Screening for and Quantification of Fungicide Dithiocarbamates using QuEChERS Methodology

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Summary

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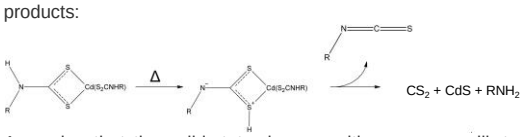
- Screening markers for monoalkyl *bis*-dithiocarbamates are amenable to QuEChERS and GC-MS techniques: (a) ethylene-*bis*-isothiocyanate, (b) propylene-*bis*-isothiocyanate.
- The screening approach enlarged both: the number of CS_2-positives in all types of commodities and the number of CS_2 MRL-violations in our lab.

Introduction

For pesticide residue laboratories, analysis of dithiocarbamates (DTCs) is very challenging since the physicochemical properties of various representatives belonging to this compound class lead to analytical difficulties. The polymeric DTC fungicides (e.g. mancozeb, propineb) are macromolecular metal-coordinated complexes of variable size and are thus virtually impossible to analyze directly. Moreover they are practically insoluble in aqueous and organic solvents. Therefore, routinely applied multiresidue methods, involving partitioning into organic solvents, are not applicable for the analysis of intact DTCs. Most routine pesticide labs apply a methodology that is based on the chemical cleavage of DTCs by a mixture of tin(II)-chloride/ hydrochloric acid and the partitioning of the released carbon disulfide (CS_2). Finally, quantitative analysis of CS_2 is either achieved by spectrophotometry or gas chromatography (GC) with different detector options (e.g. ECD). From the practical point of view, the disadvantage of this method is that a laborious analysis has to be conducted without having any information if DTCs are present in the sample or not. A qualitative screening approach for DTCs by a routinely applied multiresidue method would be of help to reduce the likelihood of random findings by allowing the selection of positive samples. Consequently, workload and costs could significantly be reduced for the quantitative DTC-analysis.

Monoalkyl DTCs Decompose to Isothiocyanates

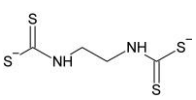
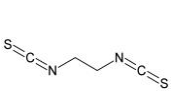
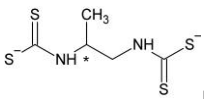
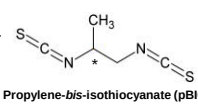
According to van Popple *et al.* monoalkyl DTCs decompose in solid-state with isothiocyanates being formed as side products:



Assuming that the solid-state decomposition process will, to some extent, also take place at room temperature in monoalkyl *bis*-DTC-based plant protection products, the occurrence of the isothiocyanate SCN-R drew our attention as such a substance could be used as a marker for purposes of a qualitative DTC screening in fruits and vegetables by applying multiresidue methods.

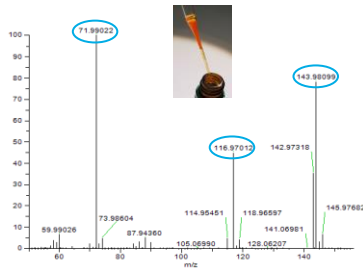
Results

Characteristic decomposition products of monoalkyl *bis*-DTCs were identified in analytical DTC-standards:

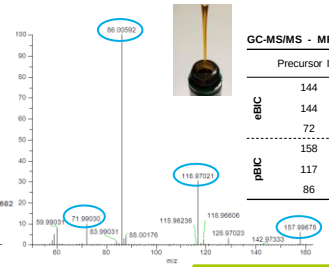
	Monoalkyl *bis*-DTC	Common organosulphur moiety	Identified product from thermal solid state decomposition
Monoethyl *bis*-DTCs	Maneb Mancozeb Mancopper Metiram Nabam Zineb		 **Ethylene-*bis*-isothiocyanate (eBIC)**
Monopropyl *bis*-DTC	Propineb		 **Propylene-*bis*-isothiocyanate (pBIC)**

Experimental procedure: 5 mg of each analytical DTC-standard were extracted with 5 ml acetonitrile. After centrifugation, the extracts were used for analysis by GC-Orbitrap. eBIC and pBIC are not LC-MS (ESI pos/ESI neg) amenable.

GC-EI Spectrum of eBIC



GC-EI Spectrum of pBIC



GC-MS/MS - MRM Transitions

	Precursor Ion	Product Ion	CE [eV]
eBIC	144	72	10
	144	88	10
	72	45	20
pBIC	158	86	5
	117	86	5
	86	60	15

Achievements of Qualitative DTC-Screening

QuEChERS-extracts of numerous cryogenically homogenized samples of plant origin were screened for eBIC and pBIC by GC-MS/MS (and GC-Orbitrap). Positive samples were reanalyzed by the chemical cleavage approach involving CS_2-release for confirmatory and quantitative purposes. Results achieved:
(A) Enlargement of commodity scope with more CS_2-findings and a good correlation of eBIC/pBIC-positives and CS_2-results:

1376 samples screened:

	# of positive samples
eBIC	101 (7 %)
pBIC	4 (0,3 %)

Commodity[1]	# of samples screened	# of eBIC positives	thereof # of CS_2 positives	# of pBIC positives	thereof # of CS_2 positives
Table Grapes	46	3	3	4	1 + 3 <LOQ
Green Beans	43	6	4 + 2 <LOQ	0	-
Orange	25	8	3 + 5 <LOQ	0	-
Tomato	36	3	2 + 1 <LOQ	0	-

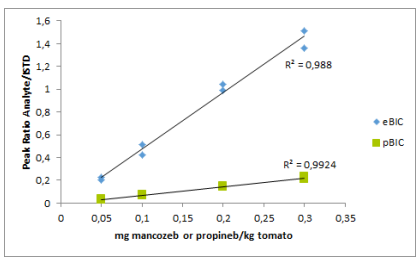
[1] List of some exemplary commodites; LOQ for CS_2 using GC-ECD: 0.05 mg/kg

Samples which were first analyzed by the chemical cleavage method and identified positive for CS_2, were detected positive in the eBIC/pBIC-screening in the overall majority of cases. No clear correlation was observed yet between eBIC-/pBIC-concentration and CS_2-concentration.
(B) Detection of CS_2 EU MRL-violations which would have been missed without the qualitative screening approach:

Commodity	eBIC-screening	CS_2-finding [mg/kg]	EU-MRL CS_2 [mg/kg]
> MRL (non compliant)			
Figs	✓	4.0	0.05
Raspberry	✓	0.3	0.05
Parsley	✓	24.5	5.0
> MRL, but compliant due to uncertainty interval			
Basil	✓	6.0	5.0
Head lettuce	✓	6.7	5.0
Passion fruit	✓	0.076	0.05

Quantitative Approach for Monoalkyl *bis*-DTCs

Various non-carcinogenic derivatization reagents (e.g. $TBABr_3$, Cu_2SO_4, PFBBr, DMDC) were tested for conversion of ethylene- and propylene-DTCs to eBIC and pBIC in homogenates of fruits and vegetables. After the specific chemical reaction, potential conversion products were extracted by applying the QuEChERS methodology. Positive results were achieved with one reagent:



First experiments with positive samples from the DTC-screening indicated comparable results (calculated as CS_2) to the acid digestion/hydrolysis approach.

What's Next?

(a) Optimization of the derivatization approach; (b) Development of a screening and quantitative approach for N,N-dimethyl DTCs (ziram, thiram).

EPRW 2020

Baden-Württemberg