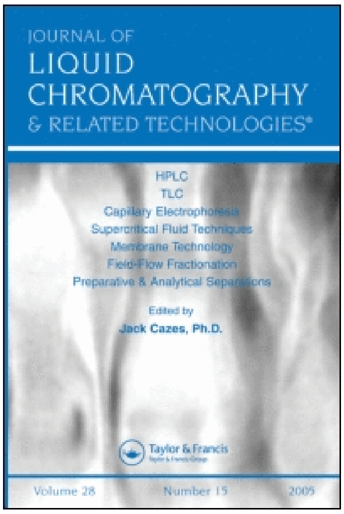

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ELECTROCHEMICAL DETECTION IN THE DETERMINATION OF SEVERAL DITHIOCARBAMATES BY REVERSE-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Amperometric and coulometric methods of detection using glassy carbon electrodes, for liquid chromatographic determination of general thiuram disulfide, salts of alkyl-dithiocarbamates and alkylenbis-dithiocarbamates were evaluated. Several chromatographic methods for separation of these compounds were proposed based on a reverse-phase column including ion-suppression for N-alkyl-dithiocarbamates and methyl derivation for N-alkyl and alkylen-bisdithiocarbamates. The effects of several experimental parameters on the separation and the sensitivity of the methods were evaluated. Optimized methods were applied to determination of Thiram and Dimethyl ethylenebisdithiocarbamate in some fruits.

INTRODUCTION

Disulfides and salts of N-alkyldithiocarbamates and alkylenbis-dithiocarbamates form the most important class of pesticides for broad spectrum control of a variety of fungal diseases on growing crops. Some of them are also used as pharmaceuticals and rubber vulcanizers.

Different types of methods have been developed for the analysis of dithiocarbamates in several samples: Gas-Chromatography,[1,2] spectrometric determination,[3-9] electrochemical techniques including potentiometric titration,[10,11] stripping voltammetry,[12] and cyclic voltammetry.[13] Except polarographic and chromatographic methods, most of these techniques are based on decomposition of the dithiocarbamates prior analysis mainly by hydrolysis to carbon disulfide and no discrimination between different dithiocarbamates is obtained.[14,15]

Disulfides are substances which can be separated easily by reverse-phase liquid chromatography. However, these methods are rather non-selective because UV detection is employed. Gustafsson et al.[16,17] have published methods for the determination of salts of alkylenebisdithiocarbamates and thiram, previous transformation into water-soluble sodium salts by RPLC, and UV detection at 272 nm. Smyth et al.[18,19] and Kirkbright et al.[20] examined ionic dithiocarbamates using ion-interaction of dithiocarbamates with transition-metal ions to form complexes or tetraalkylammonium salts respectively. Irth et al. proposed some selective methods for determination of dithiocarbamates all metal-complexes using pre- or post-column reaction-detection systems[21-23] which provide an important enhancement of the selectivity and sensitivity.

Although electrochemical detection is not used frequently in RPLC, for these types of compounds, we have employed this detection method previously in our laboratory for amperometric detection of Thiram on a carbon paste electrode with a detection limit of 2.7 ng.[24]

In this work we want to establish conditions for Liquid Chromatographic determination of a Thiuram Disulfide (Thiram) and several dialkyl-dithiocarbamates (Figure 1) using electrochemical detection in amperometric and coulometric mode, in order to obtain a more sensitive and selective method to analyze pesticide residues in several environmental samples.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Gilson Model 302 pump, a Gilson Model 802C manometric module equipped with a membrane damper, and a Rheodyne Model 7510 injector equipped with a 20 µL loop. A Gilson Model 116 UV detector and a Metrohm Model 641-VA with a 656 flow cell for amperometric detection and an ESA Model 5010 analytical cell for coulometric detection, were used. A glassy carbon electrode (Metrohm Model

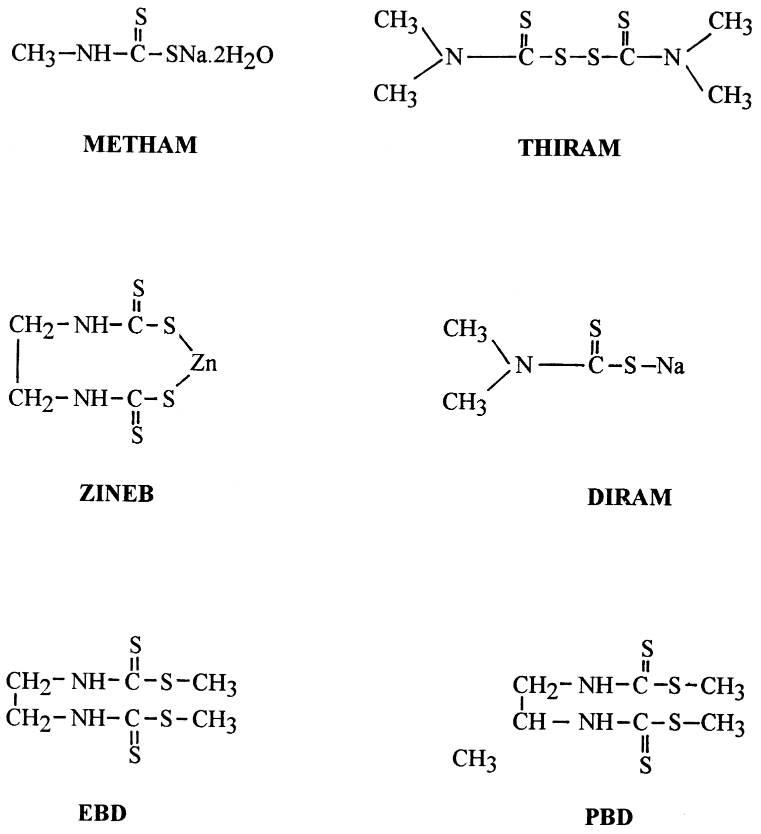

Figure 1. Structure of studied compounds.

6.0805.010) with a surface area of 7 mm^2 was used as amperometric working electrode. The chromatograms were registered with a Spectra-Physics mod. SP-4290 integrator. Cyclic voltammograms were obtained with a BAS CV-27 voltammograph, equipped with a glassy carbon rotating disk electrode Metrohm 628-10 (7mm^2 surface area). An Ag/AgCl reference electrode and a platinum counter electrode were also used.

To obtain better responses in amperometric detection the electrode was polished during 60 s on filter paper and then, a potential of +1.4 V was applied for 1 minute before to set the working potential. In coulometric detection it is necessary to chemically clean the surface electrode with 5-10 mL of HNO3 3N and to apply a potential of +1.4 V during 1 minute before to set the working potential in order to obtain a good response for several days.

Reagents

All reagents were of analytical-reagent grade. The LC solvents were of special solvent grade for liquid chromatography (Carlo Erba, Milan, Italy). Thiram (tetramethylthiuram disulfide), Diram (sodium N,N-dimethyl-dithiocarbamate) (Aldrich, Germany), Metham (sodium, N-methyl-dithiocarbamate), Zineb (zinc, ethylenebisdithiocarbamate) (Aragonesas, S.A. Spain), and Nabam (sodium, ethylenebis-dithiocarbamate) (Dr. Ehrenstofer, Lab., Germany) were used as received. Methyl iodide (RPE, Merck. Germany), carbon disulfide (PA, Panreac), and tetrabutylammonium hydrogen sulfate (Carlo Erba, Milan Italy) were used to prepare methyl derivatives. Thiram and methyl derivatives stock solutions were made up in methanol, whereas stock solutions of Diram and Metham were prepared in ultrapure water. All solutions were stored in darkness, under refrigeration, and removed weekly.

Dimethyl ethylenebisdithiocarbamate (EBD) and dimethyl propylene-bisdithiocarbamate (PBD) were prepared in our laboratory.

Procedures

The preparation of Dimethyl-ethylenebisdithiocarbamate (EBD) and dimethyl-propylenebisdithiocarbamate was made according to the procedure described by Gustafsson and Thompson,[16] which consists of the preparation of a mixture of 1,2 diaminoethane or 1,2 diaminopropane and carbon disulfide. The precipitate obtained was treated with methyl iodide and the product recrystallized twice from chloroform. These products obtained were characterized and checked for both Mass Spectrometry and IR Spectroscopy.

The extraction procedure for determination of Thiram residues in apples and grapes was as follows: The samples of pulp of apples or grapes were ground and, after, were fortified with a commercial product. Each sample was extracted with 3x15 mL of chloroform and after filtration through a glass fiber filter. The filtered sample was evaporated till dryness, the residue was dissolved in 1.0 mL of mobile phase, and 20 µL were injected in the chromatographic system.

The extraction of Zineb from fruit samples as methyl derivative, was made according to the extraction procedure of Gustafsson et al.;[16] the sample, treated with a known amount of Zineb, was extracted with EDTA in sodium hydroxide to form the ethylenebisdithiocarbamate. The aqueous part was extracted with a solution which contain tetrabutylammonium hydrogen sulfate and methyl iodide in chloroform-hexane, the volatile part was stripped off, and the residue was diluted in 1.0 mL of mobile phase.

RESULTS AND DISCUSSION

A 150 x 3.9mm stainless-steel pre-packed reversed-phase column, containing 5 µm Spherisorb ODS-2 (Tracer, Spain) was used in all separation methods. Three mobile phases were used in the described methods. Thiram is poorly soluble in water but easily soluble in methanol. For this compound a methanol percentage of 60% containing ammonium acetate buffer (pH 5.0) was chosen.

Dithiocarbamates and alkylenebisdithiocarbamates are ionic compounds which present great solubility in both water and methanol. Whereas, the sodium and ammonium salts of these compounds are weakly retained on reverse-phase columns. Alkylenbisdithiocarbamates behave as strong acids and it is necessary to transform them into methyl derivatives as previously proposed.[16,17] A mobile phase methanol-water 45:55 (v/v) containing ammonium acetate buffer (pH 5.0) was adequate for the separation of methyl derivatives of nabam and sodium propylenbisdithiocarbamate, with good resolution and retention time for the last compound detected. The pH value of the solution did not affect the resolution and retention time, for values ranging between 2 and 8.

In both eluents proposed, the concentration of buffer was 5 mM in amperometric detection mode and 0.5 mM in coulometric detection mode.

Diram and Metham are weak acids and can be separated using a mobile phase methanol-water 55:45 (v/v) containing 0.02 M of ortophosphoric acid. However, in acidic media Metham can suffer a slow degradation.[25,26] This problem can be overcome by injecting the compound at neutral pH without modifications in retention time and peak parameters.

All proposed solutions as eluents were filtered through a membrane filter of 0.45 µm (Durapore, Waters) and de-aerated by stirring under vacuum for 10 min. The flow rate used was 0.9 mL/min.

Electrochemical Studies

In Figure 2A, it is observed that Thiram is oxidized on the glassy carbon electrode in the chromatographic conditions, reaching a maximum at a potential of +1.1 V. As the electrolyte concentration in eluent is an important factor in electrochemical detection, we studied the effect of electrolyte concentration on an analytical signal. A better signal-to-noise ratio was observed at an electrolyte concentration of 5 mM for amperometric detection. In coulometric detection, the electrochemical detector was equipped with a coulometric cell with a porous glassy carbon electrode, which increased the oxidation efficiency of the detector.

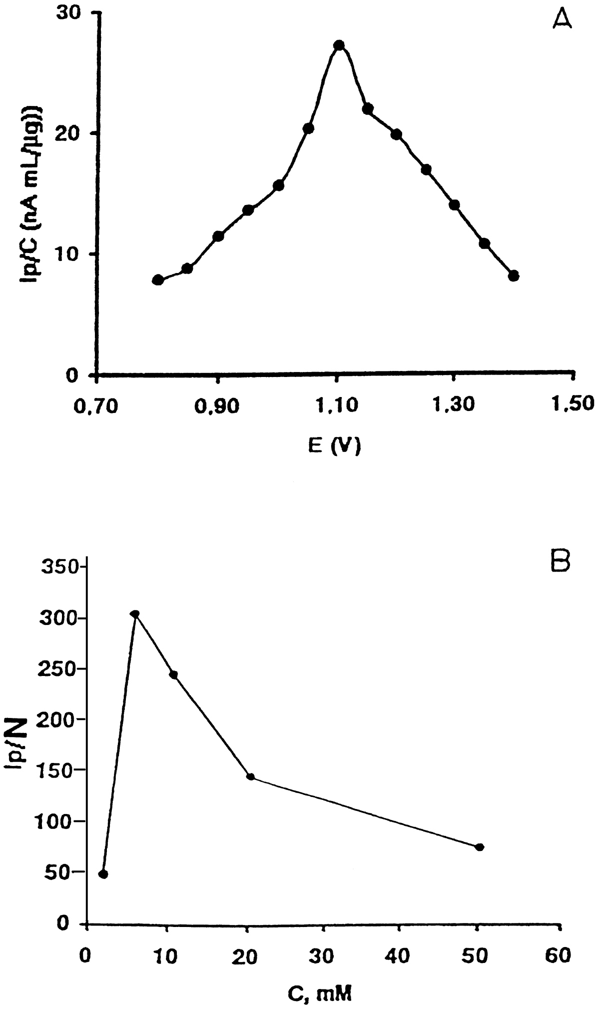

Figure 2. (A) Hydrodynamic voltammograms of Thiram in 60% of MeOH, 0.01 M AcH/AcNH$_4$ pH 5.0. Thiram = 4.84 µg/mL. (B) Variation of relation signal to noise of Thiram vs. electrolyte concentration. Conditions: 60% MeOH, 0.01 M AcH/AcNH$_4$ pH 5.0 using Amperometric Detection: Thiram = 10.1 µg/mL. (i_p/C = Peak intensity vs Concentration; i_p/N = Peak intensity vs signal noise).

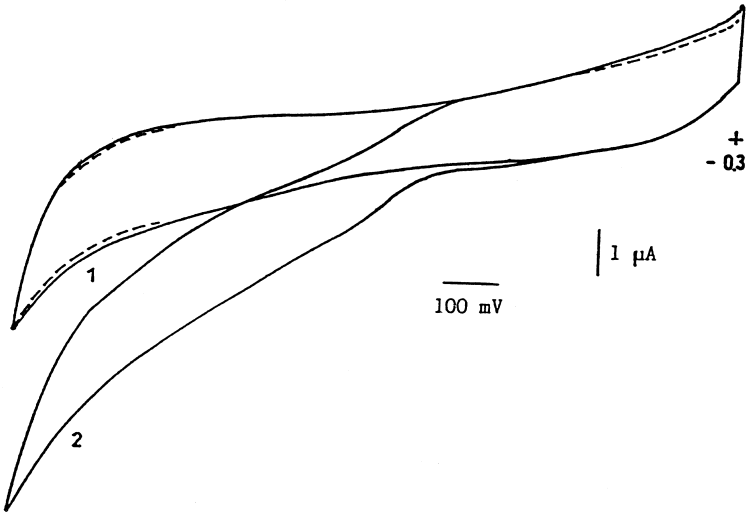

Figure 3. Ciclovoltammograms of Diram (1) and Metham (2) on glassy rotating disk electrode at 1500 rpm. Conditions: 55% MeOH, 0.02 M phosphoric acid Diram = 1.06 x 10^{-4} M; Metham = 8.38 x 10^{-5}M. Scan rate = 100 mV/s. (Dashed line = background)

Because of this it was necessary to work at lower electrolyte concentrations than in amperometric detection mode, in order to obtain adequate signal-to-noise ratio. In these conditions an ammonium acetate buffer concentration of 0.5 mM was chosen.

The electrochemical behaviour of Metham and Diram was characterized by cyclic voltammetry in the chromatographic conditions. It can be observed in Figure 3 that Metham shows a well defined and stable oxidation wave with a semi-wave potential of 0.32 V. The oxidation of Diram was only observed at pH values higher than 5.0.

In optimum chromatographic conditions for Diram and Metham no signal was observed for Diram. Electrochemical detection was only useful for Metham detection in these separation conditions and Diram was not included afterwards. A potential of +0.7 V was chosen for Metham determination from its hydrodynamic current potentials curve (not shown).

No literature has been reported about the oxidation of methyl derivatives of alkylenebis- and alkyldithiocarbamates. The hydrodynamic current potential curves show that these compounds are oxidized on glassy carbon electrode with a maximun signal at +1.1 V.

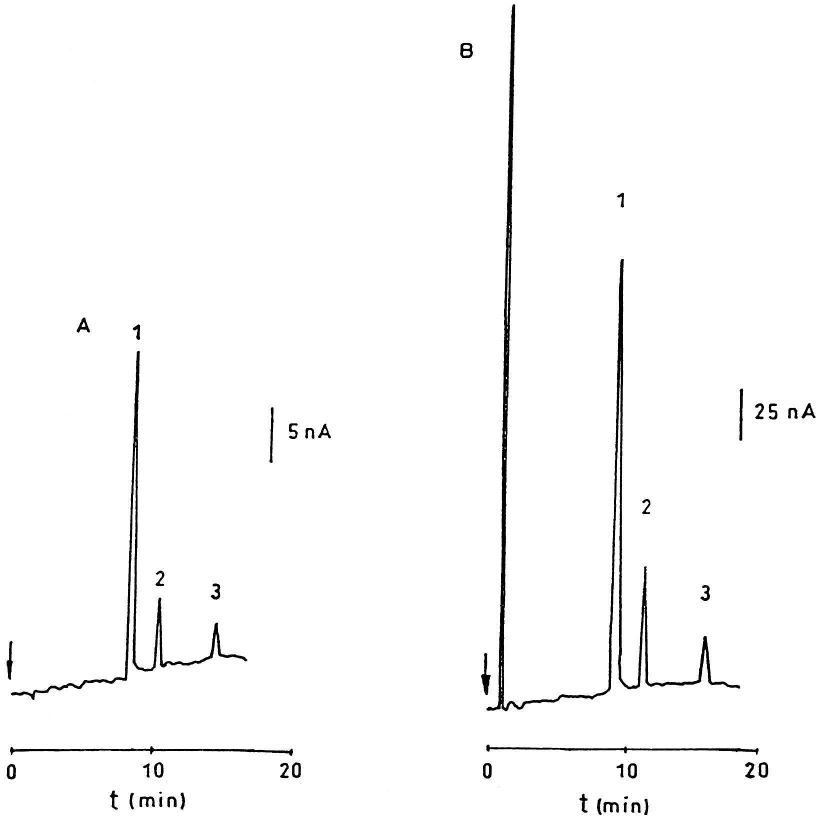

Figure 4. Chromatograms of a mixture of Thiram, EBD and PBD in a glassy carbon electrode at + 1.1 V with: A: Amperometric Detection. (Reference electrode: Ag/AgCl). B: Coulometric Detection. (Reference electrode: Pd) Chromatographic peaks: (1) Thiram, 0.5 µg/mL (2) EBD, 0.5 µg/mL (3) PBD, 1.0 µg/mL.

For methyl derivative of dimethyldithiocarbamate (Diram) no oxidation was observed, probably due to absence of hydrogen in the nitrogen atom of the molecule. The voltammetric results indicated that oxidative amperometric detection can be applied in the determination of the pesticides studied (EBD and PBD) with the detector operating at +1.1 V vs Ag/AgCl electrode.

The study of electrolyte concentration effect on analytical signal indicates better ratio for electrolyte detection respectively using a working potential of +0.9 V vs Pd electrode in coulometric detection mode. A typical chromatogram is shown in Figure 4.

Table 1

Statistical Data of Calibration Graphs and Limits of Detection*

Compound	**Technique**	**Potential (v)**	**Sensitivity (µA mL/µg)**	**L.O.D. (ng)**	**k'**
EBD	Amperometry	1.1	0.028	4	9.70
	Coulometry	0.9	0.120	0.06	
PBD	Amperometry	1.1	0.039	2	12.27
	Coulometry	0.09	0.089	0.08	
Thiram	Amperometry	1.1	0.014	0.7	1.58
	Coulometry	0.90	0.947	0.1	
Metham	Amperometry	0.7	0.021	0.2	1.04
	Coulometry	0.5	0.231	0.04	

* (S/R = 3). (Injection volume = 20 µL).

Analytical Data of Calibration Graphs and Limits of Detection

The statistical data of the calibration graphs for all standard solutions of compounds, chromatographic methods, and detection techniques studied are compared in Table 1. The linearity of the calibration graphs is shown by the correlation coefficients ranging from 0.991 to 0.999 for concentrations up to 10.0 µg/mL in amperometric detection and 1.0 µg/mL in coulometric detection.

As we expected, better detection limits were obtained in coulometric detection than amperometric detection, due to better efficiency in the oxidation processes. Although we obtain better sensitivity in coulometric detection, there is a characteristic problem with this technique: the deactivation on the surface electrode due to the great number of samples injected. We solved this problem cleaning the coulometric cell by procedure described previously.

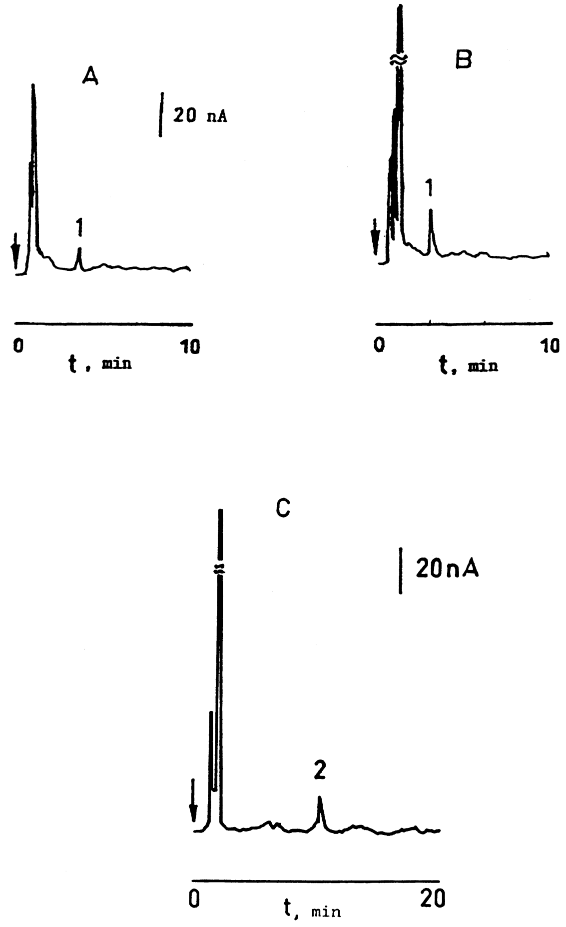

Figure 5. Chromatograms obtained in Amperometric Detection mode on glassy carbon electrode at +1.1 V. (Sample size = 10 g; Number of samples analyzed = 4) A**:** 0.016 µg/g of Thiram (1) in Apple pulp. B**:** 0.031 µg/g of Thiram (1) in Grapes. C: 0.007 µg/g of EBD (2) in Apple pulp.

Applications

To check the efficiency of the extraction and the accuracy of the proposed method, samples of fruits were spiked with known amounts of Thiram and EBD as commercial formulations, extracted by the procedure described by Gustafsson and Thompson[16] and the pesticide residues were measured by amperometric detection mode.

Fruits samples of apples and grapes were spiked with a known amount of ZZ Fernide (ICI-Zeltia, S.A. Spain) which contain an 80% of Thiram. The concentration of Thiram obtained from fortified samples of apple were 0.203 ± 0.009 µg/g with an extraction recovery of 79.3%. The amount of Thiram obtained from fortified samples of grapes was 0.180 ± 0.007 µg/g with an extraction recovery of 83.0%. The minimum quantification level of this method (S/N ratio=10) is 2 ng/g using amperometric detection and 0.1 ng/g with coulometric detection with a sample size of 10 g and a final volume of 1 mL.

Determination of EBD in fruits samples was made after formation of methyl derivative of Zineb according to the procedure described by Gustafsson et al.[16] The extraction recovery of Zineb as EBD in apples was 51.9%, lower than the recoveries obtained by Gustafsson et al.[17] in fruit samples. The limits of detection were not optimized in this case. Typical chromatograms of samples are shown in Figure 5.

CONCLUSIONS

The results obtained show that electrochemical detection is useful as a detection method for these types of compounds. Using electrochemical detection it is possible to increase the selectivity and sensitivity of the determination of compounds investigated in comparison with the results reported in the bibliography[16,18,24,27] This proposed method allows the easy determination of compounds studied without previous treatment, hence, decreasing the time of analysis.

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