Ion Mobility Spectrometry after Supercritical Fluid Chromatography

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A capillary supercritical fluid chromatograph with on-column UV absorption detection and ambient pressure ion mobility spectrometry was constructed. Preliminary experiments using a series of benzoate and methyl esters were conducted to determine the basic compatability of supercritical fluid chromatography (SFC) and ion mobility spectrometry (IMS). IMS after capillary SFC was accomplished by using CO_2 as both the chromatographic mobile phase and the ion mobility drift gas. Ion mobility scans at appropriate chromatographic retention times produced reproducible and distinct spectra for each of the test compounds. With this particular design, significant clearance time problems were observed, creating serious solvent contamination throughout the chromatographic run and limiting this design to the detection of lower molecular weight compounds. Nevertheless, the successful production of ion mobility spectra in CO_2, the enhanced sensitivity of ion mobility detection over "on-column" UV absorption detection, and the ease with which the interface can be achieved demonstrated that the combination of SFC with IMS deserves further attention.

The use of capillary supercritical fluid chromatography (SFC) for low-temperature separation of compounds has expanded significantly over the last few years (*1*, *2*). Carbon dioxide, which becomes a supercritical fluid under relatively mild conditions, has been found to be an especially convenient mobile phase for SFC. Its relatively high polarizability provides better solvating characteristics than might be expected for such a nonpolar compound, while its high ionization potential and inert character make it ideally suited for interfacing with sensitive ambient-pressure ionization detectors commonly used with gas chromatography. Flame ionization detectors have been the primary ionization detection method of choice with SFC, but other ionization detectors have also been investigated (*3-5*).

This paper investigates and discusses the potential of ion mobility spectrometry (IMS) as a detection method for capillary SFC. IMS is an atmospheric-pressure ion separation technique that separates gas-phase ions according to their differing diffusivities (*6*). Coupled to a capillary gas chromatograph it can be used to obtain quantitative and quasi-qualitative information about the compounds being separated. Normally, ion mobility spectrometry is conducted with the ions being separated in either air or nitrogen. However, when CO_2 is used as the mobile phase for SFC, the use of CO_2 as the drift gas in the ion mobility spectrometer might reduce complications that could occur by mixing CO_2 from the chromatographic column with the nitrogen or air drift gas of the ion mobility spectrometer.

Recently, we have reported results obtained when carbon dioxide was used as the drift gas in IMS (*7*). This study showed that, while product ions of the test compounds drifted with longer times in CO_2 than in N_2, ion mobility patterns were similar and there was a possibility of using IMS after SFC under conditions where CO_2 was employed as the supercritical fluid mobile phase and also for the drift gas. Since the two gases produced ions with drift times quite different from one another, it did seem that mixing the two gases in the ion mobility spectrometer may cause difficulty in reproducing spectra.

The purpose of this project was to construct a supercritical fluid chromatograph-ion mobility spectrometer and to evaluate its operation when CO_2 was used both as the mobile phase for the chromatography and as the drift gas in the ion mobility spectrometer.

EXPERIMENTAL SECTION

Instrumentation. Figure 1 is a block diagram of the SFC-IMS system that was assembled for this project. Details of each of the blocked sections are described below in the order that they are presented in the diagram. Standard purity CO_2 was purchased from Iwatani, Co., Kyoto, Japan. The CO_2 tank was fitted with a siphon tube in order to deliver liquid CO_2 to fill the syringe pump.

The pump used for the supercritical chromatograph was a Japan Spectroscopic Co., Ltd. (Jasco Model LCP-350) syringe-type pump with 200-mL capacity. The maximum pressure obtainable was 35.0 MPa with constant displacement rates that were variable from 1 μL/min to 3 mL/min. At room temperature the pump would fill to about 50% capacity (100 mL of liquid CO_2). It could be more completely filled if the stainless-steel reservoir was chilled during the filling process. Normally, 100 mL was quite sufficient for a day of operation and chilling the reservoir was not necessary.

A SPF-W 1-Mm stainless-steel prefilter for liquid chromatography was purchased from Umetani Seiki Co., Osaka, Japan, and inserted into the solvent delivery line between the pump and the preheater. Without this filter in the line, the system would plug periodically during chromatographic runs.

The CO_2 mobile phase was passed through a 40-cm $\times$ $^1/_8$-in. stainless-steel tube that was coiled around a 9-mm-diameter aluminum rod and placed in the column oven. In this manner the CO_2 was preheated prior to entering the injection loop.

The sample was injected into the CO_2 stream via a Model C14W 60-nL injection loop purchased from Valco Instruments Co., Inc., Houston, TX. Optimal temperature for injection for supercritical fluid chromatography is still debatable. Theory would say that liquid injection would provide the best transport properties for the sample. Yet in some cases, supercritical fluid injections have been found to be more efficient (*8*). In our case, we elected to mount the injection port just outside of the oven; thus, the injector was warm but not above critical temperature and injection was accomplished under the conditions of a warm liquid rather than a supercritical fluid.

From the injection loop the sample was transferred directly to the capillary chromatographic column located in the column oven of a Shimadzu capillary gas chromatograph, Model GC mini 2. The column was a 100-μm-diameter fused-silica open tubular column coated with cross-linked SPB-1 prepared by Supelco Co., Inc., Supelco Park, Bellefonte, PA. The length of the column used in this work was 10 m.

The capillary chromatographic column was butt connected to a capillary flow cell for UV detection. This flow cell was constructed from 190-μm fused-silica tubing by burning off part of

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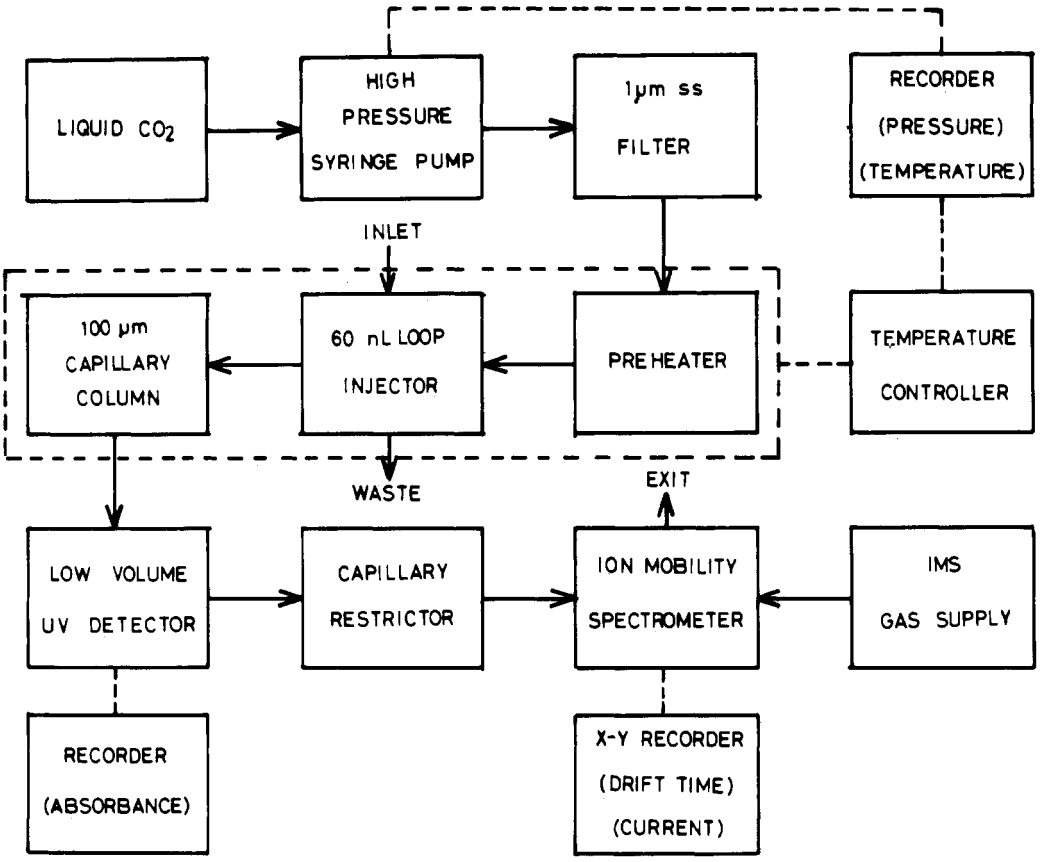

Figure 1. Block diagram of supercritical fluid chromatograph with UV detector and ion mobility spectrometer.

the surface polyimide coating. For strength and protection the outside of the flow cell was covered by a stainless-steel tube, which was fitted with a window in the region of the light path. The stainless-steel protector was fastened to the capillary flow tube with epoxy resin. To reduce background light, a slit approximately 0.1 mm wide was constructed with two thin stainless-steel razor blades and placed behind and on axis with the cylindrical microflow cell. The actual size of the flow cell was 0.2 mm i.d. × 5 mm in length for an internal volume of about 150 nL. This entire flow cell assembly was placed in the light path of a Jasco UVIDEC 100-II UV detector. A fixed wavelength of 210 nm was used to monitor absorption as the chromatographic effluent passed through the flow cell. The detector housing was located outside of the column oven and was not heated. Thus, detection occurred at pressures above the critical pressure but at temperatures below the critical temperature. Under these conditions the samples were dissolved in liquid CO_2.

After the UV detector the sample was transferred to the ion mobility spectrometer via a capillary restrictor. This restrictor consisted of an uncoated fused-silica capillary column that was 45 cm long with an internal diameter of 10 μm. Decompression occurred across this restrictor as CO_2 changed from a liquid to a gas. In order to prevent condensation of the sample, the decompression region of the restrictor was heated to 250 °C, the inlet temperature of the ion mobility spectrometer.

The restrictor was inserted directly into the inlet of a Phem-to-Chem 100 ion mobility spectrometer purchased from PCP, Inc., West Palm Beach, FL. Except for the manner of injection, no physical modifications were made to the instrument. This ion mobility spectrometer consists of a series of metal rings that are insulated from one another forming a segmented tube 14 cm long. A ^{63}Ni foil, located at one end of the tube, served as the ionization source for the spectrometer. The ion collector, at the other end of the segmented tube, was directly coupled to a multichannel analyzer. The carrier gas enters the tube from the ion source end of the segmented tube, and the drift gas enters the tube from the ion collector end of the tube. In this study the effluents from the supercritical fluid chromatograph were introduced into the carrier gas, which transferred the neutral species of interest to the ionization chamber of the spectrometer. After the compound of interest was ionized, the product ions along with the remaining neutrals were swept down the tube by the force of the carrier gas. This region in which ions and neutrals exist together in the spectrometer is called the reaction region. The ionization region and the reaction region made up the first 5 cm of the tube. The drift region where the ions were separated made up the last 8 cm of the tube. At the beginning of the drift region an electrostatic ion gate controls the timing and size of the ion pulses that were introduced into the drift region. Neutrals were swept away from the product ions by a countercurrent of drift gas that flowed in direct opposition to the carrier gas. The two gases, along with the neutral sample species, exited the spectrometer near the center of the tube. The opposing gas flows of this instrument coupled with the open segmented tube design produced unacceptably long residence time in the spectrometer. Moreover, due to the single ion gate and multichannel analyzer approach to data collection, continuous monitoring of a given ion species was not possible. Nevertheless, the instrument served to adequately investigate the fundamental question of whether or not ion mobility spectrometery was feasible after supercritical fluid chromatography, when carbon dioxide is used not only as the mobile phase for the chromatography but also as the carrier and drift gas in the ion mobility spectrometer.

Experiments. Because the flow patterns of the ion mobility spectrometer were such that heavy molecular weight compounds introduced into the spectrometer would have many opportunities to condense on surfaces within the spectrometer, we decided that for initial experiments we would use only compounds similar to those which have produced ion mobility spectra in the past. For the studies presented in this paper two classes of compounds were chosen: a series of benzoates for which UV detection was possible and a series of methyl esters which did not contain chromaphors. The first test mixture consisted of methyl, ethyl, *n*-propyl, *n*-butyl, isoamyl, *n*-amyl, n-hexyl, and benzyl benzoates dissolved in cyclohexane. This mixture was separated by both isobaric and pressure-programmed techniques, and responses were evaluated in both the UV detector and the ion mobility spectrometer. A series of methyl esters was also investigated, consisting of methyl caprate, methyl laurate, methyl myristate, and methyl stearate.

Operating Conditions. Typical supercritical fluid chromatographic conditions used in this work were the following: The mobile phase was carbon dioxide passing over a 10-m × 100-μm-i.d.

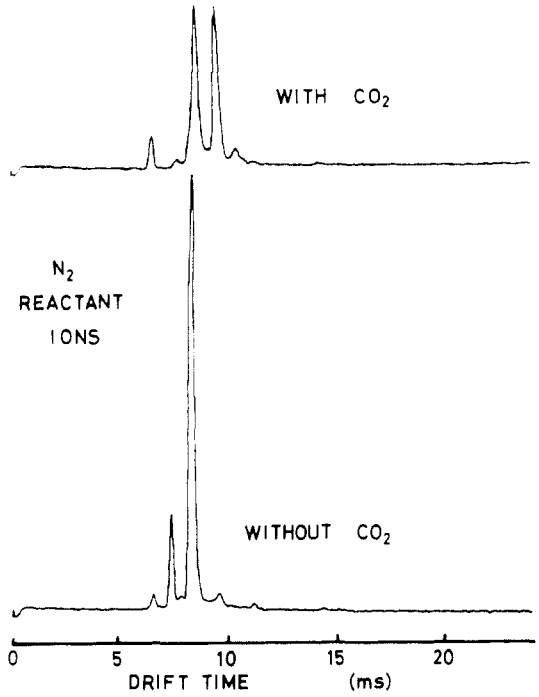

Figure 2. Ion mobility spectra of reactant ions in nitrogen with and without carbon dioxide. The lower ion mobility spectrum shows the normal pattern observed for nitrogen drift gas. The upper tracing demonstrates the shift in the reactant ion pattern when small quantities of CO_2 are introduced into the spectrometer through the chromatographic column.

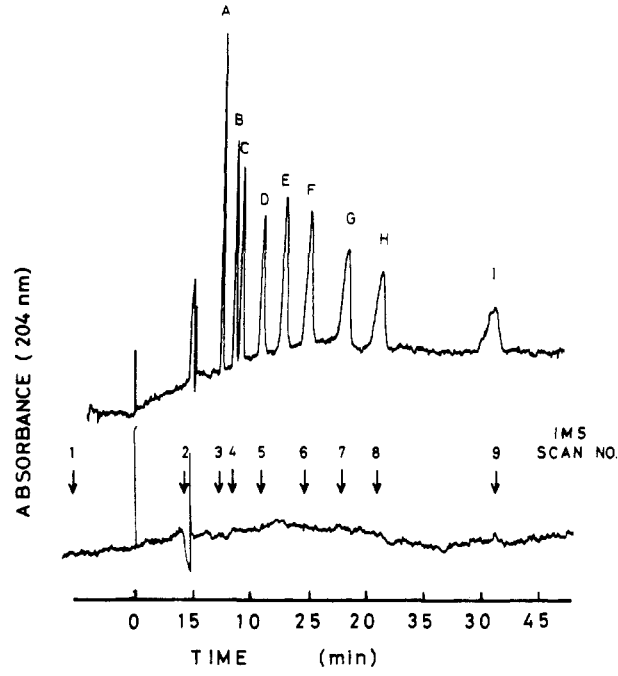

Figure 3. Isobaric supercritical fluid chromatograms of benzoates with UV detection.

fused-silica open tubular column coated with cross-linked SPB-1. The pressure was varied from 2.0 to 12.0 MPa, depending upon the conditions of the experiment. The column temperature was also varied somewhat from chromatographic run to chromatographic run although never varied during a chromatographic run. The injection temperature as stated above was always around room temperature, and the UV detection temperature was also around room temperature.

Typical conditions used for the ion mobility spectrometer were as follows: electric field, 214 V/cm; CO_2 carrier gas flow, 250 cm^3/min; CO_2 drift gas flow 350 cm^3/min; ambient pressure, 752 ± 6 torr; spectrometer temperature, 220 °C; repetition period, 24 or 60 ms; dwell time, 24–60 μs; gate width, 0.2 ms; autostop, 1024; vertical display, X; horizontal display, 1024 points; volts full scale, 2 V; input filter, 20; and ADC resolution, 9 bits.

RESULTS AND DISCUSSION

In general, ion mobility spectrometery has been conducted with either nitrogen or air as the drift gas. When traditional chromatographic carrier gases are introduced either directly or through a gas chromatograph into an IMS, the nature of the reactant ions does not change. For example, if small amounts of helium, hydrogen, or nitrogen are introduced into the ionization region of the spectrometer as a result of 1 mL/min flow through a capillary GC column, no change can be observed in the reactant ions. However, if CO_2 is used as mobile phase in SFC then interferences may occur. We know from earlier work that when nitrogen is replaced with CO_2 as the drift gas, reactant ions continuously change in drift time until they stabilize in pure CO_2 at much longer drift times than the nitrogen reactant ions exhibit (7). However, what about small quantities of CO_2 introduced through a capillary column after supercritical fluid chromatography? Would this affect the character of the reactant ions?

Figure 2 compares reactant ions obtained when the carbon dioxide mobile phase of a capillary supercritical fluid chromatograph is introduced into an ion mobility spectrometer. The lower spectrum is typical of that observed when only nitrogen is present in the spectrometer, with the pattern and the mobility constants agreeing well with those normally reported for these conditions. When CO_2 was introduced into the spectrometer, the reactant ions shifted to positions shown in the upper tracing of Figure 2. The exact position and pattern of the reactant ions are dependent on the rate of introduction of the carbon dioxide. In SFC the mobile-phase flow rate is not held constant when the chromatography is pressure or temperature programmed, and thus the reactant ions can be expected to change during the course of an SFC separation. When CO_2 was used as both the mobile phase for the chromatography and the ion mobility drift gas, no change in the reactant ions during a pressure-programmed SFC separation was observed.

Figure 3 shows an example of the separation that was achieved with supercritical fluid chromatography for a mixture of nine benzoate esters used as the test compounds. The benzoates were chosen as the initial test compounds because their chromatographic behavior is well-known in gas chromatography and because they could be readily detected by UV absorption methods. In order of elution, the test compounds were as follows: (A) 0.6 μg of methyl benzoate, (B) 0.6 μg of ethyl benzoate, (C) 0.6 μg of isopropyl benzoate, (D) 0.6 μg of *n*-propyl benzoate, (E) 0.9 μg of isobutyl benzoate, (F) 0.9 μg of *n*-butyl benzoate, (G) 0.9 μg of isoamyl benzoate, (H) 0.9 μg of *n*-amyl benzoate, (I) 0.9 μg of *n*-hexyl benzoate, and (J) 0.9 μg of benzyl benzoate. The solvent used for this mixture was cyclohexane, which, as can be seen from the chromatogram, did not produce a UV absorption response.

Because IMS is such a sensitive technique, the quantities of benzoates used to obtain the chromatogram in Figure 3 were so large that they saturated the spectrometer. Thus, in order to obtain meaningful ion mobility data, the sample mixture had to be diluted by 2 orders of magnitude. The lower chromatogram shown in Figure 3 represents the UV detector tracing of this diluted mixture. As is clear from the chromatogram, the compounds present in the diluted mixture fell below the detection limit of the UV detector. The numbered arrows associated with this chromatogram indicate when ion mobility scans were taken during the chromatographic run. Ion mobility spectra that were obtained as a result of these successive scans are shown in Figure 4.

The most obviously significant difference between the ion mobility spectra shown in Figure 4 and those found in most

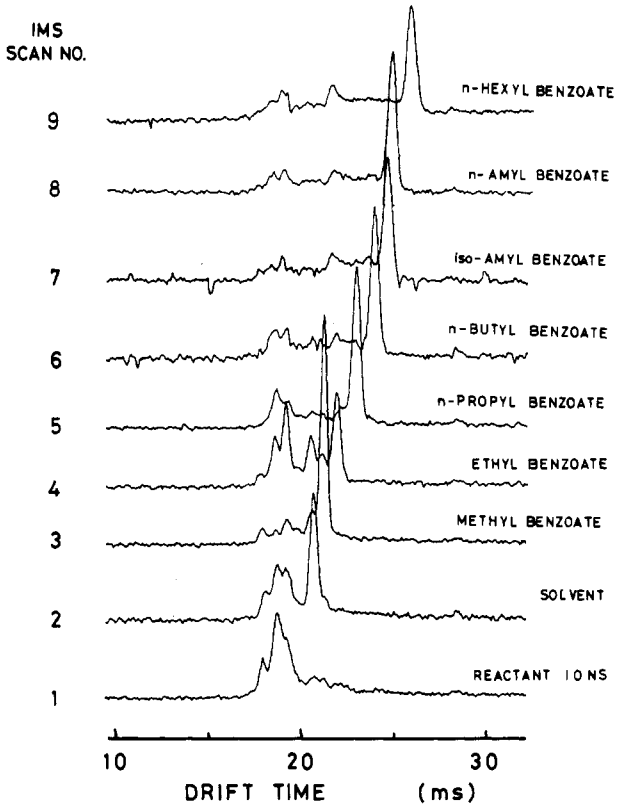

Figure 4. Ion mobility spectra of benzoates with UV detection.

of the literature is the inordinately long drift times that were observed for the product ions of the benzoate compounds. Only extremely large ions have ever demonstrated such long drift times in previous work where nitrogen or air was used as the ion mobility drift gas. Furthermore, the reactant ions did not appear to have the same pattern as when generated in a purely nitrogen or air environment. While the spectra collected in this study from compounds separated by SFC are significantly different from those commonly observed in nitrogen or air IMS, they are in complete agreement with the data collected in our previous study on ion mobility spectrometry with CO_2 as the drift gas (*7*).

In this earlier study, reactant ions produced from a ^{63}Ni ionization source in carbon dioxide were found to be more complex and to drift with much slower velocities than those produced from nitrogen. Separation of product ions from reactant ions and other product ions was also demonstrated in this initial CO_2 IMS study even though velocities were extremely low. One product ion in this study took 57 ms to traverse the 8-cm drift region. The primary difference between the result reported here and those reported earlier is that all of the spectra shown in Figure 5 were obtained from a single supercritical fluid chromatographic run, while in the former case each compound was introduced directly into the spectrometer without the benefit of chromatographic separation. As in the previous study, Figure 4 confirms that product ions produced in a carbon dioxide environment can be separated from reactant ions and from other product ions, providing meaningful information in the form of unique CO_2 ion mobility spectra.

Although the exact nature of the reactant ions formed in carbon dioxide is not known, the general ionization mechanism that occurs in CO_2 IMS can be assumed to be a form of protonation of the netural organic species of interest, forming the positive product ion. Since many organic compounds of interest have at least one proton-accepting site, the technique of ion mobility spectrometry, when using an ionization source which generates proton-donating reactant ions, is innately a sensitive one. Figures 3 and 4 illustrate that even for the benzoates, which have a UV-absorbing chromaphor, the IMS is a more sensitive detection method than the UV detector. Of course some sensitivity may be gained for the UV detector if careful attention had been paid to optimizing the various detector parameters, but the point is still a valid one. This is especially true if the compounds of interest do not have chromaphors for absorbing UV radiation.

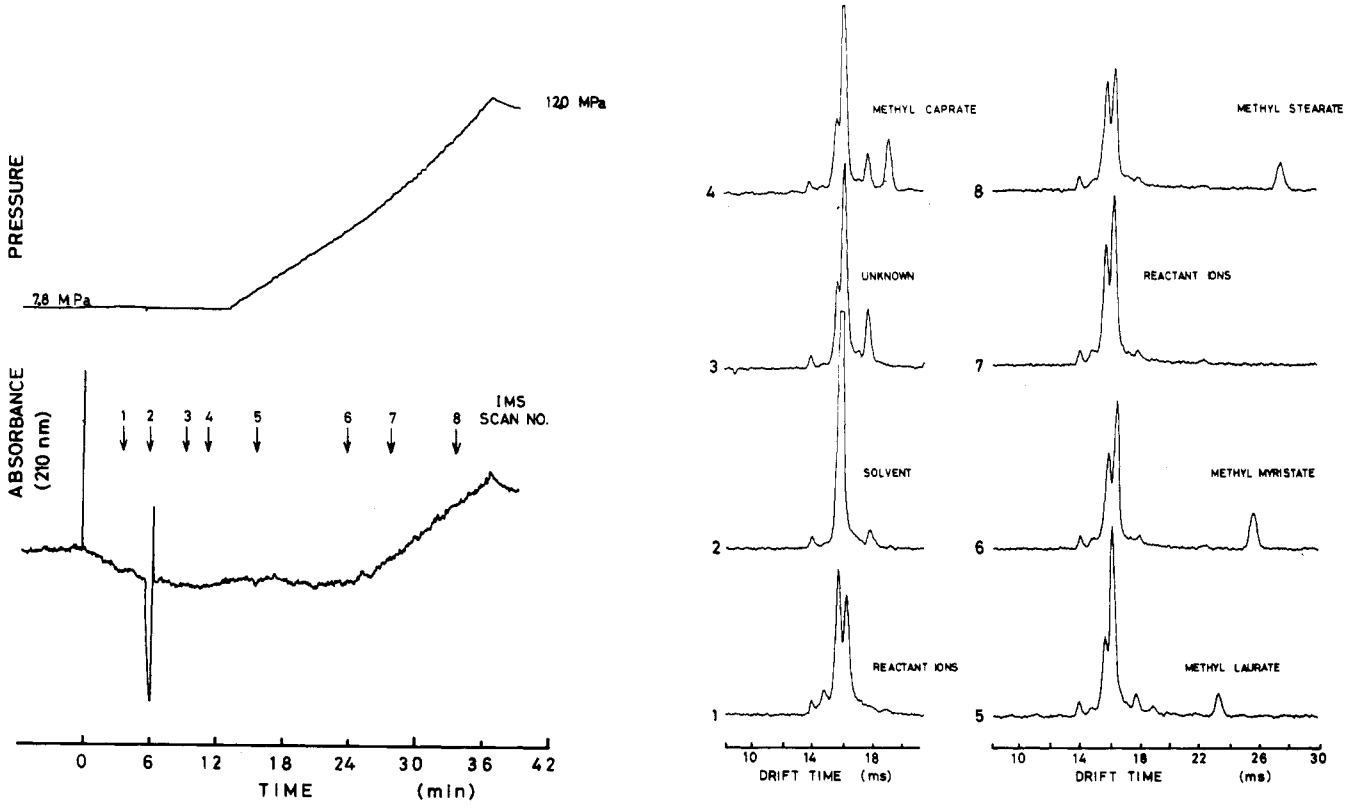

Figure 5. Pressure-programmed supercritical fluid chromatogram of methyl esters with UV detection and selected ion mobility spectra.

The lower left portion of Figure 5 is the UV absorbance tracing at 210 nm of a supercritical fluid separation of a mixture of methyl esters that do not have strong molar absorptivities at this wavelength. Thus, for quantities that did not saturate the column, the methyl esters could not be detected by the UV detector. The chromatographic conditions for this separation were an isothermal oven temperature of 95 °C and a pressure program where the pressure was held constant at 7.8 MPa for the first 12 min then increased at a rate of 1.75 MPa/min, to a final pressure of 12.0 MPa. The components of the mixture along with the amounts injected were as follows: 0.1 ng of methyl caprate, 0.1 ng of methyl laurate, 0.1 ng of methyl myristate, and 0.9 ng of methyl stearate.

As before the numbered arrows indicate the position in the chromatogram that a scan was taken, and the spectra which resulted from these scans are shown in Figure 5 along with identifying numbers.

Scan 1, located in the lower right-hand corner of Figure 5, is of the CO_2 reactant ions just after the injection and just prior to the elution of the solvent. The pattern shown in this scan was typical of the complex pattern normally observed when the IMS was operated under these conditions. Scan 2, loacted just above scan 1, was taken as the solvent eluted from the column. The reactant ions have been completely depleted and replaced by a major peak that has a drift time similar to the reactant ions. Scan 3 was taken just after the solvent but before the components of the mixture began to elute. From this spectrum it appears that all of the solvent has not yet been flushed from the spectrometer. While the reactant ions appear to be reestablishing in the ion source (note the shoulder on the left of the major peak), the major peak still appears to be primarily due to the solvent, and the presence of a contamination peak, the peak labeled unknown, is now clearly noticeable.

With scan 4 the methyl esters begin to elute. Although the reactant ions still appear to be contaminated with the product ion of the solvent, the product ion from methyl caprate can clearly be seen in this spectrum. Scan 5, located in the lower right-hand corner of Figure 5, was taken as methyl laurate eluted from the column. Because we could not monitor the chromatogram with the UV detector it was difficult to always judge the ion mobility scan such that the maximum quantity of the compound would be in the spectrometer during the scan. Thus, it is difficult to make observations about the quantitative nature of the detection of one compound over another in this study. Qualitatively it was clear that we were obtaining reproducible ion mobility spectra for each of the compounds in our mixture. Thus, the small peak at about 23.5 ms represented the product ion for methyl laurate, and the peak at about 26 ms in scan 6 represented the product ion for methyl myristate.

Scan 7 is another scan of only the reactant ions but was taken about half way into the pressure program to determine the effect that changing pressure had on the character of the reactant ions. From this scan it appears that pressure has little effect on the makeup of these ions. Comparing scan 7 with scan 1 it was obvious that the pattern had changed, but this change was noted after the introduction of the solvent to the spectrometer and before the pressure had been changed. In fact when a blank pressure program is run and the reactant ions are monitored, no differences in the reactant ions at high or low chromatographic column pressure can be observed. The final scan, scan 8, shows the product ion of a reasonably heavy molecule methyl stearate. Methyl stearate was chromatographed and detected with relatively little difficulty, indicating that the use of SFC with ion mobility detection may have significant application for the separation and detection of small quantities of high molecular weight compounds.

While the primary results of this study are encouraging by demonstrating that ion mobility spectrometry can be used as a detection method after supercritical fluid chromatography when CO_2 is the ion mobility drift gas, it should be noted that all of the test compounds in this study have significant vapor pressures. Although much higher temperatures are required, these compounds can also be separated by gas chromatography. In fact, they were selected for the initial SFC–IMS tests because their behavior in both gas chromatography and ion mobility spectroscopy is well-known.

Even with these volatile compounds, long residence times in the spectrometer were found to be a significant problem. As shown in Figure 5, the solvent swamped the spectrometer leaving residual amounts of the solvent in the spectrometer throughout the chromatographic run. Also, in Figure 4 an ion mobility spectrum for *n*-propyl benzoate, which was free of contamination from the previously eluting compound ethyl benzoate, could not be obtained. These two examples illustrate a potentially serious disadvantage to using ion mobility spectrometry after SFC. As higher molecular weight compounds are investigated, adsorption and/or condensation in the system may cause serious problems if efficient methods are not found for "sweeping" the ionization region clean of previously eluted compounds. Currently, more efficient ionization cell designs are being investigated for the detection of high molecular weight compounds after separation by SFC.

A second difficulty with the system reported in this work is that it was unable to continuously monitor a specific ion drift time. With the spectrometer available, only complete mobility spectra could be obtained. Since the objective of this study was to determine ion mobility behavior of compounds after SFC, complete spectra were necessary. For routine analytical use, however, we believe that ion mobility spectrometry is best used as a tunable, selective, ambient-pressure, detection method for chromatography, rather than as a qualitative spectrometric method. With the exception of certain isomer identifications, mass spectrometry can provide more detailed qualitative information than can ion mobility spectrometry. When compared to other ambient pressure chromatographic SFC detection methods, such as flame ionization, thermionic and UV detection, ion mobility detection offers a wide range of detection capabilities that cannot be achieved by any other single ambient-pressure device. Based on the results of this study, investigation and development of the ion mobility spectrometer for a continuous-monitoring detection method for SFC seems warranted.

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RECEIVED for review March 10, 1986. Accepted August 11, 1986. This work was supported in part by the Japan Society for the Promotion of Science and by the Public Health Service (No. R01-GM29523).