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Simultaneous determination of maneb and its main metabolites in tomatoes by liquid chromatography using diode array ultraviolet absorbance detection

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Abstract

Liquid chromatography (LC) with diode array ultraviolet absorbance (DAD UV) detection is used for the simultaneous determination of the fungicide maneb and its main metabolites (ethylenethiourea—ETU, ethylenebis (isothiocyanate) sulfide—EBIS, and ethyleneurea—EU) in tomatoes. The identity of EBIS, one of the main UV degradation products of maneb, was verified by both DAD UV detection and mass spectrometry. The analytes were extracted three times with 3 mL of 1:1:1 acetonitrile–dichloromethane–chloroform by 2 min of mechanical shaking and separated on a C-18 column by gradient elution with an acetonitrile–methanol–aqueous 100 mM sodium dodecylsulfate (SDS) mixture. The quantification limits of 0.45, 0.04, and 0.35 mg kg^{-1} obtained for maneb, ETU, and EU, respectively, show that the proposed method is suitable for their determination in tomatoes.

Keywords: Tomatoes; Pesticides; Maneb; Ethylenethiourea; Ethylenebis (isothiocyanate) sulfide; Ethyleneurea

1. Introduction

Maneb is an ethylene bisdithiocarbamate fungicide (EBDC) used in agriculture for the control of early and late blights in potatoes and tomatoes, as well as many other diseases in fruits, vegetables, field crops and ornamental plants [1]. The toxic effects of EBDCs are usually associated with ethylenethiourea (ETU) and ethylenebis (isothiocyanate) sulfide (EBIS), the main metabolites of their hydrolysis and photolysis. Ethyleneurea (EU) and glycine are degradation products of EBDCs, ETU, and EBIS [2]. ETU is known to have thyreotoxic, teratogenic, and carcinogenic effects and EBIS causes peripheral paralysis and thyroid dysfunction in rats [3,4].

There is an increasing interest in developing analytical methods for simultaneous determination of the amount of the fungicide and its metabolites in a single assay. The complexity of simultaneous extraction is due to the different polarity of the metabolites with respect to maneb, as well as to the instability of the latter during extraction. The majority of reported methods for determining EBDCs are based on the quantification of carbon disulfide [5–8] or ethylenediamine using different techniques [9–11]. Several attempts have been made to analyze EBDCs by liquid chromatography (LC) using UV [6,12,13], electrochemical [14], and, more recently, mass spectrometry detectors [15]. However, all these methods require derivatization steps, and most of them are insufficiently specific to distinguish between residues of individual EBDCs. LC techniques coupled with UV [16–19], MS [20] or electrochemical detection [21,22] have been reported for ETU determination. Aprea et al. [23] have individually determined maneb and ETU using gas and liquid chromatography, respectively. However, there are no reliable methods for EU or EBIS despite the high toxic properties of the latter (no standards of EBIS are available). Thus, the main aim of this investigation was to develop a simple, rapid and precise LC method for the simultaneous extraction, separation, identification

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and quantification of maneb and its degradation products in tomatoes.

2. Materials and methods

2.1. Chemicals and solvents

Solid stock standards of ETU and EU were obtained from Sigma–Aldrich (Steinheim, Germany). Maneb was obtained from Riedel-de-Häen (Seelze, Germany). EBIS solution was obtained from the degradation of maneb in a 1:1 deionized water–acetonitrile solution using a UV lamp (CN-6T Vilber Lourmat, France) at a wavelength of 312 nm. The identity of the product was confirmed by elemental chromatographic analysis and mass spectrometry. Analytical grade sodium dodecylsulfate (SDS) was purchased from Merck (Darmstadt, Germany). Acetonitrile, chloroform, dichloromethane and methanol supergradient HPLC grade were also obtained from Sigma–Aldrich. All reagents used were of analytical grade or better. Deionized water was obtained using a Milli-Q water system (Millipore Ibérica, Madrid, Spain).

2.2. Instrumentation

Experiments were performed using an HP 1050 Series liquid chromatographic system (Hewlett Packard, Palo Alto, CA, USA) equipped with a quaternary pump, a column compartment, a vacuum degasser and a diode-array detector. The instrument control and data processing utilities included Hewlett Packard CHEMSTATION software (Hewlett Packard). The stainless steel analytical column used was packed with LiChrosorb RP-18 5 μm (25 cm × 9.6 mm i.d.) from Supelco (Bellefonte, PA, USA). Samples were injected through a sample injection valve (Rheodine Inc., Model 7725; Hewlett Packard) fitted with a 20 μL loop.

2.3. Preparations of standards and of spiked tomatoes

Standard stock solutions of maneb (250 mg L^{-1}) were prepared by dissolving 12.5 mg of the fungicide in 50 mL of acetonitrile. Standard stock solutions of ETU and EU (1000 mg L^{-1}) were prepared by dissolving 10 mg of each analyte in 10 mL of acetonitrile. The EBIS solution concentration (approximately 7.4 mg L^{-1}) was calculated as the difference between the total amount of maneb and the rest of the degradation compounds (ETU and EU) after UV degradation of a 25 mg L^{-1} maneb solution in 1:1 water–acetonitrile. Solutions were stored in amber glass bottles at −20 °C in the dark. Working solutions were prepared daily in ultrapure Milli-Q water by appropriate dilution. For recovery determinations, some samples were spiked with 500 μL of diluted standard solutions containing all compounds and homogenized by shaking. These spiked samples were maintained at room temperature for 30 min before extraction to allow the solution to penetrate the test material.

2.4. Sample extraction

Raw tomatoes were finely chopped using a knife. One gram subsamples were then transferred into a glass beaker. The analytes were extracted in 3 mL of 1:1:1 acetonitrile–dichloromethane–chloroform by 2 min of

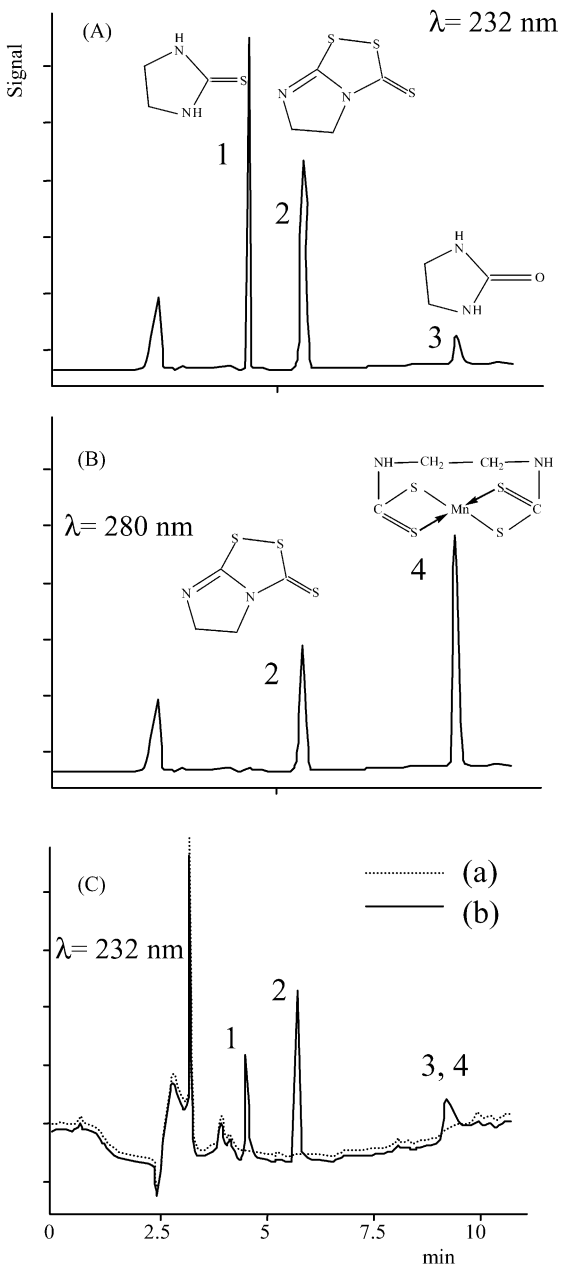

Fig. 1. Chromatograms obtained at optimum conditions corresponding to: (A) standard mixture of maneb and metabolites at 232 nm: (1) ETU, (2) EBIS, (3) EU; (B) standard mixture of maneb and metabolites at 280 nm: (2) EBIS, (4) maneb; (C) blank tomato extract (a) and tomato sample spiked with maneb and metabolites (b).

mechanical shaking. The suspension was filtered through filter paper into a Büchner funnel and rinsed with 2 mL of the extractant. Two milliliters of methanol were then added to the filtrate and the mixture evaporated to dryness under a gentle stream of argon at room temperature. This extraction procedure was repeated three times. Finally, the residues were redissolved in 500 μL of 1:1 water–acetonitrile and filtered through a Millipore 0.45 μm nylon syringe filter (Whatman International, Maidstone, England) before being directly injected into the chromatographic system.

2.5. LC–DAD UV analysis

Analyte separation was performed by gradient elution at a flow rate of 1 mL min^{-1}. The initial conditions were 95% 100 mM SDS in aqueous solution plus 5% acetonitrile isocratic for 5 min followed by a linear gradient to 30% 100 mM SDS in aqueous solution, 33% methanol and 37% ACN within 1 min, and a postrun time of 5 min. Quantitative measurements of peak areas by LC–UV were usually carried out at 232 nm (ETU, EBIS, EU) and 280 nm (maneb), but other wavelengths were occasionally used.

3. Results and discussion

3.1. LC separation and identification

Optimization of elution gradient programs using both binary and ternary mixtures of acetonitrile, methanol, and SDS (0–100 mM) in aqueous solution was carried out. SDS increased the sensitivity of the method and improved the detection limit (DL) of maneb up to five-fold (<0.2 mg L^{-1}). Under the optimized conditions given in Section 2.5, separation of ETU, EBIS and maneb was achieved, but EU coeluted with maneb. Due to the different spectra of both analytes, their individual quantification at 232 nm (EU) and 280 nm (maneb) was possible (Fig. 1A and B). The presence of EBIS was confirmed by monitoring the ions *m*/*z* 177 (molecular ion $M + H^+$) and 179 ($M + H^+ + 2$) using LC–ESI–MS in positive mode (Fig. 2).

3.2. Sample extraction

Preliminary tests were performed with 2 mL of spiked distilled water. Several extractants, dichloromethane, chloroform, acetonitrile and methanol, were tested. Results obtained with methanol were unsatisfactory. Therefore,

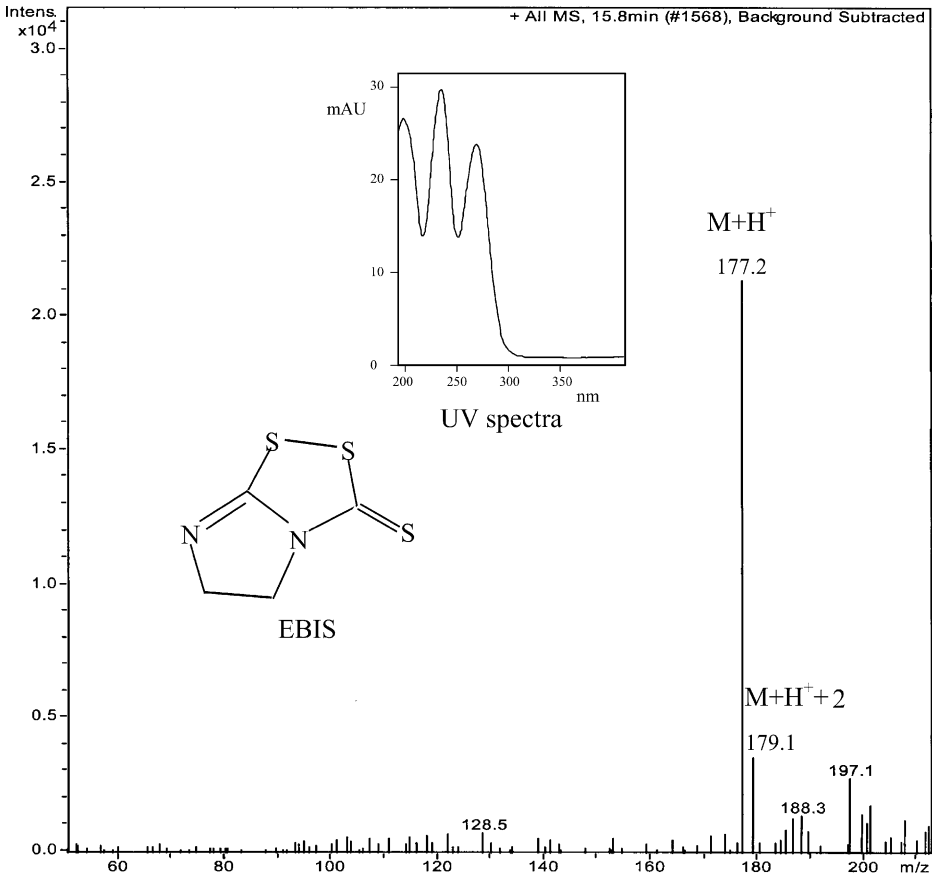

Fig. 2. Identification of EBIS verified by electron mass impact mass spectrometry and UV spectrum. The chromatographic system consisted of an Esquire-LC 00126 Bruker (Bremen, Germany) ion trap mass spectrometer coupled to LC equipment by an electrospray ionization (ESI) interface.

Table 1
Analytical characteristics of maneb and metabolites from spiked tomatoes

Compound	Concentration range (mg L^{-1})	Regression equation	R^2	R.S.D.[a] (%)	LOQ (mg kg^{-1})
Maneb	0.1–5.0	$y = 72.33x - 5.66$	0.9993	4.9	0.45
ETU	0.025–5	$y = 251.17x - 2.84$	0.9997	3.8	0.040
EU	0.1–3.0	$y = 97.63x - 6.54$	0.9990	6.2	0.35

[a] For concentrations of 0.2, 0.3 and 0.2 mg L^{-1} for maneb, ETU, and EU, respectively ($n = 3$).

dichloromethane, chloroform and acetonitrile were individually tested with spiked tomatoes.

Mechanical and ultrasonic assisted modes of extraction were tested. Around 1–2 min of mechanical shaking were required to achieve the highest recoveries for all analytes, as is shown in Fig. 3. Ultrasonic irradiation led to an important reduction in the amounts extracted in all cases, but especially of maneb and EU (recoveries lower than 10%). This was probably due to the decomposition of dichloromethane with ultrasonic irradiation and long mechanical shaking. This behavior has already been reported by several authors [24,25] who suggest that unsaturated non-aromatic compounds react with radicals formed during the decomposition of dichloromethane. Therefore, mechanical shaking was selected for further experiments. The sample mass and solvent volume ratio were optimized using 1 g of sample and 2–10 mL of dichloromethane; 3 mL provided the best recoveries and this volume was used in further work.

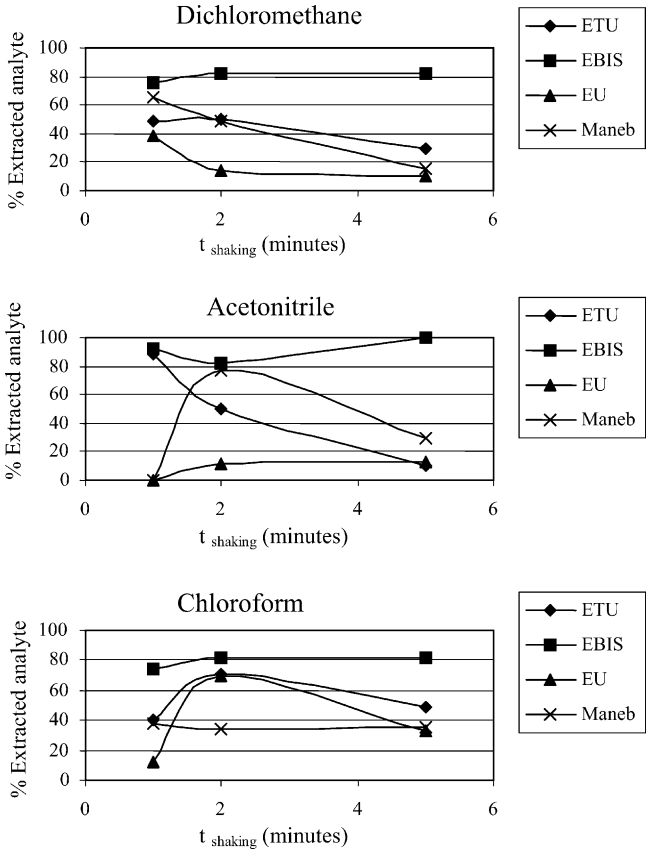

Fig. 3. Effect of shaking time on the extraction of analytes from spiked tomatoes (0.1 mg kg^{-1} for ETU and 0.9 mg kg^{-1} for maneb and EU using different solvents ($n = 2$)).

In order to improve the recoveries from tomatoes, experiments were performed with a mixture of dichloromethane, chloroform and acetonitrile. To determine the optimum proportion of each, a central composite design was followed, with a total volume of solvent of 3 mL, a sample weight of 1 g and a shaking time of 2 min, which left only the volume of the three solvents to be optimized. The results showed that the region of maximum recovery was in the range of 1–1.4 mL of acetonitrile, and 0.8–1 mL of dichloromethane and chloroform for ETU and maneb; similar behaviour was observed for EBIS and EU. Therefore, a 1:1:1 acetonitrile–dichloromethane–chloroform mixture was chosen for use with the tomatoes.

3.3. Analytical performance and application

Calibration curves were obtained by plotting peak area against concentrations of the spiked tomatoes (in triplicate). Relevant data are summarized in Table 1, which also includes the correlation coefficients and the precision of the method expressed as relative standard deviation (R.S.D.). The quantification limits (LOQ) were calculated as the lowest concentration where the R.S.D. was less than 5%. Taking into account the proposed sample preparation, LOQs found to be 0.45, 0.040, and 0.35 mg kg^{-1} for maneb, ETU, and EU, respectively, in tomatoes. Fig. 1C shows a chromatogram of a tomato sample spiked at concentrations close to the LOQ. The maximum residue limit (MRL) established by the European Union for maneb in tomatoes is 3 mg kg^{-1} (expressed as mg kg^{-1} of CS_2), corresponding to 10 mg kg^{-1} maneb equivalent [26]. The LOQs achieved by this method are comparable to MRL values, thus making the method suitable for routine analysis of these analytes in tomatoes.

In conclusion, a successful analytical method for simultaneous determination of maneb and its main metabolites, ETU, EBIS, and EU in tomatoes at concentrations level lower than those required by current legislation is proposed.

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References

[1] R.T. Meister (Ed.), Farm Chemicals Handbook'92, Meister Publishing Company, Willoughby, OH, 1992.
[2] E. Downing, Environmental Fate of Maneb, Environmental Monitoring and Pest Management, Department of Pesticide Regulation, Sacramento, CA, 2000.
[3] World Health Organisation, Environmental Health Criteria 78. Dithiocarbamate Pesticides, ETU and PTU: A General Introduction, WHO, Genova, Switzerland, 1988.
[4] Joint Meeting of the FAO, Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, Rome, 1980.
[5] T.K. McGhie, P.T. Holland, Analyst 112 (1987) 1075.
[6] M.J.M. Jongen, J.C. Ravensberg, R. Engel, L.H. Leenhers, J. Chromatogr. Sci. 29 (1991) 292.
[7] N. Ahmad, L. Guo, P. Mandarakas, V. Frarah, S. Appleby, T. Gibson, J. AOAC Int. 79 (1996) 1417.
[8] Z. Vryzas, E.N. Papadakis, E. Papadopoulou-Mourkidou, J. Agric. Food Chem. 50 (2002) 2220.
[9] G.E. Keppel, J. Assoc. Off. Anal. Chem. 54 (1971) 528.
[10] W. Schwack, S. Nyanzi, J. AOAC Int. 78 (1995) 458.
[11] G. Zweig, J. Sherma (Eds.), Analytical Methods for Pesticides and Plant Growth Regulators, Updated General Techniques and Additional Pesticides, vol. XI, Academic Press, New York, 1980, p. 197.
[12] K.H. Gustafsson, S.H. Fahlgren, J. Agric. Food Chem. 31 (1983) 461.
[13] K.H. Gustafsson, R.A. Thomson, J. Agric. Food Chem. 29 (1981) 729.
[14] V. Lishaut, W. Schwack, J. AOAC Int. 83 (2000) 720.
[15] Y. Hanada, T. Tanizaki, M. Koga, H. Shiraishi, M. Soma, Anal. Sci. 18 (2002) 441.
[16] E.A. Hogendoorn, P. Van Zoonen, U.A. Th. Brinkman, Chromatographia 31 (1991) 285.
[17] N. Ahmad, L. Guo, P. Mandarakas, V. Frarah, S. Appleby, T. Gibson, J. Assoc. Off. Anal. Chem. 78 (1995) 1238.
[18] P.C. Nascimiento, D. Bohrer, S. Garcia, A.F. Ritzel, Analyst 122 (1997) 733.
[19] S. Kontou, D. Tsipi, V. Oreopoulou, C. Tzia, J. Agric. Food Chem. 49 (2001) 1090.
[20] D.R. Doerge, C.J. Miles, Anal. Chem. 63 (1991) 1999.
[21] L. Bolzoni, A. Sannino, M. Bandini, Food Chem. 47 (1993) 299.
[22] M. Maruyama, Fresenius J. Anal. Chem. 348 (1994) 324.
[23] C. Aprea, A. Betta, G. Catenacci, A. Colli, A. Lotti, C. Minoia, P. Olivieri, V. Passini, I. Pavan, C. Roggi, R. Ruggery, G. Sciarra, R. Turci, P. Vannini, V. Vitalone, Sci. Total Environ. 203 (1997) 167.
[24] E. Leonhardt, R. Stahl, Anal. Chem. 70 (1998) 1228.
[25] A.R. Cassella, R.J. Cassella, S. Garrigues, R.E. Santelli, R.C. de Campos, M. de la Guardia, Analyst 125 (2000) 1829.
[26] Official Journal of the European Community, Commission Directive 98/82/EC, 27 October 1998.