Analysis of Fumigants in Cereals and Dry Fruits Applying GC-MS/MS

Initiator/Institution: EURL-SRM

Version 1 (last update: 27.03.2014)

Brief description:

The intension was to develop a multi-method to analyze residues fumigants in cereals and dried fruits together with carbon disulfide (hydrolysis product of dithiocarbamates) by GC-MS/MS. The fumigants were extracted with n-hexane. The extracts were filtered and subjected to gas chromatography quadrupole mass spectrometry (GC-MS/MS). The isotopic labeled substance chlorobenzene D5 was used as internal standard.

Compounds included: (carbon tetrachloride, trichloroethylene, ethylene chlorobromide, 1.3-dichloropropene (cis+trans), tetrachloroethylene, 1,1,2,2-tetrachloroethane, p-dichlorobenzene, naphthalene, 1.2-dibromo-3-chloropropane, dichlorvos, p-nitrochlorobenzene, azobenzene, chloropicrin and ethylene dibromide).

Table 1 lists the MRLs for the compounds.

Table 1 MRLs of fumigant residues

Substance	Maximum residue level (mg kg^{-1})*
1,2-Dibromo-3-Chloropropane	0.01**
1,3-Dichloropropene	0.01 (products of animal origin)
Azobenzene	0.01**
Carbon Tetrachloride	0.01**
Chloropikrin	0.02 (tea. spices), 0.01 (cereals)
Dichlorvos	0.01
Ethylene Chlorobromide	0.01**
Ethylene Dibromide	0.01
Naphthalene	0.01**
p-Dichlorobenzene	0.01**
p-Nitrochlorobenzene	0.01**
Tetrachlorethane	0.01**
Tetrachloroethylene	0.01**
Trichlorethylene	0.01**

* (European Commission Directorate - General Health & Consumers (SANCO) I)

** Default MRL according to article 18 (1B) REGULATION (EC) NO 396/2005 (14)

Materials:

n-hexane of EMPLURA® grade (Merck Darmstadt, Germany)
helium 5.0 and argon 5.0 (Praxair) for GC-MS/MS
carbon disulfide (pro analysi grade) (Merck Darmstadt, Germany),
carbon tetrachloride (purity ≥ 99.5 %), trichloroethylene (purity ≥ 99.6 %), ethylene chlorobromide (purity ≥ 99.5 %), 1.3-dichloropropene (cis+trans), (purity ≥ 92 %), tetrachloroethylene (purity ≥ 99 %), 1,1,2,2-tetrachloroethane (purity ≥ 98.5 %), p-dichlorobenzene (purity ≥ 99.5 %), naphthalene (purity ≥ 99.5 %), 1.2-dibromo-3-dhloropropane (purity ≥ 98.5 %), dichlorvos (purity ≥ 98 %), p-nitrochlorobenzene (purity ≥ 99.5 %) and azobenzene (purity ≥ 98.5 %) (Dr. Ehrenstorfer GmbH Augsburg, Germany), chloropicrin (purity ≥ 99 %) and ethylene dibromide (purity ≥ 99.6 %) (Sigma-Aldrich Munich, Germany)
internal standards chlorobenzene D5 (purity ≥ 99 %) and 1,1,2- tribromoethane (purity ≥ 99 %) (Sigma-Aldrich Munich, Germany).

The stock solutions of all standards (1 mg mL^{-1} in n-hexane) were diluted to 10 µg mL^{-1} and 1 µg mL^{-1} (working solutions) with n-hexane. All solutions were stored in the dark at 4 °C.

Instrumentation:

Geno Grinder 2010 (SPEX Sample Prep, Metuchen, USA)
centrifuge Rotanta 460 (Hettich Tuttlingen, Germany
electronic pipettes applicable for volumes of 10–100 µL and 100–1000 µL, respectively, and manual pipettes applicable for volumes of 1–10 mL (Eppendorf Hamburg, Germany)
volumetric pipettes (10 mL; 0,1 DIN B Ex 20 °C; Hirschmann Laborgeräte, Eberstadt, Germany).
50 mL PP (114×28 mm) single-use tubes with screw caps (Sarstedt Nümbrecht, Germany).

1.5 mL GC autosampler vials (Klaus Ziemer GmbH Langerwehe, Germany)
6 mL single-use syringes from (Henke Sass Wolf Tuttlingen, Germany)
disposable polyester syringe filters (0.45 µm pore size, 15 mm diameter) from (Machery-Nagel Düren, Germany).

ThermoScientific Trace 1310 GC system (ThermoScientific, Waltham, USA) combined with the mass spectrometer ThermoScientific TSQ 8000 (ThermoScientific, Waltham, USA), run in EI positive mode was used for the analysis of the fumigant extracts. The GC system was connected to a TriPlus RSH autosampler (ThermoScientific, Waltham, USA).

Sample Extraction:

Dry samples (e.g. wheat grains) were directly weighed without any milling and without adding water.

For the extraction 5 g ± 0.1 g of the samples were weighed into 50 mL PP tubes. 5 mL of n-hexane followed by 50 µL of the internal standard working solution (1 µg mL^{-1}) were added to the samples. Two isotopic labeled standards (chlorobenzene D5 and 1,1,2-tribromoethane) were used. The tube was closed and shaken by a mechanical shaker for one minute. The tube was centrifuged for 5 min at 4000 rpm afterwards filtered through a syringe filter (0.45 µm) into 50 mL PP tubes. 1 mL of the extract was transferred into vials. The extracts were applied GC-MS/MS measurement.

GC-MS/MS analysis

Injection: split-mode, split ratio of 1:5 (split flow: 5 mL/min) injection volume 2 µL.

	Rate (°C/ s)	Temperature (°C)	Time (min)
Injection		120	0,1
Transfer	14	250	5
Cleaning	10	300	10

Flow: helium with a constant flow rate of 1 mL/min

Column: 30 m; 0,20 mm; 1,12 µm Agilent HP VOC column (Agilent, Waldbronn, Germany)

Pre-column 10 m; 0,25 mm Fused-Silica (Agilent, Waldbronn, Germany).

Oven program:

Ramp	Rate (°C/min)	Temperature (°C)	Hold Time (min)
Initial		45	2
1	5	80	5
2	125	280	10

MS/MS parameters: EI ionization: positive mode, MS/MS detection selected reaction monitoring (SRM) mode. transferline 350 °C, source temperature 280 °C

The mass transitions for each compound are shown in the Appendix.

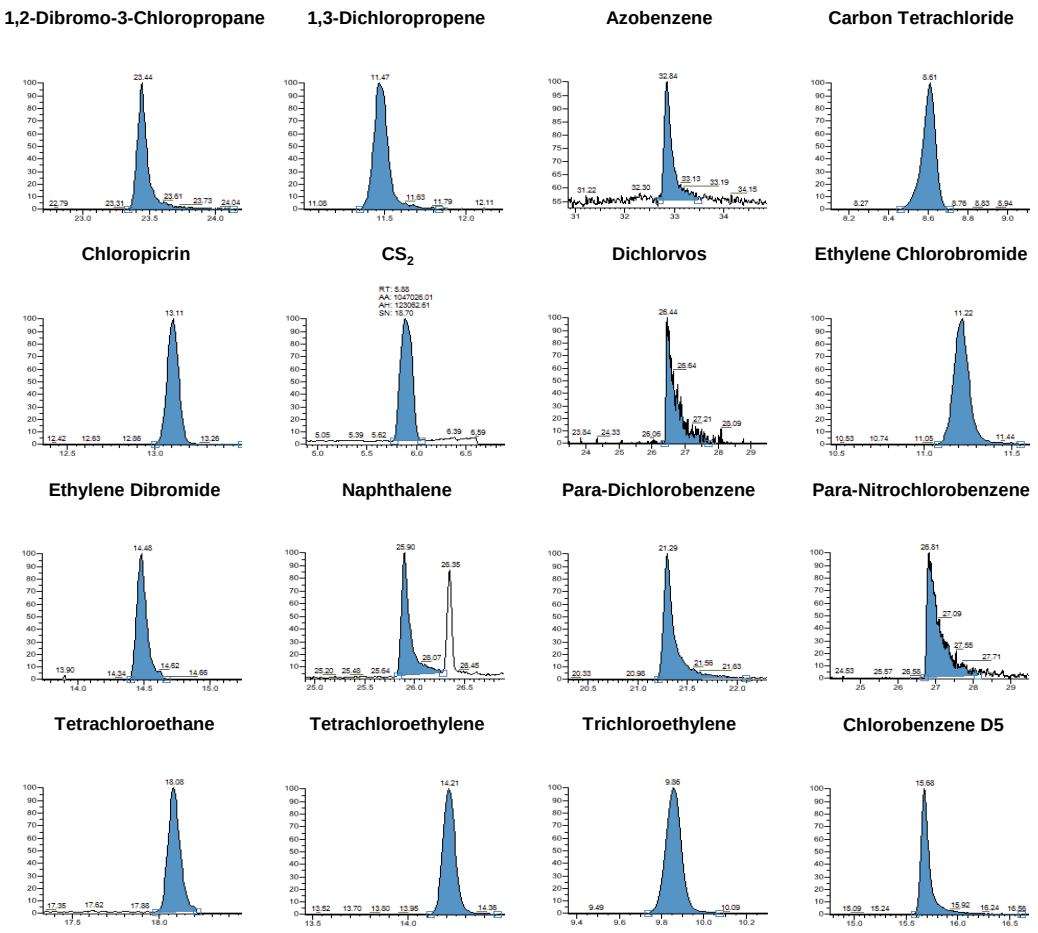

Figure 1 Chromatograms of the fumigants on wheat matrix, 0.1 mg kg^{-1}

Validation:

The validation in wheat and raisin matrix (n=5) showed satisfying recoveries between 70–120 % and relative standard deviations (RSDs) well below 10 % for all compounds (see tables 2 and 3). except CS_2. The substance is very volatile and therefore discriminated. An internal standard with similar losses such as CS2 would be beneficial.

Table 2 Recoveries for Raisin

Compound	Raisin						
	Recovery (%)					Average (%)	RSD (%)
1,2-Dibromo-3-Chlorpropane	103	98	102	102	101	**101**	**2**
1,3-Dichloropropene	95	93	92	92	93	**93**	**1**
Azobenzene	106	102	114	127	144	**118**	**13**
Carbon Tetrachloride	86	85	85	85	88	**86**	**1**
Chloropicrin	96	95	94	95	97	**95**	**1**
CS_2	52	54	51	53	53	**52**	**2**
Ethylene Chlorobromide	92	91	95	92	93	**93**	**2**
Ethylene-Dibromide	96	97	99	98	99	**98**	**1**
Naphthalene	102	97	101	99	95	**99**	**3**
p-Nitrochlorobenzene	108	104	102	98	98	**102**	**4**
p-Dichlorobenzene	107	99	101	106	107	**104**	**3**
Tetrachloroethane	103	92	98	95	101	**98**	**4**
Tetrachloroethylene	100	98	98	100	100	**99**	**1**
Trichloroethylene	91	90	88	90	91	**90**	**1**

Table 3 Recoveries for Wheat

Compound	Wheat						
	Recovery (%)					Average (%)	RSD (%)
1,2-Dibromo-3-Chlorpropane	97	96	98	95	98	**97**	**1**
1,3-Dichloropropene	79	83	83	90	82	**83**	**4**
Azobenzene	97	95	97	89	92	**94**	**3**
Carbon Tetrachloride	77	81	79	85	75	**79**	**4**
Chloropicrin	87	87	89	91	85	**88**	**3**
CS_2	52	58	53	61	51	**55**	**7**
Ethylene Chlorobromide	82	78	80	85	80	**81**	**3**
Ethylene-Dibromide	85	87	89	87	89	**87**	**2**

Naphthalene	94	103	98	101	99	**99**	**3**
p-Nitrochlorobenzene	90	98	92	91	97	**94**	**4**
p-Dichlorobenzene	97	99	101	90	95	**96**	**4**
Tetrachloroethane	91	91	93	94	94	**93**	**1**
Tetrachloroethylene	90	91	91	93	91	**91**	**1**
Trichloroethylene	79	84	82	87	79	**82**	**4**

The limit of quantification (LOQ) and the limit of detection (LOD) were estimated from the un-diluted extracts as the level showing a signal-to-noise ratio of at least 10 to 1 (LOQ) respectively 3 to 1 (LOD). **Table** shows the LOQ and LOD of the compounds for solvent and matrix.

Table 4 LOQs and LODs of the fumigants in solvent and matrix

Fumigant	Matrix	LOQ (mg kg^{-1})	LOD (mg kg^{-1})
1.2-Dibromo-3-Chloropropane	n-Hexane	0.01	0.005
	Raisin/Wheat	0.01	0.005
1.3-Dichloropropene	n-Hexane	0.05	0.01
	Raisin/Wheat	0.05	0.01
Azobenzene	n-Hexane	0.01	0.01
	Raisin/Wheat	0.01	0.01
Carbon Tetrachloride	n-Hexane	0.001	0.001
	Raisin/Wheat	0.005	0.005
Chloropicrin	n-Hexane	0.005	0.005
	Raisin/Wheat	0.01	0.005
	Wheat	0.01	0.005
CS_2	n-Hexane	0.05	0.05
	Raisin/Wheat	0.05	0.05
Dichlorvos	n-Hexane	0.1	0.05
	Raisin/Wheat	0.1	0.1
Ethylene Chlorobromide	n-Hexane	0.05	0.05
	Raisin/Wheat	0.05	0.05
Ethylene Dibromide	n-Hexane	0.05	0.01
	Raisin/Wheat	0.05	0.05
Naphthalene	n-Hexane	0.05	0.01
	Raisin/Wheat	0.01	0.01
Para-Nitrochlorobenzene	n-Hexane	0.05	0.05
	Raisin/Wheat	0.05	0.05
Para-Dichlorobenzene	n-Hexane	0.01	0.005

Fumigant	Matrix	LOQ (mg kg^{-1})	LOD (mg kg^{-1})
	Raisin/Wheat	0.01	0.005
Tetrachloroethane	n-Hexane	0.01	0.005
	Raisin	0.01	0.005
	Wheat	0.1	0.005
Tetrachloroethylene	n-Hexane	0.001	0.0005
	Raisin/Wheat	0.005	0.0005
Trichloroethylene	n-Hexane	0.001	0.005
	Raisin/Wheat	0.005	0.005

Discussion and Conclusions:

Since the analytes are volatile it is recommended to prepare the calibration solutions daily, and to analyze the extracts directly after extraction.

Linear range of the majority of the fumigants is between 0.005 to 2 ppm. The slopes of the calibration curves on matrix tend to be lower compared to the calibration curves on solvent (see **Fehler! Verweisquelle konnte nicht gefunden werden.**). An exception is trichloroethylene, where the slope of the calibration curve on matrix is in a similar range as the solvent.

Not for all fumigants LOQs were low enough to control the MRLs. An up scaling of the extraction conditions might be advantageous in this case.

Appendix:

Table 2 Mass transitions of the compounds

Compounds	Retention Time (min)	Precursor Mass (u)	Product Mass (u)	Collision Energy (eV)
CS_2	5.6	77.7	77.9	10
		75.9	75.9	10
		75.9	44	40
		77.7	45.8	40
Pentafluorobenzene	8.0	99	79.6	25
		168	99	20
Carbon Tetrachloride	8.2	116.9	81.9	28
		118.9	83.9	28
		120.9	83.9	28
Trichlorethylene	9.3	94.9	60	25
		129.9	95	10
		131.8	96.9	10
Ethylene Chlorobromide	10.6	65	65	0
		143.9	63	5
		63	63	0
Methylisothiocyanate (MITC)	11.9	71.8	45	15
		73	45	10
1.3-Dichloropropene	12	110	75	5
		111.9	77.2	5
Chloropicrin	12.5	116.9	82	30
		120.9	83.9	30
		118.9	81.9	30
Tetrachloroethylene	13.5	128.9	93.9	20
		130.9	95.9	15
		163.9	128.9	15
		165.8	130.9	10
Ethylene-Dibromide	13.8	187.8	107	5
		106.9	106.9	0
		108.9	108.9	0

Compounds	Retention Time (min)	Precursor Mass (u)	Product Mass (u)	Collision Energy (eV)
Chlorobenzene D5 (ISTD)	15.8	82	54	15
		117	82.1	15
		118.8	82.1	15
Tetrachloroethane	17.2	132.6	97	15
		165.8	83	5
		82.8	82.8	0
P-Dichlorobenzene	20.0	111	75.1	10
		112.8	75	15
		146	111	15
		147.9	113	15
1.2-Dibromo-3-Chloropropane	22.0	154.9	75	5
		154.9	92.9	25
		156.8	75	5
		234	155	5
1.1.2-Tribromoethane (ISTD)	22.0	184.9	104.9	15
		186.9	104.9	15
		186.9	106.9	15
		188.8	106.9	15
Naphthalene	24.4	127.9	77.7	20
		128	128	0
Dichlorvos	25.2	109	79	5
		185	93.1	10
		185	108.9	15
Para-Nitrochlorobenzene	25.5	111.1	75.1	10
		156.9	99	15
1-Bromo-4-Nitrobenzene	28.4	154.9	75.5	15
		156.8	75.7	15
		170.9	143	10
Azobenzene	29.7	105	77.1	5
		182.1	105.1	5