Journal of Chromatography, 400 (1987) 307–316
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1165

ANALYTICAL APPLICATIONS OF CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY–MASS SPECTROMETRY

H. T. KALINOSKI, H. R. UDSETH, B. W. WRIGHT and R. D. SMITH*
Chemical Methods and Separations Group, Chemical Sciences Department, Pacific Northwest Laboratory, Richland, WA 99352 (U.S.A.)*

SUMMARY

Capillary chromatographic analyses of thermally labile, less volatile and higher molecular weight compounds can be accomplished using supercritical mobile phases due to the combination of solvating powers and attractive mass transport properties. Recent advances in the use of small bore (25–50 μm) capillary columns, rapid pressure programming methods, and polar mobile phases have yielded high-sensitivity and high-speed analyses with chromatographic resolution approaching that of conventional capillary gas chromatography. Mass spectrometry (MS) provides a nearly universal detector for supercritical fluid chromatography (SFC), and the ease with which capillary SFC can be directly coupled with the mass spectrometer avoids the complications inherent in liquid chromatography (LC)–MS interfaces. Capillary SFC can be interfaced with both electron impact and chemical ionization (CI) modes of operation, and high selectivity and sensitivity as well as structural data can be obtained through appropriate choice of CI reagents. Recent developments in capillary SFC–MS and applications to several compound classes are described.

INTRODUCTION

The analytical potential of capillary supercritical fluid chromatography (SFC)[1] and its combination with mass spectrometry (SFC–MS)[2] is currently being realized. The first successful applications of SFC–MS were demonstrated relatively recently[3–5], although the first reports on SFC appeared in the 1960s[6,7]. Progress in liquid chromatography (LC) and the subsequent focus on LC–MS interfacing, combined with the complexity of early SFC instrumentation, are among the reasons for the slow development of SFC–MS. However, perceived limitations in LC–MS interface methods and enhanced SFC performance possible with the introduction of capillary columns prompted further development of SFC–MS. In the course of this development, it has become obvious that SFC (and SFC–MS) serves as an excellent complement to both gas chromatography (GC) and LC, and particularly their combinations with MS. This complementary role derives from the physical properties of

* Operated by Battelle Memorial Institute.

0021-9673/87/$03.50

supercritical fluids and the ability to readily vary their solvent power by pressure control. The densities of supercritical fluids are variable between the limits of the normal gas and liquid by pressure control, and, to a first approximation, the solvent power of the fluid is proportional to density. Under typical SFC conditions, where fluid densities range from *ca.* 0.1 to *ca.* 0.8 of liquid density, diffusion coefficients are substantially greater than in liquids. Viscosities of typical supercritical fluids are 10–100 times less than for liquids[8]. The various fundamental interactions which define the limits of applicability of SFC are beyond the scope of this paper and are described in greater detail elsewhere[9,10]. However, it must be understood that supercritical fluid phase solubility and fluid phase behavior play vital roles in SFC and SFC–MS and that an understanding of these phenomena is often necessary for their successful application.

A few general statements should be made in discussing SFC and SFC–MS. The lower viscosities and higher diffusion coefficients of supercritical fluids relative to liquids results in significantly enhanced chromatographic efficiency per unit time compared to liquids. More than 3000 and 12 000 effective theoretical plates per meter can be obtained with 50-μm and 25-μm I.D. columns, respectively[11]. In SFC, mobile phase temperature is maintained somewhat above the critical temperature of the fluids, and in general, it is advantageous to utilize the highest temperature compatible with the SFC system and the material being analyzed. Mild thermal conditions (determined by the critical temperature of the chosen mobile phase) allow application to labile compounds, that cannot be analyzed by GC. The negligible pressure drop at normal linear velocities and column dimensions of open tubular columns, allows full exploitation of pressure programming techniques. Pressure programming in capillary SFC imparts many of the advantages of gradient elution in LC, and allows very rapid pressure programming to effect high speed separations[12]. Further, because the flow-rates in capillary columns are lower than those in packed column SFC or LC, interfacing to MS is easier. However, at times, the use of higher flow-rates in SFC is preferred. This includes (a) very high speed separations where only few effective plates are required, (b) utilization of packed or microbore columns to exploit the wider range of stationary phases currently available and (c) situations where greater sample loading is necessary.

The solvating powers of supercritical fluids extend the applicability of chromatography to analyses that are difficult by GC due to insufficient volatility or thermal lability or by LC due to insufficient chromatographic resolution or selectivity. Thus, SFC–MS is now applicable to mixtures of moderately polar compounds that are thermally labile or of relatively high molecular weights. SFC–MS is not a general replacement for LC–MS or GC–MS, but it may permit analysis of otherwise intractable mixtures. In cases where mixtures can be analyzed by either SFC–MS or LC–MS, SFC–MS offers significant advantages. The analyte molar concentration in the mobile phase is, to a reasonable approximation, inversely proportional to the density in the mobile phase and directly proportional to peak width. Thus, both chromatographic resolution and sensitivity, given the same ionization process, are expected to be in the order GC > SFC > LC. Either resolution or sensitivity can be improved at the expense of the other. Recent applications of SFC–MS are briefly described in the following sections.

Broad implementation of SFC requires a sensitive and selective detector with

universal applicability; the mass spectrometer provides such a capability. In contrast to other methods of detection in SFC, the mass spectrometer is compatible with a wide range of mobile phases, including polar fluid mixtures. A broad range of analytes is also compatible with MS detection, and the mass spectrometer, with the ability to acquire both chemical ionization (CI) and electron ionization (EI)[13] spectra, provides the basis for obtaining both high sensitivity and selectivity or structural information for the identification of unknown materials.

EXPERIMENTAL

The apparatus used for capillary SFC–MS has been described in detail previously[3–5,14]. A syringe pump under computer control generates a pulse-free flow of high purity supercritical solvent with fluid pressures programmed to increase the fluid solvating power. The temperature of the capillary column is maintained to the point of injection with a gas chromatograph oven and an air-heated direct fluid injection (DFI) probe[3–5]. The DFI interface probe tip has been designed to allow heating of the capillary pressure (flow) restrictor, independent of the oven, probe and ion source[15]. A schematic diagram of one design of this interface, for interface to an Extranuclear (Extrel, Pittsburgh, PA, U.S.A.) "simultaneous" dual EI–CI source, is shown in Fig. 1. The optimum temperature of the heated region is generally not a highly sensitive variable, but might be anticipated to increase for less volatile compounds[15]. A modification of this design permits application to high fluid flow-rates by incorporation of a direct pumping region just prior to the CI source volume[10]. This modification also provides for efficient analyte transfer to the ionization volume, particularly for less volatile materials by maintaining high fluid densities at the restrictor exit[10].

Fused-silica capillary columns, typically 25, 50 and 100 μm I.D., coated with bonded and cross-linked polysiloxane stationary phases, were used for chromatography. Generally 50-μm (I.D.) columns were employed with cross-linked 5% phenyl polymethylphenylsiloxane (SE-54 type) stationary phases. Sample introduction with a Valco C14W HPLC injection valve was followed by ambient temperature flow splitting. Chromatography on packed columns or 100-μm I.D. capillary columns with thick stationary phase coating (used with the high flow interface) was performed with splitless injection[10]. A fused-silica capillary restrictor, reproducibly drawn in a manner similar to that described by Chester *et al.*[16], was employed to allow depres-

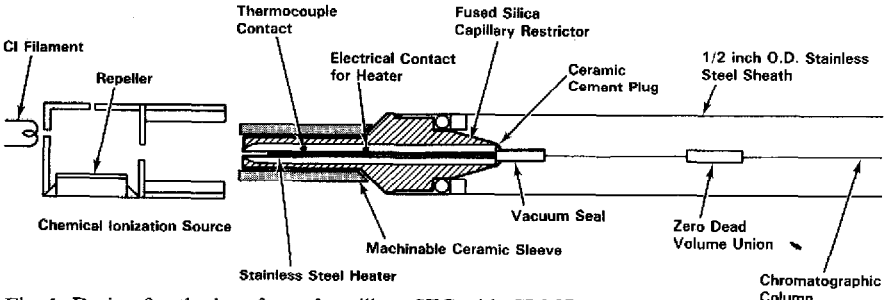

Fig. 1. Design for the interface of capillary SFC with CI-MS.

surization of the fluid and was connected to the terminus of the chromatographic column by a zero dead volume union.

CI mass spectra, obtained following SFC, with ammonia, methane, propane, isobutane, pentane and other common CI reagents, and the interface to an EI source has also been described earlier[13]. A large number of supercritical solvents have been employed in SFC–MS, including carbon dioxide, nitrous oxide, pentane, ethane, propane and fluid mixtures such as carbon dioxide with methanol, 2-propanol and water, or hydrocarbons with alcohol modifiers. In the present work a 5% (v/v) 2-propanol solution in carbon dioxide was used at temperatures between 110–125°C. The mixture was prepared by charging the syringe pump with an appropriate volume of 2-propanol (Burdick and Jackson, Muskegon, MI, U.S.A.) and filling the remaining volume with purified (passed through an activated charcoal and alumina adsorbant trap) carbon dioxide (99.99% anaerobic grade, Airco, Vancouver, WA, U.S.A.).

RESULTS AND DISCUSSION

Complex mixture analysis

The characterization of complex mixtures requires both high separation effi-

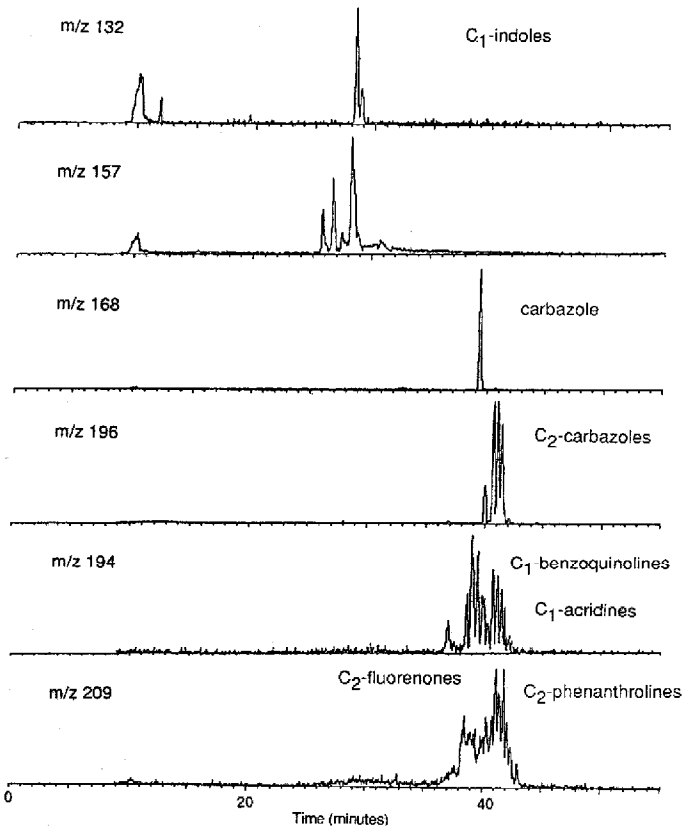

Fig. 2. Selected ion chromatograms obtained in capillary SFC–MS of a diesel fuel fraction with a carbon dioxide–2-propanol supercritical mobile phase and methane as the CI reagent.

ciencies and universal detection with good sensitivity. The SFC–MS separations of a polar diesel fuel fraction shown in Fig. 2 illustrate the high resolution achievable by capillary SFC, the use of polar modified fluids for transport of polar compounds, and the selectivity offered by mass spectrometric detection. The selected ion chromatograms are given for the analysis of a polar fraction from a marine diesel fuel fractionated on alumina[17,18]. Carbon dioxide supercritical mobile phase containing 5% (v/v) 2-propanol was used with a 50-μm I.D. capillary column for the separation and methane CI for MS. The complex fraction, including many nitrogen-containing heterocyclic compounds, was not completely resolved on this relatively short (< 10 m) column. The compounds were efficiently transported to the CI source of a single quadrupole mass spectrometer, and tentative identification was made on the basis of molecular weights. The relative complexity of some of the selected ion chromatograms, such as m/z 194 and m/z 209, indicated more than one type of compound contributed to the signal of these mass-to-charge ratios. Identifications of other compounds could not be made on the basis of molecular weight information alone, *e.g.*, the compounds at m/z 157, nor could assignment of specific isomers of the various alkyl substituted heterocycles be made. The added dimension of molecular weight information did aid in the characterization, but more advanced techniques, such as tandem MS, are clearly necessary for more complete characterization of such complex mixtures[19].

Polymeric materials

Complete characterization of polymeric materials requires information on the molecular weight of oligomers as well as the relative abundance of each oligomer. SFC techniques have found application in the characterization of polymeric mixtures

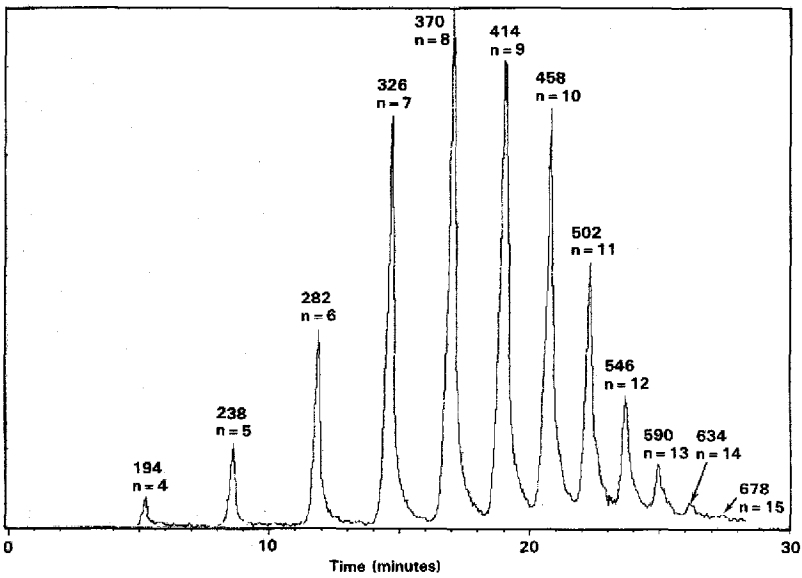

Fig. 3. Total ion chromatogram (TIC) for the capillary SFC–MS separation of a polyethylene glycol (PEG) sample with an average molecular weight of 400. Ammonia was used as the CI reagent. Mobile phase, carbon dioxide–isopropanol (95:5); temperature 125°C.

consisting of oligomers in excess of 5000 dalton[1,2,20–30].The ability to obtain mass spectra on-line following SFC is of great benefit in polymer characterization in contrast to off-line methods for molecular weight determination or desorption/ionization techniques. Fig. 3 shows the total ion chromatogram (TIC) of a polyethylene glycol (PEG) sample with an average molecular weight of 400, separated with a supercritical carbon dioxide–2-propanol mixture as the mobile phase. Relatively mild thermal conditions (125°C), combined with pressure programming in capillary SFC, permit-

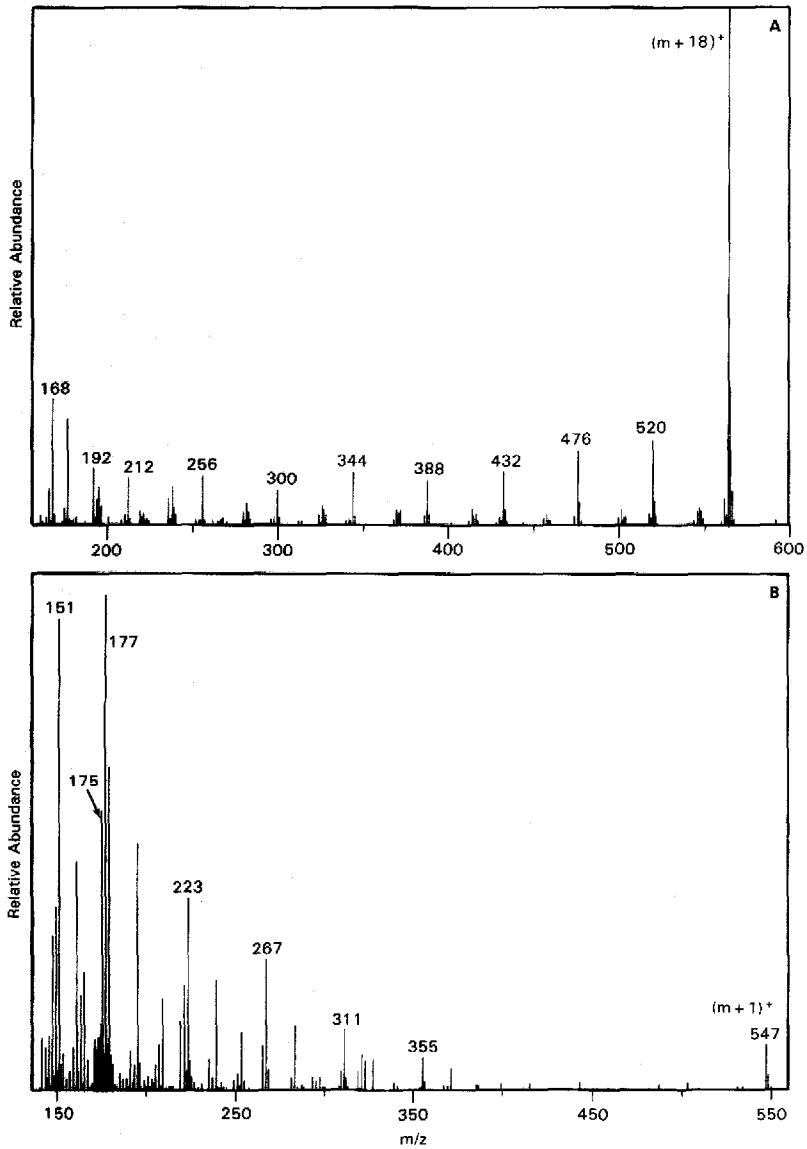

Fig. 4. Ammonia (A) and methane (B) CI mass spectra of the $n = 12$ oligomer of PEG (molecular weight 546).

ted elution of all oligomers from a 10 m × 50 μm I.D. column in less than 30 min. Polyethylene glycol samples of up to 1000 average molecular weight have been analyzed by SFC–MS with both quadrupole and magnetic sector mass spectrometers[31].

The mass spectra shown in Fig. 4 are the ammonia (A) and methane (B) CI spectra of the $n = 12$ (molecular weight 546) oligomer of PEG obtained on a VG ZAB magnetic sector instrument, modified for SFC–MS[31]. The ability to choose a reagent gas (or ionization mode) in SFC–MS allows either molecular weight or structural information to be obtained (although the structural information will be more limited than that afforded by EI). The ammonia CI spectrum (A) is characterized by an abundant ammonium adduct ion $[M + 18]^+$ and fragment ions, similar to those seen for FAB ionization of PEG samples[32]. The much more energetic methane CI gives rise to numerous structurally significant fragment ions and a much smaller contribution due to the protonated molecule $[M + H]^+$ at m/z 547 (Fig. 4B). As information regarding relative oligomer abundance is obtained from the chromatographic peak area, fragmentation following ionization in SFC–MS does not skew the apparent oligomer distribution of polar polymers, as is the case with some desorption/ionization methods[32,33]. The use of mass spectrometers with greater mass range and new interface designs promises to extend the range of materials that can be analyzed by SFC–MS.

Labile and less volatile compounds

The analysis of labile and less volatile compounds is particularly benefited by

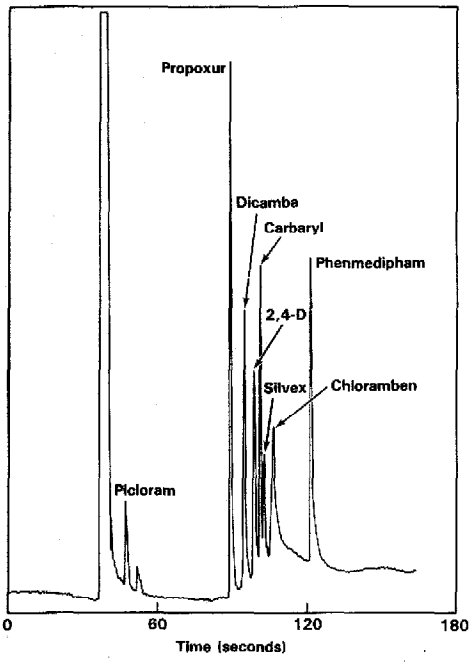

Fig. 5. Fast SFC separation of a mixture of eight acid and carbamate pesticides with carbon dioxide as the supercritical mobile phase. Column, 1.5 m × 25 μm I.D.; temperature, 100°C; pressure, 75 atm, 100 atm/min.

SFC–MS. The chromatogram of eight acid and carbamate pesticides, shown in Fig. 5, was obtained by using carbon dioxide as the supercritical mobile phase and a 25-μm I.D. capillary column with a fast pressure program. The eight compounds in this mixture were rapidly analyzed (in slightly over 2 min). Flame ionization was used for detection in this particular example, but the ammonia and methane CI mass spectra of all compounds in this mixture have been obtained following SFC[34].

Examples of mass spectra obtained by SFC–MS of thermally labile herbicides are shown in Fig. 6. The methane CI mass spectra of all three compounds (linuron,

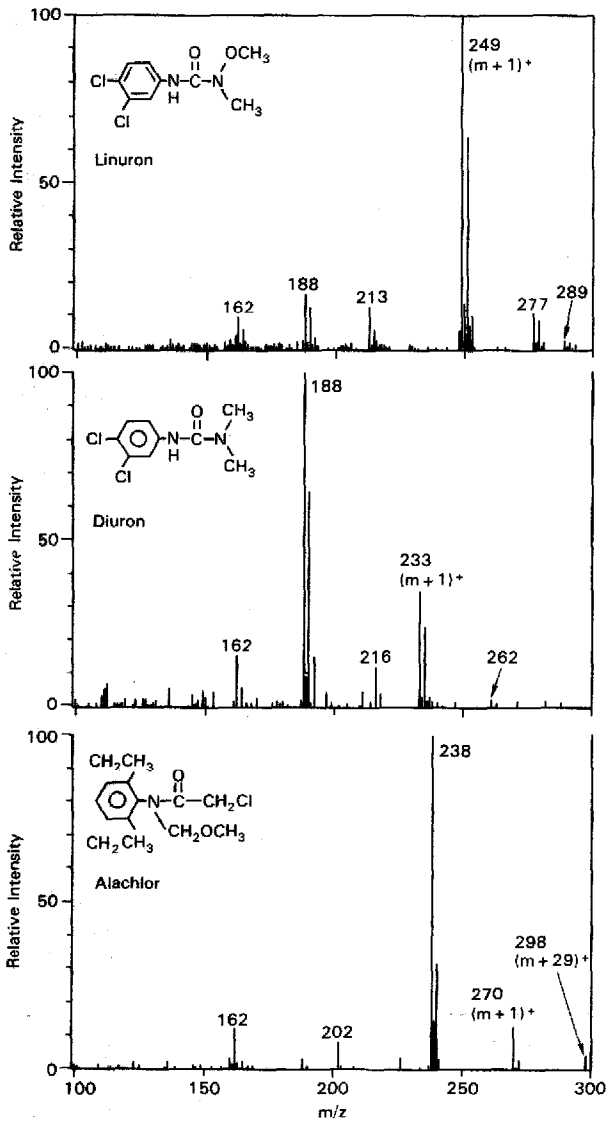

Fig. 6. Methane CI mass spectra of the herbicides linuron (MW 248), diuron (MW 232) and alachlor (MW 269) following SFC.

diuron and alachlor) are characterized by the protonated molecule and structurally characteristic fragment ions. The urea herbicides, linuron and diuron, are fragmented to form a common ion (*m/z* 188), which may arise due to thermal decomposition of the parent urea. Analysis by SFC–MS under mild thermal conditions limits the amount of this type of fragmentation for labile compounds. This facile decomposition is unlikely in alachlor, where the base peak corresponds to loss of methanol from the protonated analyte molecule (*m/z* 270) (Fig. 6, bottom).

The choice of mobile phase has not been found to affect the mass spectra of compounds analyzed by SFC–MS, even when modifiers are used[2,10,13,31,34–37]. In fact, the modifier can be employed as the reagent gas for CI mass spectrometry[37].

CONCLUSION

The nature and properties of supercritical fluids allow chromatographic techniques to be extended to materials that are difficult or impossible to characterize by either GC or LC. SFC serves a valuable complementary role to these more mature analytical separation techniques. The combination of SFC with MS has furthered the applicability of the technique due to the sensitivity, selectivity and the essentially universal nature of mass spectrometric detection. A more complete understanding of the principles underlying the SFC and the SFC–MS interfacing methods will aid in the fulfillment of the promises of this new technique.

ACKNOWLEDGEMENT

This work was supported by the U.S. Department of Energy, Morgantown Energy Technology Center, under Contract DE-AC06-76RLO-1830.

REFERENCES

1 C. M. White and R. K. Houck, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 4.
2 B. W. Wright, H. T. Kalinoski, H. R. Udseth and R. D. Smith, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 145.
3 R. D. Smith, W. D. Felix, J. C. Fjeldsted and M. L. Lee, *Anal. Chem.*, 54 (1982) 1883.
4 R. D. Smith, J. C. Fjeldsted and M. L. Lee, *J. Chromatogr.*, 247 (1982) 231.
5 R. D. Smith and H. R. Udseth, *Anal. Chem.*, 55 (1983) 2266.
6 S. T. Sie, W. Van Beersum and G. W. A. Rijnders, *Sep. Sci.*, 1 (1966) 459.
7 M. N. Myers and J. C. Giddings, *Sep. Sci.*, 1 (1966) 761.
8 M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction*, Butterworths, Boston, 1986.
9 C. R. Yonker, S. L. Frye, D. R. Kalkwarf and R. D. Smith, *J. Phys. Chem.*, 90 (1986) 3022.
10 R. D. Smith, H. T. Kalinoski and H. R. Udseth, *Mass Spectrom. Reviews*, in press.
11 B. W. Wright and R. D. Smith, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 73.
12 R. D. Smith, E. G. Chapman and B. W. Wright, *Anal. Chem.*, 57 (1985) 2829.
13 R. D. Smith, H. R. Udseth and H. T. Kalinoski, *Anal. Chem.*, 56 (1984) 2971.
14 R. D. Smith, H. T. Kalinoski, H. R. Udseth and B. W. Wright, *Anal. Chem.*, 56 (1984) 2476.
15 R. D. Smith, J. L. Fulton, R. C. Petersen, A. J. Kopriva and B. W. Wright, *Anal. Chem.*, 58 (1986) 2057.
16 T. L. Chester, D. P. Innis and G. D. Owens, *Anal. Chem.*, 57 (1985) 2243.
17 R. D. Smith, H. R. Udseth and R. N. Hazlett, *Fuel*, 64 (1985) 810.
18 D. W. Later, M. L. Lee, K. D. Bartle, R. C. Kong and D. L. Vassilaros, *Anal. Chem.*, 53 (1981) 1612.
19 H. T. Kalinoski, H. R. Udseth, B. W. Wright and R. D. Smith, *Anal. Chem.*, 58 (1986) 2421.
20 R. Jentoft and T. H. Gouw, *J. Polym. Sci., Polym. Lett. Ed.*, (1969) 811.

21 J. A. Nieman and L. B. Rogers, *Sep. Sci.*, 10 (1975) 517.
22 E. Klesper and W. Hartmann, *J. Polym. Sci., Polym. Lett. Ed.*, 14 (1976) 77.
23 E. Klesper and W. Hartmann, *J. Polym. Sci., Polym. Lett. Ed.*, 15 (1977) 9.
24 E. Klesper and W. Hartmann, *J. Polym. Sci., Polym. Lett. Ed.*, 15 (1977) 707.
25 E. Klesper and W. Hartmann, *J. Polym. Sci., Polym. Lett. Ed.*, 15 (1977) 717.
26 E. Klesper, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 738.
27 Y. Hirata, *J. Chromatogr.*, 315 (1984) 39.
28 T. L. Chester, D. P. Innis and G. D. Owens, *Anal. Chem.*, 57 (1985) 2243.
29 T. L. Chester and D. P. Innis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 209.
30 F. P. Schmitz and H. Hilgers, *Makromol. Chem. Rapid Commun.*, 7 (1986) 59.
31 E. K. Chess, H. T. Kalinoski and R. D. Smith, *Anal. Chem.*, submitted for publication.
32 R. P. Lattimer, *Int. J. Mass Spec. Ion Processes*, 55 (1983) 221.
33 R. P. Lattimer and H. R. Schulten, *Int. J. Mass Spectrom. Ion Processes*, 67 (1985) 277.
34 H. T. Kalinoski, B. W. Wright and R. D. Smith, *Biomed. Environ. Mass Spectrom.*, 13 (1986) 33.
35 B. W. Wright, H. T. Kalinoski and R. D. Smith, *Anal. Chem.*, 57 (1985) 2823.
36 A. J. Berry, D. E. Games and J. R. Perkins, *J. Chromatogr.*, 363 (1986) 147.
37 R. D. Smith, B. W. Wright and H. T. Kalinoski, in H. Parvez, M. Yoshiota and S. Parvez (Editors), *Progress in HPLC*, Vol. 4, V.N.U. Science Press, Utrecht, The Netherlands, 1987.