EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compound(s)**: Dithiocarbamates, such as maneb, mancozeb, metiram, propineb, thiram and ziram
- **Commodities**: Plant origin (except of high oil-content) and milk
- **Extraction Method(s)**: Reductive cleavage with HCl/$SnCl_2$ followed by partitioning of CS_2 into isooctane
- **Instrumental analysis**: GC-MS/MS (or -ECD)

Analysis of residues of dithiocarbamate fungicides in low-oil content food of plant origin involving cleavage into carbon disulfide, partitioning into isooctane and measurement by GC-MS/MS or GC-ECD

Version 3.1 (last update: June 2024)

1. Introduction and background information

This document describes an adjusted procedure for the analysis of dithiocarbamate residues in fruits, vegetables and cereals via the common moiety (carbon disulfide = CS_2) approach[1] involving cleavage with HCl/$SnCl_2$, partitioning of CS_2 into isooctane, and measurement by GC-techniques.

The procedure described in the previous version (V2) had to be adjusted for several reasons including the following:

i) it provided satisfactory recovery rates for thiram, but rather insufficient recovery rates[2] for certain polymeric dithiocarbamates (such as metiram and propineb), see **Figure 1**;

ii) the consumption of chemicals was considered too high; and

iii) it didn't include the use of GC-MS/MS for measurement.

[1] As required by Reg. (EC) No. 396/2005.

[2] Outside of the 80-120% range required by the AQC-document for not requiring correction of results for bias (https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf)

The new procedure described below entails the following:

a) an extended hydrolysis time, from previously 2 h to 3 h;

b) a slightly higher reaction temperature, from previously 80 °C to 85 °C;

c) a reduced sample weight, from previously 25-50 g to 5-10 g (taking into account that subsampling variability portion sizes in the range of 5 to 10 g still ensure satisfactory portion-to-portion variability when homogenates are used);

d) an increased reagent-to-sample ratio, from previously 3:1 (150 mL HCl/$SnCl_2$ reagent for 50 g sample) to 7.5:1 (75 mL reagent for 10 g sample), but still a 2-fold reduction of reagent amount per sample;

e) the use of smaller reaction vessels (100 mL) compared to the previous 250 mL vessels (allowing faster energy/temperature transfer to the reaction mixture within the bottles, thus allowing faster heating and cooling, but also reducing the space required); and

f) the use of a GC-MS/MS instrument (with GC-ECD being maintained as an alternative)

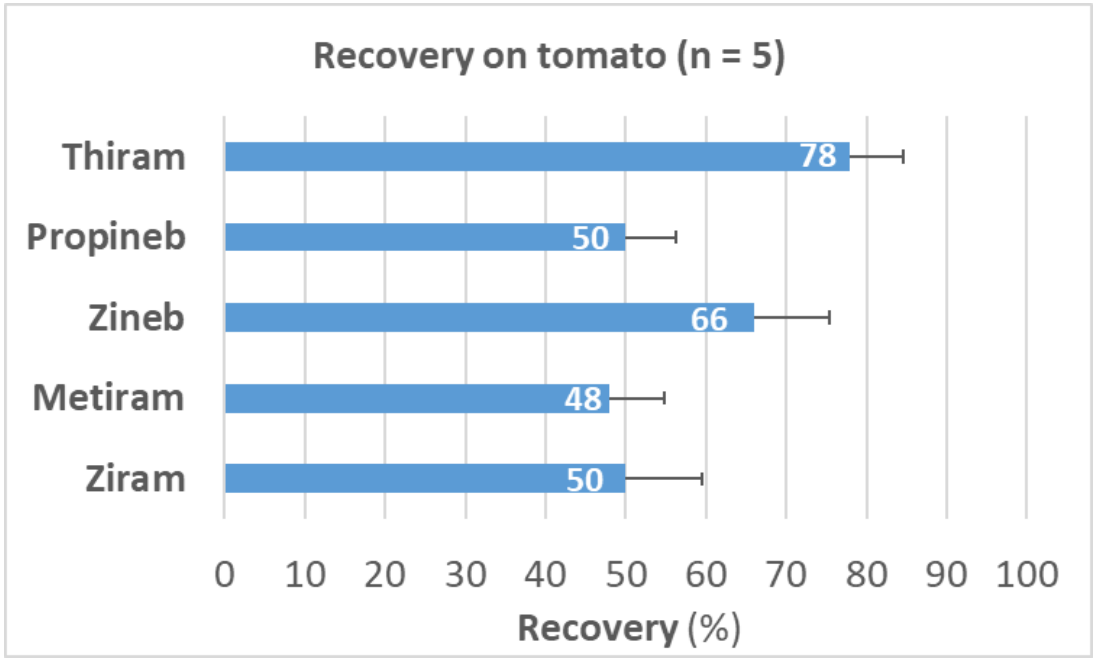

Figure 1: *Recovery of five dithiocarbamate fungicides spiked on comminuted tomato using the reductive cleavage conditions of the previous version of the document (50 g sample weight, 150 hydrolytic agent of 0.1 M /$SnCl_2$ in 4 M HCl, cleavage conducted at 80 °C for 2 h) (each n = 5).*

2. Analyte properties

Physicochemical properties and additional information on carbon disulfide are given in **Table 1**.

Table 1: *Chemical properties of carbon disulfide*

Carbon disulfide (CAS: 75-15-0), **Synonyms: Carbon disulphide, methanedithione**		
Parameter	Value/Notes	
Molecular Mass	76.13 g/mol	
Formula	CS_2	
Boiling point	42.2 °C (at 997-998 hPa)	
pKa	No ionizable atoms present	
LogP	Chemicalize.com (computed): 1.95	
Water solubility	Up to 2.9 g/L	
Stability	Chemically stable but take measures to minimize evaporation losses!	
Residue definition (EU)	1) Carbon disulfite (refers to the use of CS_2 as a fumigant) 2) Dithiocarbamates (dithiocarbamates expressed as CS_2, including maneb, mancozeb, metiram, propineb, thiram and ziram)	
Approved in …	The use of CS_2 as such (as a fumigant) is currently **not** approved within the EU; The following dithiocarbamate fungicides, having CS_2 as common moiety, are approved within the EU: • Metiram: AT, BE, BG, CY, CZ, DE, EL, ES, FR, HR, HU, IT, LU, NL, PL, PT, RO, SI, SK (MS shall withdraw PPP authorizations containing metiram by 28 May 2024. Grace period shall expire by 28 Nov. 2024). • Ziram (AT, BE, CY, CZ, EL, FR, HR, HU, IT, MT, PL, PT, RO, SI, SK (expires 15 Mar. 2025) Note that numerous dithiocarbamate fungicides (of all three types, i.e.: ethylene-bis, propylene-bis and dimethyl) are still approved in many third countries	
Toxicity	Flam Liq. 2; Acute Tox. 4; Skin Irrit. 2; Eye Irrit. 2; Repr. 2; STOT RE 1	
Other sources	• Dithiocarbamates are also widely used in other areas, e.g. as vulcanisation accelators in the rubber industry. Therefore, contaminations with CS_2-precursors in the lab, due to the use of rubber-containing vessels or contaminated solvents, may be possible. • Natural precursor compounds, contained in certain crop types (e.g. brassica and allium crops), are known to generate CS_2 during the stated cleavage. Natural CS_2 formation has also been reported in shiitake mushrooms. The EURLs have prepared a collection of data concerning the CS_2 background levels in various crops[3].	

3. Chemicals and Consumables

Where water is indicated, de-ionized water is to be used.

3.1 Isooctane (e.g. Merck, EMSURE® ACS, Reag. Ph Eur, art. no.: 1.04727.2500)

3.2 Toluene (e.g. Merck, EMSURE® ACS, ISO, Reag. Ph Eur, art. no.: 1.08325.1000)

3.3 Hydrochloric acid, fuming (36.5% = 12 N) (e.g. by Thermo Scientific, art. no.: 33257)

3.4 Tin(II)-chloride (e.g. Merck, dihydrate for analysis, art. no.: 1.07815.0100)

3.5 Carbon disulfide (CS_2), density 1266 mg/mL at 25°C, (e.g. Merck, EMSURE® ACS,Reag. Ph Eur, art. no.: 1022141000)

3.6 Acetonitrile (e.g. Merck, suitable for HPLC, gradient grade, ≥99.9%; art.-No. 34851).

[3] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/2022_Poster-EPRW_Carbon-disulphide_PM-06.pdf

3.7 Thiram (e.g. Dr. Ehrenstorfer, art. no.: C17570000)

3.8 Chloroform, density: 1.49 g/cm³ at 25°C (e.g. Sigma Aldrich, art. no.: C2432);

3.9 Internal standard stock solution: e.g. 10.0 mg/mL in isooctane:

14.8 mL of isooctane (see 3.1) are filled into a 20 mL screw-cap glass tube and 100 µL (= 149 mg) of chloroform (see 3.7) is added. Close the vessel immediately and shake well. Keep the solution in a fridge or freezer for long-term storage.

Note 1: *If a 20 mL volumetric flask is used, add 134 µL of formic acid to 19 mL isooctane and fill up to volume with isooctane. Shake well. Transfer the solution into a well sealable (e.g. screw cap) vessel for long-term storage.*

Note 2: *Pneumatic pipettes may get contaminated with chloroform fumes and will need to be aerated well before further pipetting to prevent cross-contamination. Air or nitrogen stream will accelerate aeration. Check the behavior of your pipette to assess the contamination potential and adjust the aeration procedure.*

Note 3: *Pipette contamination is avoided by weighing the chloroform. For a 10 mg/mL stock solution add 10 mL of isooctane into a screw cap bottle, close the bottle with the cap and tare the balance. Open the vial, add 5 drops of chloroform using a Pasteur pipette, corresponding to roughly 80-100 µL (roughly 120-150 mg), close the vial immediately and weigh again. Depending on the weight of chloroform added, calculate the volume of the remaining isooctane that needs to be added as follows: remailing volume of isooctane to be added [in mL] = 0.1*X -10 (where X = mg of chloroform weighed into the bottle)*[4].

3.10 Internal standard working solutions:

Internal standard working solution 1 (IS-WS1): 100 µg/mL in isooctane: Transfer 19.80 mL of isooctane (see 3.1) is pipetted into a 20 mL screw-cap glass tube and 200 µL of internal standard stock solution (at 1 mg/mL, see 3.9) is added. Close immediately and shake well.

Internal standard working solution 2 (IS-WS2): 10 µg/mL in isooctane: Transfer 18.00 mL of isooctane (see 3.1) is pipetted into a 20 mL screw-cap glass tube and 2.00 mL of internal standard working solution 1 (100 µg/mL) is added and shaken well.

3.11 CS_2 stock solution at 10.0 mg/mL in isooctane:

12.55 mL of isooctane (see 3.1) is filled into a 20 mL screw-cap glass tube; and 100 µL (=126.5 mg[5]) of CS_2 (see 3.5) is added. Close the vessel immediately and shake well.

[4] This formula is simplified (volume and purity of chloroform disregarded) and as the absolute concentration of the IS is of secondary importance

[5] Assuming 99,9% purity

Note 1: *Since CS_2 is volatile, it has the potential of cross-contamination. When handling with pure CS_2, highly concentrated working standards and CS_2–contaminated waste, make sure that low-concentrated standards and sample extracts are not contaminated with CS_2 fumes.*

Note 2: *Pneumatic pipettes may be used for preparing CS_2 stock and working solutions, but the pipettes used for pure CS_2 or highly concentrated working standards should not be immediately reused for pipetting low-concentrated calibration solutions, internal standard solutions or sample extracts, as there is a high risk of cross contamination potentially leading to false positives and/or to artificially raised calibration curves. Let contaminated pipettes to aerate well before reusing them. Flushing pipettes with a stream of air will speed-up aeration. Conduct cross-contamination experiments to establish a suitable aeration protocol for your lab.*

Note 3: *Pipette contamination can be avoided by weighing the CS_2. For a 10 mg/mL stock solution add 10 mL of isooctane into a screw cap bottle, close the bottle with the cap and tare the balance. Open the vial, add 5-8 drops of CS_2 using a Pasteur pipette, corresponding to roughly 100-150 µL (roughly 110-180 mg)[6], close the vial immediately and weigh again. Depending on the weight of CS_2 added, calculate the volume of the remaining isooctane that needs to be added as follows: remailing volume of isooctane to be added [in mL] = 0.1*X * CS_2 purity – (10+X/1266) (where X = mg of CS_2 weighed into the bottle).*

Note 4: *For long-term storage of stock solutions, use well sealable screw cap containers[7], making sure that the headspace volume remains small[8]. To avoid tempering the stock solution to room temperature for preparing further working solutions from it, see notes under 3.12.*

3.12 CS_2 working solutions

Working solution 1 (WS1) at 100 µg/mL in isooctane: Transfer 19.8 mL of isooctane (see 3.1) into a 20 mL screw-cap glass tube and add 200 µL of CS_2 stock solution at 10 mg/mL (see 3.11). Close the bottle and shake well.

Working solution 2 (WS2) at 10 µg/mL in isooctane: Transfer 18.0 mL of isooctane (see 3.1) into a 20 mL screw-cap glass tube and add 2.0 mL of WS1 at 100 mg/mL (see 3.11). Close the bottle and shake well.

Working solution 3 (WS3) at 1 µg/mL in isooctane: Transfer 18.0 mL of isooctane (see 3.1) into a 20 mL screw cap bottle, add 2.0 mL of WS2 solution at 10 µg/mL, close bottle and shake well.

Working solution 4 (WS4) at 0.1 µg/mL in isooctane: Transfer 18.0 mL of isooctane (see 3.1) into a 20 mL screw cap bottle, add 2.0 mL of WS3 solution at 1 µg/mL, close bottle and shake well.

[6] Check the approximate weight in a pre-experiment to make sure that the weight will range between 100 and 200 mg

[7] Avoid using volumetric flasks, but rather vials with screw caps and good quality seal.

[8] If parts of a stock solution or of a highly concentrated working solutions need to be discarded, take measures to minimize cross contamination of sample, sample extracts and solvents.

Note 1: *You may proceed with the preparation of working standards either using the freshly prepared stock solution or after cooling it down before use. Cooling the stock solution to refrigerator temperature before use will reduce CS_2 fumes. In this case, any dilutions for the preparation of working solutions will need to be done with isooctane of the same temperature (i.e. refrigerator cold).*

Note 2: *For long-term storage of working solutions, store them into well sealable screw cap containers, making sure that the headspace volume is small[9]. Store these solutions in a refrigerator or a freezer. If stored in a freezer temperate to refrigerator temperature before reuse, again using isooctane of the same temperature for any dilutions. This way you avoid the need to temperate the solution(s) to room temperature, thus reducing evaporation and cross-contamination in the lab.*

3.13 CS_2 calibration standards:

Prepare calibration standards at e.g. 1.0; 0.5; 0.2; 0.05, 0.02, 0.01 µg/mL CS_2 in isooctane following the pipetting scheme in Table 2.

Table 2: Pipetting scheme for exemplary CS_2 calibration solutions.

Calibration solution CS_2	1.0 µg/mL	0.5 µg/mL	0.2 µg/mL	0.05 µg/mL	0.02 µg/mL	0.01 µg/mL
Volume of internal standard working solution 10 µg/mL *(see 0)*	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Volume of isooctane *(see 3.1)*	800 µL	850 µL	700 µL	850 µL	700 µL	800 µL
Volume of CS_2 working solutions *(see 3.12)*	100 µL of WS2 10 µg/mL sol.	50 µL of WS2 10 µg/mL sol.	200 µL of WS2 10 µg/mL sol.	50 µL of WS3 1 µg/mL sol.	200 µL of WS3 1 µg/mL sol.	100 µL of WS4 0.1 µg/mL
Total volume	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL

Note 3: It is recommended to prepare many series of calibration solutions in one go and to store them in the freezer until use. Calibration solutions are prepared directly in well sealable GC-vials. If stored in the freezer, calibration solutions filled into GC-vials, may be used for at least 3 months.

[9] If parts of a stock solution or of a highly concentrated working solutions need to be discarded, take measures to minimize cross contamination of sample, sample extracts and solvents.

3.14 Stock solution thiram: 1 mg/mL in toluene:

A stock solution of thiram in toluene (3.2) at a concentration of 1.00 mg/mL is prepared. Volume of solvent (mL) = weight of thiram (mg) x purity. Note: 1.00 mg of thiram theoretically generates 0.6333 mg CS_2

3.15 Working solution thiram: 0.100 mg/mL in isooctane:

In a 20 mL screw cap vessel, mix 1.00 mL of thiram stock solution (see 3.10) with 9.00 mL of isooctane and shake well.

3.16 Hydrolysis reagent:

Weigh 30 g of tin(II) chloride dihydrate (see 3.4) into a 2 L volumetric flask add 666±10 mL HCl conc. (see 3.3) and fill up to volume with water. Shake well to dissolve the salt.

3.17 Xanthan gum (e.g. Sigma Aldrich, art.-no. G1253).

3.18 Water/acetonitrile (95/5, v/v) mixture:

Add 5 mL of acetonitrile (3.6) into a 100 mL glass volumetric flask and fill up to the mark with deionized water.

3.19 Mixture of water/acetonitrile/xanthan gum (95/5/0.2 V/V/W)[10]:

Weigh a portion of 0.2 g of xanthan gum (3.17) into e.g. a 500 mL glass beaker and add 100 mL of the water/acetonitrile (95/5, v/v) mixture (3.18). Mix well e.g. by using an immersion blender. Any formed air bubbles may be removed by ultra-sonication. This solution can be stored for several weeks in a fridge and pipetted by standard automatic pipettes (4.7).

3.20 Stock and working suspensions for other dithiocarbamate active substances

Stock suspensions for the dithiocarbamate active substances (other than thiram) are following the procedure presented by Zipper et al.[10]. For this, a certain amount of the analytical standard is mixed with an appropriate volume of the prepared water/acetonitrile/xanthan gum (95/5/0.2 V/V/W) mixture (3.19) to achieve the desired concentration. The purity of the respective standard substance is taken into account. Working suspensions are prepared by diluting stock suspensions using the same water/acetonitrile/xanthan gum mixture (3.19). Stock and working suspensions are stable for only a short time and should be preferably used within 60 min following preparation.

[10] According to the procedure presented by Zipper *et al.* of the EURL-SRM: https://www.eurl-pesticides.eu/userfiles/file/EurlFV/Joint2021/Wachtler-Zipper.pdf

4. Apparatus and Glassware

4.1 20 mL screw-cap glass tube (e.g. Klaus Ziemer GmbH, art.-no. 1.300160 and 2.301273)

4.2 Cleavage vessels: 100 mL Duran® glass bottles with a screw cap suitable for high-temperature applications (glass fiber-containing PBT with PTFE-coated silicone septum), obtained from DWK Life Sciences (purchased via Carl Roth GmbH + Co. KG, Product-No. KCN6.1).

4.3 High speed mill (e.g. Stephan UM 5 universal CUT or Thermomix TM5 or equivalent)

4.4 Shaking water bath with thermostat (e.g. GFL, type 1083)

4.5 Vials amenable to GC autosampler with plastic septum, free of CS_2-emitting components

4.6 Solvent dispensers (10 - 50 mL)

4.7 Pipettes: Automatic pipettes (50 - 1000 µL and 5 -100 µL), pipette tips (100 µL and 1000 µL)

4.8 Ultra-sonicator

5. Sample Preparation

A homogenized sample portion of 10.0 g, or 2.0 g in case of dried herbs and spices, is weighed into a cleavage vessel (see 4.2) and 10 mL of isooctane (see3.1) are added. Then, 75 mL of hydrolysis reagent (0.066 M tin (II)-chloride in 4 M hydrochloric acid, see 3.15) and 100 µL of internal standard working solution 100 µg/mL (see 3.10) are added and the vessel is immediately closed with a screw-cap with septum. The vessels are put into a shaking-water bath for 3 hours at 85°C. Within 2-5 minutes after placing the bottles into the water bath, tighten the screw cap further. After 10-20 min shake the vials giving emphasis on samples that show signs of coagulation. Thereafter shake the samples every approx. 60 min, making sure to wash down any sample parts that could have possibly stuck to the cap. After the 3 hours have passed, cool down the bottles to preferably <10°C (but at least to < 20°C), by placing them into a cooling water bath[11]. Once the cooling process after extraction is completed, shake the container before opening and withdrawing the aliquot of isooctane. This is to ensure that CS_2 that may have enriched in the headspace is absorbed into the isooctane. The isooctane aliquots may be transferred directly into GC-vials. Make sure that the aliquot occupies almost the entire volume of the storage container to reduce the volume of the headspace. In the case of GC-vials, transfer e.g. 1.5 mL of the isooctane-phase into a well sealable GC vial[12]. The isooctane extract is directly subjected to GC-MS/MS measurement. If the vials are not measured within 1-2 days, store them in a freezer[13].

[11] The cool-down can be accelerated by adding ice cubes into the water bath. Placing the precooled bottles into a freezer for 15 min or a refrigerator for 30 min will also accelerate the process.

[12] Consider filling up one or more GC-vials with the same extract, as a backup, e.g. in case the CS_2 level of the sample falls out of the calibration range or in case problems in measurement come up, requiring re-injection.

[13] The use of a coolable autosampler is preferred.

6. Measurement

Exemplary GC-MS/MS and –ECD conditions are given in **Table 3** and **Table 4**.

Table 3: *Instrumentation and method details for GC-MS/MS analysis (Injector: Gerstel MPS, GC: Agilent 8890; MS/MS: 7010B GC/TQ)*

Instrument parameters	Conditions			
Injection volume	1 µL			
Injection temperature	200 °C (constant)			
Split	1:10			
Pre-column	HP-VOC Agilent (approx.. 1 m, 0.20 mm ID, 1.12 µm film thickness)			
Column	HP-VOC Agilent (30 m, 0.20 mm ID, 1.12 µm film thickness)			
Carrier gas and gas flow	Helium, 1.0 mL/min (constant flow)			
Oven temperature program	**Rate (°C/min)**	**Temperature (°C)**	**Hold Time [min]**	
	-	35	2	
	50	150	0	
	120	260	10	
Transfer Line temperature	250 °C			
Ion Source Temperature	250 °C			
Ion source mode and voltage	EI positive, 70 eV			
Acquired mass transitions (m/z)	**Compound**	**Mass transitions and their MS-parameters**		
		Q 1 (m/z)	**Q 3 (m/z)**	**Collision Energy (V)**
	Carbon disulfide (CS_2)	76	44	40
		76	76	5
		78	46	40
		78	78	5
	Chloroform (Internal Standard)	85	49	40
		85	47	40

Table 4: *Instrumentation and method details for GC-ECD analysis (Injector: Gerstel MPS, GC-ECD: Thermo Scientific Trace 1310)*

Instrument parameters	Conditions		
Injection volume	2 µL		
Injection temperature	200 °C (constant)		
Split	1:10		
Pre-column	HP-VOC Agilent (approx.. 1 m, 0.20 mm ID, 1.12 µm film thickness)		
Column	HP-VOC Agilent (30 m, 0.20 mm ID, 1.12 µm film thickness)		
Carrier gas and gas flow	Helium, 1.0 mL/min (constant flow)		
Oven temperature program	**Rate (°C/min)**	**Temperature (°C)**	**Hold Time [min]**
	-	45	2
	12	95	0
	125	260	8

7. Quality Control and Critical Steps:

It is advisable to run a recovery check with each series of samples. The respective dithiocarbamate working solution (thiram) or working suspension (polymeric dithiocarbamates) should be spiked onto the, preferably still frozen, analytical portion before the addition of isooctane and hydrolysis agent.

In principle, the initial temperature of the blank homogenate to be spiked as well as the waiting time between spiking and the start of the reaction should reflect the routine procedure. But it is considered beneficial to cryogenically mill fresh produce and to keep the homogenates frozen till the start of the above-described procedure. Extensive exposure of the dithiocarbamate compound with defrosted matrix may result in the formation of degradation products that may not be transformable to CS_2 during the procedure, and consequently to reduced recovery rates.

Ideally, the average recovery rate following conversion to CS_2 and partitioning of the later to isooctane should be between 80 and 120 % with the RSD being ≤20% (see Document Nº SANTE/11312/2021).

Significantly overestimated mean recoveries may be an indication of a too low CS_2 concentration in the calibration standards (e.g. due to evaporation losses). Poor recovery rates may have various reasons, including the following: low purity of the dithiocarbamate compound spiked, too large particle sizes of the dithiocarbamate compound spiked, evaporation losses of CS_2 due to leaking reaction bottles or careless handling after the reaction (normally the internal standard should partly correct such losses), too weak reaction conditions (temperature, time, reagent), too complex matrix. A critical step in the procedure is the cooling down of the reaction bottles prior to opening them. Adding the internal standard (chloroform) to the reaction bottle after the addition isooctane is also essential, as it reduces evaporation losses.

Make sure that the rims of the cleavage vessels as well as the seals of the caps are intact. Leaks may lead to evaporation losses during the reaction process.

Conversion factors for the calculation of spiked dithiocarbamate compounds to CS_2—concentration are shown in **Table 5.**

__Table 5__: Conversion factor for seven dithiocarbamate active substances for the calculation of the spiked concentration into the corresponding CS_2-concentration.

DTC-Compound	Molecular weight (g/mol)	No. of CS_2-moieties per molecule[14]	Molecular weight of CS_2 (g/mol)	Resulting conversion factor	0.1 mg CS_2/kg sample corresponds to the following levels of DTC-compound in the sample
Thiram	240.44	2	76.139	0.633	0.158
Ziram	305.83	2		0.498	0.201
Metiram	1088.7	8		0.559	0.179
Maneb	265.31	2		0.574	0.174
Mancozeb	541.07	4		0.563	0.178
Propineb	289.79	2		0.525	0.190
Zineb	275.76	2		0.552	0.181

[14] Di- or oligomers are used for the calculation. In the polymer the situation may be slightly different.

8. Validation data:

Validation experiments for several dithiocarbamate active substances were conducted using matrices representing high water-content, high acid-content and dry plant commodities[15] as well as milk. The analytes were spiked in quintuplicate to the respective weighed portions of the sample homogenates with freshly prepared working suspensions according the previously described in chapter 3.20[10].

The conducted validations at 0.02 mg/kg CS_2 were successful for at least three mass traces of all matrix-active substance-combinations according the criteria stated in Document Nº SANTE/11312/2021 V2, see **Table 6.**

Table 6: *Recoveries (Rec.), relative standard deviations (RSD) for the validation of several dithiocarbamate active substances in banana, wheat flour, milk, potato and tomato at 0.02 mg/kg CS_2, each n = 5.*

Pesticide spiked	CS_2 mass traces[16]	Banana (10 g)		Wheat Flour (10 g)		Milk (10 g)		Potato (10 g)		Tomato (10 g)	
		Avg. Rec. (%)	RSD (%)	Avg. Rec. (%)	RSD (%)	Avg. Rec. (%)	RSD (%)	Avg. Rec. (%)	RSD (%)	Avg. Rec. (%)	RSD (%)
Metiram	MRM 1 (76/76)	85	3.4	88	3.2	100	2.8	80	2.8	90	3.0
	MRM 2 (76/44)	79	4.9	86	3.2	98	1.2	76	4.5	90	1.5
	MRM 3 (78/78)	93	9.2	83	11.0	103	6.1	102	7.2	100	10.1
	MRM 4 (78/46)	76	2.4	99	18.3	113	7.2	74	5.6	92	10.0
Propineb	MRM 1 (76/76)			96	9.3	100	7.4			99	4.1
	MRM 2 (76/44)			98	8.6	101	7.8			98	4.9
	MRM 3 (78/78)			93	8.2	102	6.7			111	7.3
	MRM 4 (78/46)			102	15.0	104	10.3			92	8.7
Thiram	MRM 1 (76/76)	108	3.7					103	4.1		
	MRM 2 (76/44)	111	3.6					101	4.3		
	MRM 3 (78/78)	*128*	5.4					105	14.1		
	MRM 4 (78/46)	108	3.5					103	10.7		
Zineb	MRM 1 (76/76)			89	3.4	111	3.4			107	4.8
	MRM 2 (76/44)			79	13.4	105	8.2			97	15.0
	MRM 3 (78/78)			90	*21.3*	120	14.2			119	5.0
	MRM 4 (78/46)			88	4.1	109	4.1			107	5.8
Ziram	MRM 1 (76/76)			94	8.6	97	14.0			104	6.8
	MRM 2 (76/44)			95	11.0	*129*	41.6			107	10.7
	MRM 3 (78/78)			99	15.9	90	19.3			101	6.7
	MRM 4 (78/46)			93	8.6	97	14.1			103	8.5

9. Miscelanneous hints

- When analyzing plant material with sulphur-containing components (e.g. brassica crops, allium crops, papaya) high sample blank values have to be taken into account. Blank values are higher if homogenized samples are left standing at room temperature. A compilation of background levels can be found online on the EURL-SRM website.[3]
- Vulcanized latex gloves can contain traces of carbon disulphide and, should therefore not be used**.**

[15] According of the grouping of commodities in Document Nº SANTE/11312/2021 V2;

[16] In the order of signal intensity

10. Document History

Action	When	Changes / Actions	Document Version
Publication of V3.1	June 2024	- Chapter 3.4: the unhydrous tin(II)chloride salt was changed to tin(II)chloride dihydrate which is actually used for the hydrolysis reagent (3.16) - Chapter 3.16: the necessary amount of tin(II)chloride is changed to 30 g as the previous 75 g were actually related to a volume of 5L instead of 2 L of the hydrolysis agent; the correct dihydrate salt was included - Section 5: the exakt concentration ot tin(II)chloride in the hydrolysis agent is included - removal of text errors and typos	V3.1
Publication of V3	Dec. 2023	- Various changes regarding the cleavage conditions replacing sections 5.2, 6.1.1 and 6.1.2 of V2; - Implementation of GC-MS/MS conditions amending section 6.5 of V2; - Introducing validation data of several dithiocarbamate active substances using the optimized cleavage conditions - General updates throughout the document	V3
Optimisation of method	In 2016 and in 2022-2023		
Publication of V2	Dec. 2009	- Addition of exemplary chromatograms - Improvement of sample preparation part - removal of text errors	V2
Publication of V1	March 2009		V1
Drafting the document	2008-2009		V1
Elaboration of method	2008		