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Ionization mechanisms in capillary supercritical fluid chromatography–chemical ionization mass spectrometry

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ABSTRACT

Ionization mechanisms have been studied for supercritical fluid chromatography (SFC) with mass spectrometric (MS) detection. One of the problems associated with SFC–MS is the interference of mobile phase constituents in the ionization process, which complicates the interpretation of the resulting mass spectra. This interference can be reduced by adding a reagent gas to the ion source. It was found that the properties and the pressure of this reagent gas control the ionization process. In this study ammonia was used as a chemical ionization (CI) reagent gas. An increase in the reagent gas pressure generally resulted in higher abundances of the protonated molecular ion. The presence of an excess of reagent gas suppresses charge exchange processes between the mobile phase constituents and the solutes. Charge exchange causes a more pronounced fragmentation than proton transfer in CI processes. The spectra obtained by charge exchange ionization, with helium as the reagent gas at moderately high pressures, are comparable to electron ionization spectra from standard MS libraries.

INTRODUCTION

The combination of capillary supercritical fluid chromatography (SFC) and mass spectrometry (MS) offers a powerful analytical technique. With SFC it is possible to analyse thermally labile and high-molecular-weight components which cannot be analysed directly by gas chromatography (GC) [1]. The properties and the low flow-rate of the SFC mobile phase (*e.g.*, carbon dioxide) allows direct interfacing with MS; liquid chromatography (LC) is more difficult to couple with MS. The application of MS provides a sensitive and general detection technique which gives structural information for unknown components. Organic mobile phase modifiers (*e.g.*, alcohols) preclude the use of flame ionization detection (FID), but are compatible with MS [2].

Prerequisites for the analysis of unknown samples are a good chromatographic separation efficiency and mass spectral integrity, yielding interpretable mass spectra. Obtaining a good separation efficiency can be a problem whenever the sample components interact with the column wall. In addition, the properties of the mobile phase are sometimes not favourable for dissolving the solutes. In both instances this may lead to deformed peaks and thus to a decreased separation efficiency. Adding polar

mobile phase modifiers can have a positive effect on the separation. The modifier molecules can give rise to a deactivation of the active sites on the column wall and to an increase of the mobile phase polarity and density [3].

For the identification of the separated solutes, information about the molecular weight can be obtained from the mass spectrum produced whenever the molecular ion is stable enough. The spectrum can be compared with electron ionization (EI) mass spectra in computer databases or can be interpreted by the fragmentation pattern. In this way, unknown substances can be identified.

The identification process for SFC–MS can be complicated. The mobile phase constituents are ionized and collide with the sample molecules. Charge exchange ionization can occur between the ionized mobile phase constituents and the solutes [4,5] and, in addition, protic mobile phase modifiers can give rise to chemical ionization (CI) of the solutes [6]. The mass spectrum obtained thus depends on the composition and the pressure of the mobile phase in the ion source, both of which can change during the analysis.

To reduce the interference of the mobile phase in the ionization process two different approaches can be followed. One is to open the ionizer to improve pumping in this section and to effectively remove the mobile phase constituents. This approach leads to a reduced sensitivity; the reason for the reduction is not yet well known [6,7]. The improved pumping probably also causes the removal of sample constituents.

The other approach is to use a reagent gas to achieve relatively high-pressure conditions in the ionizer. The excess of reagent gas in the ionizer suppresses charge exchange and CI reactions between the solutes and the mobile phase constituents. In this way the solutes are primarily ionized by collisions with the reagent gas ions. The high-pressure conditions in the ionizer also break up clusters of solutes, which would otherwise lead to a decrease in sensitivity [8]. This cluster formation is caused by the rapid expansion of the supercritical fluid to low-pressure conditions. The expansion causes adiabatic cooling of the flow restrictor (Joule–Thompson cooling), necessitating heating of the restrictor [8]. Under high-pressure conditions the clusters formed will be broken up because an average molecule undergoes about 10^5 collisions before ionization [8]. Under low-pressure conditions the solutes may not have enough time to volatilize prior to ionization [8].

There are several reports of SFC–MS in the CI and charge exchange mode of operation. However, systematic studies on the influence of the ion source pressure on the ionization processes have not yet been published. This paper reports experiments which were carried out to acquire a better insight into the ionization processes occurring in SFC–MS. Methods to reduce the influence of the mobile phase constituents on the ionization process are described. It is shown that these methods are useful in obtaining mass spectra which are less dependent on the composition and the pressure of the mobile phase during SFC analysis.

EXPERIMENTAL

Supercritical fluid chromatography

All experiments were conducted on a Carlo Erba SFC 3000 instrument (Milan, Italy) equipped with a dual-syringe pump system. This system allows the pressure, temperature, density and composition of the mobile phase to be programmed. In the

pump system one of the two syringe heads was cooled to 5°C to facilitate filling with carbon dioxide. Carbon dioxide of 99.996% grade purity (Intermar, Breda, Netherlands) was used. An activated carbon filter was installed between the carbon dioxide bottle and the pump to further purify the mobile phase [9]. In the case of a modified mobile phase, carbon dioxide of 99.5% grade purity (Hoekloos, Schiedam, Netherlands) was used with ethanol (p.a. grade, Merck, Darmstadt, Germany) as the modifier.

The samples were dissolved in dichloromethane at concentrations of about 1 g/l. These samples were introduced onto the column by a switching valve (NI4W, Valco, Schenkon, Switzerland) actuated by helium. This valve was operated at 30°C with an injection time of 200 ms. The internal loop of the valve had a volume of 100 nl. In this way about 10 ng of the solute were introduced onto the column. No extra flow splitting was applied to reduce the amount of sample.

For small percentages of modifier, the volumetric flow-rate of the mobile phase modifier was too low to operate the pump under stable conditions. Therefore, the total flow was increased by incorporating a flow splitter before the injection valve.

The separation column was an 8 m × 50 μm I.D. fused-silica capillary, coated with a 0.25-μm film of SB-Methyl 100 stationary phase (Lee Scientific, Salt Lake City, UT, USA). The separations were performed at 100°C, except where indicated otherwise. A polished integral restrictor [10] was fabricated at the end of the column to depressurize the SFC mobile phase.

Interface design

Fig. 1 shows the laboratory-made SFC–MS interface for Finnigan MAT 4000/4500/4600 instruments (Sunnyvale, CA, USA) which required only minor modifications of the mass spectrometer inlet system.
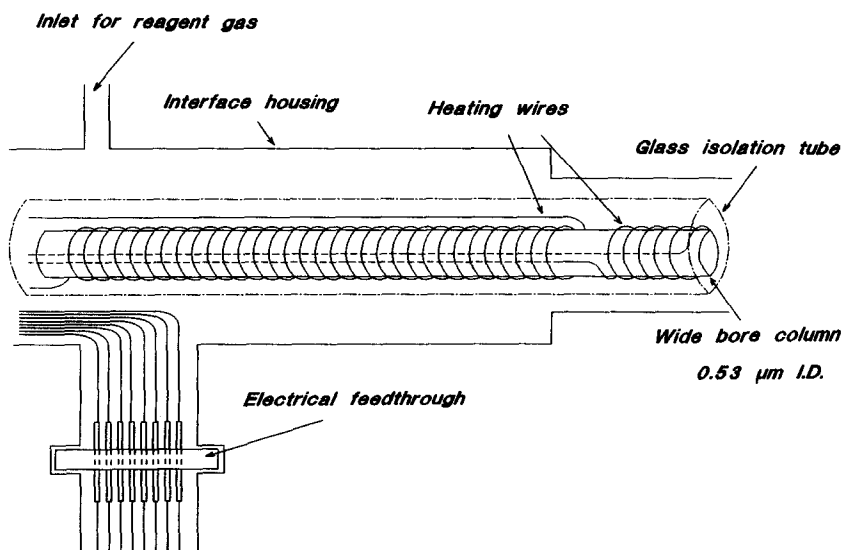

Fig. 1. Schematic diagram of the laboratory-made SFC–MS interface.

The heart of this interface is a 0.53 μm I.D. uncoated fused-silica column, wrapped with 0.2 mm O.D. Kanthal heating wire. The heating wires are electrically isolated by glass tubes (0.1 mm wall thickness) which are placed around the coil. The standard GC–MS inlet system incorporates a 5 cm × 2 mm I.D. tube, which can be switched to close the ion source (CI mode). The dimensions of this tube restrict a part of the SFC–MS interface to < 2 mm O.D.

The interface consists of two separately heated sections. A section of 55 cm maintains the SFC oven temperature up to the ion source housing, and is heated by a 50-V power supply (Delta Electronics TPS 050-5, Zierikzee, Netherlands). A 2-cm-long section controls the temperature of the restrictor and compensates for the Joule–Thompson cooling effect [8]. This section is heated by a 30-V power supply (Delta Electronics E 030-1) and is kept at a temperature of 350°C.

A glass plate in which molybdenum pins are embedded forms a vacuum feed-through for the electrical connections. To obtain a vacuum seal between this plate and the metal housing of the interface, two Kalrez rings (DuPont, Wilmington, DE, USA) were used on both sides of the plate.

Mass spectrometry

Mass specta were acquired on a Finnigan 4000 instrument with a dual electron ionization (EI)–CI source. The ion source can be switched between the EI and the CI mode by automatic mechanical and electrical readjustment. The quadrupole mass filter has a mass range of 4–1000 dalton. The vacuum system consists of two diffusion pumps, backed up by a 300 l/s mechanical forepump. The ion source and the quadru-pole analyser are differentially pumped to ensure high vacuum conditions in the analyser section.

The mass spectrometer was operated in the positive-ion chemical ionization mode with a gastight ion source. The electron energy and emission current were held at 75 eV and 0.3 mA, respectively. The electron multiplier was operated at − 1900 V. Under CI conditions ammonia (10% in methane) was used as the reagent gas. Under charge exchange conditions helium was used as the reagent gas. Both reagent gases were fed coaxially to the SFC effluent. In this way better results were obtained com-pared with feeding the gases perpendicularly to the SFC effluent.

The MS data were acquired and processed using a Data General NOVA 4/S system (Southboro, MA, USA) with custom-written software.

RESULTS AND DISCUSSION

Fig. 2 shows the influence of the CI reagent gas pressure on the ionization process. Partial mass spectra of the polymer additive Irgafos 168 [tris(2,4-di-*tert*.-butylphenyl) phosphite] are plotted as a function of the pressure in the mass analyser. This pressure was applied by adding ammonia to the ion source. The pressure in the mass analyser was used as an indication of the pressure in the ion source. From Fig. 2 it can be seen that the abundance of the protonated molecular ion, at m/z 647, increases with increasing reagent gas pressure. At low pressure the solutes are partly ionized by charge exchange, with carbon dioxide. The relative contribution of charge exchange ionization with carbon dioxide decreases when more reagent gas is in-troduced into the ion source. A maximum in the abundance of the protonated molec-
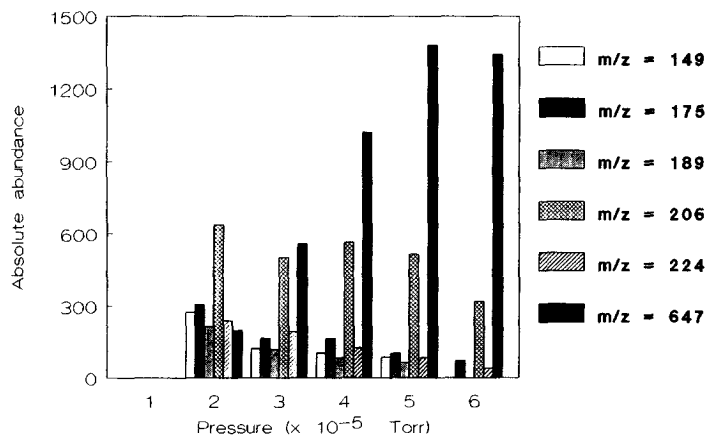
Fig. 2. Partial mass spectra of Irgafos 168 as a function of the reagent gas pressure in the mass analyser. SFC pressure programme, 150 bar (5 min), ramp of 10 bar/min to 320 bar, isobaric for 5 min. The MS was scanned at a rate of 2 s per scan with a mass range of 100–1000. The ion source temperature was 200°C.

ular ion was found at a pressure of $5 \cdot 10^{-5}$ Torr in the mass analyser. This pressure corresponded with a pressure of 0.3 Torr in the ion source (as measured with a Pirani gauge). These results show that the pressure of the reagent gas in the ion source controls the ionization and fragmentation process. During the course of an SFC pressure programme, increasing the mobile phase pressure will change the conditions in the ion source continuously. This indicates that at lower mobile phase pressures, CI spectra are obtained, but that higher mobile phase pressures result in mixed charge exchange–CI spectra. However, Fig. 2 indicates that CI conditions can be obtained for the component Irgafos 168, which eluted at a SFC mobile phase pressure of about 310 bar.
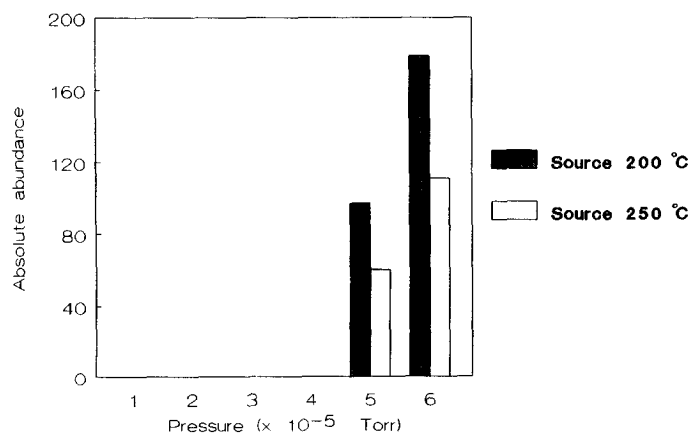

Fig. 3. Abundance of the protonated molecular ion of Tinuvin 770 as a function of the reagent gas pressure in the mass analyser. The ion source was held at 200 and 250°C, respectively. Other experimental conditions as in Fig. 2.

For some types of samples, relatively high-pressure conditions are necessary to detect the protonated molecular ion. The energy transferred at the ionization of the sample molecules should be low enough to prevent extensive fragmentation. For the polymer additive Tinuvin 770 [bis(2,2,6,6-tetramethyl-4-piperidinyl) sebacate], no protonated molecular ions, at m/z 481, were found at pressures lower than $5 \cdot 10^{-5}$ Torr in the mass analyser (see Fig. 3). Lowering the source temperature also resulted in more abundant molecular ions and less fragmentation as is also shown in Fig. 3.

One way to obtain mass spectra similar to EI spectra, which can be found in libraries, is to use charge exchange [11]. In the charge exchange process the sample constituents are ionized by collisions with a reagent gas that does not contain protons. Carbon dioxide [4,5], helium [6], nitrogen [11] and air [12] have been used as reagent gases. During the ionization process a relatively high amount of energy is transferred to the solutes [13]. This means that charge exchange ionization causes a high degree of fragmentation, comparable to the fragmentation process in EI. For SFC–MS this implies that spectra similar to those seen with EI can be produced while relatively high-pressure conditions are maintained in the ion source [6]. A comparison between the spectra obtained with SFC–MS with helium as the reagent gas and GC–EI-MS is given in Figs. 4–6. This comparison was made by transferring the SFC–charge exchange MS data from the NOVA 4/S computer to a VAX 8530 computer. Using MASS-LIB (Max-Planck-Institut für Kohlenforschung, Mülheim/Ruhr, Germany), a software package for library searching of mass spectra, the SFC–charge exchange MS spectrum was matched with the EI spectra available in the
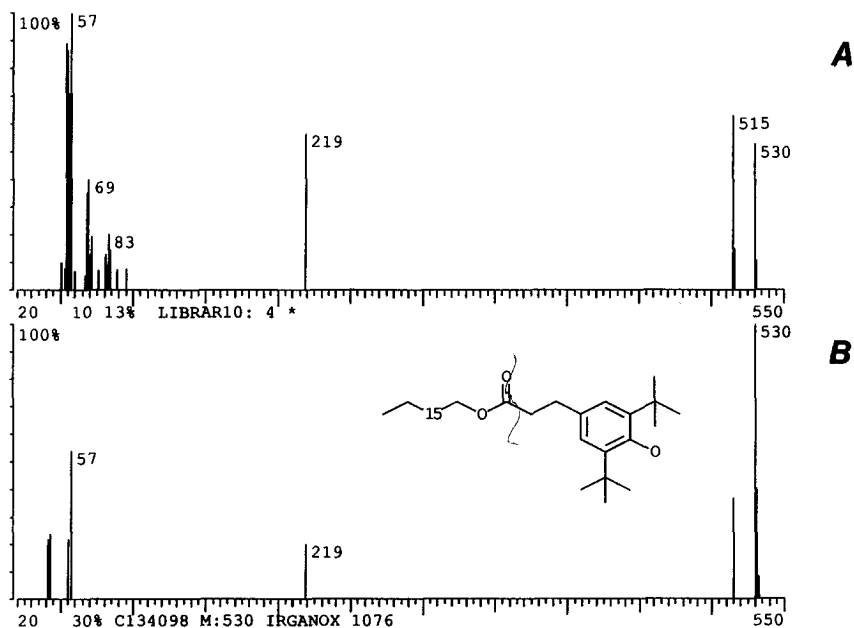

Fig. 4. Mass spectra of Irganox 1076 (A) under SFC–charge exchange MS conditions and (B) from the MASS-LIB library (best hit). The pressure in the analyser under SFC–charge exchange MS conditions was $2.5 \cdot 10^{-5}$ Torr. Other experimental conditions as in Fig. 2.
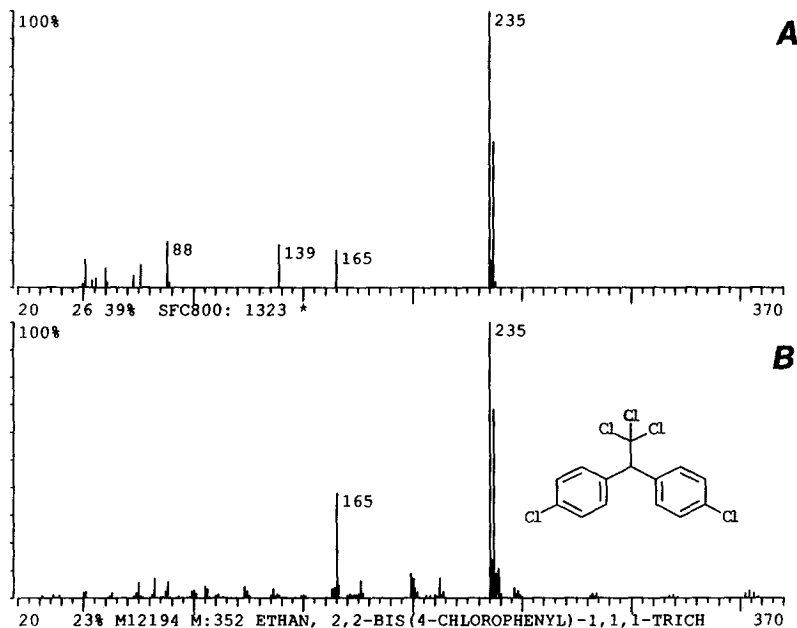
Fig. 5. Mass spectra of *p,p'*-DDT (A) under SFC–charge exchange MS conditions and (B) from the MASS-LIB library (best hit). SFC pressure programme, 100 bar (5 min), ramp of 10 bar/min to 320 bar. The MS was scanned at a rate of 1 s per scan with a mass range 50–500. The pressure in the analyser was 2.5 · 10^{-5} Torr.
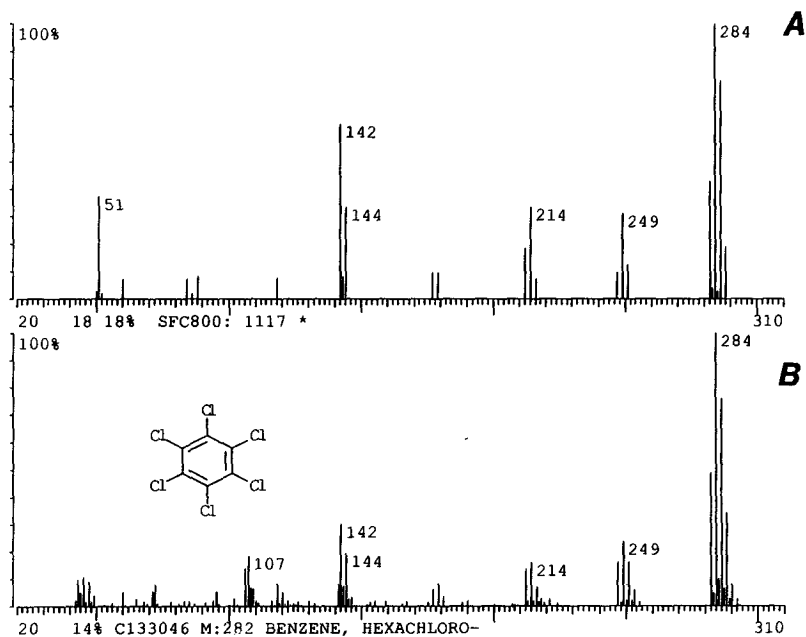

Fig. 6. Mass spectra of hexachlorobenzene (A) under SFC–charge exchange MS conditions and (B) from the MASS-LIB library (best hit). Experimental conditions as in Fig. 5.

libraries. The unknown compound can be identified by finding the spectrum with the highest similarity index ("best hit"). In Fig. 4 the spectra of the polymer additive Irganox 1076 [octadecyl-3-(3,5-di-*tert.*-butyl-4-hydroxyphenyl) propionate] are shown. Figs. 5 and 6 give the results for *p,p'*-DDT and hexachlorobenzene, compounds of environmental interest. The SFC–charge exchange MS spectra differ from the GC–EI-MS spectra in two ways, namely in the relative intensity of the different ions and in the presence of ions produced from mobile phase constituents. The relative intensity of the different ions can be tuned by selecting the pressure and the properties of the charge exchange reagent gas. It was found that SFC–charge exchange MS with helium as the reagent gas resulted in a higher degree of fragmentation than that found with GC–EI-MS. The degree of fragmentation decreased when more helium was introduced into the ion source. At the same time, a decrease in sensitivity was found. Good results were obtained at a moderately high pressure of $2.5 \cdot 10^{-5}$ Torr in the mass analyser (ion source pressure 0.2 Torr). In contrast, contributions found from mobile phase impurities (such as hydrocarbons) can only be reduced by the use of more highly purified carbon dioxide [9].

CONCLUSIONS

In coupled SFC–MS, the ionization process can be controlled by applying a relatively high reagent gas pressure to the ion source. In this way ionization primarily occurs by collisions between the reagent gas ions and the solute molecules. The influence of the composition and the pressure of the SFC mobile phase on the ionization process is thus reduced. The properties and the pressure of the reagent gas control the ionization process resulting in charge exchange spectra similar to EI, or in CI spectra.

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