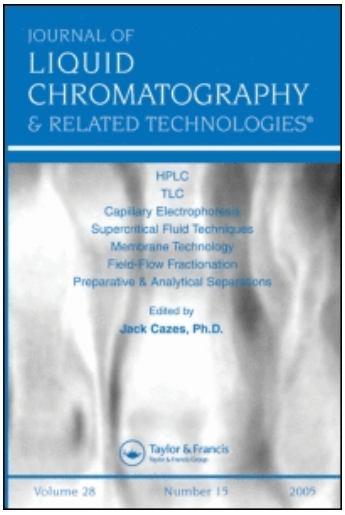

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Liquid Chromatographic Characteristics of Ethylenethiourea with HPLC Carbon, Chiral, Polymer and Reverse-Phase Bonded Silica Columns

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LIQUID CHROMATOGRAPHIC CHARACTERISTICS OF ETHYLENETHIOUREA WITH HPLC CARBON, CHIRAL, POLYMER AND REVERSE-PHASE BONDED SILICA COLUMNS

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ABSTRACT

Of the columns investigated, the graphitised carbon column provided the best chromatographic characteristics for the highly water-soluble compound ethylenethiourea (ETU). The stability of the carbon column in strongly acidic media permitted the incorporation of the phosphoric acid electrolyte into the 5% acetonitrile-in-water mobile phase. ETU eluted from the column in 200 s as a sharp symmetrical peak at a mobile phase flow rate of 1 mL/min and a column temperature of 35°C. The k' value was 1.72. ETU peak retention times and responses showed excellent repeatability with coefficients of variation of 0.28 and 1.40%, respectively, for 6 replicates with the high performance liquid chromatographic-electrochemical system using the graphitised carbon column. Although ETU eluted as a sharp symmetrical peak with the cyclodextrin chiral columns, their instability at low pH required post-column addition of the phosphoric acid electrolyte solution. ETU chromatographed poorly or degraded on the polymer columns. The chromatographic separation of ETU on the C-8 reverse-phase bonded silica column appeared to be due mainly to residual silanol groups. With the NH_2 bonded silica column ETU eluted immediately after the injection solvent.

INTRODUCTION

High performance liquid chromatography (HPLC) has become an important analytical tool for the determination of many analytes, especially polar water-soluble compounds such as ethylenethiourea (ETU). In 1977 Onley et al. (1) reported the application of HPLC to the determination of ETU, a metabolite of the ethylenebisdithiocarbamate fungicides; they used a normal-phase column and a UV detector. Farrington (2) and Hanekamp et al. (3) in 1979 reported using reverse-phase columns to chromatograph ETU. Farrington chromatographed ETU on a C-18 column using water as the mobile phase and monitored ETU with a UV detector. Hanekamp et al. chromatographed ETU on a C-8 column using a 1% methanol-aqueous mobile phase containing 0.01 M potassium nitrate and 0.02 M nitric acid as electrolytes for the dropping mercury electrode detector. Other researchers (4-8) later reported using C-18 and C-8 reverse-phase HPLC columns to chromatograph ETU. Lawrence et al. (5) and Krause and Wang (8) did not use an organic modifier in the mobile phase. Kobayashi et al. (7) used a mobile phase containing 5% methanol, the highest percent of organic modifier reported, to chromatograph ETU. ETU retention times reported in the literature vary from 2.5 to 11 min.

An HPLC column was desired that would provide the best chromatographic characteristics for the highly water-soluble ETU and permit the use of the simplest liquid chromatographic system compatible with the electrochemical (EC) detection technique previously reported (8). Chromatographic characteristics of ETU were investigated with carbon, chiral, polymer and reverse-phase bonded silica columns.

MATERIALS AND INSTRUMENT PARAMETERS

Chemicals

Acetonitrile (UV grade), methanol and water were distilled-in-glass grade from Burdick & Jackson Laboratories, Inc. (Muskegon, MI). Phosphoric acid (85%) was HPLC grade from Fisher Scientific (Fair Lawn, NJ). The Environmental Protection Agency (Research Triangle Park, NC) provided the ETU reference standard, which was dissolved and diluted to appropriate concentrations with water from Burdick & Jackson.

Apparatus

The mobile phase and aqueous electrolyte solutions were contained in Ultraware HPLC solvent reservoirs (Kontes, Vineland, NJ) and degassed with helium (99.995%) that was purified with in-line Hydro-Purge II and Oxy-Purge traps (Alltech Associates, Inc./Applied Science Labs, Deerfield, IL). These solutions were delivered with Model SP8700XR pumps (Spectra-Physics, San Jose, CA). Injections were made into the column with a Spectra-Physics Model SP8700XR autosampler fitted with a 20 μL loop. The HPLC columns investigated are listed in Table 1. The temperature of the columns was controlled by a Model 2080 HPLC column oven (Varian Associates, Inc., Palo Alto, CA). For the carbon and polymer columns, the aqueous phosphoric acid electrolyte solution

TABLE 1

HPLC Columns Investigated

Particle		Column		Manufacturer
Type	Size (μm)	Name	Length[a] (cm)	
Graphitised carbon	7	Hypercarb	10	Shandon Scientific Ltd., Cheshire, UK
Polystyrene-divinyl-benzene copolymer	10	PRP-1	15	Hamilton Co., Reno, NV, USA
Polystyrene-divinyl-benzene copolymer	5	PLRP-S	15	Polymer Laboratories Inc., Amherst, MA, USA
Polystyrene-vinyl pyridine copolymer	11	ACT-2	15	Interaction Chemicals Inc. Mountain View, CA, USA
β-cyclodextrin/silica	5	Cyclobond I	25	Advanced Separation Technologies Inc., Whippany, NJ, USA
α-cyclodextrin/silica	5	Cyclobond III	25	same
C-8/silica	5	Zorbax C-8	25	DuPont Co., Wilmington, DE, USA
NH_2/silica	5	Zorbax NH_2	25	same
CN/silica	5	Zorbax CN	25	same

[a]All columns were 4.6 mm i.d.

was made a part of the mobile phase. For the bonded silica columns (C-8 and chiral), the electrolyte solution was added to the column eluate by using a 0.5 mm i.d. stainless steel tee (Upchurch Scientific, Oak Harbor, WA). ETU was detected in the flowing stream with a Model 8490 UV detector (Spectra-Physics) or an EC detector (Bioanalytical Systems, Inc., West Lafayette, IN). The EC detector consisted of a Model LC-17 thin-layer EC cell equipped with a Au/Hg working electrode, a Ag/AgCl reference electrode and a stainless steel block auxiliary electrode. A 5 μm gasket was used between the EC cell halves. The potential was applied with a Model LC-4B amperometric detector controller. All chromatograms were recorded on a Spectra-Physics Model 4200 computing integrator.

HPLC Operating Parameters

The column oven was operated at 35°C unless otherwise stated. A flow rate of 1 mL/min was used except for the C-8, CN and NH_2 columns, for which a flow rate of 1.5 mL/min was used. The UV detector was set at a wavelength of 240 nm to monitor ETU. For the EC detector, a potential of +0.350 V was applied to the working electrode, and a time constant of 0.3 Hz was used. When the HPLC-EC system was not used overnight, the flow rate of the mobile phase was reduced to 0.5 mL/min, the cell control was turned to "stby" and the detector was turned off.

RESULTS AND DISCUSSION

The most common HPLC columns used today are the reverse-phase columns containing spherical silica particles to which has been bonded a given functionality such as an octyl (C-8), cyanopropyl (CN) or aminopropyl (NH_2) group. Table 1 lists the columns investigated that contained these packing materials. ETU eluted just after the injection solvent (k' = 0.14) with the NH_2 column when water was used as the mobile phase. The general rule of thumb is that the analyte should have a k' value of at least 1; therefore, the NH_2 column was not considered acceptable. The k' values obtained for ETU with the CN and C-8 columns were 1.39 and 1.68, respectively, with a mobile phase of 10% acetonitrile in water at 1.5 mL/min and a column temperature of 35°C. Although the CN column produced the sharpest ETU peak with the least amount of tailing, the C-8 column is more stable than the CN column according to the manufacturer and produced a somewhat larger k' value for ETU. Thus, the C-8 column was selected to test the suitability of the HPLC-EC technique to

determine ETU in the presence of crop coextractives (8). The referenced work used a column temperature of 60°C to sharpen the ETU peak and reduce tailing. ETU peak retention time was 210 s, corresponding to a k' value of 2.58, with the C-8 column (serial no. L7656) and water as the mobile phase. In subsequent work it was observed that the k' values were significantly smaller with the newer C-8 columns from the same manufacturer. The k' values for ETU were 1.11 for column L9801 and 0.27 for column L12209. The author was informed after an inquiry to the manufacturer that the manufacturing process had been improved to reduce the number of residual silanol groups. Therefore, it appeared that the chromatographic separation of ETU on the C-8 column was a function of the number of residual silanol groups. Chromatography based on residual silanols was considered undesirable.

Several polymer columns were investigated to eliminate the difficulties encountered with the bonded silica columns and also to permit the aqueous phosphoric acid electrolyte solution to be added directly to the mobile phase rather than to the column eluate as reported previously (8). The polymer columns investigated are listed in Table 1 and are reported to be stable in the pH range of 1-13. The ACT-2 (vinyl pyridine) and PRP-1 (divinyl benzene) columns produced a rather broad ETU peak having a tailing front edge with an indication of a shoulder. These columns were not considered satisfactory. The second divinyl benzene column (PLRP-S) produced a sharp peak (k' = 2.32) for ETU using an aqueous 0.05 M H_3PO_4 mobile phase. There was a small but noticeable rise and fall in the baseline prior to the ETU peak with the column at 35°C. It was observed that as the column temperature was increased the ETU peak height decreased and the height of the earlier eluting peak increased. Example chromatograms are shown in Figure 1. This phenomenon was also observed when only water, *i.e.*, water without phosphoric acid, was used. Thus, the polymer columns investigated were not satisfactory for chromatographing ETU.

Cyclodextrin bonded silica columns have been used to separate a variety of compounds, especially isomers (9-11). The separation mechanism is based on the degree of inclusion complexing of the analytes within the cyclodextrin molecule. Although the particles are silica, it was thought that the bulkiness of the cyclodextrin molecules bonded to the silica would shield the ETU molecules from residual silanol groups. Thus, the β- and α-cyclodextrin columns (Cyclobond I and III, respectively) were investigated for their ability to chromatograph ETU.

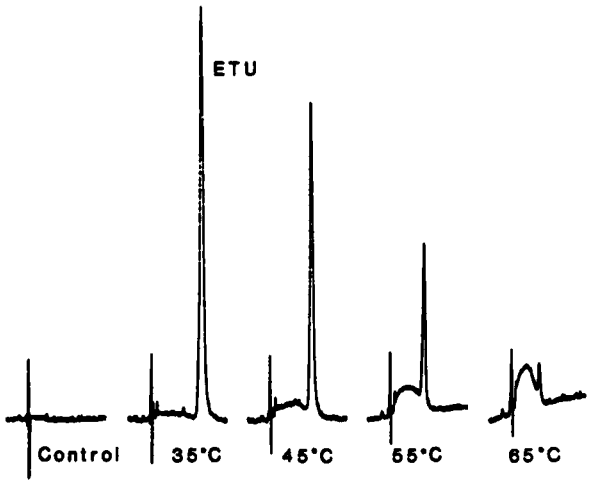

FIGURE 1. Effect of column temperature on the degradation of ETU on the PLRP-S 20 ng ETU injected using the HPLC-UV system.

Each column was found to produce a very sharp symmetrical peak for ETU. A 10% acetonitrile-in-water mobile phase appeared optimum for both columns with a flow rate of 1 mL/min. ETU eluted in 213 s (k' = 0.18) and 241 s (k' = 0.34) from the Cyclobond I and III columns, respectively. The aqueous phosphoric acid electrolyte solution was added to the column eluate because cyclodextrin columns are not stable below a pH of 3.5, according to the manufacturer. The Cyclobond III column was selected for further evaluation because it produced a slightly larger k' value and sharper peak for ETU. This column was studied in conjunction with the extraction and coextractive removal procedures of the ETU method being developed. It was observed that ETU retention time decreased during the course of the study. In discussions with the manufacturer, it was learned that chlorinated hydrocarbon solvent molecules become tightly bound in the cavity of the cyclodextrin molecule, causing a decrease in the retention time of the analyte of interest. Methylene chloride is a major component used in the coextractive removal procedure, and although it is evaporated, traces of this chlorinated hydrocarbon solvent may be present in the injected solution. Thus, not only does this column not permit the addition of phosphoric acid to the mobile phase because of its limited stability at low pH, but it is also incompatible with traces of methylene chloride which may be present in the injected solution of the purified sample extract.

FIGURE 2. Effect of conditioning carbon column on ETU chromatography; 5 ng ETU injected using the HPLC-UV system.

TABLE 2

ETU Peak Retention Time and Response Repeatability for the HPLC-EC System with the Carbon Column

	Retention time (s)	Response (area units)[a]
	197	69066
	198	69873
	198	70720
	198	70880
	197	71619
	197	70718
Av.	197.6	70432
SD	0.55	984
CV	0.28	1.40

[a]Computing integrator area units.

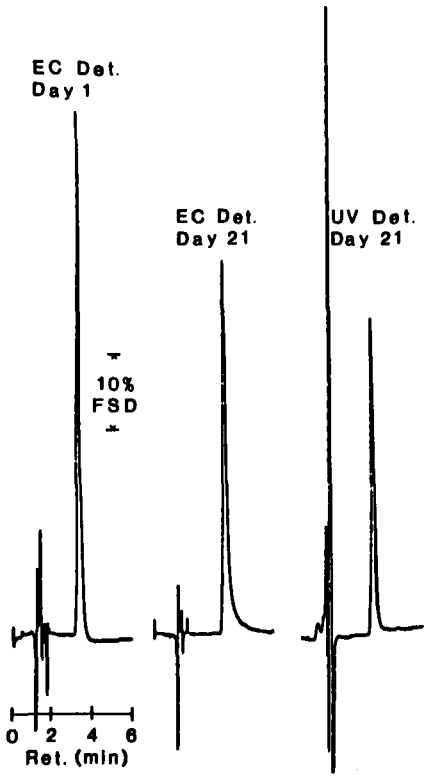

FIGURE 3. Gold/mercury electrode response (peak shape) to ETU, with comparison to UV chromatogram; 5 ng ETU injected.

Recently, an HPLC graphitised carbon column became commercially available. Knox et al. (12) developed the chromatographic material and have described its chromatographic, physical and chemical properties. The spherical 7 μm particles are reported to act as a strong hydrophobic adsorbent, and the column can be used under typical HPLC conditions of pressure and flow rate. The material is reported by the manufacturer to be stable in both strongly alkaline and acidic solutions. The chromatographic characteristics of ETU with the carbon column were investigated. ETU chromatographed as a sharp symmetrical peak with the carbon column when a 5% acetonitrile-in-water mobile phase containing phosphoric acid at 0.025 N was used at a flow rate of 1.0 mL/min and a column temperature of 35°C. ETU eluted from the column in

approximately 200 s, which corresponds to a k' value of 1.72. Experience has shown that the columns should be preconditioned overnight with 0.5 mL acetonitrile/min to remove impurities and improve column efficiency. Figure 2 shows example chromatograms of ETU chromatographed on a column before and after conditioning. Figure 3 shows ETU chromatograms obtained using the UV and EC detectors. The gold/mercury amalgam electrode tends to produce a tailing peak for ETU after 3 weeks of use. The HPLC-UV chromatogram of ETU obtained after the 21 day HPLC-EC chromatogram shows that the tailing effect is due to a phenomenon of the EC detector and not the carbon column. ETU peak retention times and responses are given in Table 2. Both showed excellent repeatability with coefficients of variation of 0.28 and 1.40, respectively, when the HPLC-EC system with the carbon column was used.

CONCLUSION

The graphitised carbon column meets the desired criteria for the determination of ETU. Because of the stability of the carbon column at low pH, the HPLC system requires only one HPLC pump because the phosphoric acid electrolyte solution can be added directly to the mobile phase. The carbon column produces a sharp symmetrical peak with a k' value >1 but small enough to enable many injections to be made throughout the day.

The reader is advised not to conclude that the carbon column is superior for all applications. It has been the experience of this worker that each type of column has unique properties that will make it equal or superior to other column types for a given application.

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