Applications of Packed-Column SFC Using Light-Scattering Detection

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Abstract

The application of packed-column supercritical fluid chromatography using ultraviolet and evaporative light-scattering detection in series for the analysis of a range of polar, nonchromophoric analytes is reported. Cyano-, amino-, silica, and β-cyclodextrin columns were used with carbon dioxide–methanol, carbon dioxide–methoxyethanol, or chlorodifluoromethane–methanol mobile phases. The performance of the system is dependent on the geometry of the nebulizer spray device in addition to the temperature and pressure (density) of the mobile phase and the percentage of solvent entrainer used. However, this may be optimized by adjustment of the nebulizer gas pressure and drift tube temperature of the detector. The technique offers a means of separating and detecting a wide range of organic compounds not normally directly amenable to high-performance liquid chromatography or supercritical fluid chromatographic analysis using ultraviolet detection and provides a rapid means of confirming optimum component separation and the purity of samples. The applicability of the technique is illustrated by analysis of mixtures of steroids, underivatized amino acids, underivatized carbohydrates, and ionophores, by the separation of plant pigments within a leaf extract, and by the confirmation of the purity of a peptide standard.

Introduction

During the past two decades, the performance and application of supercritical fluid chromatography (SFC) using either open-tubular capillary or conventional packed high-performance liquid chromatographic (HPLC) columns have been investigated for the analysis of a wide range of organic compounds. During this period, the relative advantages and properties of packed and capillary columns have been extensively addressed (1–3); comprehensive accounts and reviews of both approaches, including lists of applications, are available (4–7). The use of conventional packed columns for SFC offers more rapid analysis and higher efficiency with unit time than the use of open-tubular capillary SFC or HPLC columns; the attainment of 220,000 plates using eight packed columns in series has recently been reported (8). In addition, the technique can be more versatile because of the wide choice of column packings available and the various types of solvent modifiers/entrainers that may be used. The addition of a polar solvent is often required to increase the solvating power of the primary mobile phase (normally carbon dioxide) above that which is obtainable at maximum density and to modify the free active sites that are present on silica-based packing materials (4,9,10). The presence of solvents in supercritical carbon dioxide in quantities greater than 5% renders the eluent incompatible with flame-ionization methods of detection. For this reason, ultraviolet (UV) detection has been most widely used for packed-column SFC. The disadvantage with this approach is that compounds lacking chromophoric functionalities that exhibit low molar absorptivities in the UV region are excluded.

Originally designed as a nonselective detection method for HPLC, the evaporative light-scattering detector (LSD) used in combination with SFC has received much interest. Here, detection is based on the mass of a relatively nonvolatile compound rather than the capability of that particular compound to absorb light of a specific wavelength. The principal of operation for most commercially available evaporative LSDs is similar. It consists of the following steps: nebulization of the column eluent with an inert gas; evaporation of solvent from droplets formed during nebulization in a heated tube; illumination of the solute particles formed from solid substances or the small droplets formed from nonvolatile liquids; and finally, measurement of the intensity of light, scattered at either 120° to a beam of light produced by a tungsten source or 90° to a helium–neon laser.

One of the first accounts of a successful coupling of packed-column SFC with LSD was by Carraud and co-workers (11), who reported a fivefold increase in sensitivity over HPLC–LSD using a detector in which the conventional nebulizer was replaced by a length of fused-silica capillary tubing. Significant improvements in sensitivity were later achieved by directly heating the nebulizing interface (12). A number of teams have performed SFC–LSD with minimal or no modification to existing instrumentation. Evidently, this is dependent on the design of the detector and the size of SFC column used. Favorable

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results have been generated with standard-sized columns (4.6-mm i.d.) using a section of crimped stainless steel tubing as a fixed back pressure regulator that introduces column eluent into the nebulizer of the LSD (13,14). By reducing the length of the drift tube of the detector, improvements in sensitivity have been achieved with this approach, thus effecting a decrease in pressure-induced baseline drift (15). The use of packed capillary SFC–LSD has been reported (16,17). Here, it was necessary to construct a modified restrictor/nebulizer; the system allowed the analysis of low concentrations of mono- and triglycerides that could not be analyzed by HPLC or packed capillary SFC with modifier using flame-ionization detection.

This paper describes the use of packed columns with supercritical carbon dioxide and chlorodifluoromethane for the analysis of a range of polar, non-UV-absorbing compounds using UV–LSD in series.

Experimental

The system used for these studies is very similar to that described by Loran and Cromie in their studies on SFC–LSD of steroids (13). SFC was performed on a dual-pump Gilson HPLC system that had been modified for SFC operation. The primary mobile phase, either carbon dioxide (BOC) or chlorodifluoromethane (Arcton 22-ICI) was delivered by a Gilson 303 pump that was cooled to –20°C using a refrigerating recirculating bath (RTE-4Z, Neslab Instruments; Portsmouth, NH). A second Gilson 303 pump was used at room temperature to deliver various organic modifiers to the primary mobile phase. These were mixed under high pressure using a dynamic solvent mixer (Gilson 802). The pumps were configured to perform accurate modifier gradients. Heating of columns and mobile phase was achieved using a gas chromatographic oven (Carlo Erba), and sample injections were made via a Rheodyne 7125 valve injector fitted with a 25-µL loop. Concomitant UV detection was accomplished by placing a Kratos Spectraflow 757 variable wavelength detector fitted with a high-pressure flow cell (0.5-µL volume, 1-mm path length) in series between the column outlet and a Model 750/14 LSD (Applied Chromatography Systems). The design and mode of operation of this system has been described in detail previously (14).

The UV detector was connected to the LSD using a stainless steel transfer line (1.6-mm o.d, 250-µm i.d.) via a low dead volume pressure relief valve (Rheodyne 7037) that acted as a variable back pressure regulator and provided a means of controlling the density of the supercritical fluid within the column. A length of the same stainless steel tubing (76 mm) was connected to the outlet port of the pressure relief valve; this was inserted into the nebulizer orifice of the evaporation chamber of the LSD such that the valve made close thermal contact with the heated nebulizer block. The outlet of this tubing was crimped to give a moderate point restriction. This was to facilitate a constant vertical spray of column eluent droplets down the evaporation chamber without affecting the back pressure provided by the upstream pressure relief valve. In this configuration, 100% of column eluent was introduced into both the ultraviolet and light-scattering detectors.

The following chromatographic columns were used: a stainless steel column (100 mm or 250 mm × 4.6-mm i.d.) packed with 3-µm Spherisorb silica or 5-µm Spherisorb propylamine (Phase Separations), a 5-µm Hypersil nitrile column (Shandon), and β-cyclodextrin (Technicol). All modifier percentages used in this work are stated volume-to-volume.

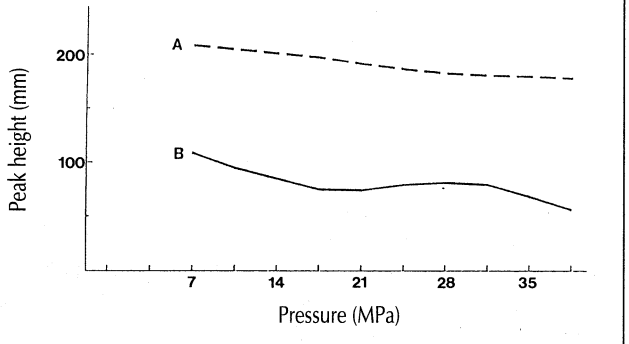


Figure 1. Effect of supercritical fluid pressure on relative response for cholestane as a solute: A, Chlorodifluoromethane and B, carbon dioxide (70°C, 30 MPa). Conditions: flow rate, 4 cm³/min; nebulizer pressure, 0.14 MPa; drift tube temperature, 40°C; injection, 10 µL of a 250-ppm solution in methanol.

Results and Discussion

Nebulization of the mobile phase

Our preliminary investigations indicated that the configuration of the crimp at the base of the nebulizer spray device significantly influenced the sensitivity of the detector. It is believed that the configuration modifies the atomization of the eluent at the nebulizer, which in turn effects a change in the spray particle size and the direction and velocity of particles within the drift tube. Optimum sensitivity was obtained using a crimping tool that fashioned a 4-mm long compression perpendicular to the tubing axis, terminating 3 mm from the stainless steel restrictor outlet. Additionally, use of the tool greatly improved reproducibility of performance between individual nebulizer spray devices.

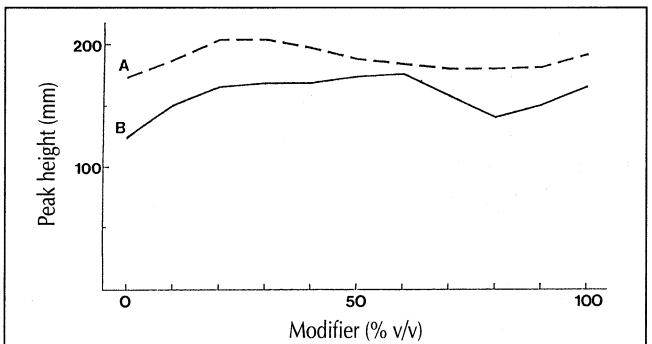


Figure 2. Effect of modifier concentration (% v/v methanol) on relative response for cholestane as solute. Conditions: flow rate, 4 cm³/min; nebulizer pressure, 0.14 MPa; drift tube temperature, 40°C; injection, 10 µL of a 250-ppm solution in methanol.

Chromatographic variables and detector response

A fundamental operational variable of the LSD Model 750/14 is nebulizer gas pressure. In HPLC mode, a jet of compressed air is required to convert large volumes of column eluent into a spray. The pressure (volumetric flow rate) of this supply has a significant effect on system sensitivity. In SFC operation, the eluent is self-nebulizing as it emerges from the tip of the spray device; thus, the only requirement of additional nebulizer gas is to carry the expanding eluent through the drift tube. As with observations reported in previous articles (12,14,15), our initial studies indicated that air pressure also influenced sensitivity in the SFC mode. Here, optimum sensitivity was achieved with a 0.14-MPa setting, in contrast to the findings of Upnmoor and Brunner (14), who observed lower pressures to be more favorable. This discrepancy was probably created by the use of different spray device geometries.

Mobile phase density is a major experimental variable in SFC, although the effect is more pronounced in capillary than in packed SFC. It is used by both approaches to form density gradients that will affect the elution and optimum resolution of analytes. Supercritical fluid chromatographs lacking in-line flow transducers, such as those used here, are incapable of dynamic flow correction. This results in significant pressure-induced flow fluctuations. Ultimately, an increase in mobile phase density (pressure) causes a subsequent increase in the volumetric flow into the drift tube. Figure 1 shows the relative response of the LSD at increasing pressures of supercritical carbon dioxide and chlorodifluoromethane using the steroid cholestane as a solute. The study was performed using a flow injection analysis technique in which the packed column was replaced by a length of steel tubing (250 mm × 250-µm i.d.). The rest of the system remained configured for standard SFC–UV–LSD operation. Clearly, chlorodifluoromethane yields a higher overall response and a lower response variation than carbon dioxide throughout the experimental pressure range of 6.9–34.5 MPa. Significantly, the 50% response variation of carbon dioxide precludes the use of density programming; this is not considered a limitation of the system because gradient addition of solvent modifier can be used as an alternative approach. The significant response variation observed between the two fluids is probably related to differences in their physical properties. The enthalpy of vaporization of carbon dioxide is higher than that of chlorodifluoromethane; thus, the magnitude of the Joule–Thomson cooling effect will be higher for carbon dioxide on decompression of column eluent at the nebulizer. It is believed that this variation in temperature at the nebulizer tip significantly influences solute particle size, which directly affects a variation in detector response.

Upnmoor and Brunner (14) reported a drop in detector sensitivity to a minimum using carbon dioxide containing between 5 and 10 wt % of methanol modifier. Our studies have shown a similar effect; however, the sensitivity minimums occur at much higher modifier concentrations. Again, this probably reflects the use of different spray device geometries. Figure 2 shows the relationship between modifier concentration (methanol) as a percentage of total flow and relative response for cholestane as a solute. Carbon dioxide and chlorodifluoromethane were used as primary mobile phase components with a drift tube temperature of 40°C. Evidently, there is a notable influence throughout concentration range 0–100%, and sensitivity minimums occur at 80% for both fluids. This variation is not disadvantageous because packed-column SFC rarely uses modifiers in excess of 20% and commonly uses modifiers within the range of 1–10%, corresponding to response variations of 9% and 12% for chlorodifluoromethane and carbon dioxide, respectively.

Many workers in this field have, in previous articles

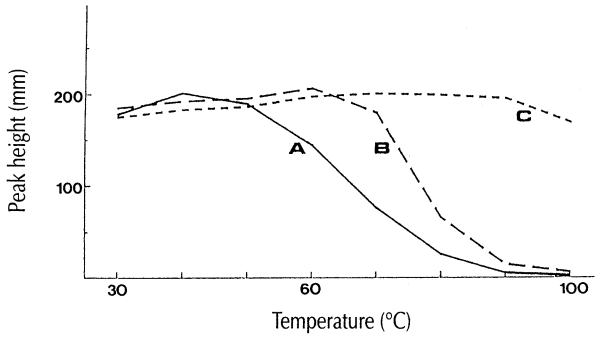


Figure 3. Effect of drift tube temperature on relative response for cholestane (A), pregnenolone (B), and hydrocortisone (C) as solutes. Conditions: column, Hypersil CN (250 mm × 4.6-mm i.d., 3-µm particle size); mobile phase, carbon dioxide–10% methanol (70°C, 20 MPa); flow rate, 4 cm³/min; nebulizer pressure, 0.14 MPa; injection, 10 µL containing 250 ppm of each compound in methanol.

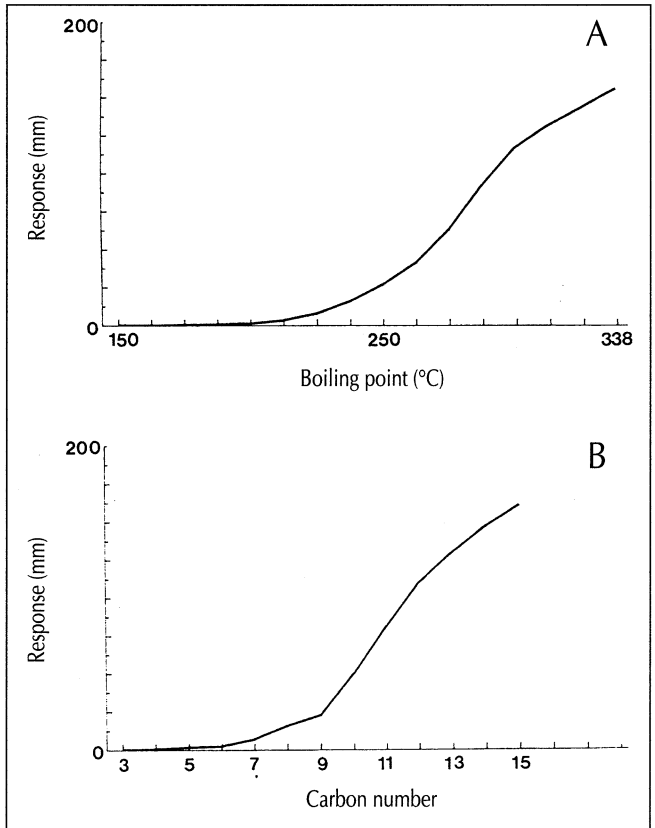


Figure 4. Relationship between response and solute boiling point (A) and effect of solute carbon number on response (B). Conditions: carboxylic acids, C_2–C_{15}; mobile phase, carbon dioxide; flow rate, 4 cm³/min; nebulizer pressure, 0.14 MPa; drift tube temperature, 40°C; injection, 10 µL containing 250 ppm of each compound in methanol–dichloromethane (1:1).

(12,14,15,18), described the significant effects that drift tube temperature can have on detector sensitivity and signal-to-noise levels. We performed similar studies investigating the influence of temperature on the relative response of a mixture of steroids containing cholestane, pregnenolone, and hydrocortisone. The results summarized in Figure 3 agree with previous findings in that a relationship between temperature and analyte response does exist when a temperature range of 30–50°C is used, which is optimum for this particular instrument. Additionally, it is evident from this data that the degree of influence is compound-dependent. This variation in response factors for individual compounds can be attributed to their different physical properties, which directly influence the shape and size of the solute particles formed within the drift tube of the detector (19,20).

Solute-related variables and applications

Detector response and solute volatility

LSDs generally provide nonselective detection for nonvolatile compounds. Analytes with lower boiling points can remain in the vapor phase and pass through the heated drift tube undetected. From their studies using mixtures of fatty alcohols and fatty acids, Nizery and co-workers (12) concluded that analyte volatility had a significant influence over LSD response; the more volatile solutes produced lower signals for a given set of operating conditions. We have investigated this selective behavior by examining the effect of solute volatility on relative response using a homologous series of 14 monocarboxylic acids ranging from ethanoic acid (C_2; melting point [mp], 16°C; boiling point [bp], 117°C) to pentadecanoic acid (C_{15}; mp, 52°C; bp, 340°C) using chlorodifluoromethane (10% methanol) as the mobile phase. A strong correlation between solute carbon number or boiling point and response is evident in Figures 4A and 4B. The lowest member of the series detected under the given conditions was the C_4 acid, butanoic acid (mp, –4.6°C; bp, 162°C), which is a comparatively volatile solute; however, the relative response of this compound was 2 orders of magnitude lower than that of the C_{15} acid. The substantial range of responses exhibited in Figure 4 indicates that the LSD could be inappropriate for quantitative packed-column SFC unless calibration curves are prepared for all potential analytes. Investigations using carbon dioxide as the primary mobile phase and lower modifier concentrations produced similar results: C_4 was the lowest detectable homologue. This marked improvement in the detection of the more volatile components may be explained by the fact that this particular LSD can respond to light scattered through both reflective and refractive mechanisms (photomultiplier 120° to light source) and, therefore, can detect light scattered by both solid and liquid particles. Failure to detect the more volatile solutes

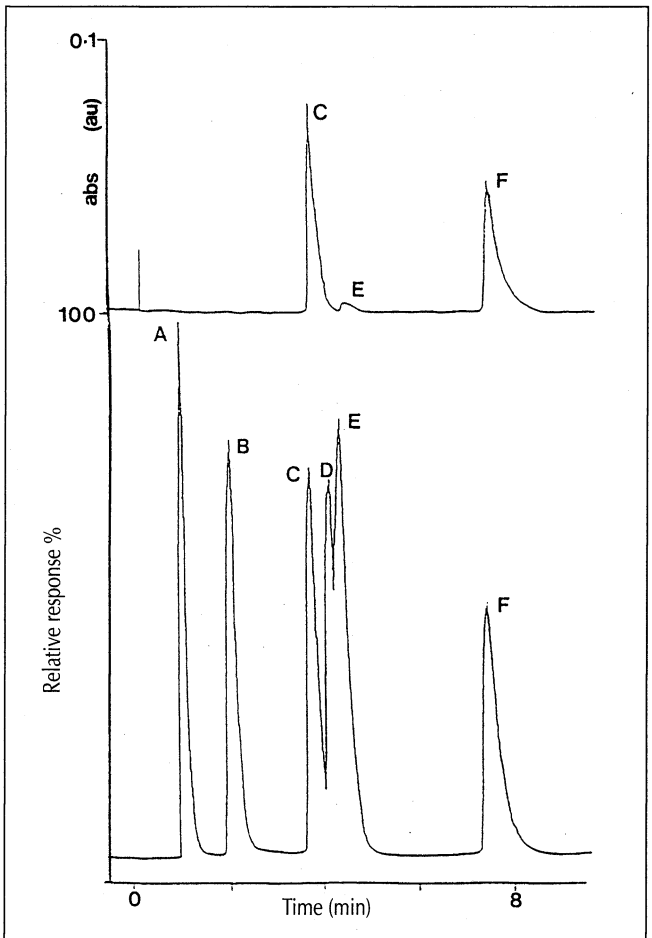


Figure 5. SFC–LSD–UV of six steroids. Conditions: column, Spherisorb silica (250 mm × 4.6-mm i.d., 3-µm particle size); mobile phase, carbon dioxide–10% methanol (70°C, 21 MPa); flow rate, 4 cm³/min; drift tube temperature, 40°C; nebulizer pressure, 0.14 MPa; injection, 10 µL of 300 ppm of each compound in methanol. Peaks: A, cholestane; B, 5β-pregnane-3,20-dione; C, progesterone; D, 5β-pregnane-3α-ol,20-dione; E, pregnenolone; and F, testosterone.

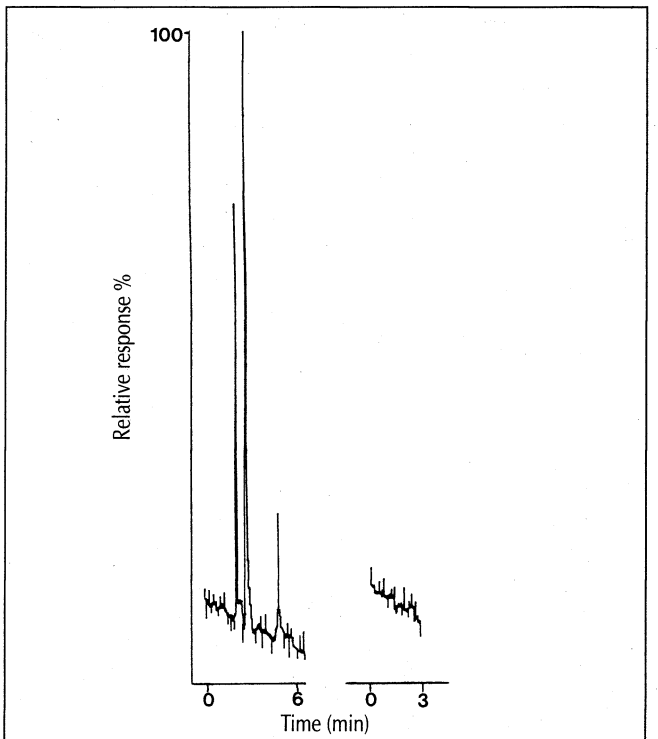


Figure 6. SFC–LSD of three steroids. Conditions: column, Spherisorb silica (250 mm × 4.6-mm i.d., 3-µm particle size); mobile phase, carbon dioxide–10% methanol (70°C, 21 MPa); flow rate, 4 cm³/min; drift tube temperature, 40°C; nebulizer pressure, 0.14 MPa; injection, 15 µL in methanol. Peaks: A, cholestane (200 ng); B, pregnenolone (400 ng); and C, hydrocortisone (200 ng).

is an inherent disadvantage of the evaporative process; however, the data presented here represent a substantial improvement on results that we obtained using HPLC–LSD. In that instance, the lowest homologue detected was octanoic acid (C_8), and the overall sensitivity achieved was only 20% of that afforded by SFC. The magnitude of volatility induced selectivity of the LSD is insignificant in comparison with that inherently derived by UV detection. This is well-demonstrated in Figure 5, which shows the SFC separation of six steroids using UV–LSD in series. Here, UV has completely failed to detect three components of the mixture at 254 nm. The separation was not optimized, but it serves to demonstrate the effective analysis of components in a mixture that possess different UV adsorption maximums.

Minimum detection limits

The minimum detection limit (MDL) of LSDs has progressively improved over recent years. Carraud and co-workers (11) originally reported MDLs of 75 and 40 ng with their system, and this was later improved to 12 ng (12) for a signal-to-noise ratio of 3. The lowest MDL reported to date, which was obtained on a packed capillary column using a laser-based detector, is 2 ng for both Irgafos-168 and trimyristin (9). We have conducted MDL studies on our American Chemical Society (ACS) system using a mixture of three steroids: cholestane, pregnenolone, and hydrocortisone. Figure 6 shows the separation of the mixture with on-column loading of 200 ng for both cholestane and hydrocortisone and 400 ng for pregnenolone using carbon dioxide modified with 10% methanol. Accordingly, the MDL for cholestane has been calculated as 40 ng at a signal-to-noise ratio of 3, for pregnenolone it was 180 ng, and for hydrocortisone it was 340 ng. These values represent an improvement on values obtained using HPLC (800 ng for cholestane) and on previously reported MDLs using the same detector in SFC mode (13,14). However, these calculated values are relatively difficult to reproduce because they are significantly dependent on instrumental operating conditions. For higher concentrations (greater than 1 µg), such as those used for detector response studies, reproducibility improved to the point that a relative standard deviation of 7% over three injections was calculated. The background signal instability of the detector was found to be due to inherent short-term electrical noise. This is evident in Figure 6, which shows a baseline trace obtained using identical settings but without introduction of column eluent or nebulizer gas. Here, the magnitude of noise is comparable with that manifested during on-line operation. A reduction in this electrical noise would significantly improve limits of detection. The long-term negative baseline drift also evident in the figure is believed to be caused by a gradual heating effect on the photomultiplier within the LSD; this is only detectable at high-sensitivity settings.

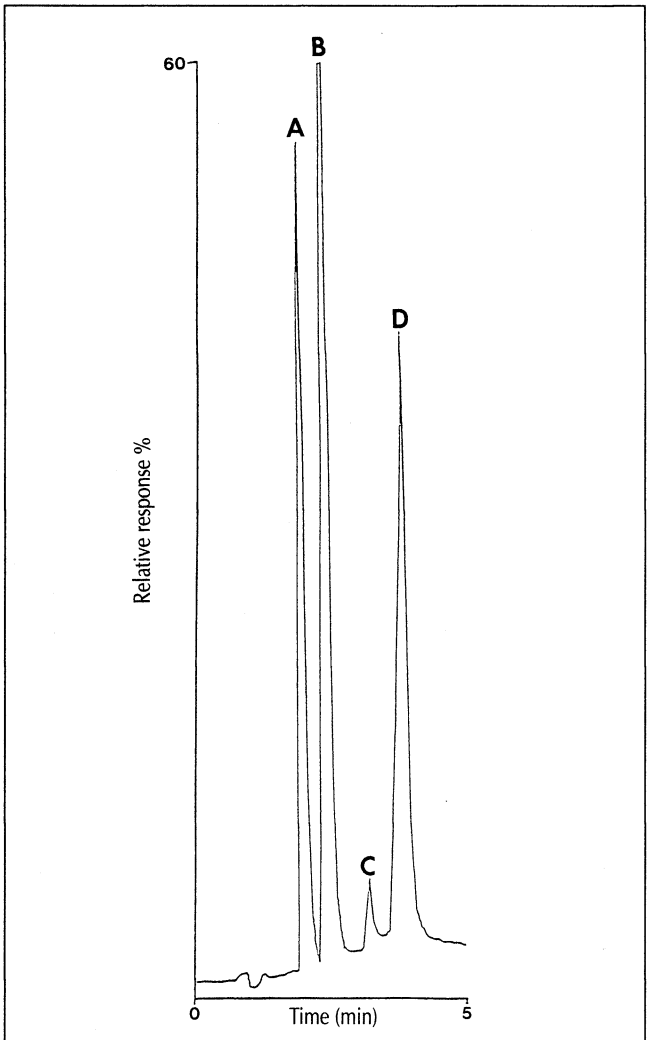


Figure 7. SFC–LSD of four amino acids. Conditions: column, Spherisorb amine (100 mm x 4.6-mm i.d., 3-µm particle size); mobile phase, chlorodifluoromethane–methanol (3% TFA); gradient, 5–20% in 5 min (110°C, 30 MPa); flow rate, 4 cm^3/min; drift tube temperature, 45°C; nebulizer pressure, 0.14 MPa; injection, 10 µL (1.5 µg for arginine and cysteine, 2.0 µg for aspartic acid, and 1.0 µg for glutamic acid in methanol–3% TFA). Peaks: A, arginine; B, aspartic acid; C, glutamic acid; and D, cysteine.

Underivatized amino acids

Amino acids are a group of polar solutes that exhibit poor UV absorption. The less polar PTH- amino acids and FMOC derivatives have been investigated by SFC with UV detection (21–23) and ultraviolet with mass spectrometry (MS) (24). More recently, Lafosse and co-workers (25) reported the effective separation of three of the less polar underivatized amino acids using a mixture of carbon dioxide–methanol–water–triethylamine as the mobile phase and SFC–LSD. Our investigations into SFC of the more polar amino acids have revealed that a primary fluid more polar than carbon dioxide is required as a constituent of the mobile phase. Figure 7 presents results obtained using chlorodifluoromethane modified with 10% methanol containing 3% trifluoroacetic acid (TFA) using an amine column. The chromatogram shows the separation of four underivatized amino acids—arginine, aspartic acid, glutamic acid, and cysteine—in less than 5 min. A moderate increase in baseline height is a direct effect of the rapid modifier gradient used throughout the run (5–20% methanol containing 3% TFA). Attempts to eliminate this effect by adjustment of the drift tube temperature were unsuccessful. The addition of TFA as a tertiary modifier was necessary to maintain optimum chromatographic peak geometry, without which the peaks were broad and unsymmetrical because of severe tailing. The addi-

tion of TFA effects an increase in solubility of analytes within the mobile phase. This is achieved without any permanent solvent–solute reaction and has been confirmed in our investigations using combined SFC–MS. Studies are continuing for the purpose of optimizing the separation of all members of this group of solutes using multiple columns in series with modifier additives that are less corrosive.

Underivatized carbohydrates

Carraud and co-workers (11) first reported the analysis of fructose and sucrose by SFC–LSD using an ODS column with carbon dioxide modified with methanol (7.5%). Later, using a wide range of sugars, Herbreteau and co-workers (26) performed a detailed study in which the selectivity of different columns was investigated. They concluded that the use of supercritical carbon dioxide with a modifier gradient (methanol) in combination with cyano, diol, and nitro bonded-silica columns could provide greater selectivity than HPLC. Recently, a method was reported (25) in which four sugars were separated in less than 11 min using a modifier gradient that did not induce baseline drift. Using our system, we have investigated cyclodextrin and cyano-bonded columns for the separation of carbohydrates. Figure 8 shows the effective separation of eight sugars on both column types in less than 12 min using carbon dioxide with gradient addition of modifier (methanol). It is evident from these chromatograms that both columns provide class separation of carbohydrates; the eight compounds elute as three distinct groups: the mono-, di-, and trisaccharides. The cyclodextrin column affords higher resolution and superior peak symmetry of the later-eluting di- and trisaccharides; however, resolution of the monosaccharides is inferior to that offered by the cyano column. The differences in relative response for components B, C, E, and F (Figure 8) between the two columns cannot be explained, although we believe this behavior to be related to the presence of water in the injection solvent, which is known to affect column selectivity under supercritical conditions. The cyclodextrin column appears to be more retentive under the given conditions. This retention is

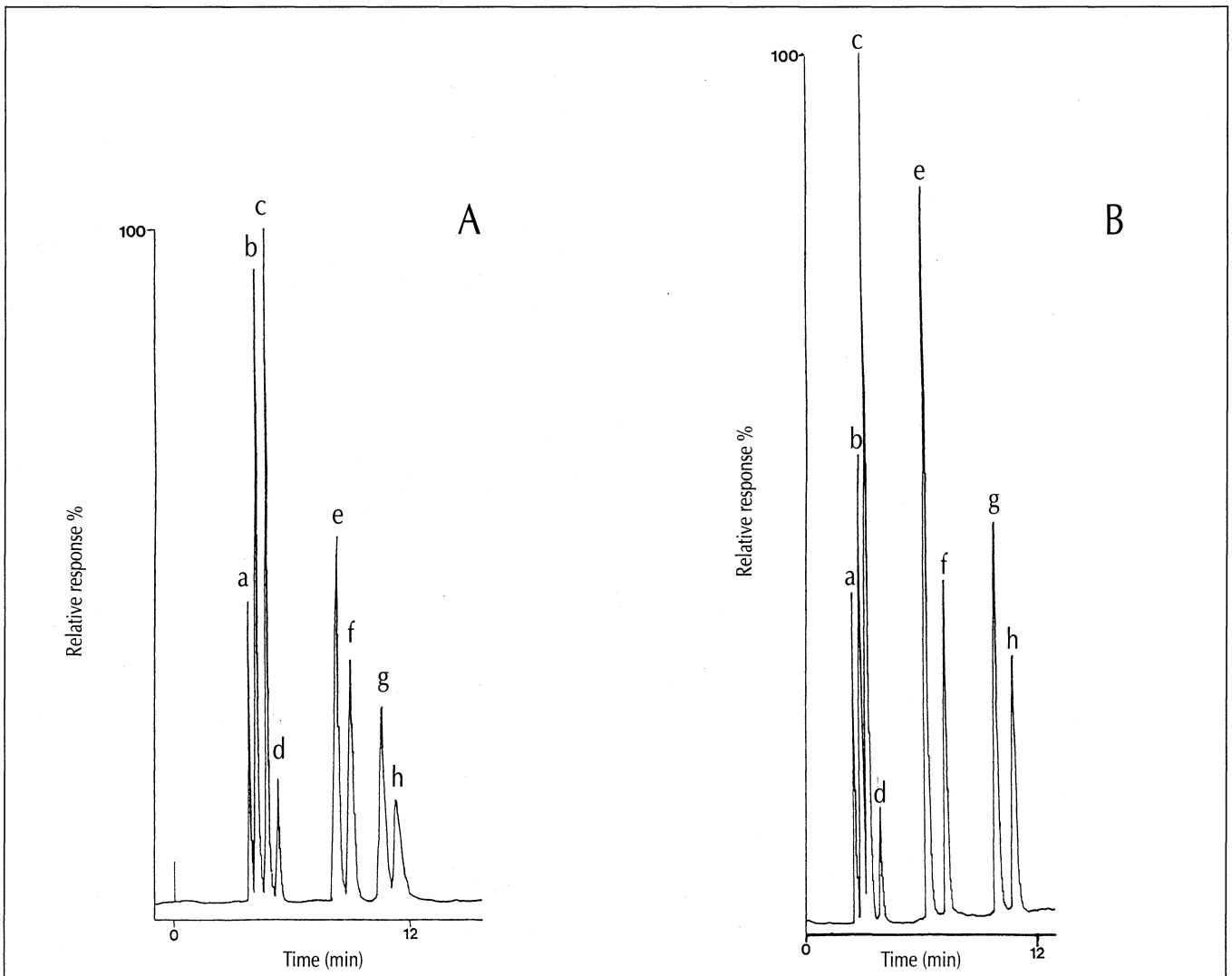


Figure 8. SFC–LSD of eight underivatized carbohydrates. Conditions for A: column, Hypersil CN (250 mm x 4.6-mm i.d., 5-µm particle size); mobile phase, carbon dioxide–methanol; gradient, 8–18% in 4 min (85°C, 22.5 MPa); flow rate 4 cm^3/min. Conditions for B: column, β-cyclodextrin (250 mm x 4.6-mm i.d.); mobile phase, carbon dioxide–methanol; gradient, 20–30% in 4 min (95°C, 25 MPa); flow rate, 4 cm^3/min; drift tube temperature, 35°C; nebulizer pressure, 0.1 MPa; injection, 20 µL in methanol–water (9:1). Peaks: a, ribose; b, xylose; c, fructose; d, glucose; e, sucrose; f, maltose; g, melezitose; h, raffinose.

demonstrated by the need to use a higher concentration of methanol modifier: 20–30% (4 min) compared with 8–18% (4 min) to elute the solutes. It is apparent from the figure that use of elevated quantities of modifier causes baseline drift; however, this does not create a detrimental effect on the quality of separation and represents a significant improvement in class selectivity and sensitivity over results gained using HPLC–LSD.

Peptides

Modifier gradient elution was not necessary for separating the protected peptide hippuryl-L-histidyl-L-leucine hydrate, a substrate for the angiotensin converting enzyme, from an impurity. Figure 9 shows the resulting chromatogram obtained by packed-column SFC–LSD using chlorodifluoromethane modified with 10% methanol (0.5% TFA) at 110°C. The separation of these two components had previously presented a problem as the later-eluting impurity does not contain a chromophore and could not be detected using HPLC–UV or HPLC–LSD because of poor sensitivity. This separation demonstrates the potential of the technique as a rapid purity-screening method.

Ionophores

The antimicrobial and growth-promoting activities of carboxylic polyether antibiotics have influenced their prevalent use as veterinary drugs in the poultry and beef farming industries. Members of this group, which include monensin, salinomycin, and narasin (Scheme 1), exhibit ionophoric properties and are administered in the form of their sodium salts within animal feeds. Because these compounds are toxic in amounts greater than their recommended dosage levels, the analysis of individual ionophores is necessary for monitoring concentrations within animal feeds and meat products. Published methods for the determination of ionophores have been reviewed (27). Because many ionophores do not possess strong chromophores, many of the HPLC methods involve precolumn (28) or post-column derivatization (29,30) procedures to facilitate the effective use of UV detection. Figure 10 shows the SFC–LSD separation of a mixture of the sodium salts of monensin ($C_{36}H_{61}O_{11}Na$), salinomycin ($C_{42}H_{69}O_{11}Na$), and narasin ($C_{43}H_{71}O_{11}Na$), in which separation is effected in less than 5 min with high efficiency without prior derivatization.

The aim of this study was to assess the value of "mass selectivity" offered by the LSD as a comparable means of detection for off-line methods development before final on-line analysis using SFC–MS. Experience indicates that conditions for a method that has been developed and optimized using UV are often inappropriate for separating the same sample using mass selectivity because of the difference in selectivity. Our findings confirm that the development of an SFC method using mass selective detection facilitates method transfer to on-line SFC–MS by minimizing parameter changes that are often required for reoptimization of a separation originally acquired using UV.

Plant pigments

The levels of chlorophylls within leaf tissue can be indicative of plant condition. Chlorophyll surveillance programs are being used to monitor forest vitality in the many areas exposed to atmospheric pollution. Many of the current analyses of these pigments are performed using reversed-phase HPLC methods (31–33), and

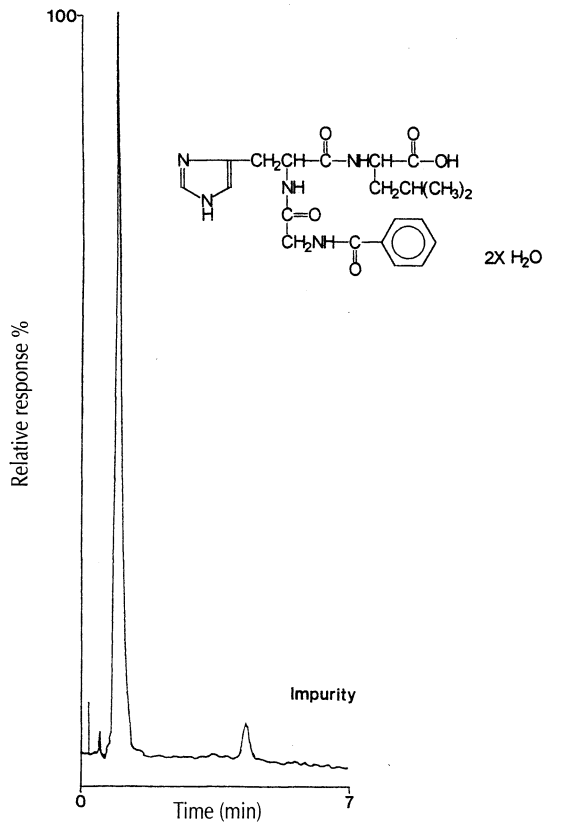
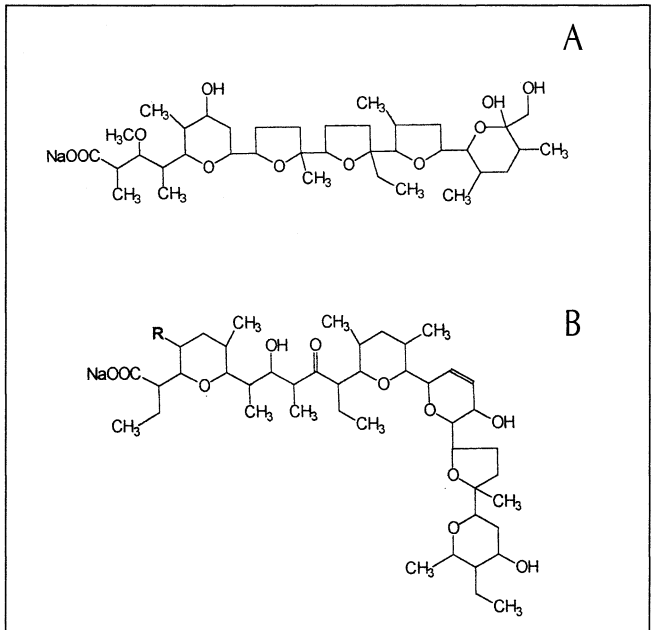


Figure 9. SFC–LSD of an impure standard of hippuryl-L-histidyl-L-leucine hydrate. Conditions: column, Spherisorb amine (100 mm × 4.6-mm i.d., 3-µm particle size); mobile phase, chlorodifluoromethane–10% methanol (0.5% TFA) (110°C, 30 MPa); flow rate, 4 cm³/min; drift tube temperature 45°C; nebulizer pressure, 6.9 × 10^4 Pa; injection, 20 µL of 150 ppm in methanol–3% TFA.



Scheme 1. Structures of monensin (A) and salinomycin (R = H) and narasin (R = CH_3) (B).

more recently there have been reports describing the separation of the yellow-orange carotenoid pigments using SFC (34,35). Recently, we have investigated the potential of SFC as an alternative method for quantitative chlorophyll analysis using UV detection; the findings will be reported in a future publication. As part of this study, leaf pigment extracts were analyzed by SFC–LSD. The extraction was necessary for confirming the separation of chlorophylls from endogenous coextractants by mass selectivity, in contrast to UV detection at 436 nm, which was potentially compound-selective and, therefore, discriminative. Figure 11 shows the effective separation of chlorophylls a and b within a leaf extract using an amine column with carbon dioxide modified with methoxyethanol and ethanal (80:19.4:0.6) as the mobile phase at 130°C. It is evident from the figure that mass separation of chlorophylls from coextractants was successfully achieved.

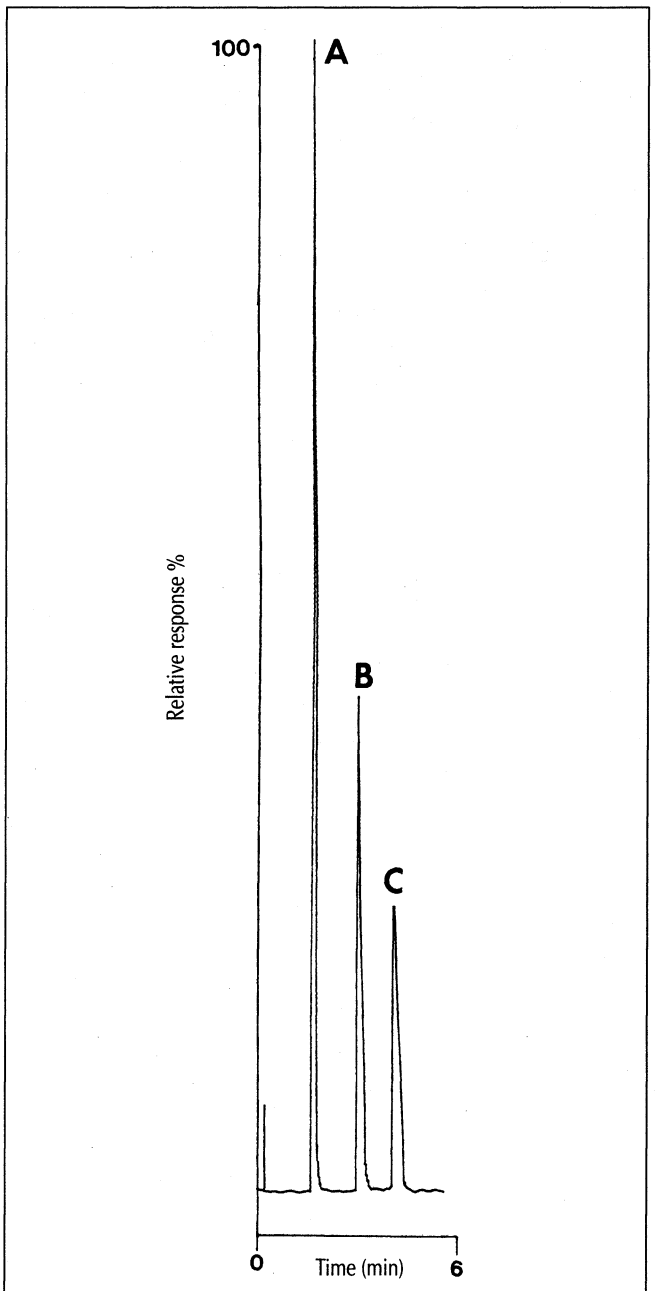


Figure 10. SFC–LSD of the sodium salts of monensin (A), salinomycin (B), and narasin (C). Conditions: column, Alphasil amine (250 mm x 4.6-mm i.d., 5-µm particle size); mobile phase, carbon dioxide–15% methanol (80°C, 30 MPa); flow rate, 4 cm^3/min; drift tube temperature, 55°C; nebulizer pressure, 0.1 MPa; injection, 10 µL of 200 ppm in methanol.

Conclusion

This work demonstrates of the value of the use of evaporative LSD in combination with packed-column SFC for the analysis of mixtures of compounds with weak chromophores. The detection of a wide variety of compounds is possible without modification of this particular design of LSD, although nebulizer spray device geometry, which in this case is used as a secondary restrictor, has a significant influence on sensitivity. The design of the device used in these studies affords better sensitivity than that previously reported using the ACS detector. The technique allows a more rapid analysis of steroids, carbohydrates,

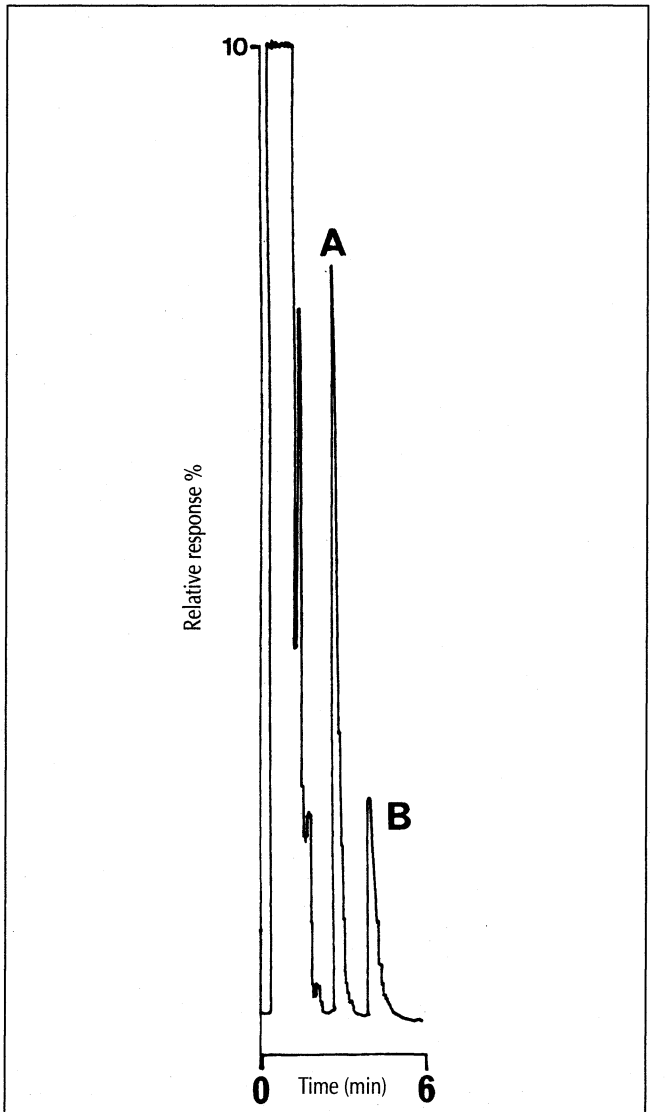


Figure 11. SFC–LSD of a leaf pigment extract containing chlorophylls a and b. Conditions: column, Alphasil amine (250 mm x 4.6-mm i.d., 5-µm particle size); mobile phase, carbon dioxide–methoxyethanol–ethanal (80:19.4:0.6) (130°C, 25 MPa); flow rate, 5 cm^3/min; drift tube temperature, 40°C; nebulizer pressure, 0.14 MPa; injection, 10 µL in methanol.

ionophores, and chlorophylls and shows sensitivity and selectivity superior to that of HPLC. It is an effective screening method for standard preparations that potentially contain non-UV-absorbing impurities.

One of the major disadvantages of the system is that the LSD is a destructive detector and, therefore, cannot be readily used in the preparative mode unless the column eluent is split. Here, a minor portion of total eluent would have to be channeled to the detector for monitoring purposes, whereas the majority would be transferred to an appropriate spray deposition collection device, which is a configuration that would further decrease the sensitivity of the system. These studies have shown that the LSD is compatible with both supercritical carbon dioxide and chlorodifluoromethane using a range of secondary and tertiary modifiers. Chlorodifluoromethane allows the elution of polar solutes not normally amenable to SFC with carbon dioxide; we are currently investigating the use of alternative chlorofluorocarbon mobile phases that are environmentally acceptable. In addition to this, our future studies will center on the improvement of detector sensitivity by reduction of the inherent electrical noise within the instrument and the investigation of the effects of using a resistively heated nebulizer device that could potentially provide a direct means of influencing column eluent nebulization efficiency.

Acknowledgment

We gratefully acknowledge Amersham International, Cardiff, U.K., for the donation of equipment and initial financial support.

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Manuscript accepted November 28, 1995.