Analysis of Dithiocarbamates

Stewart Reynolds

Head of Analytical Services

Food Science Group

Central Science Laboratory, York, UK

SELAMAT Workshop, 5-7 July 2006, Bangkok

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 ethylenebisdithiocarbamates 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

Dimethyldithiocarbamates

Thiram

Salts

Ferbam (Fe)

Ziram (Zn)

Alkylenebis(dithiocarbamates)

Ethylenebis(dithiocarbamates)

Maneb (Mn)

Mancozeb (Mn/Zn)

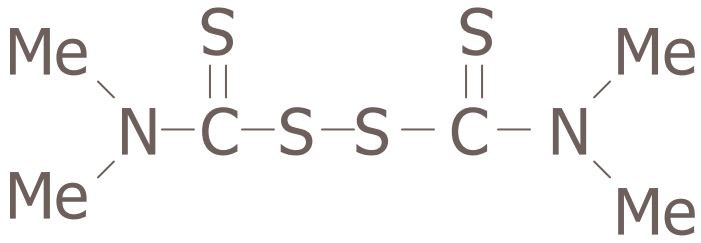
Nabam (Na)

Zineb (Zn)

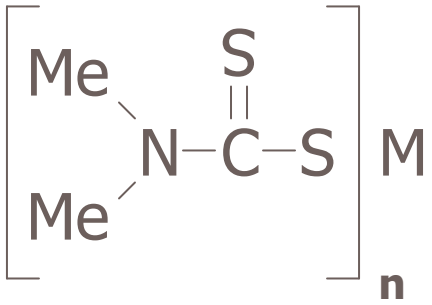
Propylenebis(dithiocarbamate)

Propineb (Zn)

Dimethyldithiocarbamates - thiram



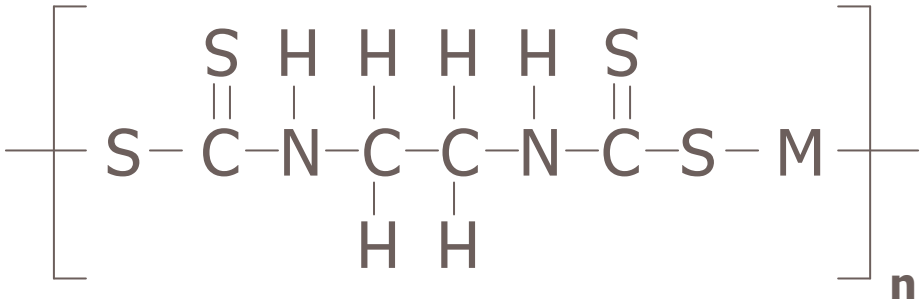
Dimethyldithiocarbamates



Ferbam: M = Fe, n= 3

Ziram: M = Zn, n = 2

Ethylenebisdithiocarbamates

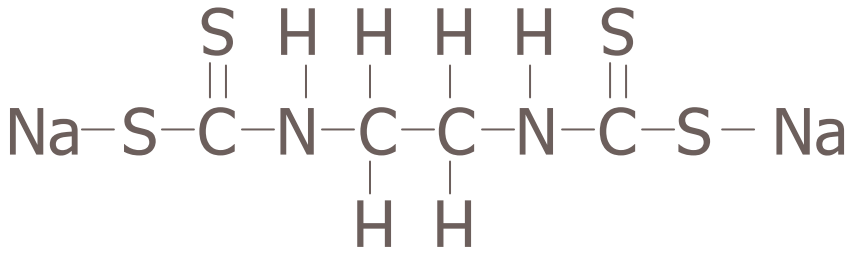


Maneb: M = Mn

Zineb: M = Zn

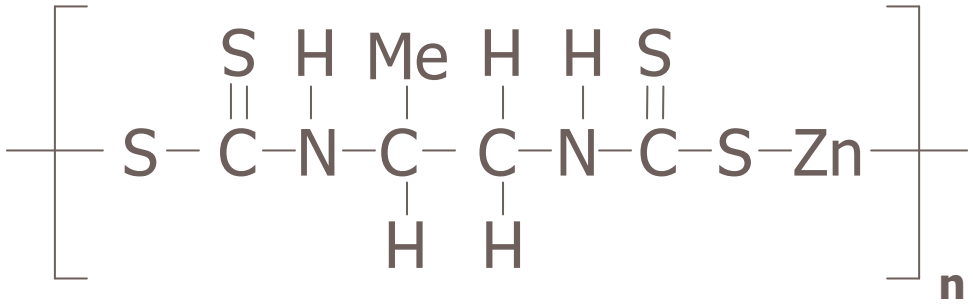
Mancozeb: M = Mn/Zn

Ethylenebisdithiocarbamates



Nabam

Propylenebisdithiocarbamate



Propineb

Sub-sampling

- 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 particualr 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 (CS_2) that is liberated by the action of Zn and HCl

- The liberated CS_2 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_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^{o}C +/- 5^{o}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
-

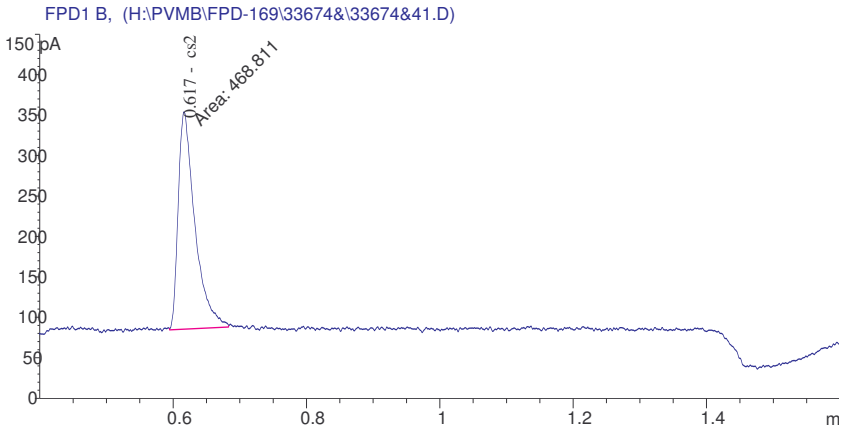
Gas Chromatography

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

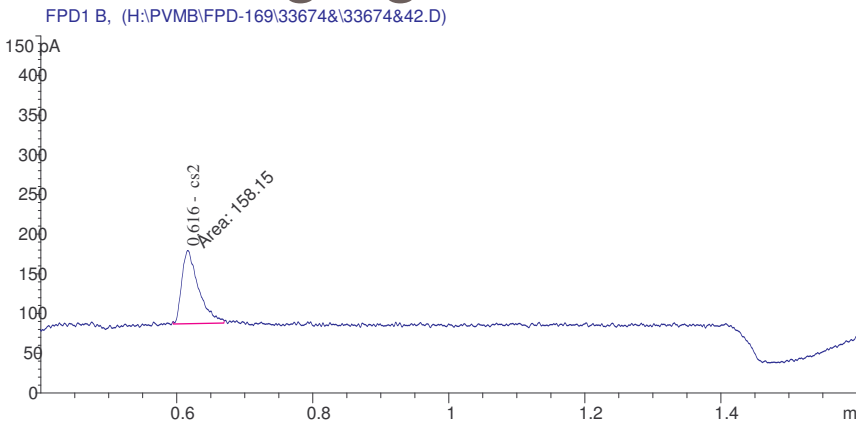
- Inject 1-3 µl aliquots onto 30 m x 0.32 mm CP Sil5 CB column of 4 µm film thickness

Example chromatograms of CS_2

Calibrant @ 0.05 mg/kg

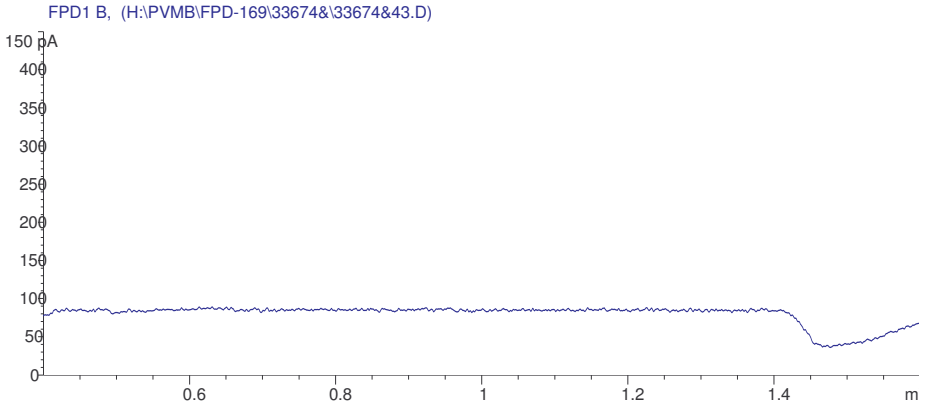


Calibrant @ 0.025 mg/kg

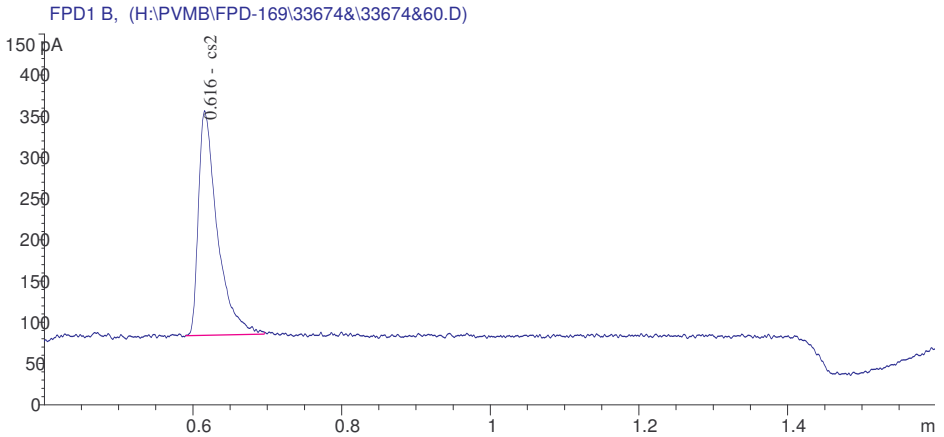


Example chromatograms of CS_2

Blank (Apple)



Spike @ 0.1 mg/kg Thiram (equivalent to 0.062 mg/kg CS_2)



GC-MS

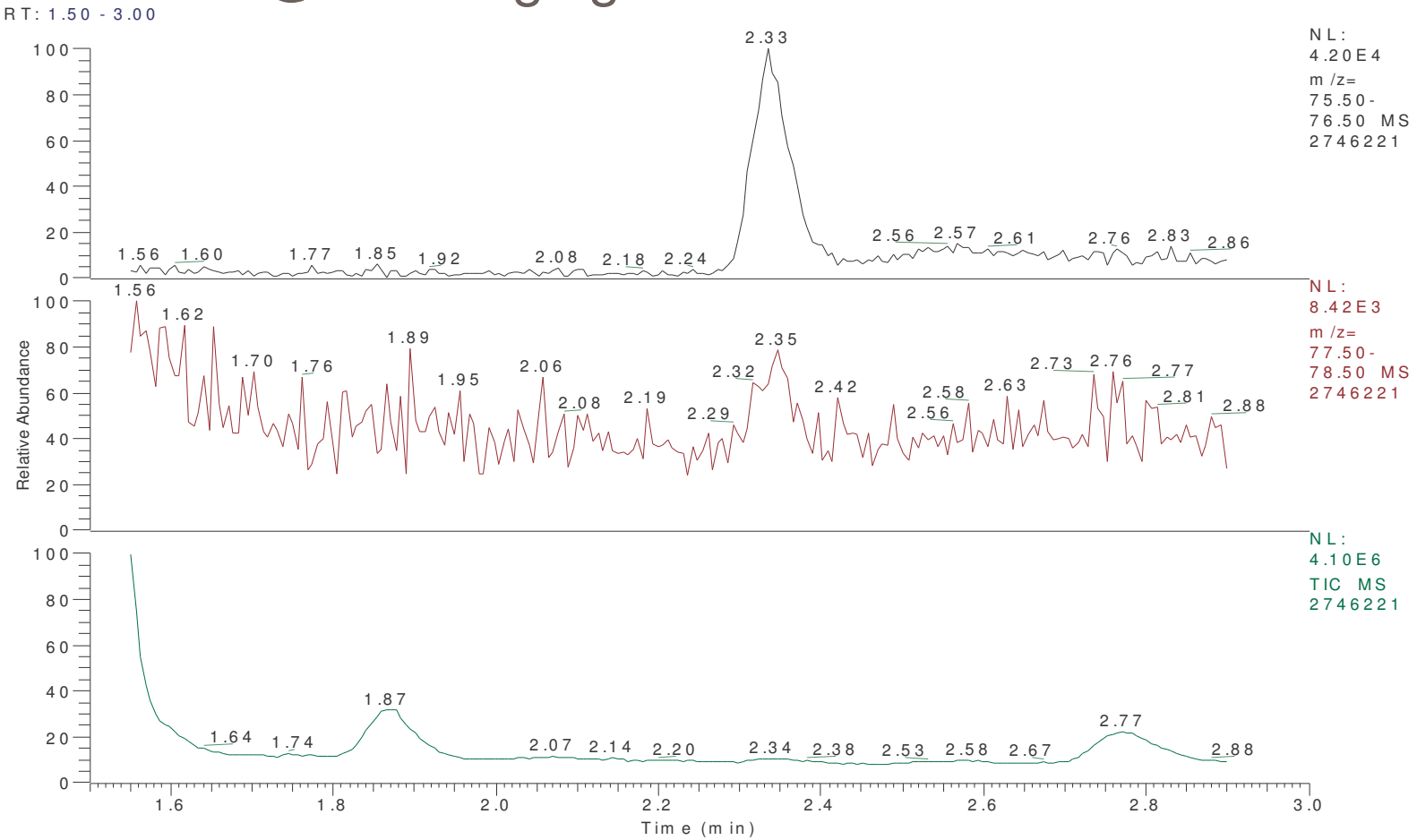
Monitor for ions

***m/z* = 76** & *m/z* = 78 (S-34 isotope)

m/z = 78 around 10% abundance of *m/z* = 76

GC-MS SIM of CS_2

Calibrant @ 0.05 mg/kg



Problems associated with this approach

- Cannot tell which dithiocarbamate is present
- CS_2 can be can be produced by natural precursors in in certain commodities, eg cruciferacae
- 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

[-SCSNHCH$_2$CH$_2$NHCSS-] chain after derivatisation

- Extract with EDTA, cysteine and iodomethane

  The EDTA breaks the polymeric chain and the iodomethane methylates the EBDC to form:

$CH_3SCSNHCH_2CH_2NHCSSCH_3$ - 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

LC-MS/MS

- 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 maneb, 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

Stephen Brewin et al
Huntingdon Life Sciences,
Eye
Suffolk IP23 7PX
UK

www.huntingdon.com

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