at the pump, 2.0 mL/min; decompressed CO_2 diverted to the ELSD by an integral restrictor, 138 mL/min; oven temperature, 60°C; makeup gas flow rate, 0.89, SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in. Conditions for the high decompressed CO_2 flow rates (B and D): 1% methanol-modified CO_2 (0.5-min hold) to 20% methanol-modified CO_2 (2-min hold) at 1%/min; pressure, 280 bar CO_2 ; liquid CO_2 measured at the pump, 3.0 mL/min; column, Deltabond cyanopropyl derivatized silica; decompressed CO_2 diverted to the ELSD, 858 mL/min; oven temperature, 60°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in. Peak identity: P, progesterone; T, testosterone; HP, 17-α-hydroxyprogesterone; HC5 hydrocortisone.

(Fairlawn, NJ). The ethanol was supplied by AAPER Alcohol and Chemical (Shelbyville, KY). Progesterone, testosterone, 17-α-hydroxyprogesterone, and hydrocortisone were purchased from Sigma (St. Louis, MO). The PEG samples were obtained from Proctor and Gamble (Cincinnati, OH). The ginkgolide samples were provided by Teris van Beek (Wageningen Agriculture Institute, The Netherlands).

Results and Discussion

The purpose of this work was to investigate whether a range of decompressed CO_2 flow rates (150–1000 mL/min) could be used with SFC–ELSD from 2% (v/v) to 20% (v/v) methanol-modified carbon dioxide. The flow rates were obtained with a post-column split diverting the desired flow to the ELSD via an integral restrictor. The remaining flow was sent to the UV detector. Several detector parameters (nitrogen makeup gas flow rate, orifice size, and drift tube temperature) were investigated by a 3^3 factorial design. Unfortunately, one level of the 3^3 factorial did not yield a response and was left out of the data analysis. Subsequently, a $3 \times 3 \times 2$ factorial was used in place of the 3^3 factorial. A detailed discussion of the factorial experiment is beyond the scope of this paper, but the factorial experiment is based on the approach of Winer (16). Each parameter was assigned three levels and tested (Table I) at both low (150 mL/min) and high (1000 mL/min) decompressed CO_2 flow rates and at 2% (v/v) and 20% (v/v) methanol-modified CO_2. The response was based on area counts of progesterone, testosterone, and 17-α-hydroxyprogesterone on a Deltabond cyanopropyl derivatized silica column. The conditions were as follows: column, 250 × 4.6 mm; particle size, 5 µm; pressure, 200 bar CO_2; temperature, 50°C; liquid flow rate measured at the pump, 2 mL/min CO_2; and oven temperature, 60°C for $n = 3$. This enabled three-way ANOVA to be performed to determine the significance of each parameter. The results of the ANOVA are shown in Tables II and III. Each parameter had a statistical effect at both 2% and 20% methanol-modified CO_2 and at both flow rates (150 and 1000 mL/min decompressed CO_2).

The orifice size had the smallest effect on the response of the detector. At 150 mL/min decompressed CO_2, no trend could be determined at either 2% or 20% (v/v) methanol-modified CO_2. At 1000 mL/min decompressed CO_2, the smaller orifice size provided the highest response for progesterone, testosterone, and 17-α-hydroxyprogesterone. It was reasoned that the smaller orifice provided better mixing of the nitrogen makeup gas with the decompressing CO_2, which afforded better evaporation of the mobile phase.

The drift tube temperature had a significant effect on the response of the detector. When the detector temperature was increased from 50°C to 70°C, the signal response was found to

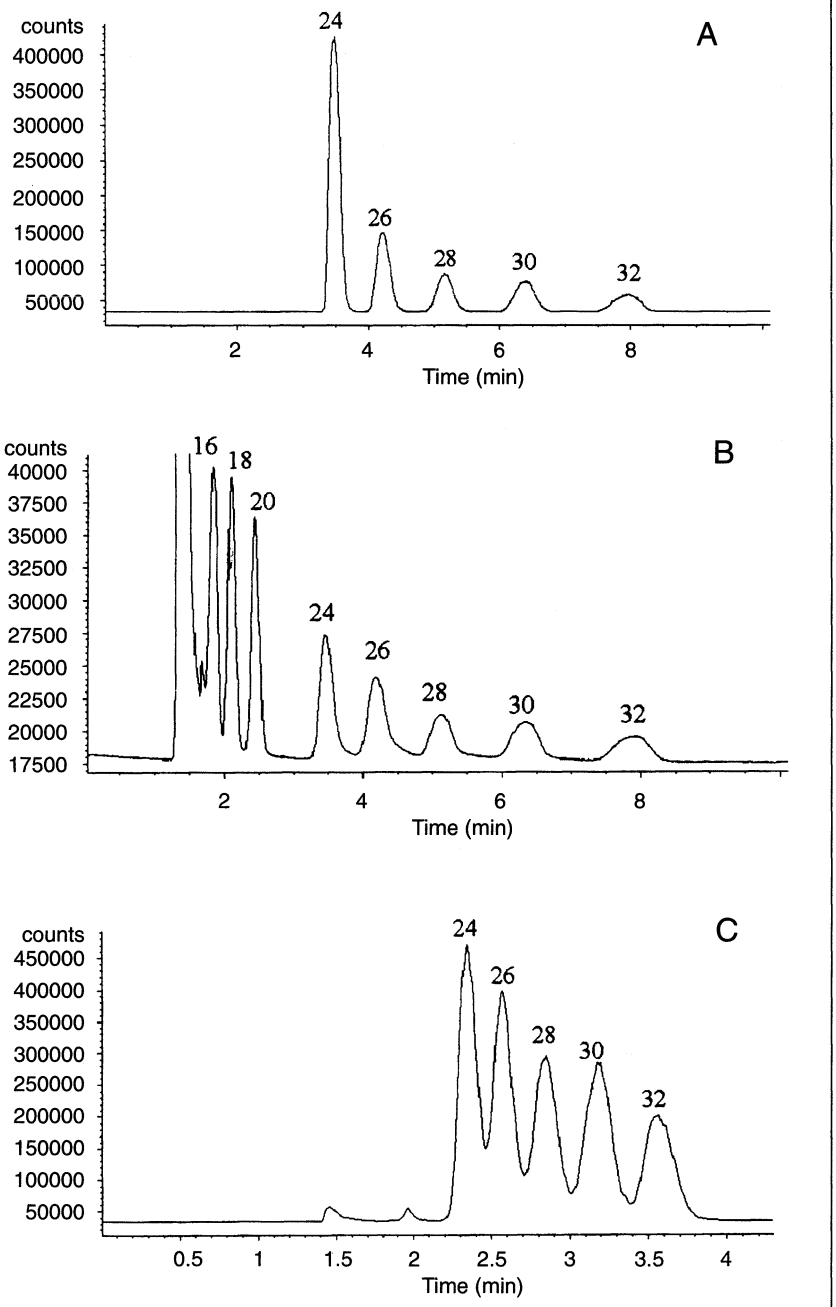

Figure 6. Effect of *n*-alkane volatility on the performance of the ELSD. Conditions: liquid CO_2 measured at the pump, 1.5 mL/min; pressure, 112 bar CO_2; column, Deltabond cyanopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 600 mL/min; oven temperature, 75°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in. An ELSD was used for A and C, and an FID was used for B. Numbers over the peaks correspond to the carbon numbers.

decrease at both modifier concentrations and decompressed CO_2 flow rates. This decreased signal response was thought to be caused by the solute vaporizing at the increased temperatures. A more detailed investigation was performed to study the effect of detector temperature. Moderate conditions (800 mL/min decompressed CO_2 and 5% methanol-modified CO_2) were chosen. The response for progesterone, testosterone, and 17-α-hydroxyprogesterone was found again to decrease as the detector temperature was increased. Above 70°C, the response of the analytes was reduced even more when methanol was used as the modifier. The decreased signal was attributed to solute vaporization at the higher detector temperatures (2). When the modifier was changed to either ethanol or isopropanol, the signal response for progesterone did not change with increasing detector temperature (Figure 2). A similar effect was observed when testosterone and 17-α-hydroxyprogesterone were used. Since ethanol and isopropanol have higher boiling points than methanol, more thermal energy would be required to evaporate these solvents.

The nitrogen gas flow rate also had a large effect on the response of the detector. As the nitrogen gas flow rate was increased, the signal response of the detector decreased at 2% and 20% methanol-modified CO_2 and at 150 mL/min and 1000 mL/min decompressed CO_2. The decreased signal was thought to be caused by a decreased residence time of the particles in the laser beam (17). Although the maximum response was obtained at the low nitrogen gas flow rate setting under all conditions, the peak width of the analyte with 2% and 20% methanol-modified CO_2 at 150 mL/min decompressed CO_2 was found to be twice that of the peak width observed with the UV detector. The decreased nitrogen flow rate was thought to allow the particles to broaden while they descended the drift tube to the laser beam. The band-broadening of the peaks was reduced to that observed in the UV detector when the nitrogen gas flow rate was increased to 0.75 standard liters per min (SLPM). At this flow rate, however, the signal response of the analyte was reduced. On the other hand, the peak-widths at 1000 mL/min decompressed CO_2 with 2% and 20% methanol-modified CO_2 were the same as that found in the UV detector at all nitrogen gas flow rates.

It was later believed that the total flow rate of the detector controlled both the response and the peak width of the analyte. The total flow rate of the detector was defined as the combination of the nitrogen gas flow rate and the decompressed CO_2 flow rate. When the total gas flow rate was plotted against the response of the detector, a good correlation was observed at 2% and 20% methanol-modified CO_2. In an effort to understand the relationship of CO_2 gas flow rate, several integral restrictors (100 µm) were fashioned to deliver flow rates from 100 to 1000 mL/min. A plot of signal response versus CO_2 flow rate was made using 5% methanol-modified CO_2. The total gas flow rate of the CO_2 and nitrogen gas was set to equal or exceed 1000 mL/min. Since the mobile phase effluent was split between the UV and ELSD, the concentration of progesterone, testosterone, and 17-α-hydroxyprogesterone was changed at each flow rate to maintain a constant mass delivered to the ELSD. From Figure 3, it can be seen that the response of the detector did not change by orders of magnitude as a function of CO_2 flow rate as previously reported (6). Since our detector was less dependent on the CO_2 flow rate, pressure programming could be used without affecting the performance of the detector.

Table IV. Limit of Detection for Steroids

	Progesterone (ng)	Testosterone (ng)	17-α-Hydroxyprogesterone (ng)
2% CH_3OH 98% CO_2	9	7	7
10% CH_3OH 90% CO_2	10	6	6
20% CH_3OH 80% CO_2	4	5	5

Pressure gradient programming

A pressure gradient program was used to discover if the baseline noise and resulting chromatography were affected by the changing mobile phase pressure at both low and high decompressed CO_2 flow rates. The baseline peak-to-peak noise did not increase as the pressure was increased, but some spiking was observed at both low and high decompressed CO_2 flow rates (Figure 4A and 4B). This increased spiking was thought to be caused by the analytes precipitating on the restrictor tip. The spiking was eliminated when a new integral restrictor was made for both flow rates. The baseline noise level increased slightly as the pressure was increased; however, the baseline interferences did not affect the chromatography of the steroid separation at both low and high decompressed CO_2 flow rates, as shown in Figure 4C and 4D.

Table V. Calibration Curve Results*

	2% CH_3OH– 98% CO_2	10%CH_3OH– 90% CO_2	20% CH_3OH– 80% CO_2
Progesterone			
b	1.80	1.74	1.65
a	2.00	2.48	1.65
r^2	0.998	0.994	0.9998
s_b	0.044	0.052	0.013
s_a	0.28	0.32	0.081
Testosterone			
b	1.83	1.68	1.50
a	1.88	2.38	3.06
r^2	0.998	0.993	0.9994
s_b	0.035	0.023	0.021
s_a	0.25	0.16	0.12
17-α-Hydroxyprogesterone			
b	1.69	1.79	1.52
a	2.18	2.18	2.91
r^2	0.998	0.9996	0.995
s_b	0.033	0.017	0.057
s_a	0.22	0.098	0.36

* Where *b* is the slope, *a* is the intercept, r^2 is the correlation coefficient, s_b is the standard error in the slope, and s_a is the standard error in the intercept.

Modifier gradient programming

A modifier gradient program was used to ascertain if the baseline noise and resulting chromatography were affected by the changing mobile phase composition at both low and high decompressed CO_2 flow rates. The baseline peak-to-peak noise was found to increase as the modifier concentration was increased at the low decompressed CO_2 flow rate (Figure 5A); the baseline at the high decompressed flow rate remained stable. Random spiking was observed at both decompressed CO_2 flow rate conditions. The noise and spiking could be reduced and/or eliminated by increasing the nitrogen gas flow rate or increasing the drift tube temperature. Increasing the nitrogen flow rate would aid in the evaporation of the mobile phase effluent, but it would also decrease the residence time of the particles, which would reduce the response of the detector for the analyte. Similarly, the increased drift tube temperature would aid in the evaporation of the mobile phase, but the higher temperature may vaporize the analytes and reduce the sensitivity of the detector. Therefore, the noise associated with the nebulization process cannot be eliminated without potential signal loss. Fortunately, the spiking and noise becomes an issue only at low sample mass (<50 ng sample injected). The overall baseline noise level increased slightly as the methanol modifier was increased at both low and high decompressed CO_2 flow rates. The baseline interferences did not affect the chromatography of the steroid separation at both low and high decompressed CO_2 flow rates, as shown in Figure 5C and 5D.

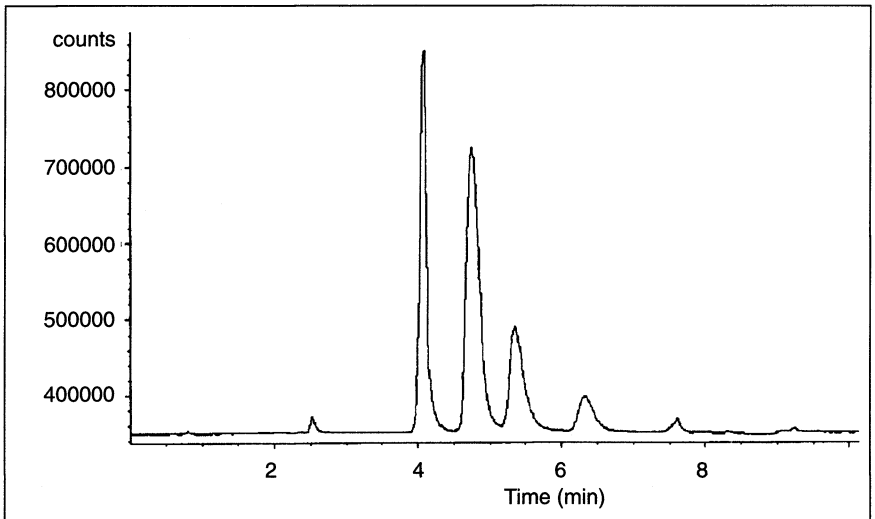

Figure 7. Separation of PEG 200. Conditions: 15% (v/v) methanol-modified CO_2; liquid CO_2 measured at the pump, 4.0 mL/min; pressure, 200 bar CO_2; column, LiChrospher aminopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 600 mL/min; oven temperature, 50°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in.

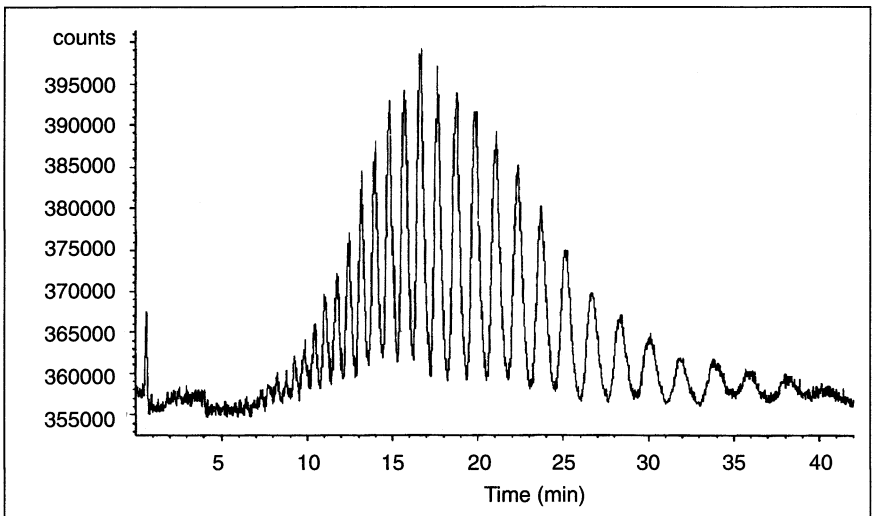

Figure 8. Separation of PEG 2000. Conditions: 20% (v/v) methanol-modified CO_2 with 1% (v/v) triethylamine and 5% (v/v water); liquid CO_2 measured at the pump, 4.0 mL/min; pressure, 200 bar CO_2; column, LiChrospher aminopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 1000 mL/min; oven temperature, 50°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in.

Solute volatility

Nizery and co-workers (7) found that the volatility of the analyte had a significant effect on the response of the detector. The more volatile analytes produced lower signal responses than the higher boiling analytes. We have investigated this behavior by studying a homologous series of hydrocarbons (C_{16}, C_{18}, C_{20}, C_{24}, C_{26}, C_{28}, C_{30}, and C_{32} each present at 500 ng at the ELSD) with 100% CO_2 and 5% methanol-modified CO_2. The lowest member of the series detected by ELSD was C_{24} (Figure 6A), but the FID was able to detect all eight of the hydrocarbons with 100% CO_2 as the mobile phase (Figure 6B). When 5% methanol modified CO_2 was used, C_{24} was still the lowest hydrocarbon that could be detected by the ELSD (Figure 6C). Although the methanol did not aid in the detection of the more volatile analytes, the ELSD was able to detect the higher molecular weight hydrocarbons in the presence of methanol. The FID, on the other hand, could not be used due to the presence of organic modifier.

Detector performance

The limit of detection for progesterone and testosterone was determined using SFC–ELSD with methanol-modified CO_2 (2% and 20%) under the following conditions: 60°C, 0.4 SLPM N_2 makeup gas, Deltabond cyanopropyl-derivatized silica column (150 × 4.6 mm × 5 µm), 2.0 mL/min (liquid CO_2), 200 bar CO_2, 50°C oven temperature, 0.0165-in. orifice, and 700 mL/min decompressed CO_2 diverted to ELSD (Table IV). Calibration curves (peak area versus concentration injected) were based on an average ELSD response ($n = 4$) in the concentration range of 100–5000 ppm (Table V). Since the response of the

ELSD is nonlinear (1), the calibration curves were plotted using double logarithmic coordinates according to the following equations (3):

$$y = am^b \quad \text{Eq 1}$$

$$\log(y) = a + \log(m) + b \quad \text{Eq 2}$$

where y is the response of the detector, m is the sample concentration, a is the slope, and b is the intercept.

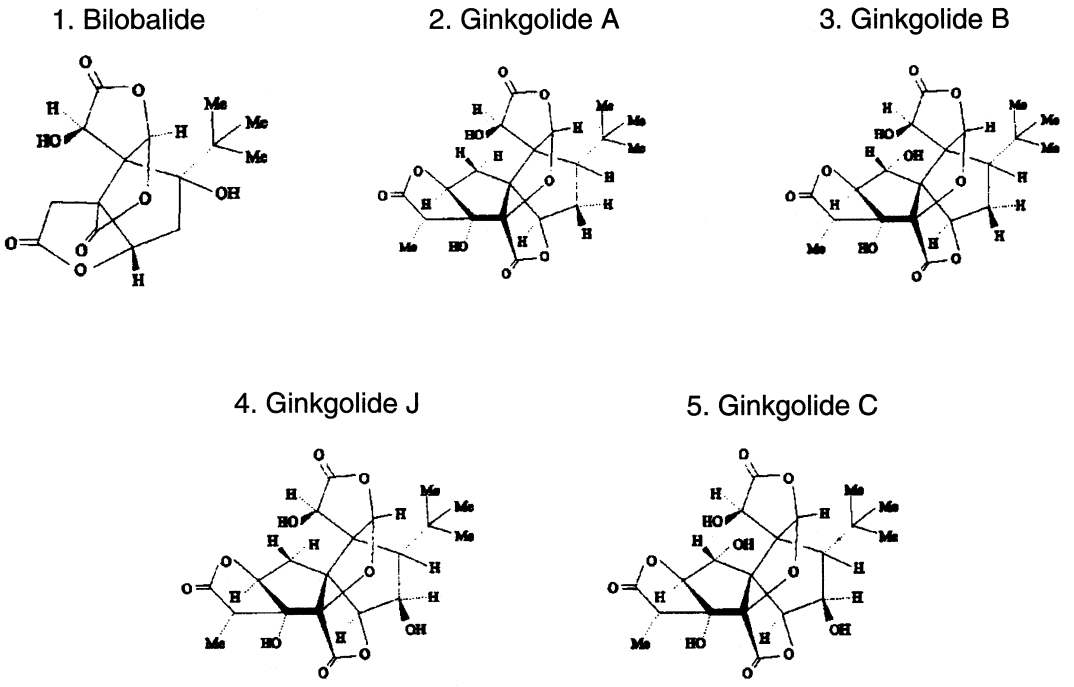

Figure 9. Structures of the five main components of the *Ginkgo biloba* leaf.

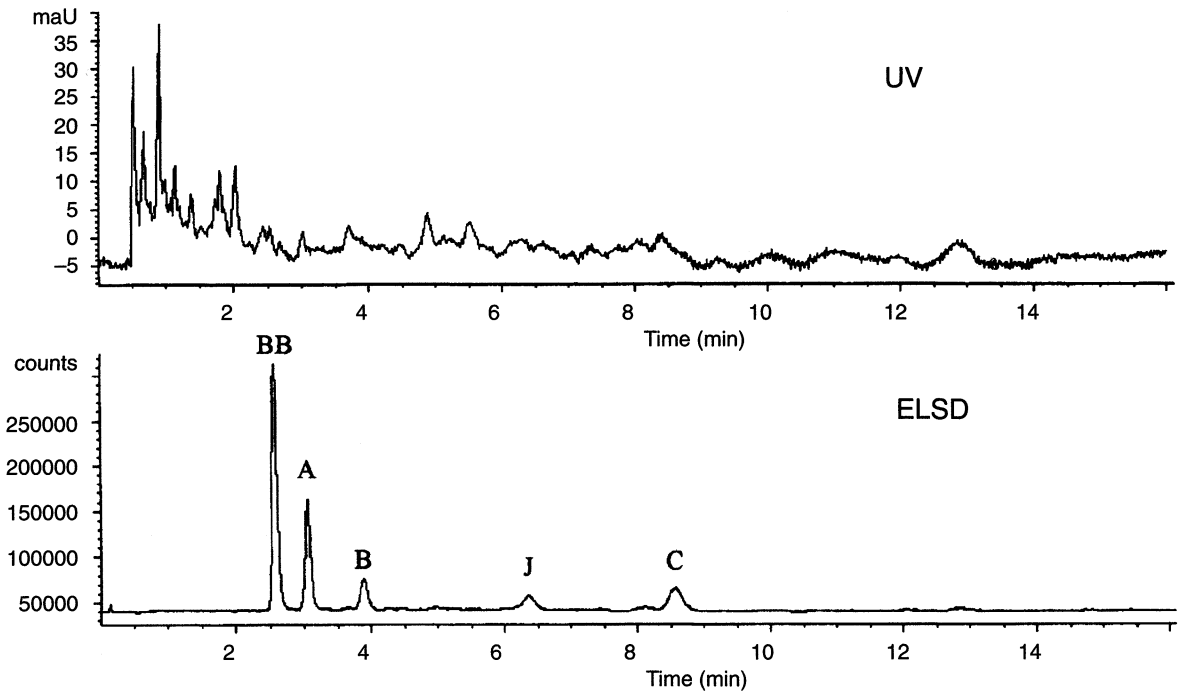

Figure 10. Separation of the Ginkgolide extract from the *Ginkgo biloba* leaf. Conditions: 12% (v/v) methanol-modified CO_2; liquid CO_2 measured at the pump, 4.0 mL/min; pressure, 280 bar CO_2; column, Deltabond aminopropyl derivatized silica; decompressed CO_2 diverted to the ELSD by an integral restrictor, 600 mL/min; oven temperature, 40°C; makeup gas flow rate, 0.4 SLPM N_2; drift tube temperature, 65°C; and orifice size, 0.0165 in.

The limit of detection of progesterone, testosterone, and 17-α-hydroxyprogesterone has been determined to be 10 ng or lower (signal-to-noise ratio of 3) at all modifier concentrations. These limit of detection values were in good agreement with previous results by other researchers (6,7). Furthermore, progesterone and testosterone had a lower limit of detection at 20% (v/v) methanol-modified CO_2 than at 2% (v/v) methanol-modified CO_2. It was theorized that the additional methanol produced larger droplets that resulted in larger particles and a larger response by the ELSD.

Applications

To demonstrate the selectivity and detectability of the SFC–ELSD system, the separation of polyethylene glycols (PEG) and ginkgolides was undertaken. The analysis of PEG samples by SFC has been difficult due to the polar nature of the polymer. The polarity of the polymer can be reduced by derivatizing the alcohol groups to less polar groups. Pinkston and co-workers (18) performed this derivatization process and used trimethylsilyl chloride to separate 33 oligomers by open-tubular SFC with 100% CO_2. This separation required a time-consuming derivatization step, and over 100 min were required to resolve the oligomers. Faster analyses can be obtained using a packed column, but modifiers are required to elute the underivatized polar polymer. Lower molecular weight PEG samples can be rapidly separately on a LiChrospher aminopropyl-derivatized silica column with 15% methanol-modified CO_2 (Figure 7). To increase the solvating strength of the mobile phase, an additive can be used. Brossard and co-workers (9) added triethylamine to the methanol–CO_2 mobile phase to elute the higher molecular weight PEGs from a diol phase. They found that water was required to reduce the activity of the column and to increase the resolution between the oligomers. Using a mobile phase similar to that used by Brossard and co-workers, they separated PEG 2000 on a LiChrospher amino column (Figure 8). Thirty-three oligomers were observed, which was in good agreement with the work of Pinkston and co-workers. However, the peak-to-peak noise was increased with this quaternary mobile phase.

Ginkgolides are prescribed for the postponement of the symptoms of old age (i.e., forgetfulness and early dementia). The ginkgolides (Figure 9) are extracted from the *Ginkgo biloba* leaf. The ginkgolides have a poor UV chromophore, which prohibits the use of a UV detector. The current assay method is usually performed using reversed-phase HPLC–ELSD with an octadecyl derivatized silica column (19). This assay method has been plagued by poor peak area reproducibility and large solvent waste. The ginkgolides could be separated and quantitated by GC–FID, but a silylation derivatization step was required (20). In an effort to improve the performance of the assay and reduce solvent waste, an SFC method was developed using a LiChrospher aminopropyl-derivatized silica column. The ginkgolide extract was separated into three peaks with 20% (v/v) methanol-modified CO_2.

As the modifier concentration was reduced to 12% (v/v) methanol-modified CO_2, the five expected ginkgolide peaks were observed but not resolved. The separation was theorized to occur by two opposing mechanisms. The first involved hydrogen bonding with the amino phase, while the second opposing mechanism involved the adsorption of the ginkgolides onto the residual silica sites. The five ginkgolide compounds were further separated on a Deltabond amino derivatized column with 12% (v/v) methanol-modified CO_2. UV detection of the ginkgolide compounds was possible at low wavelengths, but the extract contained many interfering compounds that have better chromophores than the ginkgolides (Figure 10). Since the coeluting peaks were volatile compounds, they were not detected by the ELSD.

Conclusion

The ELSD was affected by the drift tube temperature and N_2 makeup gas flow rate. The signal was found to decrease as the detector temperature was increased. The N_2 makeup gas was found to control the peak width of the analyte as well as the response of the analyte. Increasing N_2 flow rates decreased the signal but improved the peak width of the analyte when low decompressed CO_2 flow rates were used. A total gas flow rate of 1000 mL/min gave acceptable peak widths and signal response. SFC–ELSD seems to be a promising technique for assaying food stuffs, polymers, and pharmaceuticals. It would be especially useful when organic modifiers and additives are required that prohibit the use of other detectors (i.e., UV and FID).

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