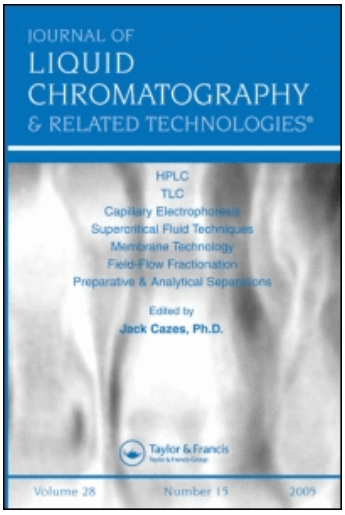

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SEPARATION OF ACROLEIN AND ITS POSSIBLE METABOLITES USING DIFFERENT MODES OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ion-exclusion, reverse phase, anion exchange and partition chromatography were used to separate acrolein and related compounds including 3-hydroxypropanal, 3-hydroxypropionic acid, 1,3-propanediol, allyl alcohol, acrylic acid, HCO_3^-, glycerol, glycidol, oxalic acid, malonic acid, propionic acid, ethanol, and propanol. The ion-exclusion chromatography employed an Interaction ORH-801 organic acid analysis column and was capable of separating these small and polar molecules. The reverse phase chromatography was used to resolve co-eluting compounds on the ion-exclusion column. The anion exchange and partition chromatography were used to separate organic acids and carbohydrates, respectively.

INTRODUCTION

Acrolein is the active ingredient in an aquatic herbicide and biocide that has been widely used to clear weeds and aquatic plants from irrigation ditches and canals and microorganisms from oil field equipments. Acrolein is also widely used as an important precursor in industrial synthesis. Acrolein was known to hydrolyze rapidly in water with first order kinetic half-lives ranging from 14 to 92 hours (1), and 3-Hydroxypropanal was identified as the major hydrolytic degradation product. Acrolein was also known to

undergo rapid biotransformation under both aerobic and anaerobic conditions to form a group of structurally related compounds. Major hydrolytic and biotransformation products included 3-hydroxypropanal, 3-hydroxypropionic acid, allyl alcohol, acrylic acid, propionic acid, HCO_3^-, glyceric acid, 1,3-propanediol, oxalic acid, propanol, and butyric acid. The metabolism of acrolein in fish (studies conducted at Springborn Laboratories, Inc., 1993) and rat (2,3) suggested the formation of various metabolic products including acrylic acid, glycerol, glycidol, and sugar conjugates. The chemistry of acrolein posed an unique challenge to analytical chemists because of its high reactivity and volatility. To understand the degradation mechanism of acrolein in the environment, an adequate analytical separation technique is essential to identify and quantify metabolites of acrolein (especially those of transient nature). The separation techniques described in this paper provided a fast and relatively simple method to monitor acrolein and its metabolites.

MATERIALS AND METHODS

Chemicals

All reference standards were purchased from commercial sources and were analytical grade. All solvents were HPLC grade. ^{14}C-Acrolein was obtained from Sigma Chemical Company, St. Louis, MO. ^{14}C-$NaHCO_3$ was obtained from New England Nuclear, Boston, MA. 3-Hydroxypropanal (^{14}C-) was synthesized by adding ^{14}C-acrolein to water and allowing the mixture to react at room temperature for approximately seven days. The 3-hydroxypropanal was the major product (approximately 95%) of this reaction. The identification of this major product was achieved by derivatization with pentafluorophenylhydrazine (Aldrich Chemical Company) and subsequent analysis of the derivative by particle beam LC/MS using electron impact ionization. HPLC standards of each compound were prepared in reagent grade water.

High Performance Liquid Chromatography (HPLC)

HPLC chromatograms were collected using following instrumentations: a Waters model 510 solvent pump, a Hewlett Packard model 1050 autosampler, a Radiomatic model A-280 radiometric detector with data acquisition systems, a FIAtron CH-30 column heater, a Hewlett Packard model 1047A refractive index detector, a Hewlett Packard model 1040A photo diode-array detector and a Hewlett Packard model 3396A integrator. The Radiomatic A-280 radiometric detector equipped with a 500-μL liquid scintillation cell was used to monitor radiolabeled compounds (^{14}C-acrolein, ^{14}C-3-hydroxypropanal, and ^{14}C-HCO_3^-), while refractive index and photo diode-array detectors were used to monitor all non-radiolabeled compounds.

Five HPLC chromatographic systems were developed to separate acrolein and related compounds. They were one ion-exclusion system, two reverse phase systems, one anion exchange system, and one partition system. The ion-exclusion chromatographic system used an Interaction ORH-801 organic acid column (300 X 6.5 mm) at 35°C and 1 mM H_2SO_4 mobile phase at 0.8 mL/minute. The first reverse phase chromatographic system used a MetaChem Inertsil ODS-2 column (5 μm, 250 X 4.6 mm) maintained at 35°C,

and using a 0.05% H_3PO_4 mobile phase at 1 mL/minute. The second reverse phase chromatographic system used a Phenomenex Ultremex C18 (3 μm, 250 X 4.6 mm) at ambient temperature and using a 0.1 M KH_2PO_4 (pH 2.5) mobile phase at 0.8 mL/minute. The anion-exchange chromatographic system used a Phenomenex Spherex 10 SAX column (150 X 4.6 mm) at ambient temperature and using a 5/95 acetonitrile/potassium hydrogen phthalate (2 mM, pH 6.5) mobile phase at 1.5 mL/minute. The partition chromatographic system employed a Waters Carbohydrate Analysis column (10 μm, 300 X 3.9 mm) at ambient temperature and using a 80/20 acetonitrile/water mobile phase at 2 mL/minute.

RESULTS AND DISCUSSION

Acrolein is a small (molecular weight of 56), very polar, and highly water soluble compound. Most of its degradation products also bear these physical properties. The structural similarities of these small molecules made it very difficult, if not impossible, for signal-run separations using a single mode of chromatography. Integrations of various modes of chromatography were used in this work to separate these polar molecules.

The ORH-801 column is a polymer based ion-exclusion column designed specifically for separating weak organic acids, carbohydrates, alcohols, and inorganic anions. The separation using this column was satisfactory for most of the compounds tested. A list of the compounds investigated and their retention times are presented in Table I. An HPLC chromatogram showing the separation of a standard mixture is shown in Figure 1. There were three retention zones that were critical to acrolein studies. The first one (zone #1) was the early eluting region (retention time ~ 4.3 - 5.3 minutes) contained mostly strong organic acids and carbohydrates. Zone #2 (retention time ~ 8.3 minutes) contained lactic acid, glycerol, 3-hydroxypropionic acid, and glycidol. Zone #3 (retention time ~ 11 minutes) contained propionic acid, acrylic acid, and HCO_3^-.

The two reverse phase systems were developed primarily to resolve co-eluting compounds in zones #2 and #3. A list of retention times of selected compounds using these two reverse phase systems was also presented in Table I. An HPLC chromatogram showing the separation of selected compounds using the Inertsil ODS-2 system is shown in Figure 2. The Inertsil ODS-2 system separated glycerol and glycidol, while glycidol and 3-hydroxypropionic acid still co-eluted. This system could also separate acrylic acid, propionic acid, and HCO_3^-. The Ultremex C18 system was used to separate glycidol and 3-hydroxypropionic acid. Acrolein chromatographed poorly (excessive retention and severe peak tailing) on both reverse phase columns due to the 100% aqueous mobile phases. These two systems were therefore, only used to monitor acrolein metabolites.

To retain strong organic acids (zone #1), a strong anion exchange chromatographic system (4) was used.

TABLE 1

Retention Times of Selected Compounds Using Ion-Exclusion and Reverse Phase Chromatographic Systems.

Compounds	Ion-Exclusion System retention time (minutes)	Inertsil ODS-2 System retention time (minutes)	Ultremex C18 System retention time (minutes)
Oxalic acid	4.32	3.52	4.12
Maltose	4.99	4.00	
Lactose	5.11	3.83	
Lactulose	5.12	4.22	
Citric acid	5.24	10.23	
Glucuronic acid	5.30	4.04	4.11
Propiolic acid	5.30	6.60	
Malonic acid	6.11	6.90	6.20
Glyceric acid	6.83	3.66	4.81
Glyceraldehyde	7.10		4.19
Lactic acid	7.95	6.22	7.02
Glycerol	8.32	3.95	4.35
3-Hydroxypropionic acid	8.40	5.80	6.87
Glycidol	8.41	5.83	7.71
Formic acid	8.70		
3-Hydroxypropanal	9.00	4.90	
Acetic Acid	9.30	6.67	
Ethylene glycol	9.68		
1,2-Propanediol	10.10	5.80	6.98
1,3-Propanediol	10.35	5.00	6.19
Propionic acid	10.98	16.31	19.2
$NaHCO_3$	11.36	9.90	
Acrylic acid	11.41	13.32	16.2
Adipic acid	11.81		
Ethanol	12.11	7.07	8.55
Allyl alcohol	12.63	10.07	12.7
Butyric acid	13.40		
Propanol	15.13	15.80	21.6
Acrolein	15.62		

Organic acids could be easily distinguished from alcohols, since alcohols (neutral) had no retention on the anion exchange column. This anion exchange system was used primarily to confirm the formation of oxalic acid in acrolein metabolism studies.

The other class of early eluting compounds on the ORH-801 column was carbohydrate. A partition chromatography utilizing a Carbohydrate Analysis column was used to selectively retain carbohydrates. Alcohols and carboxylic acids either had no retention or excess retention on this column. A list of selected compounds and their retention times using the anion exchange and carbohydrate systems are presented in Table 2.

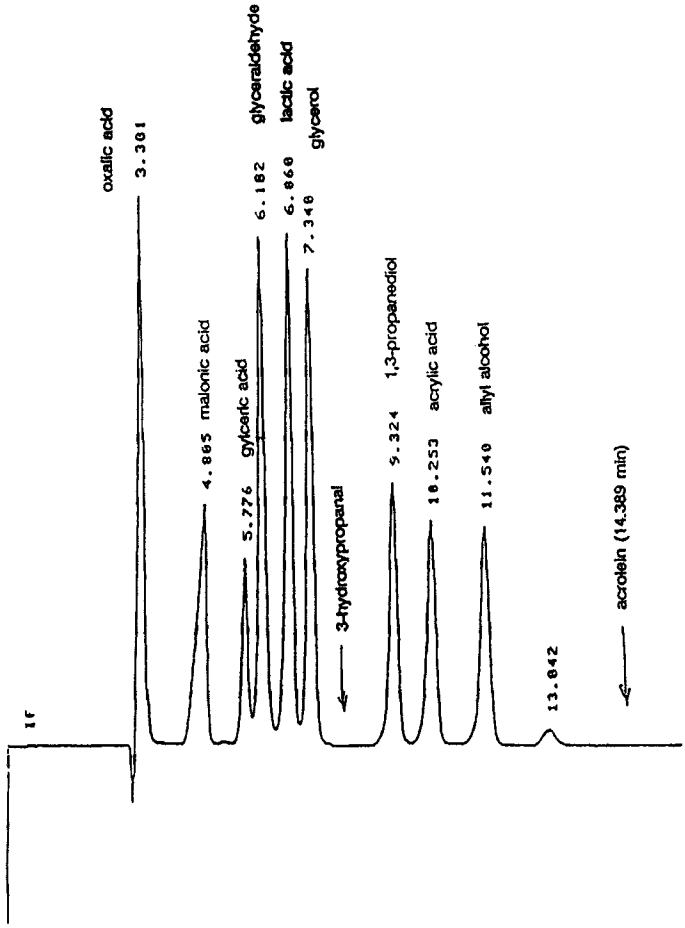

Figure 1. Separation of a standard mixture using ion-exclusion chromatography. HPLC conditions: ORH-801 column at 35°C, 1 mM H_2SO_4 at 0.8 mL/minute, RI detection. The retention times of acrolein and 3-hydroxypropanal were determined on separate runs. 3-Hydroxypropanal was detected using a radiometric detector. (Note: Retention times in this chromatogram were different from those listed in Table I due to the differences in HPLC dead volumes.)

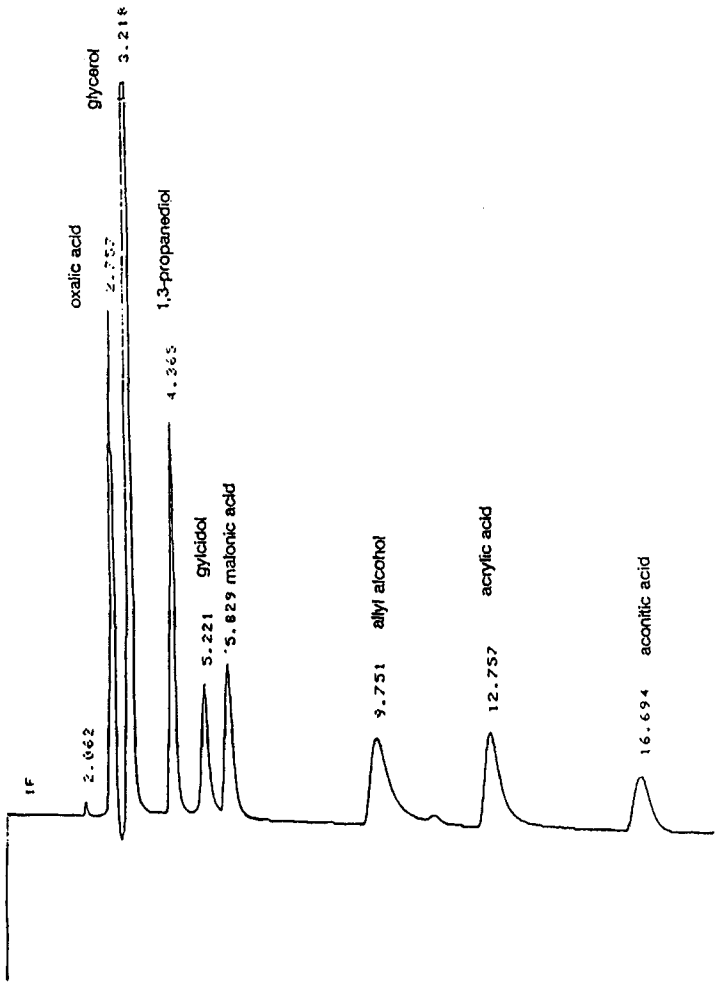

Figure 2. **Separation of a standard mixture using reverse phase chromatography. HPLC conditions: Inertsil ODS-2 column at 35°C, 0.05% H_3PO_4 at 1 mL/minute, RI detection. (Note: Retention times in this chromatogram were different from those listed in Table I due to the differences in HPLC dead volumes.)**

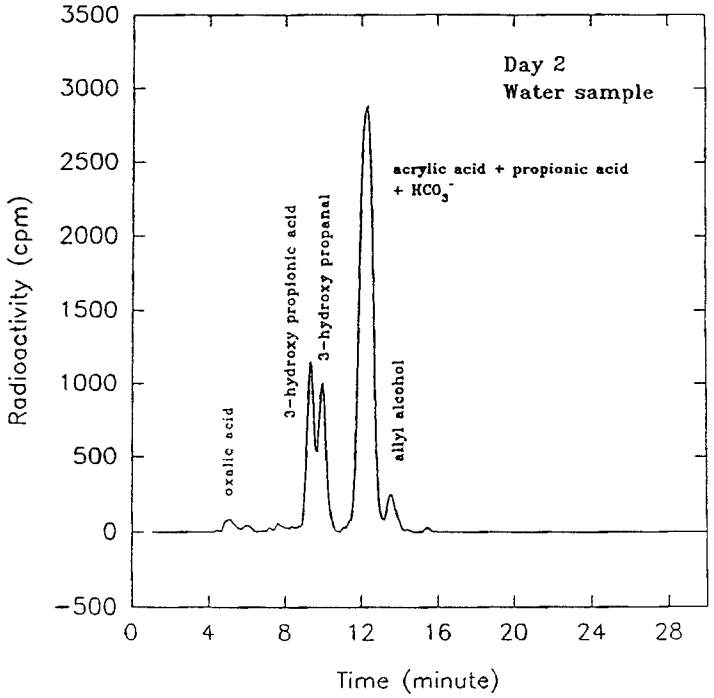

Figure 3. **HPLC radiochromatogram of a water sample collected during an aquatic metabolism study with acrolein. The separation of acrolein metabolites was achieved using the ion exclusion chromatographic system.**

TABLE 2

Retention Times of Selected Compounds Using Anion Exchange and Partition Chromatographic Systems.

Compounds	Spherex SAX System retention time (minutes)	Carbohydrate Analysis System retention time (minutes)
Oxalic acid	6.40	
Malonic acid	6.63	
Propiolic acid	3.27	
3-Hydroxypropionic acid	2.80	
Glyceric acid	2.72	
Acetic acid	3.43	
Glucose		6.17
Lactulose		12.45
Maltose		12.36
Lactose		14.47

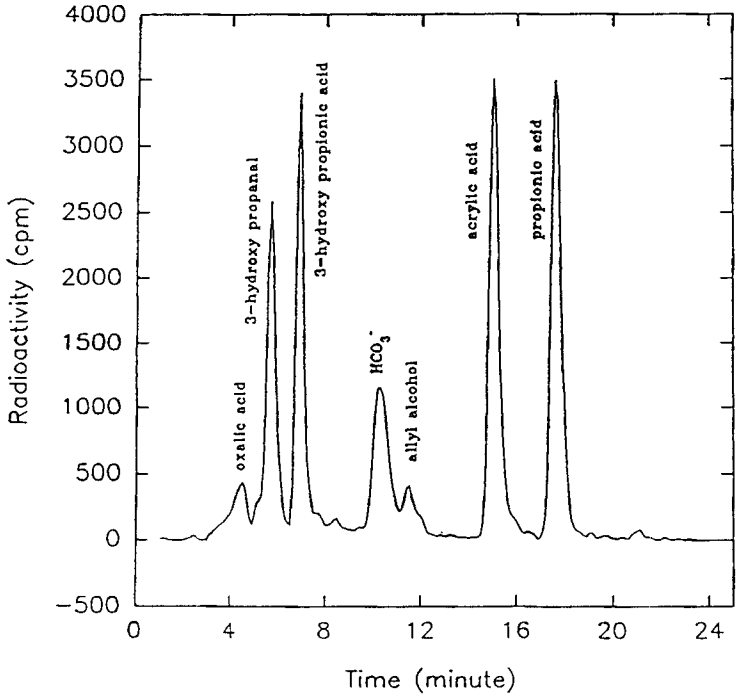

Figure 4. **HPLC radiochromatogram of the same sample as in Figure 3 using the Inertsil ODS-2 chromatographic system.**

The separation techniques developed in this work could serve as a good method reference for separating small and polar organic compounds. These techniques were applied to aquatic (aerobic and anaerobic) and fish (freshwater fish and shellfish) metabolism studies with acrolein. These chromatographic systems were proved to be adequate to separate a large number of acrolein metabolites. Aqueous samples and tissue extracts could be chromatographed directly without further manipulations (eg. derivatization, extraction). The relatively simple sample preparation and rapid analysis time permitted the identification and quantification of transitory metabolites. Two representative chromatograms collected during an aquatic metabolism study with acrolein are presented in Figures 3 and 4 showing the formation of several microbial biotransformation products. Three major metabolites, acrylic acid, propionic acid, and HCO_3^-, co-eluted on the ion-exclusion column (Figure 3), while they were separated on the reverse phase column (Figure 4).

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