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# Short Communication

# Liquid chromatographic determination of ethylenethiourea using pulsed amperometric detection

Daniel R. Doerge* and Austin B. K. Yee

*Department of Environmental Biochemistry, University of Hawaii, Honolulu, HI 96822 (USA)*

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ABSTRACT

A liquid chromatographic method was developed using pulsed amperometric detection at a gold working electrode to measure residue levels of ethylenethiourea (ETU) in crops and groundwater. Use of the sequential pulsing program eliminates electrode fouling while preserving the sensitive and selective detection of ETU. Minimum detection limits in crops were 5–10 ppb (1.25–2.5 ng on-column) and 5 ppb (0.5 ng) in groundwater. The commercial availability of the pulsed electrochemical detector and its gold working electrode that remains functional with a minimum of conditioning is an improvement in method simplicity.

## INTRODUCTION

Ethylenethiourea (ETU) is a formulation contaminant and environmental metabolite of ethylene bisdithiocarbamate (EBDC) fungicides, the most widely used fungicides worldwide [1]. The presence of ETU residues in food crops is of regulatory concern because high doses of ETU cause thyroid enlargement (goiter) and cancer (thyroid and liver) in experimental animals [2].

ETU has been determined by a variety of gas (GC) and liquid chromatographic (LC) methods [3,4]. The low volatility of ETU necessitates derivatization for analysis by GC and the low UV absorbance maximum (232 nm) makes LC with UV detection susceptible to interferences found in typical environmental samples. The most successful LC method published uses amperometric detection with a mercury–gold amalgam working electrode to determine ETU at levels $\geqslant 10$ ppb in many crops [5]. However, the gold electrode supplied by the manufacturer must be modified prior to use in the analysis. Briefly, the electrode surface was treated with liquid mercury and subsequently equilibrated in air for extended times ($\geqslant 2$ days) [5]. In addition, the modified working electrode has a finite period of consistent response ($\leqslant 21$ days), after which the electrode surface must be reconditioned as described above. These limitations provided the impetus to explore other electrochemical techniques for the sensitive and selective detection of ETU.

Pulsed amperometric detection has been used successfully to measure thiourea derivatives at low levels using gold or platinum working electrodes [6,7]. This technique is not limited, as is d.c. amperometry on these electrodes, by poisoning of the electrode surface by oxidized sulfur species and metal oxide formation. The ability to use a sequence of potential pulses allows for measurement of electron transfer at the working electrode followed by an oxidative "cleaning" potential and a subsequent reductive "reconditioning" potential to regenerate an active and stable electrode surface [8]. The commercial availability of such instrumentation led us

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to use this technique for the determination of ETU in crops and groundwater.

## EXPERIMENTAL

ETU was obtained from Aldrich (Milwaukee, WI, USA) and recrystallized from water before use. LC solvents were prepared from acetonitrile (Optima, Fisher Scientific, Fairlawn, NJ, USA) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) and were degassed by helium sparging. LC separation of ETU was performed using a quaternary gradient pump module (Dionex, Sunnyvale, CA, USA) equipped with a Rheodyne Model 7125 injector and a 25 cm × 4 mm I.D. OmniPac PAX 500 column (Dionex) using 5% acetonitrile in 25.0 m*M* $KH_2PO_4$ (Sigma, St. Louis, MO, USA), pH 7.0, at a flow-rate of 1.5 ml/min. Pulsed amperometry was measured using the potential–time waveform defined in Fig. 1 produced by a pulsed electrochemical detector (Dionex) equipped with a Ag/AgCl reference electrode and recorded on an LCI-100 recording integrator (Perkin-Elmer, Norwalk, CT, USA). The gold working electrode was used as received from Dionex following an initial polishing by hand (*ca.* 1 min) using a fine abrasive compound. A UV detector (UVIS 200, Linear Instruments, Reno, NV, USA) was used for in-line detection of ETU at 232 nm to monitor the column performance and detector response. Method validation was provided by particle beam LC–mass spectrometry (LC–MS) using [$^{13}$C]ETU as an internal standard [9].

Extraction and purification of ETU residues from papaya and banana samples obtained from commercial grocery stores were performed by using the AOAC method as revised by Krause [5]. Briefly, a 50-g portion of fruit (peel or pulp) was homogenized with aqueous methanol and filtered and an aliquot corresponding to 10 g of fruit was processed further. The bulk of the solvent was removed *in vacuo* and the remainder was applied to Gas-Chrom S. The ETU residues were eluted through a column of alumina with 2% methanol in methylene chloride, which was removed in vacuo. The samples were dissolved in water (4 ml) and filtered (0.45 μm) prior to LC analysis of 100-μl aliquots. ETU concentrations were determined from comparison of peak areas or heights with those generated by authentic standards injected immediately after the crop extract.

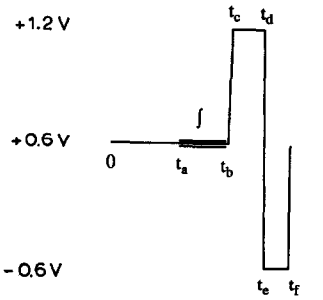


Fig. 1. Potential–time waveform used for pulsed amperometric detection of ETU. $t_a$ = 0.3, $t_b$ = 0.5, $t_c$ = 0.51, $t_d$ = 0.59, $t_e$ = 0.6, $t_f$ = 0.65 s. Current is integrated from $t_a$ to $t_b$.

## RESULTS AND DISCUSSION

Sensitive and selective detection of ETU in Hawaiian fruits and groundwater was accomplished using a commercial pulsed electrochemical detector and a polymer-based LC column. This column has been found to give reproducible retention time and peak shape for ETU in crops and groundwater with sufficient retention ($k' = 1.3$) for amperometric detection. The waveform used (Fig. 1) was based on that described by Johnson and co-workers [6,7] and the detector response to ETU standards was optimized by variation of potential and times. Integrated amperometry has been reported to give enhanced response to thioureas relative to simple potential–time waveforms such as that shown in Fig. 1 [7]. However, integrated amperometric waveforms corresponding to those previously published did not enhance the detection limit for ETU under the conditions of this study. It was also determined that varying the pH in the range 3–10 did not enhance the detector response to ETU. After several days of analyzing crop samples, an increase in the background signal and a decrease in the signal from ETU were observed. The sensitivity of the electrode was restored by polishing the working electrode surface as described above. After a short equilibration time ($\leq$30 min), the detector was ready for use.

The use of an in-line UV detector allowed the assessment of the effect of the electrochemical detector on peak asymmetry. Determination of the peak asymmetry factor ($B/A$) by measuring the ar-

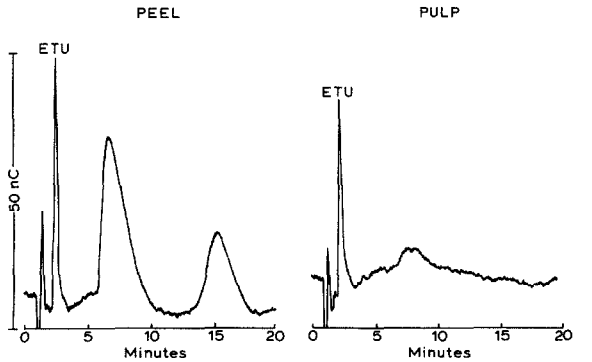


Fig. 2. Determination of ETU in papaya peel and pulp by LC with pulsed amperometric detection.

eas of the front and back portions of the peaks from both detectors showed that the electrochemical detector did not introduce significant tailing effects ($\leq 1\%$).

Fig. 2 shows the analysis of papaya pulp and peel for samples spiked with 20 ppb of ETU prior to homogenization, which corresponds to 5 ng injected on-column. The ETU detected corresponds to recoveries of 112 and 80% for the peel and pulp, respectively. These recoveries, and those from banana pulp (93%), are typical of the modified AOAC procedure and are comparable to those obtained in this laboratory from a variety of crops analyzed by a particle beam LC–MS method [9]. The detection limits (signal-to-noise ratio = 3) varied depending on the sample, but were typically 5–10 ppb (1.25–2.5 ng on-column). The major components of the total noise were baseline disturbances from unretained components and the presence of electroactive interferences, especially in papaya peel, the most complex matrix encountered. These limits compare favorably with those obtained using the mercury–gold amalgam working electrode reported by Krause (1.25–2.5 ng) [5]. While no tolerances are set for ETU levels in food crops, typical laboratory detection limits are 10 ppb.

The chromatogram in Fig. 2 shows the presence of at least two electroactive components in the papaya peel sample. The presence of sulfur-containing compounds in papaya skin has been reported previously [10]. The papaya pulp sample is essentially free from electroactive components except ETU. Chromatograms of control papaya samples showed no ETU and had similar elution profiles of coextractive components.

ETU was also determined in groundwater obtained from the Palolo section of the Pearl Harbor, Honolulu basin aquifer. Control injections showed no detectable ETU, so samples were fortified with varying amounts of ETU prior to analysis. The amperometric detector response to ETU in groundwater was linear over more than two orders of magnitude (0.5–100 ng, $r^2 = 0.9995$). The detection limit in groundwater was estimated to be 5 ppb (0.5 ng) with no sample preparation using 100-$\mu$l injections.

This study demonstrates the utility of pulsed amperometric detection for the detection of ETU in environmental samples at levels comparable to those previously obtained using d.c. amperometry with a mercury-treated gold electrode [5]. The use of the pulsed waveform permits analyte detection and the working electrode reconditioning required for extended use with typical environmental samples and minimum variation in detector response. In addition, the commercial availability of the gold working electrode used in this study, which remains functional with a minimum of conditioning, *i.e.*, occasional polishing that is easily incorporated into a daily routine, represents an improvement in method simplicity.

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