A CONTRIBUTION TO THE DETERMINATION OF 2-IMIDAZOLIDINETHIONE IN MANCOZEB-TYPE ETHYLENE-BIS-DITHIOCARBAMATE FUNGICIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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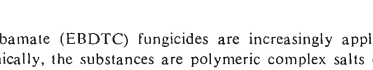
The effect of conditions on the extraction-chromatographic determination of 2-imidazolidinethione (ETU) in mancozeb was investigated. The effect of the extraction time and presence of Na_2SO_4 is not critical if the samples are analyzed immediately after the extraction with methanol, but marked changes in the content of ETU and composition of the matrix appear if the samples are allowed to stand for a long period before the chromatographic treatment. The combined effects of heat, moisture and pH of medium leading to enhanced contents of ETU were also examined.

Ethylene-bis-dithiocarbamate (EBDTC) fungicides are increasingly applied in agriculture all over the world. Chemically, the substances are polymeric complex salts of ethylene-bis-dithiocarbamic acid, *I*,



I *II*

where M is Zn (zineb), Mn (maneb), or the substance is a double salt $[(CH_2NHCSS)_2Mn]_xZn_y$ (mancozeb). With the ever-increasing number of manufacturers and suppliers of these products, the requirements placed on their specification become more stringent, particularly as concerns the content of the toxically most significant impurity, 2-imidazolidinethione (ETU, *II*), which can occur either as a technological by-product or as a metabolite or decomposition product of the fungicide[1].

The first to determine ETU in the decomposition products of EBDTC's were Clarke and coworkers[2]. Investigating the decomposition products of zineb- and maneb-based formulations by TLC on silica gel, Fishbein and Fawkes[3] and Czeglédi-Jankó[4] found ETU as the major final substance. The degree of decomposition of the commercial formulations and the content of ETU in them depends on the age, moisture content, temperature, and time of storage of the samples[4].

Since the mid-sixties, ETU in EBDTC's has been determined largely by thin layer chromatography. The first to suggest a method for the determination of ETU in methanolic extracts of EBDTC's were Bontoyan and coworkers[5,6], who tested the procedure on 28 commercial pre-

parations based on maneb, zineb, and mancozeb, and verified the identity of the ETU peak by the IR and MS techniques. The authors stress the requirement of freeing the sample from moisture prior to the analysis.

According to the EBDTC decomposition pathway[1], ETU can be formed in several ways. It can be formed from some degradation products of EBDTC intermediates, particularly from ethylenethiuram monosulphide (FTM) and disulphide (ETD). This has been corroborated by a HPLC study, in which Farrington and Hopkins[7] investigated the effect of solvent on the extractability of ETU and on the sample matrix. In parallel GLC and HPLC experiments, the former method gave systematically higher results; the authors[7] consider the results obtained by HPLC more accurate and precise and attribute the differences to the fact that at the high temperature of routine GLC, additional ETU can be formed in the injection port of the gas chromatograph as a result of decomposition of the co-extracted impurities. The appreciably lower resolving power of the GLC column as compared with HPLC may contribute to the positive error too.

When choosing between methanol and chloroform for the extraction, the authors[7] prefer the former because it extracts less potential ETU precursor impurities. Similar results were obtained by Van Damme and coworkers[8], who observed an additional increase in the ETU content on a longer (60–120 min) standing of the sample prior HPLC analysis.

In the present work, the role is investigated of some limiting factors of the determination of ETU in mancozeb, such as the extraction time, drying of the extract, or ageing of the solution; variations in the content of ETU in a product when exposed to elevated temperatures, moisture, and different pH values are also examined.

EXPERIMENTAL

ETU standards were prepared at the Research Institute of Agrochemical Technology, Bratislava. The mancozeb (Novozir MN-80) sample, of the same origin, was a 3 years old, 80% wettable powder formulation. All the other chemicals used were of analytical grade purity. Deionized water was used in all experiments.

The tentative GC determinations of ETU in mancozeb were carried out as follows: the sample (3·0 g) with Na_2SO_4 (5·0 g) was extracted by 20 min shaking with methanol (100 ml) dried with Na_2SO_4. The extracts were allowed to stand for 5 min and filtered, and 2 μl portions of filtrate were injected into a glass column (60 cm × 0·2 cm i.d.) packed with 3% neopentylglycolsuccinate (NPGS) (Lachema, Brno) on Gas Chrom Q 100/120 mesh (Carlo Erba, Italy). A Fractovap 2400 T chromatograph (Carlo Erba, Italy) equipped with a flame ionization detector and an Autolab IVB computing integrator (Spectra-Physics, U.S.A.) was used. The column, injection chamber, and detector temperatures were 210, 240, and 250°C, respectively. ETU in the samples was determined by the calibration curve method using a standard solution of 0·3 g of substance in 1 l of methanol. The peaks were evaluated by area integration.

The HPLC measurements were carried out on a Series 3 B liquid chromatograph (Perkin–Elmer, U.S.A.) fitted with a 7 125 injection valve of Rheodyne (U.S.A.) with a 6 μl injection loop, and an LC-85 UV-VIS detector (Perkin–Elmer, U.S.A.) with a 2·4 μl cell. The interfacing was accomplished by means of stainless steel capilaties, 0·144 mm i.d. A silica gel column 12·5 cm × 0·46 cm i.d., d_p 5 μm (Perkin–Elmer, U.S.A.) was used in isocratic conditions. 20% (*V*/*V*) methanol in acetonitrile served as the mobile phase; its flow rate was 0·8 ml min^{-1}, pressure 8·0 MPa. In such conditions the column performance corresponded to 12 000 theoretical plates for ETU at $k = 1{\cdot}05$. The absorbance was measured at 240 nm.

The samples of mancozeb were treated as follows: the homogenized sample (500 mg) with Na_2SO_4 (100 mg) was extracted with anhydrous methanol (5 ml) in ultrasonic bath and centrifuged at 3 000 rpm for 10 min. The supernatant (0·5 µl) was injected onto the column. The chromatograms were evaluated by peak height measurements using a calibration solution containing 0·4 g of ETU in 1 l of methanol.

RESULTS AND DISCUSSION

The GC chromatograms of the ETU standard and of the methanolic extract of mancozeb are shown in Fig. 1. The curves agree well with the data of paper[6], where polyethylene glycol (Carbowax 20M) was used as the stationary phase. We preferred NPGS to Carbowax because of its higher working temperature limit. In fact, in view of the high melting temperature of ETU (199–201°C) and its polarity, column temperatures below 200°C appear to be insufficient for an optimum chromatographic behaviour of the substance. However, even under such conditions and with the use of an inert support and an all-glass system, tailing of ETU persists. It can hardly be decided whether this effect is due to the polarity of ETU or to its partial thermal decomposition during the GLC treatment. Farrington and Hopkins[7] ar d Van Damme and coworkers[8] developped he HPLC methods for the determination of the ETU content in EBDTC's. Authors[7] used columns with chemically bonded stationary phases of CB type. According authors[7] ETU was readily eluted on the reversed

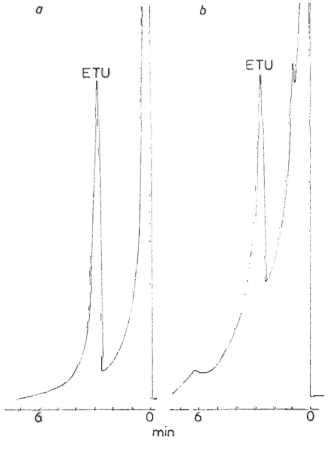


FIG. 1
GC chromatograms of ETU standard (*a*) and of mancozeb sample (*b*)

phase ODS type column, bud adequate retention could not be obtained. To the contrary Van Damme and coworkers[8] chromatographed ETU containing solutions on reversed phase Nucléosil 50 C_{18} 5 μm and/or Lichrosorb RP-18 5 μm columns without any difficulties.

In our treatment of ETU on ODS reversed-phase columns using 5% (*V*/*V*) methanol in water as the mobile phase, chromatograms such as that depicted in Fig. 2 were obtained. The ETU standard gave three peaks; this effect was observed, with minor changes only, when the pH of the mobile phase was varied over a wide region. Probably, owing to the high r. solving power of the column, individual peaks were observed of the protolysis products of ETU as precursors of various oxidative derivatives, which can form[9] in aqueous solutions of ETU. For this reason, reverse--phase HPLC was abandoned.

An open problem in the analysis of ETU in EBDTC's is the appropriate extraction of the samples. Farrington and Hopkins[7] recommend methanol as a suitable extracting agent. The effect of moisture on the formation of ETU in an extended extraction[10] of EBDTC's as well as the increase in the ETU content in ageing solutions is well known[8,9].

The content of ETU in the solutions after the extraction, in dependence on the extraction period, presence of Na_2SO_4, and time of the solutions'standing before

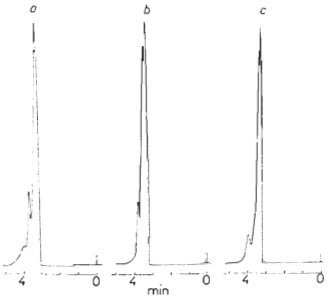


FIG. 2

Reversed-phase HPLC of ETU standard. Column: Perkin–Elmer HS 5 C_{18}, d = = 5 μm. Mobile phase: 5% (*V*/) aqueous solution of methanol, pH: *a* 3·5 (H_3PO_4 + + KH_2PO_4); *b* 5; *c* 7·5 ($NaHCO_3$)

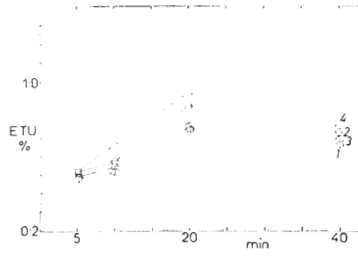


FIG. 3

Dependence of the ETU content (%) on the extraction time, presence of Na_2SO_4, and ageing of solutions. *1*, *3* solutions chromatographed immediately after the extraction, *2*, *4* solutions chromatographed after 24 h standing at room temperature

their analysis, is plotted in Fig. 3. The content of ETU reached its maximum on a 20 min extraction; no additional increase was observed if the extraction time was extended. The presence of Na_2SO_4 is no limiting factor either if the samples are chromatographed immediately after the extraction. The immediately chromato-

TABLE I

Content of ETU, as determined by HPLC, after the sample treatment

Sample treatment	Content of ETU %
Unheated	0·83[a]
Unheated	0·68
Heated without water	0·58
Heated with water at pH 5	1·0
Heated with water at pH 3 ($H_3PO_4 + KH_2PO_4$)	2·5
Heated with water at pH 7·5 ($NaHCO_3$)	1·4

[a] Determined by GLC.

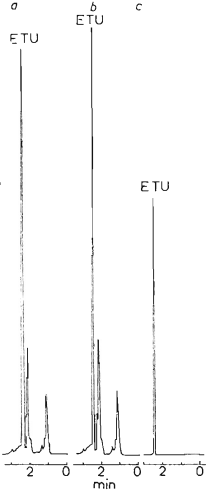


FIG. 4

Effect of Na_2SO_4 on the matrix of samples chromatographed immediately after a 40 min extraction. *a* sample extracted in the presence of Na_2SO_4, *b* sample extracted in the absence of Na_2SO_4, *c* ETU standard

graphed samples exhibit a nearly identical matrix irrespective of the extraction time and the presence of Na_2SO_4 (Fig. 4).

The situation is different if the samples are allowed to stand for a longer time. After 66 h standing at room temperature, the initially light-yellow solutions turned brown, and a coagulate, probably polymeric ethylenethiuram monosulphide[11], appeared. In samples extracted in the absence of Na_2SO_4, the ETU content was observed to increase.

For a short-term simulation of the effect of climatic conditions on the content of ETU, the samples were heated at 55°C for 2·5 h either in the absence of water or in the presence of aqueous solutions with different pH values, and further treated as given above applying a 20 min extraction without Na_2SO_4. The results are given in Table I (the values are averages of four measurements). The HPLC and GLC results for thermally untreated samples are given for a comparison too.

In agreement with papers[7,8], the results obtained from the GLC treatment were higher that those from the HPLC treatment. An elevated temperature alone has no major effect on the content of ETU (Table I); only in combination with moisture, at various pH values, it affects significantly the content of ETU as well as the composition of the solution matrix. This is evident particularly at pH below 7.

Thus, the content of ETU as well as the composition of the products of degradation of EBDTC's can vary appreciably, for one and the same product, in dependence on the previous history of the sample before the analysis.

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