Stability of dithiocarbamates during the preparation and extraction of food samples (PA90)

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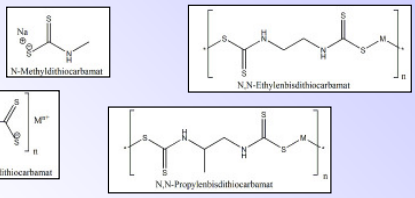
I. Introduction

Dithiocarbamates are preferred fungicides for fruits and vegetables due to their strong efficiency and their relatively low human toxicity while being produced quite cheaply.

Based on their chemistry, dithiocarbamate-fungicides are mainly subdivided in four classes: *N*-methyldithiocarbamates (NMD), *N,N*-dimethyldithiocarbamates (DMD), ethylenbisdithiocarbamates (EBD) and propylenbisdithiocarbamates (PBD).



Some dithiocarbamates are difficult to dissolve in water and organic solvents. That is why it is difficult to extract them from plant samples.

Besides, dithiocarbamates are decomposed easily and very fast in the presence of acid plant sap. Therefore they cannot be homogenized or reduced to small pieces.

Because of the poor solubility and great instability of the dithiocarbamates, there presently is no simple method for analysis available.

References: Schwack, W.; Anastassiades, M.; *Chemie in unserer Zeit 37* **(2003)** 324-335

II. Objective

The concentration of each dithiocarbamate in food has to be controlled individually, because several degradation products show gene toxicity and carcinogenicity. The last published EU regulation 2007/57/EG also underlines the need for a specific analytical method.

Using the currently available method dithiocarbamates are recorded as sum parameter; CS_2 is analysed after acidic decomposition. This does not respect the varying toxicities of the individual dithiocarbamate classes.

Until now specific methods are available for a few dithiocarbamates only. Therefore, a method of selective and sensitive detection was searched. In this regard, experiments with plant matrix were made to find reliable information about the degradation of dithiocarbamates during the clean-up procedure.

„Amtliche Sammlung von Untersuchungsverfahren nach §64 LFGB L00.00-49/1,2 and 3", Berlin: Beuth Verlag GmbH, **1999**
Hulanicki, A.; Shiskova, L.; in *Talanta 12* **(1965)** 485-490
„Amtliche Sammlung von Untersuchungsverfahren nach §64 LFGB L00.00-60", Berlin: Beuth Verlag GmbH, **1999**

III. Results

The experimental parameters of this study are based on the analytical approaches using High Performance Ion Pair Chromatography with UV detection.

These experiences and derived rules are quantitatively described by calibration functions and particular degradation curves for the particular compounds.

Within this study several experiences were made: Standard solutions should be set up every day anew (figure 1) and filters retaining (earth) alkali components have to be avoided for the sample preparation.

Dithiocarbamates are only stable in an alkaline medium. Therefore, an alkaline-stable column was used (Gemini® C6-Phenyl from Phenomenex, 25 °C). The eluent consists of an alkaline buffer (10 mmol/l, pH 10) and methanol (gradient; flow 0.4 ml/min), so that all dithiocarbamates can be separated (figure 2).

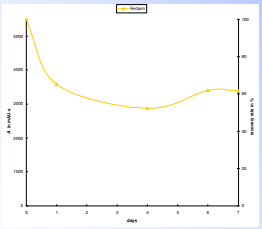


Figure 1: Stability of a Ferbam standard solution during one week

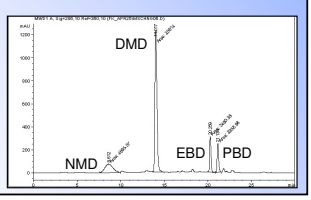


Figure 2: Chromatogram of Metam, Ferbam, Mancozeb and Propineb (each 10 mg/l, 20 µl injection volume)

Perz, R.; Schwack, W.; *Deutsche Lebensmittel-Rundschau 99* **(2003)** 137-142

IV. Results of the preparation

When processing the assays both cysteine and an alkaline pH-value of at least ten avert too fast degradation of the dithiocarbamates.

For first experiments, plant homogenats were spiked with Ziram. The amount of Ziram in these samples was measured every three hours and compared with a standard solution.
Therefore, 10 g homogenat were spiked with 100 µl of a Nabam standard solution (end concentration 10 mg/l) and flushed with a cysteine buffer solution (1 g/l cysteine, 10 mM buffer, pH 10) after a few minutes. Figure 3 shows the decreasing amounts of Ziram in plant homogenats. All food samples decompose dithiocarbamates in less than three hours.

Ziram supplies good results for the integration, so that this standard solution was used. The chromatogram of the blank was subtracted from the chromatogram of the contaminated food samples.
For further improvement we repeated these experiments with dehumidified and frozen food samples. The experimental procedure was the same.

The results on the right side are shown in figure 4 for the dehumidified food samples and figure 5 for the frozen ones. The decomposition of dithiocarbamates in frozen food samples is very fast. And, with the exception of raspberries and green pepper, the decomposition of Ziram in dehumidified food samples is slower than in fresh ones.

Summarizing, freezing the assays causes a very fast degradation of the ingredients (figure 5). Dehumidified assays (figure 4) partially offer a longer stability than fresh ones (figure 3).

Figure 6 shows the comparison of the different results. Dithicarbamates on fresh pepper are more stable than on dehumidified or frozen pepper. Dithiocarbamates added to raspberries and herbs behave vice versa.

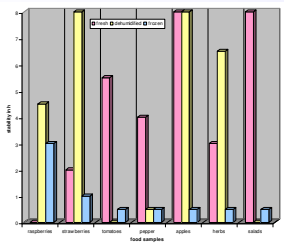


Figure 6: Comparison of the stabilities of dithiocarbamates in different food samples

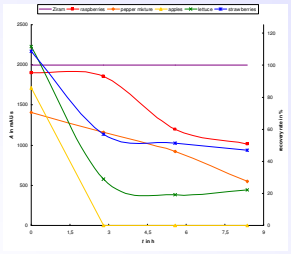


Figure 5: Stability of Ziram in frozen food samples

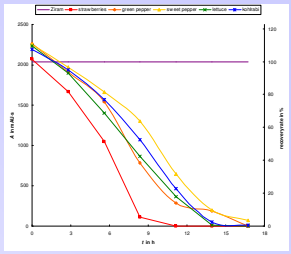


Figure 3: Stability of Ziram in fresh food samples

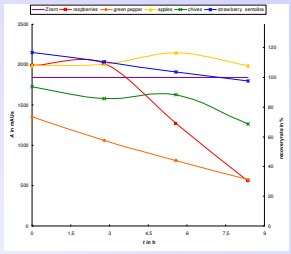


Figure 4: Stability of Ziram in dehumidified food samples

V. Results of the surface extraction

Dithiocarbamates are only on the surface of the plants, because in the inside they will be decomposed. So we decided to extract them from the surface in order to get better results.

Therefore, little pieces of a sweet pepper surface were contaminated with 100 µl Nabam (end concentration 1 mg/l) and after different times (1 minute up to 4 hours) they were extracted with an alkaline (pH 10) cystein EDTA buffer (1 g/l cysteine and 10 mM buffer) and instantly measured.

We found a time progress of the reaction as the results are shown in figure 7. Na-bam is continuously decomposed by sweet pepper. Recovery experiments are important for the analysis of real samples because they show the influence of the plant matrix.

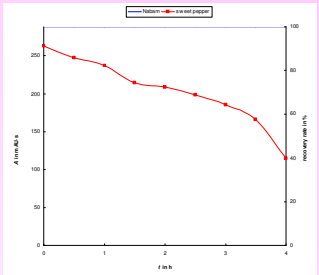


Figure 7: Stability of Nabam on sweet pepper with surface extraction

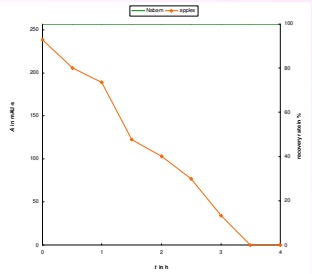


Figure 8: Stability of Nabam on apples with surface extraction

So for this it is also important that the concentration of dithiocarbamates is nearly constant to calculate the recovery rate.
We have repeated these experiments with apples (figure 8). The results are the same: a continuously decomposition of Nabam. This is due to the fact that the influence of enzymes in the food samples is very high.

Summarizing, applying dithiocarbamates onto assays in order to educe them via surface extraction leads to a continuous decomposition of the ingredients.

VI. Outlook

In summary, we can supply additional information and experiences that might be useful for the determination of dithiocarbamates. Because freezing the food samples might be successful, freeze-drying could be an option to minimize the decomposition of dithiocarbamates. Also, more plant matrices recovery studies have to be done.

Thiuramdisulfids is a very similar compound to DMD. So in the HPLC-UV-system you still have the problem that they have the same retention time. So we have also transferred our studies from the HPLC-UV-system to an LC-MS-system. The advantage is that dithiocarbamates and Thiram have a different mass and so they were separated by this in the MS-system.

In the future, the experiments with LC-MS systems have to be intensified to create a robust method for determination of dithiocarbamates.