Analysis of Dithiocarbamates

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Introduction

- What are dithiocarbamates use for
- Chemical structures
- Instability
- Occurrence of residues
- Method (no specific)
- Method (More specific)
What are EBDCs used for?

- Contact fungicides that are effective for controlling many different fungal diseases

- This means that they are extensively used on a wide range of crops
What are EBDCs?

- The ethylenebis(dithiocarbamates) are a group of compounds that exist as strong complexes with various metal ions, often in a polymeric form.

- This makes them difficult to analyse directly because of their limited solubility in most organic solvents.

- Not amenable to multi-residue methods.
# Fungicides with the dithiocarbamate moiety

## Dithiocarbamates

<table>
<thead>
<tr>
<th>Dimethyldithiocarbamates</th>
<th>Salts</th>
<th>Alkylenebis(dithiocarbamates)</th>
<th>Ethylenebis(dithiocarbamates)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thiram</strong></td>
<td><strong>Ferbam (Fe)</strong></td>
<td><strong>Maneb (Mn)</strong></td>
<td><strong>Mancozeb (Mn/Zn)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Ziram (Zn)</strong></td>
<td><strong>Nabam (Na)</strong></td>
<td><strong>Zineb (Zn)</strong></td>
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<td></td>
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<td></td>
<td>Propylenebis(dithiocarbamate)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Propineb (Zn)</strong></td>
</tr>
</tbody>
</table>
Dimethyldithiocarbamates - thiram
Dimethyldithiocarbamates

\[
\begin{array}{c}
\text{Me} \\
\text{N} \equiv \text{C} \equiv \text{S} \\
\text{Me}
\end{array} \quad \left[ \begin{array}{c} 
\text{S} \\
\text{M} \\
\text{n}
\end{array} \right]
\]

Ferbam: \( M = \text{Fe}, \ n = 3 \)

Ziram: \( M = \text{Zn}, \ n = 2 \)
Ethylenebisdithiocarbamates

\[
\left[ \begin{array}{cccccc}
S & H & H & H & H & S \\
S & C & N & C & N & C & S & M \\
H & H & & & & & \\
\end{array} \right]_n
\]

Maneb: M = Mn
Zineb: M = Zn
Mancozeb: M = Mn/Zn
Ethylenebisdithiocarbamates

\[
\text{Na-S-C=N-C-N=C-S-Na}
\]

Nabam
Propylenebisdithiocarbamate

\[
\begin{align*}
&\quad S - C - N - C - C - N - C - S - Zn \\
&\quad H - H - H - Me - H
\end{align*}
\]

Propineb
Dithiocarbamate residues are extremely heterogeneously dispersed over treated commodities.

Homogenisation at room temperature can give up to 100% loss of residue.

Duplicate analyses (CS$_2$ method) can give very poor repeatability up a factor 10 difference (Based on cutting segments from fruits and vegetables).
Cryogenic milling

- Freeze sample then mill in the presence of dry ice
- This reduces losses of dithiocarbamates spiked onto frozen samples, but losses still significant (30-60%) and are variable depending on the particular dithiocarbamate and the commodity
- In general, slightly more loss of mancozeb compared to thiram
  Limited data set for apples, tomatoes and lettuce
MRL exceedances 2000 - 2004

- Where no suitable registration data MRLs have been set at the LOD (0.05 mg/kg)

- Mangoes from Puerto Rico
- Avocado from Mexico
- Edible-podded peas from Guatemala
- Beans from Kenya
- Lettuce & parsley from UK
Method of Analysis

- Determined indirectly (and non-specifically) by measuring the amount of carbon disulfide (CS₂) that is liberated by the action of Zn and HCl

- The liberated CS₂ is now almost exclusively measured by GC-MS, or GC-FPD
Method of analysis

- 50 g of sample in a plastic coated Schott bottle
- Add 150 ml of tin(II) chloride/HCl soln
- Add 25 ml iso-octane
- Seal with screw cap fitted with 42 mm rubber septa (screw cap has a small hole in it to allow spiking)
Method of analysis

- Use an ‘organic’ (CS\textsubscript{2} free) sample of relevant commodity for preparation of two ‘blank’ samples and a ‘spike’

- Shake each bottle and put into a water bath at 80\textdegree C +/- 5\textdegree C for 60 mins

- Invert bottles ten times at 20, 40 & 60 mins

- Place in cold water bath
Method of analysis

- Transfer a portion of the iso-octane into a vial
- Transfer as much as possible of the iso-octane layer from each into a flask – this is used to make up the matrix-matched calibrant solutions – 0.025, 0.05, 0.10, 0.20 & 0.50 mg/kg CS$_2$
Gas Chromatography

- Inject 1.0 µl aliquots onto a 30 m x 0.53 mm DB-1 column of 1.5 µm film thickness

- Inject 1-3 µl aliquots onto 30 m x 0.32 mm CP Sil5 CB column of 4 µm film thickness
Example chromatograms of CS₂

Calibrant @ 0.05 mg/kg

Calibrant @ 0.025 mg/kg
Example chromatograms of CS₂

Blank (Apple)

Spike @ 0.1 mg/kg Thiram (equivalent to 0.062 mg/kg CS₂)
**GC-MS**

*Monitor for ions*

\[ m/z = 76 \& m/z = 78 \text{ (S-34 isotope)} \]

\[ m/z = 78 \text{ around 10\% abundance of } m/z = 76 \]
Problems associated with this approach

- Cannot tell which dithiocarbamate is present
- \( \text{CS}_2 \) can be produced by natural precursors in certain commodities, e.g., cruciferaceae
- Easy to contaminate the sample if in contact with rubber or latex
- Difficult to obtain representative sub-samples as dithiocarbamates unstable during normal sample processing - homogenisation
Problems with the CS$_2$ approach

- Cannot distinguish between different dithiocarbamates
- Thiram has different EU MRLs from the ethylenebisdithiocarbamates
- Non-specific method (prone to false +ves)
- Some crops contain natural precursors that produce CS$_2$ when treated with Sn/HCl
- CS$_2$ produced from accelerators used in rubbers (e.g. latex gloves)
Analysis of ethylenebisdithiocarbamates by LC-MS/MS

- Method is based on the direct quantification of [-SCSNHCH₂CH₂NHCSS-] chain after derivatisation

- Extract with EDTA, cysteine and iodomethane
  The EDTA breaks the polymeric chain and the iodomethane methylates the EBDC to form:

CH₃SCSNHCH₂CH₂NHCSSCH₃ - a stable derivative
Analysis of EBDCs

- Centrifuge and pour off the supernatant
- Clean-up on a C_{18} based SPE cartridge
- Quantification by LC-MS/MS using +ve electrospray
- Obtain the protonated molecular ion of \( m/z \) 241

- Fragmentation of \( m/z \) 241 produces a daughter ion of \( m/z \) 134

- Method has been validated down to 0.01 mg/kg for manebe, mancozeb, metiram, nabam & zineb on apples, grapes and tomatoes
Method validation data

- Individual recovery values fell between 70 - 110%
- Mean recovery values fell between 74 - 100%
- CVs of <14% for all 5 EBDCs in all 3 commodities

- The method is specific for EBDCs as dimethyldithiocarbamates and propineb will form different derivatives
Poster at EPRW 2006

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Conclusions

If using the methods based on CS$_2$ then need to take extra care:

- Ensure you are measuring CS$_2$
- Could commodity be producing CS$_2$ naturally
- Could the sample have been contaminated

Use the LC-MS/MS based more direct method if possible