Evaluation of microbore and packed capillary column chromatography with an ethylvinylbenzene–divinylbenzene polymeric packing material and supercritical ammonia as the mobile phase

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ABSTRACT

An investigation into the use of ammonia as a mobile phase for high-resolution supercritical fluid chromatography was conducted. A highly cross-linked ethylvinylbenzene–divinylbenzene polymeric packing material (5-μm diameter) in microbore stainless-steel and nickel capillary tubing demonstrated reasonable efficiencies (*ca.* 10 000–15 000 plates m^{-1}, after initial exposure to ammonia) without phase degradation as previously observed when using open-tubular capillary columns. However, ammonia treatment caused an initial rapid loss in efficiency (*ca.* 42%) for reasons as yet undetermined. The polymeric packing materials were much more inert than conventional silica-based packing materials. Separations of polar drugs, underivatized amino acids and defoliant herbicides are shown.

INTRODUCTION

Supercritical fluid chromatography (SFC) is generally performed with carbon dioxide as the mobile phase. Despite the well-documented advantages of carbon dioxide as an SFC mobile phase, at least two fundamental limitations with the use of this fluid exist. First, with carbon dioxide, moderate and highly polar materials often will not migrate through the chromatographic column. Second, supercritical carbon dioxide, like all solvating mobile phases, has limitations in the molecular weights of compounds which it can solvate. Various approaches have been taken to address these limitations, including organic modifiers, reversed micelle-containing mobile phases, ion-pairing mobile phases, and alternative fluids.

Perhaps the most polar solvent with properties conducive to use as an SFC mobile phase is ammonia. The use of ammonia as a chromatographic mobile phase has been demonstrated [1–9], however its use in capillary SFC is rather restricted since ammonia rapidly degrades most polysiloxane stationary phases [1,2] and more slowly attacks silica. Consequently, packed microcolumns which are fabricated from non-silica materials are preferred for analytical SFC with ammonia as the mobile phase. A new polymeric packing material for packed microcolumn SFC

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has been recently reported [10,11] which is resistant to the corrosive action of ammonia. A detailed evaluation of this packing material for SFC is reported in this paper.

Physical properties and high-pressure studies of ammonia

Ammonia is a polar material with physical properties compatible with the mobile phase requirements of SFC. The physical properties of ammonia are reviewed in Table I. It is considered to have a hydrogen bonding energy similar to water and a greater proton affinity than water [15]. Consequently, ammonia is a strong hydrogen bond acceptor with little propensity to donate hydrogen bonds, especially in the gas phase [3]. Ammonia possesses a high dielectric constant. Not surprisingly, supercritical ammonia was found [16,17] to have the highest solvatochromic polarity when compared with carbon dioxide, chlorotrifluoromethane, nitrous oxide and carbon dioxide + methanol. The solvating ability which results from these properties should lend itself to unique applications as an SFC mobile phase. One potential problem with the use of ammonia as a chromatographic mobile phase is its reactivity, which may be more pronounced at elevated pressures and temperatures. In chromatography, depending on the

TABLE I

PHYSICAL PROPERTIES OF AMMONIA

Data taken from ref. 12.

Molecular mass	17.031
Boiling point	−33.4°C
Critical temperature	132.4°C
Critical pressure	111.3 atm
	(1 atm = 0.10 MPa)
Critical volume[a]	72.5 ml mol^{-1}
Compressibility factor	0.242
Dipole moment (gas)	1.47 D
Dielectric constant: gas at 0°C	1.0072
liquid at −33.4°C	22.4
Acentric factor	0.250
Solubility factor at liquid density[b]	16.3 cal$^{1/2}$ cm$^{3/2}$
	(1 cal = 4.1868 J)

[a] Data taken from ref. 13.
[b] Data taken from ref. 14.

nature of the reactions, however, this may function beneficially as a type of on-column derivatization.

Although ammonia is probably the most studied non-aqueous solvent, only a few studies concerning high-pressure ammonia, especially with organic solutes or cosolvents, have been undertaken. The thermodynamic stability of ammonia in the critical region has been studied [18]. Phase equilibria studies of ammonia with nitrogen, argon, helium, water, carbon dioxide, methane, ethane, n-butane, ethylene, propylene, benzene, 2,2,4-trimethylpentane, cyclohexane, trans-decalin, o-xylene, acetylene and biphenyl + dodecane have been reported [19,20]. Potassium and silicon have been found to react with supercritical ammonia to form potassium imidonitridosilicate crystals [21]. The solubility of anthracene [22], and caffeine and theophylline [23,24] in ammonia, and near-critical (20°C) separation of 1-butene and 1,3-butadiene with 2–10% (v/v) ammonia in either ethane or ethylene [25], have been studied. Supercritical ammonia was used for the extraction of ginseng saponins [26] and the extraction of cobalt from hydrotreating catalysts [27]. In addition to these studies, the research group of Smith has used supercritical ammonia for direct fluid injection mass spectrometry [28] and for the extraction of diesel fuel marine sediments [29]. Supercritical fluid injection–time-of-flight mass spectrometry of underivatized peptides, nucleosides, and steroids was accomplished with supercritical ammonia [30].

Ammonia as a chromatographic mobile phase

Ammonia was first used as a mobile phase for SFC in 1968 [4,5] when it was shown that supercritical ammonia had solvent characteristics approximately intermediate (except acidity) between water and low-molecular-mass alcohols. It was demonstrated that the ability to form hydrogen bonds favored the solvation of compounds with hydroxylic, amino and related groups by supercritical ammonia. Solutes transported through a packed chromatographic column with ammonia included some waxes, nucleosides, sterols, amino acids, amines, di- and tripeptides, mono- and disaccharides and high-molecular-

mass polyethylene glycols, while proteins, higher polysaccharides and purines did not migrate. Additionally, material stability problems, which resulted in leaks, column plugging, and spiking in the flame ionization detector, were reported.

In capillary SFC, Grolimund et al. [6] used ammonia with fluorescence detection for the separation of fluorescent whitening agents containing free sulfonic acid groups. They also reported a variety of material compatibility problems. Kuei et al. [1,2] performed a systematic study to alleviate these problems and separated polarizable polycyclic aromatic hydrocarbons (PAHs) greater than ovalene ($M_r = 396$) in a carbon black extract with ammonia as the SFC mobile phase. Also, this mobile phase was applied to the analysis of antidepressant drugs and nucleosides. Ammonia was combined as a modifier with sulfur hexafluoride for the separation of nitrogen-containing polycyclic aromatic compounds and polar drugs [1] and imidazole derivatives [7]. Trifluoromethane with 20 mol% ammonia as the mobile phase was able to produce slightly better resolution of some basic drugs than pure ammonia [1].

Other uses of ammonia as a chromatographic mobile phase include liquid ammonia (30–40°C) for the packed-column separation of alkaloids, and a test mixture of phthalates, biphenyl and o-terphenyl [8], and as a carrier gas for capillary GC of aliphatic and aromatic amines [3] and chlorophenols [9]. Compared to nitrogen as carrier gas, ammonia gave improved peak symmetry and decreased capacity factors for primary and secondary amines on both polar (polyethylene glycol) and intermediate polarity (methylphenylcyanopropylsilicone) stationary phases [3]. The effect of ammonia was found to be more pronounced at lower temperatures. With flame ionization detection (FID), the detection limits for these amines were found to be seven to ten times lower with ammonia as the carrier gas than with nitrogen. However, in the analysis of chlorophenols, the capacity factors increased with ammonia relative to when nitrogen was used as carrier gas [9]. The more acidic dichlorophenols were more affected by ammonia than the less acidic monosubstituted ones.

The high pressures and elevated temperatures often associated with SFC have presented certain obstacles in the development of the method. These obstacles are compounded when ammonia is used as the mobile phase, since, as discussed earlier, ammonia is corrosive to many materials. Previous reports emphasized the need to use stainless-steel tubing and PTFE O-rings, gaskets and seals [4,5,8]; however, the work of Kuei et al. [1,2] has been the only systematic evaluation of materials for SFC when using ammonia as mobile phase. They found that aluminum supply cylinders provided more pure ammonia (due to fewer particulate contaminants) than when stainless-steel cylinders were used, and stainless-steel tubing should be used for supply lines between the supply cylinder and the pump, and between the pump and the SFC injector. In the assembly of the apparatus, 0.2-μm in-line filters were placed on both the inlet and outlet sides of the pump. PTFE can be used for pump cylinder piston seals; however, PTFE tends to soften in ammonia at pressures around 240 atm. A PTFE–graphite composite material for the piston seals proved to be more durable. Ferrules made of graphite or Kel-F polymer were used. For injection, Kuei et al. [1,2] used the timed-split method with a PTFE spacer in the connection to the injector body. The injector rotor was made from the standard Valcon-H material. Pinched platinum-iridium tubing was used as the restrictor. Detection was accomplished with ultraviolet (UV) absorbance since FID was found to yield significant background noise due to ionization of the ammonia mobile phase. The entire apparatus was placed in a fume hood.

Stationary phases for ammonia SFC

As previously mentioned, most of the commonly used polysiloxane stationary phases in capillary SFC are rapidly degraded by supercritical ammonia [1,2]. It was found that n-octyl- and n-nonyl-substituted polysiloxanes and polyethyleneimine were the only stationary phases studied which could resist the corrosive action of ammonia. Polymethylsiloxane stationary phases containing 50% phenyl, 50% cyanopropyl, 30% biphenyl or 5% phenyl moieties were rapidly degraded by ammonia. It was postulated that the presence of long, easily cross-linkable hydro-

carbon appendages protected the siloxane backbone from attack by ammonia.

Highly cross-linked polymers were reported [8] to be more compatible than silica-based packing materials for use with supercritical ammonia. A new packing material was recently developed [10,11] which is a highly cross-linked ethylvinylbenzene and divinylbenzene (EVB–DVB) polymer. This EVB–DVB, like other polymeric packings, should be more highly resistant to chemical attack than silica-based packings. Because this polymer is more highly cross-linked, it should be degraded less easily than other polymers. The EVB–DVB polymer has an average particle size of 5 μm, a pore size of about 60 Å, a surface area of about 300 m^2 g^{-1}, and was shown to be chemically and physically stable at temperatures and pressures up to at least 200°C and 10 000 p.s.i. (1 p.s.i. = 6894.76 Pa) [11]. The retention mechanism of polar and non-polar compounds on the EVB–DVB polymeric stationary phase was found to be governed by surface adsorption-desorption as a result of non-polar–non-polar interactions. The retention mechanism also was significantly influenced by the π–π surface interaction with π-electron-rich solutes because of the high density of π-electrons associated with the aromatic components of the stationary phase. In this paper, this EVB–DVB polymer was further evaluated for microbore and packed capillary SFC of polar compounds using ammonia as the mobile phase.

EXPERIMENTAL

For the packed microcolumn work reported here, a Hewlett-Packard Model 5790 gas chromatographic oven (Hewlett-Packard, Avondale, PA, USA), Valco Model CW14 injector (Valco Instruments, Houston, TX, USA), Chiratech Model 203 UV–Vis detector (now produced by Linear Instruments, Reno, NV, USA), and a Varian Model 8500 syringe pump (Varian Associates, Walnut Creek, CA, USA) controlled through a pressure feedback with an Apple IIe computer (Apple Computers, Cupertino, CA, USA) were used with the modifications described by Kuei et al. [1,2]. In this study,

graphite or graphitized Vespel ferrules were used. Polyether ether ketone (PEEK, Upchurch Scientific, Oak Harbor, WA, USA) tubing was used in connections, although this material is not useful at temperatures above 150°C. Adequate tightening of fittings and connections was extremely important since the polymeric materials tended to soften at elevated temperatures and pressures. Leaks were frequently encountered with fittings involving 1/32 in. O.D. (1 in. = 2.54 cm) tubing when stainless-steel ferrules were used. The use of gold-plated ferrules alleviated this problem. The fused-silica transfer lines from the injector and to the detector were minimized in length and internal diameter since the silica was weakened by the ammonia. The problems previously enumerated [1,2] due to ammonia degradation of the protective polyimide coating on exposed sections of fused-silica were not addressed. With the platinum–iridium restrictor used here, the end of the restrictor tubing was inserted into a small vial of water to collect the ammonia mobile phase and to minimize plugging of the restrictor. Occasional heating of the restrictor tip with a butane cigarette lighter restored flow when the restrictor was partially plugged.

Columns used in this study were microcolumns packed with OmniPAC μPRN-300 (Dionex, Sunnyvale, CA, USA). Initial experiments with the OmniPAC material were with test columns (15 cm × 750 μm I.D.) supplied by the manufacturer. Other columns were packed in our laboratory by the slurry packing method [31,32]. Column material of stainless steel, glass-lined stainless steel, fused silica (Polymicro, Phoenix, AZ, USA), and nickel (Valco) tubing with dimensions of 15–75 cm × 0.250–1.0 mm I.D. were used.

RESULTS AND DISCUSSION

Initially, the OmniPAC columns supplied by the manufacturer were evaluated for efficiency using carbon dioxide, before and after exposure to ammonia, following the procedure of Kuei et al. [1,2] (i.e., ammonia at 100 atm and ambient temperature for 15 h, then 200 atm and 145°C for 24 h). The measured efficiency averaged 42%

less after exposure to ammonia. Several columns used in this study were evaluated periodically during their use in the ammonia SFC system. Each column showed similar trends, *i.e.*, a 15–50% loss of efficiency after exposure to ammonia. Efficiency measurements were performed over the course of use of the columns, including after venting the column of ammonia followed by repeated exposure. From this, it appears that the loss in efficiency occurred during the initial 1–2 h following exposure to the ammonia. Minimal additional efficiency loss was observed over the period of use, including up to two weeks of continuous use and *ca.* six weeks of total use. Based on experiments involving purging and venting the columns with ammonia compared to continuous exposure, it does not seem that swelling of the packing material is a significant cause of efficiency loss. Scanning electron microscopy (SEM) of the packing material showed no gross physical changes to the OmniPAC beads due to exposure to ammonia. These SEM results, shown at 9500 × magnification, are displayed in Fig. 1. The SEM did show a wide range of particle sizes and a small degree of distortion of the particle shape, even in the material as received from the manufacturer. The erratic efficiencies obtained in ammonia SFC were verified to some extent in carbon dioxide SFC [33]. With columns supplied by the manufacturer, losses in efficiency (up to 50%) were found in most cases. After purging with organic solvents, such as acetonitrile and methanol,

some of the efficiency was regained. The highest observed efficiency in our study and in the carbon dioxide study [33] was about 20 000 plates m^{-1}. Since the loss in efficiency with these columns occurred during initial exposure, was independent of the mobile phase used (carbon dioxide or ammonia), could only be partially regenerated, and the SEM of the OmniPAC beads showed no gross physical changes due to ammonia exposure, we postulate that the efficiency loss with these columns is due to a redistribution of the packed bed upon pressurization. Swelling of the packing material during exposure to the mobile phase may also be a minor contribution to the efficiency loss.

The initial efficiencies, in ammonia, of these columns were typically 17 000–20 000 plates m^{-1} and the dimensions of the column has no apparent effect on the efficiency of packing. Fused-silica column material yielded particularly erratic efficiency results and became extremely fragile upon exposure to ammonia. Other column materials (stainless steel, glass-lined stainless steel and nickel) gave similar efficiencies. Columns packed in nickel tubing of capillary dimensions (≤500 μm I.D.) were the most reproducible, while the stainless-steel columns were the most durable. Small I.D. columns could be packed to longer dimensions, yielding greater numbers of total plates without deleterious chromatographic effects due to pressure drops. Hence, the nickel and stainless steel columns were preferred.
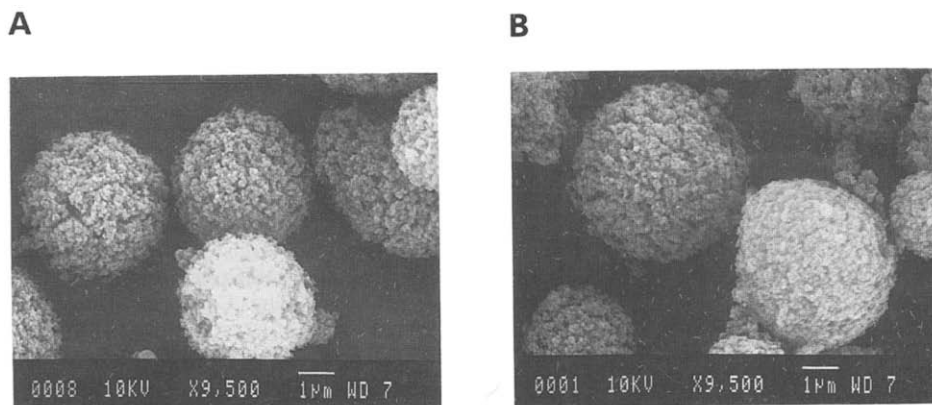
Silica-based packing materials are often in-

A   B

Fig. 1. Scanning electron photomicrographs of OmniPAC μPRN-300 packing material (A) as received and (B) after exposure to supercritical ammonia.

adequate for packed column SFC of polar materials, especially because of the high content of surface silanol groups, even after deactivation. Polymeric packing materials should be much more inert. Therefore, it was of interest to evaluate the surface activity of the polymeric OmniPAC material, especially in ammonia SFC.

Before exposing the OmniPAC material to ammonia, free fatty acids could be separated with good peak shapes [11], as displayed in Fig. 2. After the ammonia purge discussed previously, free fatty acids could not be eluted from the column in supercritical carbon dioxide, both before and after the column was additionally purged with HPLC-grade water for several hours.

To compare the OmniPAC activity in both carbon dioxide and ammonia mobile phases, a mixture of 15 explosives and related decomposition products was separated using both mobile phases. Two separate OmniPAC columns, each possessing similar efficiencies in the mobile phase in which they were used, were employed. The test mixture contained nitro- and amino-aromatics and other polar materials. The mixture components included 2,6-dinitrotoluene, 2,4-dinitrotoluene, dibenzofuran, n-nitrosodiphenylamine, 2,4,6-trinitrotoluene, 2-nitronaphthalene, benz[a]anthracene, 1-nitropyrene, benzo[a]pyrene, 1,3,5-trinitrobenzene, pyrene, phenol, 2-nitrodiphenylamine, 1-nitronaphthalene and 9-phenylanthracene. The resulting chromatograms, using FID for carbon dioxide SFC and UV detection for ammonia SFC, are presented in Fig. 3. The selectivity of the OmniPAC

Fig. 2. SFC chromatogram of a mixture of saturated free fatty acids ($C_{12}H_{24}O_2$, $C_{14}H_{28}O_2$, $C_{16}H_{32}O_2$, $C_{18}H_{36}O_2$) using carbon dioxide as the mobile phase prior to exposing the OmniPAC packing to ammonia. Conditions: carbon dioxide, 100°C, 25 cm × 250 μm I.D. OmniPAC μPRN-300, density programmed from 0.50 g ml^{-1} to 0.75 g ml^{-1} following a 5-min isobaric hold, FID. Following exposure to ammonia, the chromatographic integrity of the packing was reduced so that these free fatty acids could not again be eluted with carbon dioxide SFC.
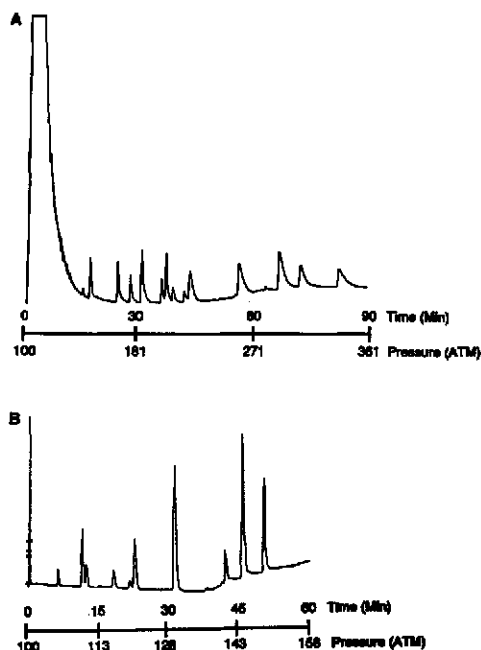

Fig. 3. SFC chromatograms of a mixture of explosives and related decomposition products using (A) carbon dioxide and (B) ammonia as the mobile phase. Conditions: (A) carbon dioxide, 145°C, 30 cm × 250 μm I.D. OmniPAC μPRN-300, pressure programmed from 100 atm to 355 atm at 3 atm min^{-1} following a 5-min isobaric hold, FID; (B) ammonia, 145°C, 25 cm × 500 μm I.D. OmniPAC μPRN-300, pressure programmed from 100 atm to 158 atm at 1 atm min^{-1} following a 2-min isobaric hold, UV at 254 nm.

material in these two mobile phases was not determined, and although no attempt was made to identify the individual peaks and the chromatograms are not directly comparable, it is obvious that the use of ammonia as the mobile phase resulted in dramatically reduced peak tailing and improved peak shape for these compounds.

The value of ammonia as an SFC mobile phase is its applicability for samples too polar to be separated by carbon dioxide SFC. To demonstrate the application to polar materials, three samples were used.

The antidepressant drugs zimelidine and nor-zimelidine present a difficult separation. These compounds, both hydrochloride salts, cannot be analyzed by GC, and separation of the compounds is difficult to perform by LC. Although zimelidine can be eluted in SFC using carbon dioxide, the formation of the "nor-" derivative imparts enough basicity to the molecule that it cannot be analyzed in the same way. A reasonable separation of these drugs using supercritical ammonia is shown in Fig. 4 with little peak tailing.

For the chromatographic analysis of amino acids, they are often derivatized, usually to their phenylthiohydantoin derivatives. The underivatized amino acids, tyrosine and tryptophan, were separated by ammonia SFC (Fig. 5). It has been shown that a wide range of amino acids can be analyzed by ammonia SFC [4,5]. To achieve a separation of a wider range of amino acids than shown in Fig. 5, a selective stationary phase will be needed, as will a more universal detector. In the ammonia SFC of amino acids, ammonia probably reacts with the carboxylic acid moiety of the amino acid to form the corresponding amide. This analysis can be considered as "on-column derivatization" since only one peak is obtained for each compound injected.

The final application is the separation of the defoliant herbicides, diquat dibromide and paraquat dichloride. These compounds are
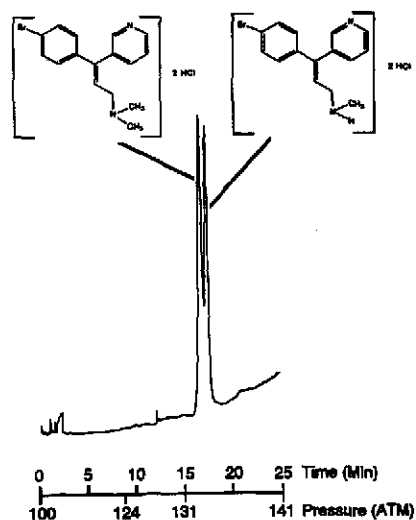

Fig. 4. SFC chromatogram of the antidepressant drugs zimelidine and norzimelidine, using ammonia as the mobile phase. Conditions: ammonia, 145°C, 15 cm × 1 mm I.D. OmniPAC μPRN-300, pressure programmed from 100 atm to 124 atm at 3 atm min^{-1} following a 1-min isobaric hold, then 1 atm min^{-1} to 141 atm, UV at 254 nm.
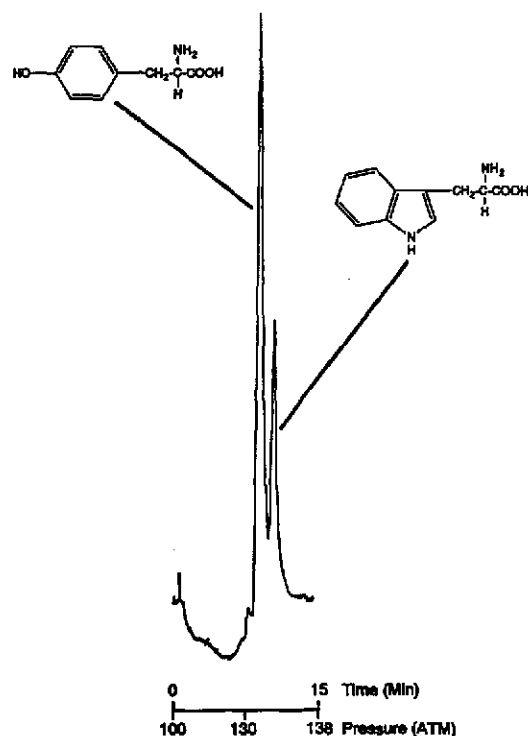

Fig. 5. SFC chromatogram of the amino acids tyrosine and tryptophan using ammonia as the mobile phase. Conditions: ammonia, 145°C, 15 cm × 1 mm I.D. OmniPAC μPRN-300, pressure programmed from 100 atm to 130 atm at 5 atm min^{-1} following a 1-min isobaric hold, then 1 atm min^{-1} to 138 atm, UV at 280 nm.
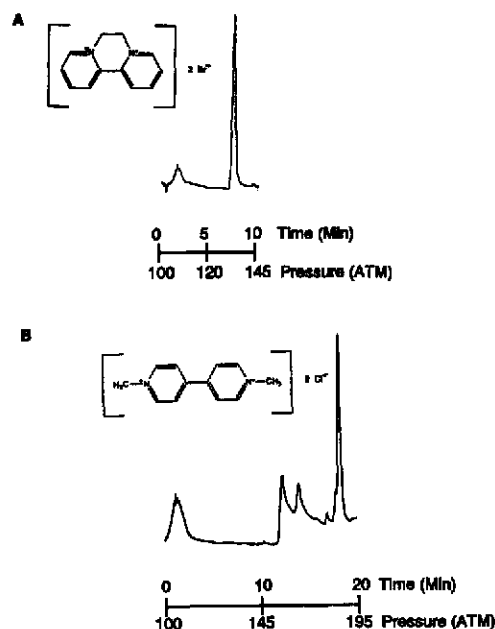
Fig. 6. SFC chromatograms of the defoliant herbicides diquat dibromide (A) and paraquat dichloride (B), using ammonia as the mobile phase. Conditions: ammonia, 145°C, 15 cm × 1 mm I.D. OmniPAC μPRN-300, pressure programmed from 100 atm at 5 atm min^{-1} following a 1-min isobaric hold, UV at 254.

quaternary pyridinium salts of great environmental concern. Due to their nonvolatile nature, these materials cannot be analyzed by GC, and LC usually involves ion chromatography or an ion-pairing mobile phase. The potential for the analysis of these compounds by ammonia SFC is demonstrated in Fig. 6. Several very polar impurities are seen in the chromatogram in Fig. 6B.

These applications have demonstrated the use of ammonia as mobile phase in SFC. While other separation methods can be used for these polar samples, the use of neat mobile phases (such as ammonia) with high efficiency stationary phases (such as OmniPAC) has advantages of simplicity that must be explored further.

ACKNOWLEDGEMENTS

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