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Review

Packed column supercritical fluid chromatography–mass spectroscopy: A review

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Abstract

The literature is reviewed with regard to packed column supercritical fluid chromatography (SFC) coupled directly with mass spectrometry (MS) using either analytical scale or microbore columns that have typically been used for liquid chromatography analysis. Efforts with direct fluid introduction, moving belt interface, thermospray, particle beam, and atmospheric pressure chemical ionization have been discussed. No longer should SFC be a last resort technique but it should be exercised to its full advantage, especially when coupled to MS. With continual advances in commercially available instrumentation, sample assay by SFC–MS should become routine and rugged. © 1997 Elsevier Science B.V.

Keywords: Reviews; Mass spectrometry; Packed columns; Interfaces, SFC–MS

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1. Introduction

Supercritical fluid chromatography (SFC) in recent years has received increasing attention, especially in the pharmaceutical, environmental, petroleum and polymer areas. SFC is an important complimentary technique for either high-performance liquid chromatographic (HPLC) or gas chromatographic (GC) analysis. Many analytes that are not amenable to GC can be separated by SFC and separations via SFC are often more efficient and faster than traditional HPLC analyses.

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Although SFC was initially demonstrated with packed columns in the 1960s, it was not until the 1980s when SFC was performed with open tubular columns that the separation technique became popular. Special columns of small inner diameter (50 μm) and highly cross-linked stationary phases were developed to enable the technique to be used. While these columns afforded a large number of theoretical plates with a minimal column pressure drop and a range of wall-coated stationary phases, the incorporation of modifiers, ultraviolet detection, variable restrictors and simple injection techniques were either not straightforward or not experimentally possible. These experimental difficulties and the need to have a broader range of stationary phases, especially for chiral separations, has called forth a resurgence in packed column SFC. One of the compelling advantages of open tubular columns with 100% CO_2 has always been the wide range of universal, element-selective and spectrometric detectors that can be interfaced to them. Considerable work is currently underway to extend this impressive list of detectors to packed column SFC with the stipulation that both modified fluids and CO_2 density programming are not restricted.

Interfacing SFC with mass spectrometry (MS) is of special interest due to the high degree of sensitivity and selectivity afforded by the MS system. Traditionally, SFC–MS has been used only in situations where either GC or HPLC was not practical, however, with the development of simple and rugged interfaces, SFC–MS has become a valuable technique in the chemist's arsenal of analytical techniques. Numerous papers and reviews have appeared that deal with open tubular columns and high resolution mass spectrometers. This review, however, will concern itself with packed columns and MS. One goal of the review will be to show that interfaces normally used with HPLC and analytical scale (4.6 mm, I.D.) columns are compatible with SFC.

Perhaps the most important aspect of SFC–MS for the scientist interested in separations is the chromatographic interface from the packed column to the mass spectrometer. Much research on the development of easy-to-use and efficient interfaces has been reported. From a chromatographic point of view, the "ideal" interface should posses several qualities: (1) Chromatographic integrity should be maintained, (2) a range of ionization methods should be possible, (3) high analyte transport efficiency and ready ionization should occur, thus producing a highly sensitive technique, (4) no thermal degradation of the analyte should occur within the interface, (5) compatibility with both different fluids under pressure programming conditions and modifiers should be possible, thereby enabling highly versatile chromatographic separations and (6) a reliable, low maintenance interface is highly desirable. No existing packed column SFC–MS interface has all the qualities desired at this time. Nevertheless, much useful information has been forthcoming from a variety of packed column SFC–MS interfaces. This review will focus only on packed column SFC–MS using either analytical scale (4.6 mm, I.D.) or microbore scale (1–2 mm, I.D.) columns that have typically been used for HPLC analysis.

2. Direct fluid introduction

Direct coupling between packed column SFC and MS was one of the earliest interfaces investigated [1,2]. This design is very simple and somewhat rugged, but is limited in the amount of fluid that can enter the MS system without losing spectrometric performance due to high ionization source pressures. This limitation often results in the need to split the column effluent prior to entering the MS system, which, in turn, reduces the sensitivity of the SFC–MS technique. This interface is attractive since little or no additional hardware is required to use it with commercially available mass spectrometers. In addition, library-searchable spectra, similar to electron impact (EI) as well as chemical ionization (CI) spectra, with differing reagent gases can be obtained very easily.

Crowther and Henion [3] published one of the earliest reports of directly coupled packed column SFC with MS. Direct coupling was possible using a heated probe and a small pinhole diaphragm to maintain supercritical conditions in the restrictor. This allowed efficient analyte transfer to the MS system, however, a post-column split of 1/12 was required to yield low source pressures where optimum sensitivity and system performance were

obtained. Detection limits were found to be near 20 ng (on column) for codeine and other alkaloids, such as cocaine and methocarbamol, utilizing chemical ionization while scanning the MS system from 149–450 m/z, even with methanol modifier concentrations of 3–10% (w/w). The desired modifier concentration was achieved by mixing pure CO_2 with a premixed 10% methanol-modified CO_2 (w/w) cylinder. Extracts of phenylbutazone from equine urine were assayed using a 200×2.1 mm I.D., 10 μm Diol column and 10% methanol-modified CO_2. Both negative and positive CI with methane reagent gas were performed, but negative CI was found to improve the sensitivity of the oxygenated compound. The overall sensitivity was expected to be enhanced compared to similar analyses performed using direct liquid insertion (DLI) LC–MS.

Later, Smith and Usdeth [4] developed the high flow-rate (HFR) SFC–MS interface (Fig. 1), which incorporated a mechanically pumped expansion region prior to the CI chamber. This allowed higher flow-rates (100–200 mg/min of decompressed CO_2) to enter the MS system without the loss of system performance. Less background contributions, ionization efficiency improvement and better sensitivity were obtained with this interface than with previous direct coupling interfaces. Several separations were demonstrated for both open tubular and microbore packed SFC–MS systems, including Triton-X-100, coal tar, a mixture of pesticides and herbicides, and a polarity test mix using isobaric, pressure programming, and fluid programming (microbore) conditions. Analysis of the polarity test mix that comprised naphthalene, acetophenone, *n*-ethylaniline, decane, *p*-chlorophenol, 1-decanol and pentadecane produced poor chromatographic results, even with the addition of 1% by volume methanol (e.g. mixed by dosing the CO_2 pump with a fixed volume of methanol) using a microbore (250×1.0 mm, I.D.) packed C_{18} column. They reported much better results with an open tubular column, but this is not the focus of our review.

Kalinoski et al. [5] employed the HFR interface for the analysis of two ionic polyether antibiotics and the cyclic undecapeptide, cyclosporin A. SFC separation was achieved using a 100×1.0 mm I.D. ODS Hypersil column and 2% (by volume) methanol was used to modify the mobile phase and to serve as the CI reagent. Cyclosporin A was eluted within 5 min, with only slight tailing, and was found to yield

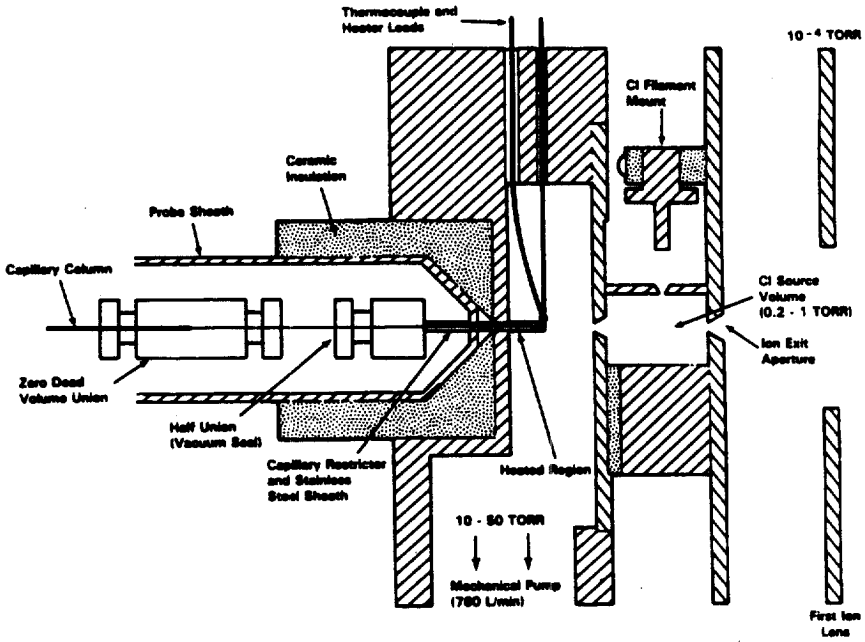


Fig. 1. Schematic illustration of the high flow-rate (HFR) interface.

almost exclusively an M+1 molecular ion using methanol as the modifier and CI reagent. The two ionic polyether antibiotics were assayed by open tubular SFC, but results were not presented for microbore SFC.

Kalinoski and Smith [6] again employed the HFR interface for the analysis of organophosphorus insecticides using microbore (1.0 mm, I.D.) packed columns. By using 2% 2-propanol-modified CO_2, prepared by dosing the CO_2 pump with a fixed volume of solvent, a 100×1 mm I.D. amino column and density programming, seven of eight insecticides were separated in less than 8 min. The use of the HFR interface allowed the entire column effluent to enter the MS system without splitting the flow. The addition of ammonia as a CI reagent gas produced good M+18 ions for all but two insecticides. The 2-propanol modifier was found to function as a CI reagent, producing almost exclusively M+H ions in the mass spectrum without the addition of additional CI gases. No change in the ion current was observed by using either NH_3 or 2-propanol as CI reagents. During selected-ion monitoring with 2-propanol as the CI reagent, 94 pg (on column) of chlorpyrifos yielded a *S/N* ratio of 2:1, while other insecticides gave similar limits of detection.

Direct fluid introduction (DFI), although a very simple interface, presents several limitations for packed column SFC–MS, with flow limitations being the most severe. Microbore separations can take full advantage of the simple design and are still being pursued. However, DFI is much more common for open tubular SFC, where the flow-rate is within working limits and modifier is rarely used.

3. Moving belt interface

The moving belt interface allows the decoupling of the SFC separation and the MS detection. By depositing the analyte on a belt, the flow-rate and composition of the mobile phase can be altered without regard to a loss in the system's performance within practical limits. This interface is also advantageous since either true EI or CI spectra can be obtained because most of the mobile phase is eliminated prior to entering the MS system. The moving belt interface is problematic, however, in that poor sensitivities are obtained, sample carry-over can occur and many non-volatile and polar molecules cannot be analyzed, due to poor analyte desorption from the belt in the ionization source.

Berry et al. [7] used a moving belt interface to study the separation of nine polyaromatic hydrocarbons (PAHs) using 5% methoxyethanol-modified CO_2 at 4 ml/min liquid flow on a 250×4.6 mm ODS column in less than 10 min. The column effluent had to be split between the spray device of the moving belt and a back pressure regulator after passing through a UV detector, however, the majority of the effluent passed through the MS interface. The back pressure regulator served to maintain pressure in the chromatographic system. Benzene was found to be absent from the MS trace and the abundance of naphthalene was greatly reduced compared to on-line UV assay. The high volatility of these analytes led to poor analyte deposition onto the belt. High quality EI spectra that gave excellent matches when searched in a computer database were obtained for all other PAHs detected. Berry et al. [8] later published the first full paper on moving belt SFC–MS. The applicability of the technique was illustrated with mixtures of xanthines and sulphonamides.

Ramsey et al. [9] investigated a moving belt interface in combination with supercritical fluid extraction (SFE)–SFC–MS–MS for the detection of veterinary drugs from freeze-dried pig kidney. The extracted drugs (trimethoprim, hexestrol, diethylstilbestrol, dienestrol and sulfamethazine) were deposited onto a 100×4.6 mm I.D. Spherisorb NH_2 column. Non-polar co-extractives passed to waste followed by elution of the drugs from the column by programming the mobile phase from 100% CO_2 to 20% methanol-modified CO_2 in 0.5 min after an 8-min initial hold time at flow-rates of 4 ml/min, with subsequent MS–MS detection. At these flow-rates, a 1:1 split of the column effluent was require prior to entering the moving belt interface. The single stage MS spectrum for trimethoprim was completely obscured because of co-eluting endogenous material. With SFE–SFC–MS–MS (Fig. 2), the daughter ion spectrum, however, allowed unambiguous detection at 10 mg of drug/kg of kidney. A technique called selected-reaction monitoring improved the sensitivity of MS–MS in a similar

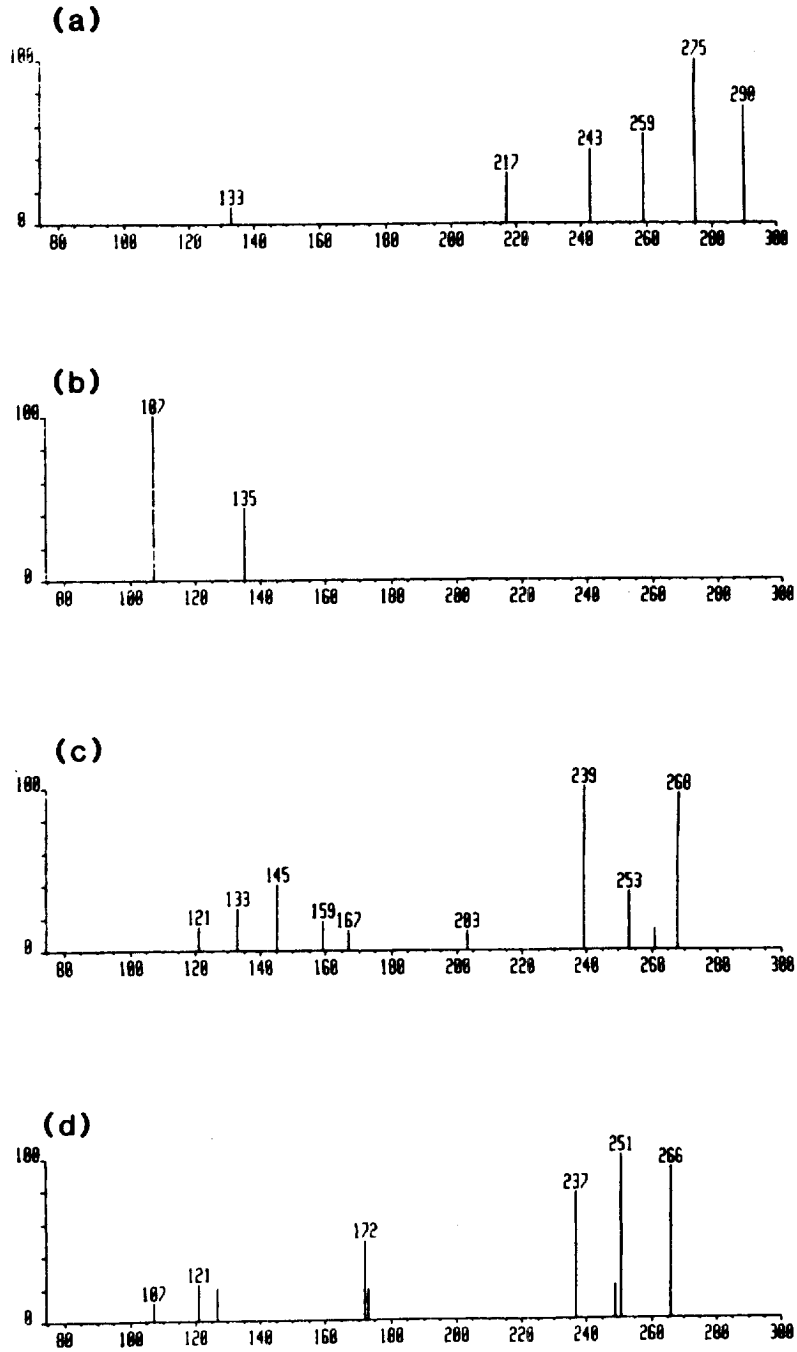


Fig. 2. Daughter ion mass spectra obtained for (a) trimethoprim, (b) hexestrol, (c) diethylstilbestrol and (d) dienestrol. Results from SFE–SFC–MS–MS analysis of kidney sample spiked with 10 mg/kg of each drug.

manner to that of selected-ion monitoring (SIM) and, in so doing, a level of 1 mg/kg (e.g. drug to matrix) of trimethoprim, dienestrol, hexestrol and diethylstilbestrol could be readily detected after extraction.

Perkins et al. [10] also used the moving belt interface to analyze sulfonamide standards in addition to sulfonamides spiked onto pig kidney. SFC–MS analysis of seven sulfonamides showed that chromatographic integrity was maintained using either 250×4.6 mm I.D. silica or amino columns at flow-rates of 4 ml/min and methanol modifier gradients of 12 to 25% compared with an SFC–UV assay, although some variability was observed for compounds eluting during the modifier gradient portion of the separation. Due to the high flow-rates employed, the column effluent was split 1:1 between a pressure regulator and the moving belt spray device. The presence of solvent in the source caused significant changes to the EI spectra. Fragments used for identification changed intensity and much simpler "CI-like" spectra occurred when solvent was present in the mobile phase. A 13-ng injection of a spiked pig kidney drug extract could be detected in an extracted ion chromatogram (*m/z* 214), but not in the total ion chromatogram. Due to the inherent poor sensitivity of the moving belt interface, thermospray MS in filament-on mode was also attempted. Although better sensitivity was obtained with a thermospray interface, badly tailing peaks were produced in the thermospray interface, which was found to be more susceptible to the mobile-phase gradient than the moving belt.

Young and Games [11] analyzed *Fusarium* mycotoxins by SFC–UV and SFC–MS using a moving belt interface with 10% methanol-modified CO_2 and either a 100×2 mm I.D. 3 μm Hypersil silica column at a flow-rate of 2 ml/min or a 250×4.6 mm I.D. Spherisorb NH_2 column at a flow-rate of 4 ml/min of liquid. The SFC–MS analysis of a liquid culture extract revealed six components, of which, four (culmorin, 7-hydroxyisotrichodermol, iso-4-deoxynivalenol and 4-deoxy-nivalenol) could be identified by comparing EI spectra with spectra from injected standards. Results were confirmed using ammonia and methane CI. Additional extracts enabled the identification of six other compounds (culmorone, sambucoin, calonectrin, 3,15-diacetyl-4-deoxynivalenol, 15-deacetylcalonectrin and 3-acetyl-4-deoxynivalenol) following spectral deconvolution procedures. The detection limits of a variety of mycotoxin standards were found to range from 10–250 ng (on-column) using the moving belt interface.

Scandola et al. [12] employed SFC with a double focusing MS system equipped with a moving belt interface to identify unknown natural products. Alkaloids from *Securidaca longipedunculata* roots were extracted using methanol and were characterized by SFC–MS. The extracts were best separated using a 250×4.6 mm, 3 μm aminopropyl column with a methanol modifier gradient ranging from 5–12% at a flow-rate of 3 ml/min of liquid. Both EI and CI (methane) modes were used to help identify and confirm the alkaloids present in the extract. Collisionally activated decomposition (CAD)–mass analyzed ion kinetic energy (MIKE) MS studies were also employed to better characterize ion fragments. Confirmation of preliminary data previously obtained on two alkaloids resulted in compound identification and produced structural information on another alkaloids present in the extract.

Games et al. [13] demonstrated a multidimensional technique combining HPLC solute focusing with SFC–MS with a moving belt interface. Solute focusing provided a means of sample concentration. Following HPLC solute focusing, the solvent was removed from the precolumn with a stream of nitrogen. The system was then pressurized with a supercritical mobile phase of 6% methanol-modified CO_2 and the compounds were eluted from a 250×4.6 mm I.D. Spherisorb NH_2 column at flow-rates of between 3 and 4 ml/min. Sample sizes as large as 1500 μl were used in the solute-focusing phase, thus greatly improving detectability. Although the moving belt interface yields inherently poor sensitivity, solution concentrations as low as 100 ng/ml of paracetamol spiked in plasma could be detected, as well as 10 μg/ml of stanozolol in a methanol–water plasma extract.

Interest in the moving belt interface has decreased with the advent of new MS interfaces. The moving belt, however, does allow EI or CI spectra to be obtained, which makes this interface attractive for semi-volatile, relatively non-polar analytes. Due to the problems associated (e.g. sensitivity and analyte

desorption) with this interface, its use is mainly relegated to specific cases and is not considered a general analytical tool.

4. Thermospray interface

The thermospray interface has previously received considerable attention, however, little work is currently being performed using this interface. The thermospray source requires "filament on" or "discharge on" modes of operation following nebulization of the column effluent from a heated interface. The thermospray suffers from several drawbacks: (1) The source is designed to operate at constant vapor throughput, corresponding to a constant flow-rate, which eliminates the possibility of density programming with a fixed restrictor unless a variable make-up gas is used (modifier programming is possible), (2) low sensitivity arises because many of the ions produced are not directed into the mass analyzer, (3) the intensities of mass fragments are not always indicative of actual mass proportion, thus causing errors in spectral interpretation and (4) thermal degradation is very possible, due to the heated nebulizer. The source does, however, allow both EI and CI ionization modes to be used without hardware alterations.

Chapman [14] reported coupling a conventional 2 mm I.D. HPLC column to a standard thermospray source equipped with a discharge ionization electrode. The column outlet was directly interfaced to the thermospray source without flow splitting. Naphthalene, fluorene, anthracene and retene were separated in under 10 min using CO_2 as the mobile phase and the separation exhibited excellent peak shape and resolution. The positive ion response was found to greatly increase when approximately 20 μl/min of methylene chloride was added coaxially to the column effluent at the probe tip. Coaxial addition of additional CI reagents was also found to alter the spectrum obtained. Elution of two disaccharide peracetates with pure CO_2 and a 20-μl/min coaxial flow of 20% diethylamine in *n*-hexane produced a quasi-molecular ion, 74 *m/z* above the molecular mass due to the addition of protonated diethylamine as well as fragment ions due to cleavage of the glycosidic bond of the disaccharide.

Berry et al. [15] investigated packed column SFC–thermospray MS in "filament on" mode with and without the presence of organic modifier. Varying the concentration of methanol modifier from 5 to 20% was found to change the mass spectrum of methiocarb following elution from a 100×4.6 mm I.D. Spherisorb NH_2 column at 4 ml/min. The high flow-rate required a 1:1 split prior to entering the thermospray source. At higher modifier concentrations, a larger methanol adduct ion was present compared to the situation where lower modifier levels were employed. The carbamate pesticide pirimicarb was detected at 64 pg injected in scan mode, but the system was not believed to be optimized and even lower levels were thought to be possible. Four carbamate pesticides were well resolved within 1 min, which compared favorably to SFC–UV using 5% methanol modified-CO_2 on a 100×4.6 mm I.D. Spherisorb NH_2 column at 4 ml/min. Elution of heptachlor, *o,p'*-DDT, *p,p'*-TDE and lindane from a 100×4.6 mm I.D. Spherisorb column with pure CO_2 was shown to produce nearly "EI like" spectra that compared favorably with Environmental Protection Agency/National Institutes of Health (EPA/NIH) library spectra.

Balsevich et al. [16] employed SFC–MS using the thermospray source to analyze indole alkaloids from the leaves of *Catharanthus rosues*. A separation of the natural product extract employed a modifier gradient of 5–15% methanol on a 100×4.6 mm I.D. Spherisorb NH_2 column. The column effluent was split (ratio was not specified) between a back pressure regulator and the spray device for both moving belt and thermospray MS, due to the high flow-rates employed. A moving belt interface was used initially to obtain pure EI spectra of the alkaloids present in the sample. By comparing reference EI spectra with spectra obtained using the moving belt interface, fourteen of the leaf's components were identified. The thermospray source in "filament on" mode was then used to enhance the sensitivity of detection compared to that of the moving belt interface. The number of alkaloids detected in the SFC–UV trace (approximately 40) increased to approximately 60 by employing SFC–thermospray MS. A typical thermo-

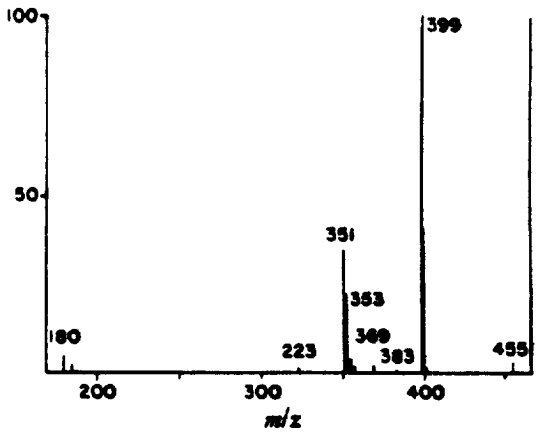


Fig. 3. Thermospray mass spectrum of eluent from SFC proximate to desactoxyvinoline peak.

spray mass spectrum of acetoxyvindoline is shown in Fig. 3. Thermospray ionization produced mainly M+1 ions, thus making the determination of the molecular mass of the major alkaloids possible, but skewing of mass fragment intensity made interpretation of the spectra difficult.

The thermospray source was used by Berry et al. [17] to assay a series of pesticides (i.e. atrazine, simazine, dimethirimol, teracil, menazin and ethirimol), using a 100×4.6 mm I.D. Spherisorb column with constant 10% methanol-modified CO_2 and a flow-rate of 4 ml/min. The high flow rate required a 1:1 split between a pressure regulator and the spray device of the thermospray source. Six pesticides were separated with near baseline resolution in under 3 min. The presence of methanol in the column effluent caused CI-like spectra to be obtained with almost exclusively M+1 ions present in the mass spectrum. However, when no methanol was present and the filament on mode was used, EI-type spectra were obtained. Since methanol was required for separation and CI-type spectra were obtained, MS–MS studies were suggested to yield more useful spectral data.

Ecdysteroids were assayed using a SFC–MS system equipped with a thermospray interface by Morgan et al. [18]. The system employed a tee that split the flow 1:1 between the thermospray and a pressure regulator, which was designed to maintain the desired conditions. The SFC method, which employed a 100×4.6 mm I.D. Hypersil column and 20% methanol-modified CO_2 at 4 ml/min, was able to separate the *R*- and *S* isomers of inokosterone, which is a very difficult separation using HPLC. Additionally, detection limits of ecdysone were found to be 10 ng (on column) using a non-optimized system. The presence of methanol in the system again caused CI-type spectra to be obtained whereas, EI-type spectra were obtained without the addition of co-solvent. The M+1 ion intensity from CI-type ionization improved the sensitivity of the system compared to highly fragmented EI-type ionization.

Anticancer drugs (Fig. 4) were assayed by SFC–CI thermospray MS by Musser and Callery [19]. A conventional thermospray probe was modified to accommodate a capillary restrictor for SFC and reagent gas for CI introduction directly into the thermospray source. CI was obtained using either "filament on" or a "discharge electrode", with the discharge producing more efficient ionization, which resulted in a ten-fold increase in signal over the "filament on" mode. Diaziquone injected at 50 ng

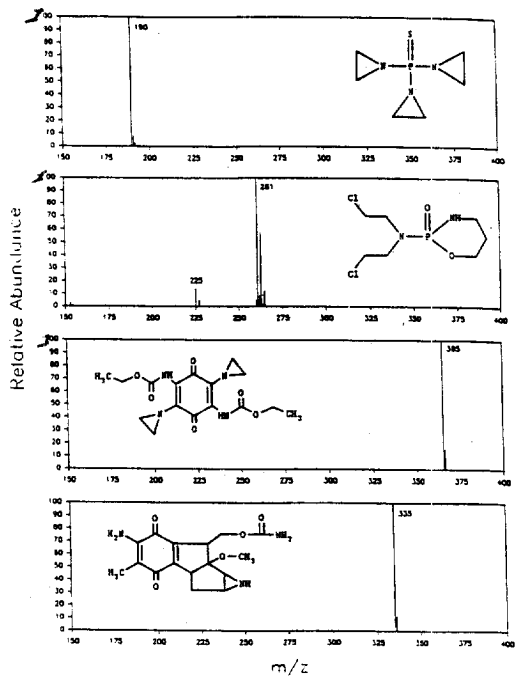


Fig. 4. SFC–CI mass spectra of (a) thiotepa, (b) cyclophosphamide, (c) diaziquone and (d) mitomycin C. SFC conditions were 400 atm, 80°C oven temperature and 5% methanol modifier. Source pressure was adjusted to 1.0 torr with methane.

was found to give a S/N ratio of 6:1 in full scan mode (150–500 a.m.u.) following elution from a 100×1 mm I.D. 3 μm Nucleosil column using 5% methanol-modified CO_2. Modifier was added to the mobile phase through a high pressure injection valve that was fitted with a 1-ml injection loop.

Conjugated bile acids were analyzed by on-line SFC–thermospray MS by Scalia and Games [20]. Their method separated four glycine and four taurine derivative bile acids in 8 min using a 250×4.6 mm I.D. Zorbax cyanopropyl column with a methanol modifier gradient from 20 to 28% at a flow-rate of 4 ml/min. The high flows were split between a pressure regulator and the spray deposition device, but split ratios were not reported. The glycine conjugates were found to be much less intense than the taurine derivatives using negative-ion MS. The cause was unknown but it was speculated to be related to acid strength. "Discharge-assisted" ionization was attempted in place of "filament on" ionization in the hope of increasing the signal of the glycine derivatives, but no enhancement was observed and the signals of the taurine derivative decreased. The negative-ion MS of the bile acids produced exclusively $(M-H)^-$ ions, even when the scan range was reduced by 300 m/z. A bile sample (from a human with chronic hepatitis) that had been purified by solid phase extraction (SPE) prior to analysis produced mass spectra that were identical to the standard negative-ion mass spectra of conjugated bile acids.

Via and Taylor [21] also employed a SFC–thermospray MS system using a 2-mm I.D. column and a fixed restrictor with pressure programming to 400 atm. Positive-ion CI was accomplished using methane and negative-ion CI could be achieved by using CO_2 alone, as a mediator. Source and nebulizer conditions were optimized for the analysis of typical propellent additives and decomposition products (Fig. 5). The source temperature was found to affect the methane–CO_2 ion profile, but little change in the mass spectra of the propellent additives was observed over the temperature range studied. Negative-ion CI was found to greatly enhance the sensitivity of three nitrated compounds, due to their high electron affinities. Using an optimized system and negative CI conditions, 0.4 pg of 2,4-dinitrotoluene could be analyzed at a S/N ratio of 20. N-Nitrosodiphenylamine could be identified by SFC–thermospray MS in positive CI mode, whereas GC–MS caused thermal breakdown of the nitroso compound. Actual propellent samples were assayed in a similar manner using both positive- and negative-ion CI. Older propellant samples showed the presence of more stabilizer breakdown products compared to a more recently prepared propellant sample.

The thermospray source, although receiving considerable attention in the late 1980s and early 1990s, has recently received little attention. Thermospray suffers from several limitations that are problematic for routine and consistent use. A lack of sensitivity is also inherent, making trace analysis difficult. This technique will mainly be relegated to established analyses where no better alternative is available.

5. Particle beam interface

The particle beam interface is similar to the moving belt interface in that it is able to decouple the chromatography from the MS analysis. This allows a much wider range of conditions to be used, since most of the mobile phase is eliminated prior to entering the MS system. The particle beam uses a two-stage momentum separator to separate analytes from the mobile phase. This causes problems when the analyte is volatile, since much of the analyte is eliminated with the mobile phase instead of entering the MS system. Highly non-volatile samples are also problematic because the analyte normally impacts the MS source where it is volatilized and ionized. If the analyte is non-volatile, the source can become contaminated and no signal will be observed. This interface also suffers from a lack of sensitivity due to the loss of the analyte in the momentum separator while trying to remove the mobile phase. The biggest attribute to the particle beam interface is the ability to obtain pure EI and CI spectra with little spectral interference from the mobile phase.

Edlund and Henion [22] were the first to evaluate a prototype particle beam interface for SFC–MS. The fixed restrictor was placed in a heated nebulizer tip in which make-up helium could be added to aid the nebulization of the column effluent. Flows of up to 2 ml/min could be handled by this interface before insufficient pumping of the momentum separator occurred (Fig. 6). The distance of the

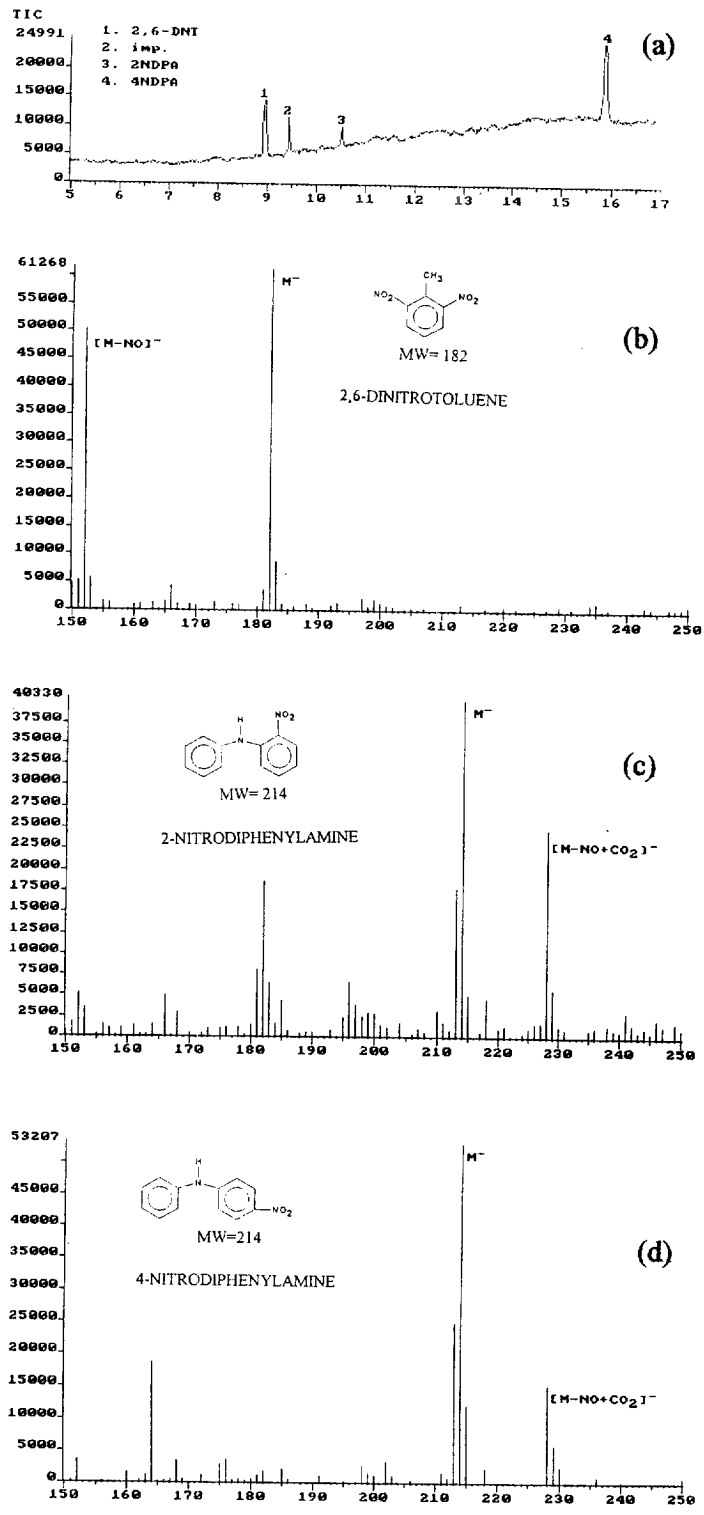


Fig. 5. TIC and CO_2-moderated NCI spectra (background-subtracted) of the propellant test mixture. Source at 366°C.

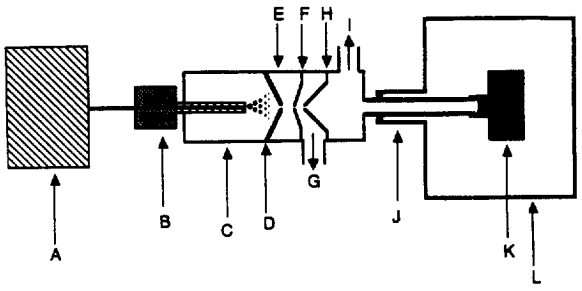


Fig. 6. Block diagram of the overall packed-column particle beam SFC–MS system: (A) HP model 1084B liquid chromatograph modified for SFC, (B) linear restrictor housed in a heated probe equipped with solvent and helium make-up tees, (C) desolvation chamber maintained at 44°C, (D) nozzle, (E) two-stage momentum separator, (F) skimmer, (G) 400 l/min rough pump on first stage of pumping, (H) skimmer, (I) 760 l/min two-stage rough pump on second stage of pumping, (K) heated dual EI–CI ion source on HP 5985B GC–MS system.

restrictor relative to the nebulizer probe tip was found to be the most important adjustment for sensitivity. Several classes of compounds were evaluated, including insecticides, pesticides, polymer additives and corticosteroids. Each chemical class produced high quality EI spectra that could be library searched. Modifier and modifier gradients could be used without sample interference and analysis of thermally labile corticosteroids and non-volatile polymer additives were easily accomplished. This method was also used to (a) monitor prednisolone in horse urine, (b) identify an unknown in a drug tablet and (c) aid the identification of an unknown metabolite of the vasodilator pentoxifylline.

Jedrzejewski and Taylor [23] further characterized the particle beam interface using a commercially available LC–MS interface. Experiments were conducted using pure and methanol-modified CO_2 as the mobile phase. The system was optimized with respect to (a) the restrictor position at the end of the nebulizer, (b) the pressure of the nebulizing gas, (c) the temperature of the desolvation chamber, (d) the distance of the nebulizer relative to the opening of the nozzle, (e) the mobile phase flow-rate and (f) the mobile phase composition. The concentration of methanol modifier was found to greatly affect the overall system sensitivity, with 4% being optimal. Using optimal conditions, the limit of detection for caffeine was found to be 40 ng (on column) using a scan mode that was 150 a.m.u. wide and 5 ng using single-ion monitoring. Separations of phenylurea pesticides (Fig. 7) and steroids using a 100×1 mm

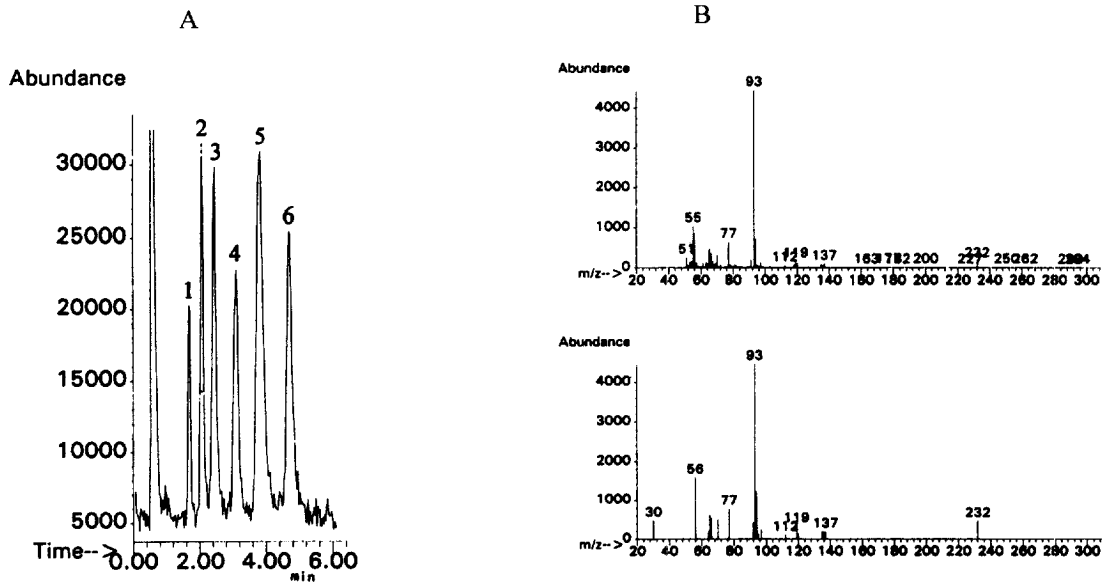


Fig. 7. (A) Separation of phenylurea pesticides. Peaks: 1=metobromuron; 2=linuron; 3=chlorbromuron; 4=monuron; 5=siduron and 6=diuron. Conditions: Dionex SFC system; column, 100×1 mm I.D. Deltabond CN, maintained at 85°C; 4% methanol-modified CO_2; 197 bar; flow-rate, 0.20 ml/min; injection, 0.5 μl of 1.0 μg/μl solution per component. (B) EI spectrum of siduron (top) and a library spectrum (bottom).

I.D. Deltabond CN column and 4% methanol-modified CO_2 were employed to demonstrate system performance. Background-subtracted EI spectra were found to be artifact-free and comparable with library spectra.

Jedrzejewski and Taylor [24] again evaluated the particle beam interface with respect to various operational parameters, including a particle-forming solvent and chromatographic parameters. A wide optimum range was observed for mobile phase flow-rate (up to 1.1 ml/min) using pure CO_2 under flow injection conditions. The addition of a small amount (20–50 μl/min) of organic solvent to the column effluent (i.e. particle-forming solvent) was found to have a profound effect on sensitivity. It was believed that the particle-forming solvent acted to sweep the volume of the connected plumbing, to foster aerosol formation and to improve analyte transport to the ion source. Addition of the particle-forming solvent reduced the dependence of sensitivity on mobile-phase composition, allowing modifier programming from 0–8% to be used without changing the sensitivity. A separation of seven carbonate pesticides on a 250×1 mm I.D. Hypersil silica column using pressure programming from 145 to 400 atm. and 4% methanol modifier with a particle-forming solvent flow of 20 μl/min methanol demonstrated the system's utility. EI spectra were artifact-free and comparable to on-line library spectra.

Due to the limitations of the particle beam interface, little research is currently being performed. Pure EI and CI spectra, however, can be obtained making the particle beam an acceptable choice in cases where sensitivity, volatility and analyte polarity are not an issue.

6. Atmospheric pressure ionization sources

Currently, atmospheric pressure ionization (API) sources, both electrospray and APCI (atmospheric pressure chemical ionization), have received a great amount of attention, especially in LC–MS. API source performance is mostly independent of flow-rate changes when pressure and temperature gradients are used, which allows more versatile chromatographic conditions to be employed. Two fundamentally different processes (i.e. gas phase ionization and liquid phase ionization) are exploited in an API source to ionize analytes following SFC separation.

6.1. Atmospheric pressure chemical ionization

APCI is gas-phase ionization using a corona discharge. When samples are present in the gas phase, they can be ionized at atmospheric pressure. This ionization method requires restrictor temperatures to be greater than 200°C in order to completely vaporize the solvent and solute. Corona discharge ionization would at first appear to be limited to volatile samples, but it has been shown to extend to the limit of masses and polarities for which SFC is operational [25]. Typically, this ionization method is termed APCI because solvent molecules are ionized in a corona discharge, where ionization occurs when there is molecular collision with sample analytes. APCI sources are versatile because the degree of fragmentation can be controlled to some extent. Drift voltage in the source can be altered, thus affecting the degree of collisions take place, therefore, "CI-like" to "EI-like" spectra can be obtained with the same source.

Matsumoto [26] used SFC–APCI-MS to determine the relative hydrogen atom affinities of acenaphthylene, fluorene, anthracene and pyrene. The system employed a 100×1 mm I.D. ODS column and pure CO_2 and the entire column effluent was directed into the MS system. System pressure was maintained via an integral restrictor, which was heated to 300°C by a vaporizer. The intensity ratios of M^+ and MH^+ of fluorene, anthracene and pyrene were obtained in SIM mode at different drift voltages. Intensity ratios can be used to produce information regarding the dissociation of MH^+ to $M^+ + H$, whose energy difference is termed hydrogen affinity (HA). A threshold voltage was found by extrapolating two linear lines of MH^+/M^+ ion ratios at low and medium drift voltages. Threshold voltages for the three PAHs were plotted against literature HA values to give a rectilinear line. HA values for unknowns were estimated using this calibration curve.

Anacleto et al. [27] employed SFC–APCI-MS

(Fig. 8) for the analysis of PAHs. The PAHs were separated with pure CO_2 on a 250×1 mm I.D. silica column with a pressure gradient from 2000 to 5000 p.s.i. The entire column effluent was directed into a triple quadrupole MS after moving through a heated (250–300°C) restrictor flowing at 80 ml/min. An additional flow of 1–2 l/min of high purity air was added as a make-up gas. Comparison of SFC–APCI MS with SFC–photoionization detection (PID) showed excellent chromatographic integrity. The addition of benzene as a CI reagent caused benz[*a*]anthracene to primarily undergo charge transfer, due to it having a lower ionization energy than benzene, however, in the presence of water, proton transfer is the primary ionization mechanism. Detection limits for benz[*a*]anthracene were estimated at around 100–150 pg (on column). Analysis of United States National Institute of Standards and Technology (NIST) coal-tar standard reference material again showed different ionization processes (i.e. the M^+/MH^+ ratio depended on the size of the aromatic moiety, furans and thiophenes showed primarily M^+ ions, and nitrogen-containing compounds exhibited only MH^+ ions). These differences were confirmed in the selected ion chromatogram for 168 *m/z* with dibenzofuran (M_r=168, M^+) and carbazole (M_r=167, MH^+) identified by comparison with individually injected standards.

Sjöberg and Markides [28] reported the SFC–APCI MS analysis of steroids. They were able to control the ionization process by using different CO_2 additives, such as water, deuterium oxide and dichloromethane. Additives were added to the CO_2 via a tee within the SFC oven prior to entering the restrictor. The addition of dichloromethane produced a $(M+Cl)^-$ ion for oleandrigenin, whereas ambient air yielded primarily $(M+H)^-$ and $(M-H-HAc)^-$ ions. Labile hydrogens could be identified using deuterium oxide-enriched air, through deuterium exchange. Differentiation of steroid isomers was possible through collision-induced fragmentation in the source or by using MS–MS to induce fragmentation (Fig. 9). Differentiation of isomers could also be enhanced by altering the selected ionization or fragmentation mode. Typically, positive-mode APCI was twice as intense as negative-mode APCI.

Huang et al. [29] also reported on SFC–MS using an APCI interface. A heated LC–MS pneumatic nebulizer equipped with a pinhole restrictor, to maintain supercritical conditions, allowed the entire column effluent to be directed into the corona discharge region of the source. A synthetic mixture

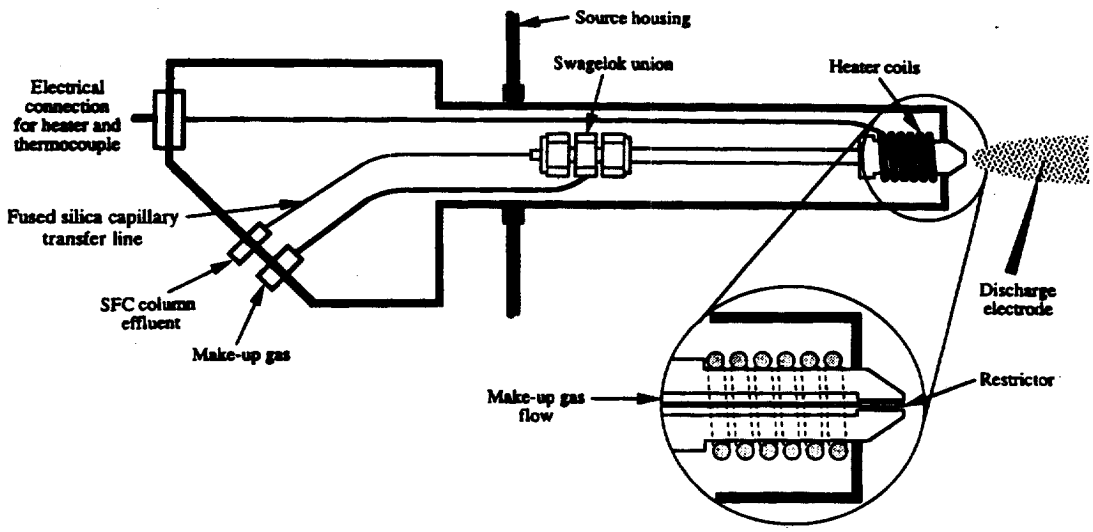
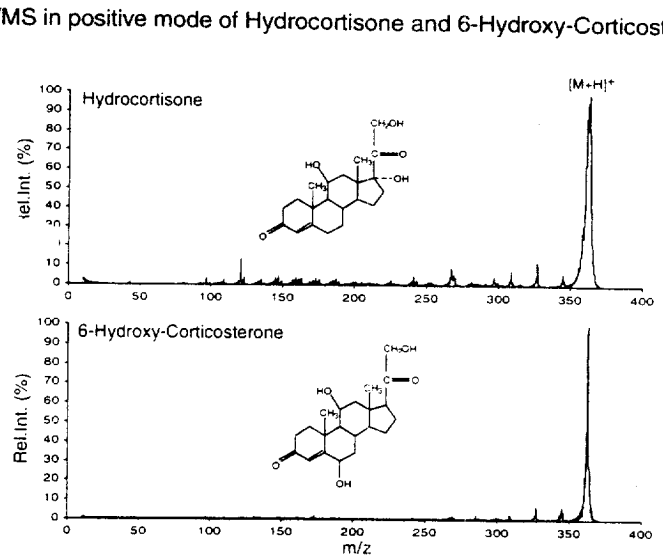


Fig. 8. Schematic diagram of the improved SFC–APCI-MS interface.



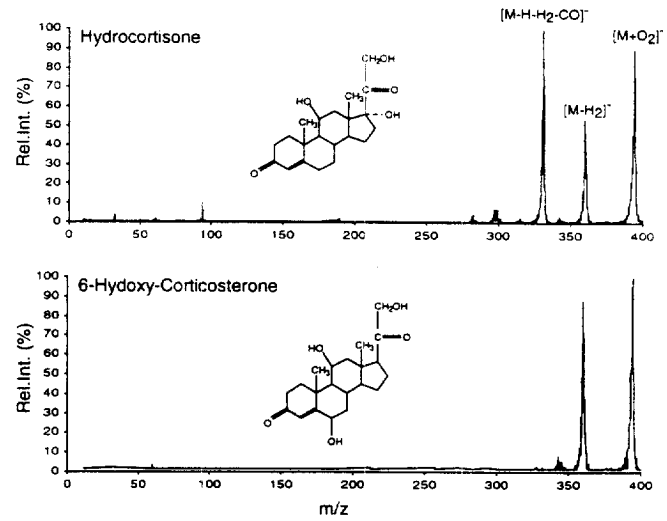


Fig. 9. SFC–APCI-MS–MS analysis of steroids in positive and negative modes.

of corticosteroids was separated using 6% methanol-modified CO_2 at a flow-rate of 2 ml/min on a 200×4.6 mm I.D. silica column with an inlet pressure of 3400 p.s.i. SFC–MS–MS studies were stated to yield more structural information, although these data were not shown. The system was found to be easily interfaced to SFC and was thought to be amenable to capillary SFC–MS, with minimum modification.

The analysis of phenols in water at the ppb level using the direct SFE of aqueous samples combined on-line with SFC–MS has been reported more recently [30]. A high pressure multivalve switching system provided a means of interfacing a direct

aqueous SFE vessel to a packed column SFC–MS system. Using full scan negative ion APCI, the combined SFE–SFC–MS instrumentation enabled the analysis of phenols in water of the 40 ppb level. The mass spectrum for each phenol was characterized by the production of an intense [M−H] negative-ion species.

6.2. Electrospray ionization

Electrospray ionization occurs in the liquid phase. Solute ions can be formed at atmospheric pressure from liquid solution aerosols in electric fields of a few kV, termed electrospray ionization. Electrospray ionization has the potential to analyze molecules with molecular masses in excess of 50 000 a.m.u., since this method allows multiple charges to be placed upon the analyte, thus generating m/z ratios in the range of typical quadrupole mass spectrometers. A very similar method, ion-spray ionization, uses a pneumatically assisted probe tip, however, ions are formed in a similar manner to that of electrospray ionization.

In a review by Arpino et al. [25], SFC–electrospray MS of four herbicides was achieved within 4 min using a methanol gradient from 0–4% with a 2.1 mm I.D. Zorbax Rx C_{18} column and a flow-rate of 1 ml/min. Electrospray ionization in the liquid droplets briefly formed in the expansion jet was observed. The limit of detection was found to be 10 pg (S/N= 3) (on column) using single ion monitoring. With restrictor temperatures below 100°C, predominately MH^+ ions, with little or no fragment ions, were observed. The restrictor temperature, however, was found to be critical. At temperatures above 150°C, vaporization of the mobile phase occurred and ionization ceased. At temperatures below 50°C, solid microparticles formed, thus stopping ionization.

Sadoun et al. [31] have reported a more detailed study on parameters affecting electrospray ionization when interfaced to a packed column SFC system. A schematic of the system is shown in Fig. 10. Due to the ion formation process, it is important that the restrictor be electrically conducting to avoid distributing charge build-up. Therefore, the last 10 cm of a fused-silica restrictor were coated with nickel-plated paint or a stainless steel crimped restrictor was used. Both mobile phase and restrictor temperature were found to affect the MH^+ ion intensity. Decreased ion abundances occurred at temperatures above 120°C, due to a decrease in ionization. A mobile phase consisting of pure CO_2 produced no ions under any conditions, whereas, at a modifier concentration of 2–3%, an optimal response for 1-chloro-2-aminopyridine MH^+ ions occurred at a flow-rate of 1 ml/min. However, 20% modifier and flow rates of 150 μl/min were found to produce an optimal response. However, at a constant modifier flow-rate, changes in the CO_2 flow-rate were found to greatly affect ion abundance. SFC–electrospray MS does however allow a much wider range of flows than those of conventional HPLC–electrospray, since the CO_2 can act as a nebulizing gas. On analysis, high molecular mass compounds were found to tail

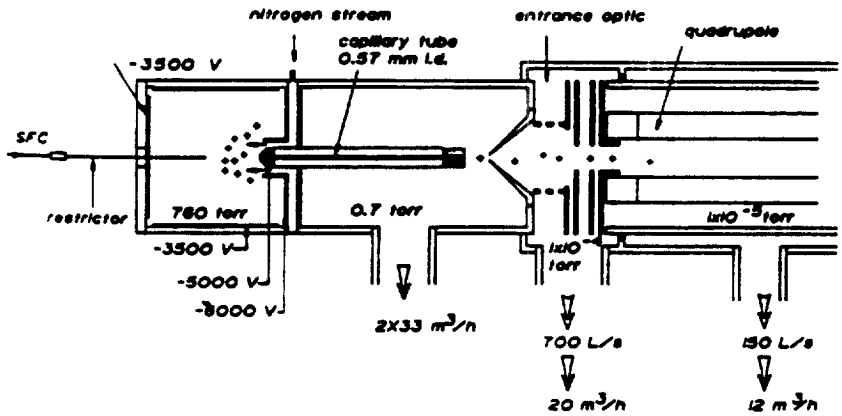


Fig. 10. Schematic diagram of the SFC–MS interface and electrospray ion source.

severely. This tailing was attributed to cold trapping at the restrictor tip. Heating of the restrictor or increasing the temperature of the mobile phase improved peak shape but decreased ion currents. Flushing the outside of the restrictor with organic modifier is thought to alleviate this problem, but differing ion formation with mobile-phase composition does, however, cause experimental difficulty.

Atmospheric pressure ionization (API) is currently the most active area of growth for both HPLC–MS and SFC–MS systems. It is believed that this trend will continue to grow as this technique becomes known. It is a powerful technique since both gas phase (APCI) and liquid phase (electrospray) ionization contribute to the overall utility of API sources.

7. Conclusions

Supercritical fluid chromatography coupled with mass spectrometry continues to advance as a valuable detection system for chemical analysis. SFC presents an attractive method for samples that are not amenable to either GC or LC, but is also useful for routine assay. No longer should SFC be a last resort technique, but it should be exercised to its full advantage, especially when coupled with mass spectrometry. With continual advances in commercially available instrumentation, sample assay by SFC–MS should become routine and rugged.

References

[1] L.G. Randall, A.L. Wahrahaftig, Anal. Chem. 50 (1978) 1703–1705.
[2] L.G. Randall, A.L. Wahrahaftig, Rev. Sci. Intr. 52 (1981) 1283–1295.
[3] J.B. Crowther, J.D. Henion, Anal. Chem. 57 (1985) 2711–2716.
[4] R.D. Smith, H.R. Udseth, Anal. Chem. 59 (1987) 13–22.
[5] H.T. Kalinoski, B.W. Write, R.D. Smith, Biomed. Environ. Mass Spectrom. 15 (1988) 239–242.
[6] H.T. Kalinoski, R.D. Smith, Anal. Chem. 60 (1988) 529–535.
[7] A.J. Berry, D.E. Games, J.R. Perkins, Anal. Proc. 23 (1986) 451–453.
[8] A.J. Berry, D.E. Games, J.R. Perkins, J. Chromatogr. 363 (1986) 147–158.
[9] E.D. Ramsey, J.R. Perkins, D.E. Games, J.R. Startin, J. Chromatogr. 464 (1989) 353–364.
[10] J.R. Perkins, D.E. Games, J.R. Startin, J. Gilbert, J. Chromatogr. 540 (1991) 239–256.
[11] J.C. Young, D.E. Games, J. Chromatogr. A 653 (1993) 374–379.
[12] M. Scandola, D.E. Games, C. Costa, G. Allegri, A. Bertazzo, J. Heterocyc. Chem. 31 (1994) 219–224.
[13] D.E. Games, J.A. Rontree, I.A. Fowlis, J. High Resolut. Chromatogr. 17 (1994) 68–71.
[14] J.R. Chapman, Rapid Commun. Mass Spectrom. 2(1) (1988) 6–7.
[15] A.J. Berry, D.E. Games, I.C. Mylchreest, J.R. Perkins, S. Pleasance, Biomed. Environ. Mass Spectrom. 15 (1988) 105–109.
[16] J. Baksevich, L.R. Hogge, A.J. Berry, D.E. Games, I.C. Mylchreest, J. Nat. Prod. 51(6) (1988) 1173–1177.
[17] A.J. Berry, D.E. Games, I.C. Mylchreest, J.R. Perkins, S. Pleasance, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 61–64.
[18] E.D. Morgan, S.J. Murphy, D.E. Games, I.C. Mylchreest, J. Chromatogr. 441 (1988) 165–169.
[19] S.M. Musser, P.S. Callery, Biomed. Environ. Mass Spectrom. 19 (1990) 348–352.
[20] S. Scalia, D.E. Games, Org. Mass Spectrom. 27 (1992) 1266–1270.
[21] J. Via, L.T. Taylor, Anal. Chem. 66 (1994) 1385–1395.
[22] P.O. Edlund, J.D. Henion, J. Chromatogr. Sci. 27 (1989) 274–282.
[23] P.T. Jedrzejewski, L.T. Taylor, J. Chromatogr. A 677 (1994) 365–376.
[24] P.T. Jedrzejewski, L.T. Taylor, J. Chromatogr. A 703 (1995) 489–501.
[25] P.J. Arpino, F. Sadoun, H. Virelizier, Chromatographia 36 (1993) 283–288.
[26] K. Matsumoto, Org. Mass Spectrom. 29 (1994) 266–268.
[27] J.F. Anacleto, L. Ramaley, R.K. Boyd, S. Pleasance, M.A. Quilliam, P.G. Sim, F.M. Benoit, Rapid Commun. Mass Spectrom. 5 (1991) 149–155.
[28] P.J.R. Sjoberg and K.E. Markides, Poster presented at the 6th International Symposium on Supercritical Fluid Chromatography and Extraction, Uppsala, Sweden, 1995.
[29] E.C. Huang, T. Wachs, J.J. Conboy, J.D. Henion, Anal. Chem. 62(13) (1990) 713A–724A.
[30] E.D. Ramsey, B. Minty, M.A. McMullagh, D.E. Games, A.T. Rees, Anal. Commun. 34 (1997) 3–6.
[31] F. Sadoun, H. Virlizier, P.J. Arpino, J. Chromatogr. 647 (1993) 351–359.