Analysis of Dithiocarbamate Residues in Foods of Plant Origin involving Cleavage into Carbon Disulfide, Partitioning into Isooctane and Determinative Analysis by GC-ECD

(**Version 2**, Document History in page 11)

1. Aim and Scope

This document describes a testing procedure for the gas-chromatographic analysis of dithiocarbamate residues in vegetable, fruit and cereal products via their common degradation product carbon disulfide (CS_2) as required by Reg. 396/2005/EC.

2. Safety Instructions

When handling the chemicals and pesticide standards used here, the relevant manufacturer safety instructions on the containers are to be taken into consideration.

3. Short Description of Procedure

Following chemical cleavage of dithiocarbamates by a mixture of tin(II)-chloride and hydrochloric acid, the released CS_2 partitions into isooctane. Determinative analysis of CS_2 is achieved by GC-ECD. The concentration of CS_2 is calculated via external calibration, with the residue being expressed as CS_2.

4. Chemicals

Where water is indicated, de-ionized water is to be used.
All reagents must have a purity level of p.a.

4.1. Toluene (e.g. Merck, art. no.: 1.08325.1000)

4.2. Isooctane (e.g. Merck, art. no.: 1.04727.2500)

4.3. Hydrochloric acid fuming (37%) (e.g. Merck, art. no.: 1.00314.1000)

4.4. Tin(II)-chloride (e.g. Merck, art. no.: 8.18150.0100)

4.5. Carbon disulfide (CS_2), density 1260 mg/mL at 25°C, (e.g. Merck, art. no.: 1.02214.1000)

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

4.7. Stock solution CS_2 : 1.0 mg/mL in isooctane:
126 mL of isooctane is filled into an iodine determination flask (see 5.1); 100 µL of CS_2 solution (see 4.5.) is added, to achieve a concentration of 1.0 mg/mL. The tube should be well shaken.
Note: if CS_2 purity is less than 100% then the isooctane volume should be adjusted accordingly (volume isooctane (mL) = 126 x CS_2 purity),

4.8. Working solution CS_2 : 40 µg/mL in isooctane:
24 mL of isooctane (see 4.2.) is pipetted into a 30 ml screw-cap glass tube and 1 mL of CS_2 stock solution is added and shaken well.

4.9. Calibration solutions CS_2 : 2.0; 0.8; 0.2; 0.08 µg/mL in isooctane:
500 µL, 200 µL, 50 µL and 20 µL each of the CS_2 working solution (see 4.8) are pipetted into separate volumetric flasks (10 mL) and filled to the mark with isooctane.

4.10. Stock solution thiram: 1 mg/mL in toluene:
A stock solution with a concentration of 1 mg/mL is produced from thiram standard.
volume of solvent (mL) = weight of thiram (mg) x purity
1 mg of thiram theoretically generates 0.6323 mg CS_2

4.11. Working solution thiram: 0.1 mg/mL in isooctane:
1 mL of the thiram stock solution (see 4.10) is pipetted into a 10 mL volumetric flask, filled with isooctane to the mark and shaken well.

4.12. Hydrolysis reagent:
75 g of tin(II)-chloride is dissolved in 5 L hydrochloric acid (4 N)

5. Equipment

5.1. 200 mL iodine determination flask (= Erlenmeyer flask with stopper)

5.2. Cleavage vessels: tightly sealable glass bottles with screw-cap and plastic septum, free of CS_2-producing components (e.g. 250 mL plastic-coated Schott bottles, art. no.: 2180536; screw-caps with plastic septum, art. no.: 1088655); other vessel sizes may be employed if the method is scaled up or down (see 6.1)

5.3. Mincer (e.g. Stephan UM 5 universal CUT)

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

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

5.6. GC autosampler (e.g. CTC Combi Pal)

5.7. GC column (e.g. Varian, CP 8781)

5.8. GC syringe (e.g. SGE, art. no.: 002981)

5.9. Solvent dispensers (10 - 50 mL)

5.10. Pipettes

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

6. Procedure

6.1. Sample preparation

Dithiocarbamate residues are typically located superficially. Thus, sample comminution (e.g. cutting, milling, grinding) is only to be performed, where this is necessary to obtain acceptable sub-sampling variability. For samples composed of small units or particle sizes e.g. grain, nuts, dry pulses, raisins and even small berries, sample comminution can be typically omitted as long as the sample portion employed for analysis is large enough and the sub-sampling variability acceptable (Note: sub-sampling variability is also a function of the analytical portion size. The bigger the portion the smaller the sub-sampling variability becomes). The method described below, refers to 50 g sample portions. In principle, however, the method can be scaled up or down (differently sized sealable glass bottles might be required in extreme cases).

Samples consisting of bigger units will require a comminution step. Most fruits and vegetables (e.g. apples, melons, tomato, iceberg lettuce) will require a thorough processing with a high speed mixer as typically employed to produce sample homogenates for multiresidue methods. Follow the instructions described in EN-15662 (QuEChERS procedure), to ensure representative sub-samples. If the laboratory sample cannot be processed in its entirety a reduction will be necessary prior to milling. In most cases opposite segments of each unit are cut out using a ceramic knife. Cryogenic milling is to be preferred to reduce the risk of CS_2 losses. For this, the representative sub-sample is put into the freezer (e.g. over night) and the comminuted in frozen condition. Cryogenic milling can be further assisted by dry ice addition. For some types of vegetables, (e.g. parsley, rucola, lambs lettuce) it might be appropriate to simply detach and mix individual leaves taken randomly from the entire laboratory sample and employ those for analysis without any further processing.

A defined portion of the homogenate (typically 50 g) is taken for analysis.

6.1.1. Vegetables, fruits and potatoes

A 50 g (± 1 %) representative portion of the sample is weighed into a cleavage vessel (see 5.2.) and 25 mL isooctane are added. Then 150 mL of hydrolysis reagent (tin (II)-chloride in hydrochloric acid (see 4.11) are added and the Schott glass is immediately closed with a screw-cap with septum. The Schott glasses are put into a shaking-water bath for 2 hours at 80º C. After half an hour the glasses are shaken upside-down in such a way that all sample parts that could have possibly stuck to the cap get contact with the hydrolysis reagent. Afterwards, the reaction mixture is cooled down to 30º C, e.g. in a cooling water bath. 1 mL of the isooctane-phase is pipetted into a GC vial for analysis.

6.1.2. Nuts, cereals, pulses, oil seeds, cereal products and dried fruits

Weigh out a 50 g (± 1 %) representative portion of the sample and add 45 mL of water and 25 mL of isooctane and continue with the cleavage reaction as in described in 6.1.1.

6.1.3. Dried herbs

Weigh out 10 g (± 1 %) of the sample and add 50 mL of water and 25 mL of isooctane and continue with the cleavage reaction as in described in 6.1.1.

6.2. Recovery

Usually a recovery is worked up with each sample sequence. A blank sample (containing no dithiocarbamates above the detection limit) is used for spiking. A specific amount of thiram working solution (see 4.10 or 4.11) is pipetted on 50 g of the blank sample.

<u>Recovery example:</u>

Spike 50 g of analytic sample with 320 µL of the thiram working solution (see 4.11) The amount of thiram added to the sample theoretically corresponds to 0.4 mg CS_2 /kg sample. Continue as in 6.1.1.

6.3. Blank Sample

As a rule, one blank sample is run within each sequence. Proceed according to 6.1.1.

6.4. Calibration solutions

Calibration solutions containing increasing CS_2 concentrations (see 4.9) are pipetted into GC vials, which are then closed.

6.5. GC Conditions

Exemplary measurement conditions are given below:

Gas Chromatograph: HP 5890

Autosampler: Combi Pal AS-CC

Column: 60 m x 0.25 mm x 1 µm, CP-SIL 8CB

Carrier Gas: helium, constant flow 1 mL/min

Injection Volume: 2 µL

Sample Injection: splitless; splitless time 0.20 min

Injector Temperature Program:	60℃, initial time: 0. 2 min
	1st ramp: heating rate: 10℃/s
	final temperature: 240℃
	keep 1 min
	2nd ramp: heating rate: 10℃/s
	final temperature: 260℃
	keep 5 min
GC Oven Temperature Program:	45℃, initial time: 0. 5 min
	1st ramp: heating rate: 5℃/min
	final temperature: 80℃
	2nd ramp: heating rate: 20℃/min
	final temperature: 260℃
	keep: 15 min

Detector: ECD

6.6. Instrument Check

The instrument check is carried out by means of the retention time, peak areas and sensitivity of the calibration solutions. The signal-to-noise ratio of the 0.08 µg/mL standard solution peak has to be at least 3:1.

6.7. Sample Sequence

As a rule, the following injections are made in every sequence:

The reagent blank, matrix blank, recovery, 4 different CS_2 concentrations (see 4.9) and sample extracts.

7. Evaluation of Results

The peak areas of the calibration solutions are used to calculate the linear regression by plotting the peak area of each standard against its CS_2 concentration. The CS_2 concentrations in the sample extracts are calculated using this calibration curve.

Calibration curve equation: y = mC + b

The CS_2 concentration is calculated as follows:

X= C x 25 (isooctane volume in mL)/ sample weight (g)

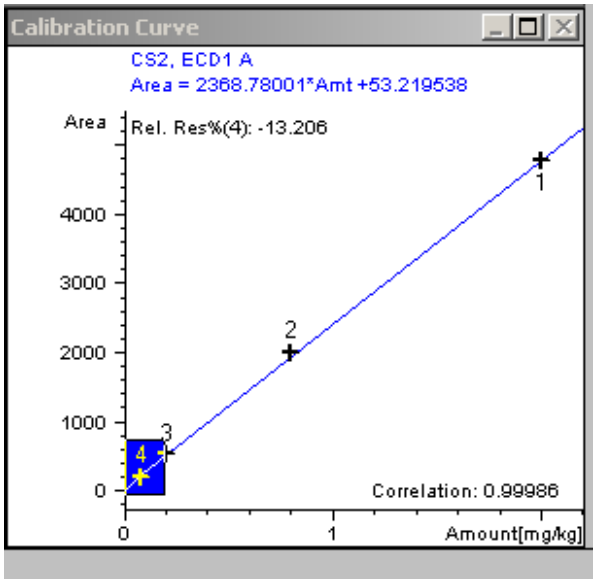
X= CS_2 concentration expressed in mg/kg

C= CS_2 concentration in the sample extracts in µg/mL calculated from the calibration curve.

In the case that the calculated CS_2 amount in the sample extracts exceeds the highest calibration level (2 µg/mL) by more than 20%, the sample extract has to be diluted appropriately so that the concentration of the extract lies within the calibration curve.

Calculation example of a real sample (cucumber):

Calibration curve resulting from the injection of the calibration standards:



Calibration curve equation:

y = mC + b

m = 2368.78

b = 53.22

C = concentration [µg/mL]

y = area

Calculation

Sample weight = 50 g

Peak area of CS_2 in cucumber extract: y = 980.22

Resulting equation: 980.22 = 2368.78C + 53.22

C = (980.22 - 53.22) / 2368.78

C = 0.39 µg CS_2 /mL (lays within calibration curve)

The calculated CS_2 content in the sample X is:

X = (0.39 µg/mL x 25 mL) / 50 g

X = 0.195 mg/kg rounded to 0.20 mg/kg

8. Quality control

As a rule, one recovery is examined in each series of samples. The average recovery rate for CS_2 has to lie between 70 and 120 %. The determination range is 0.04 - 1.0 mg/kg CS_2. The recovery chromatograms are filed away with the corresponding calibrations.

9. Advice

- 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.
- Vulcanized latex gloves can contain traces of carbon disulphide and, therefore, mustn't be used.

10. Attachment

Exemplary chromatograms: The retention time for CS_2 is 8.5 min.

Fig.1: reagent blank

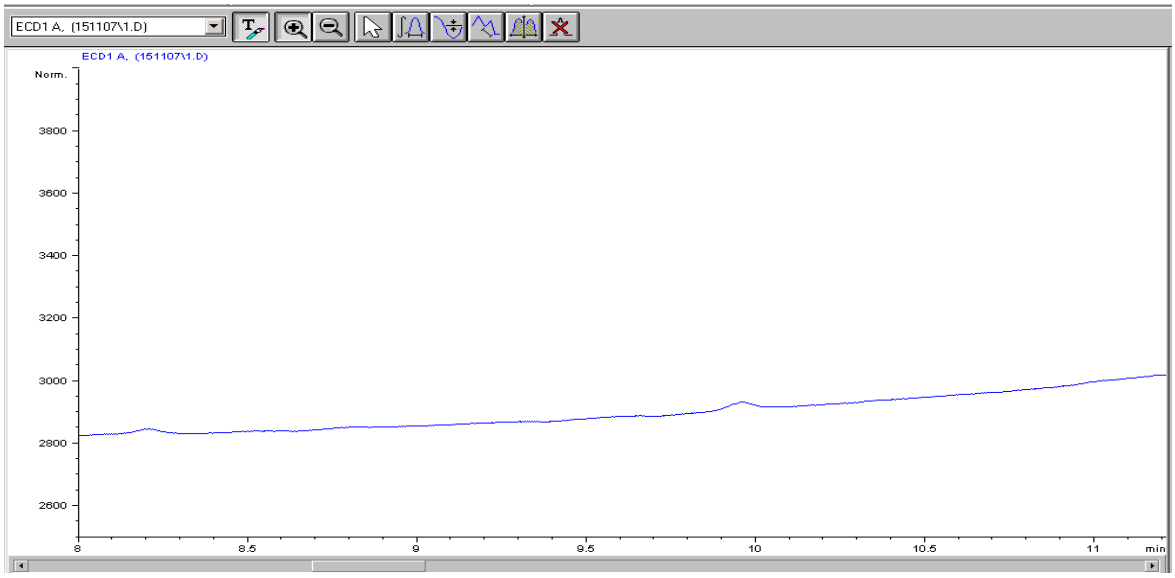


Fig.2: lowest calibration level 0.08 mg/kg CS_2

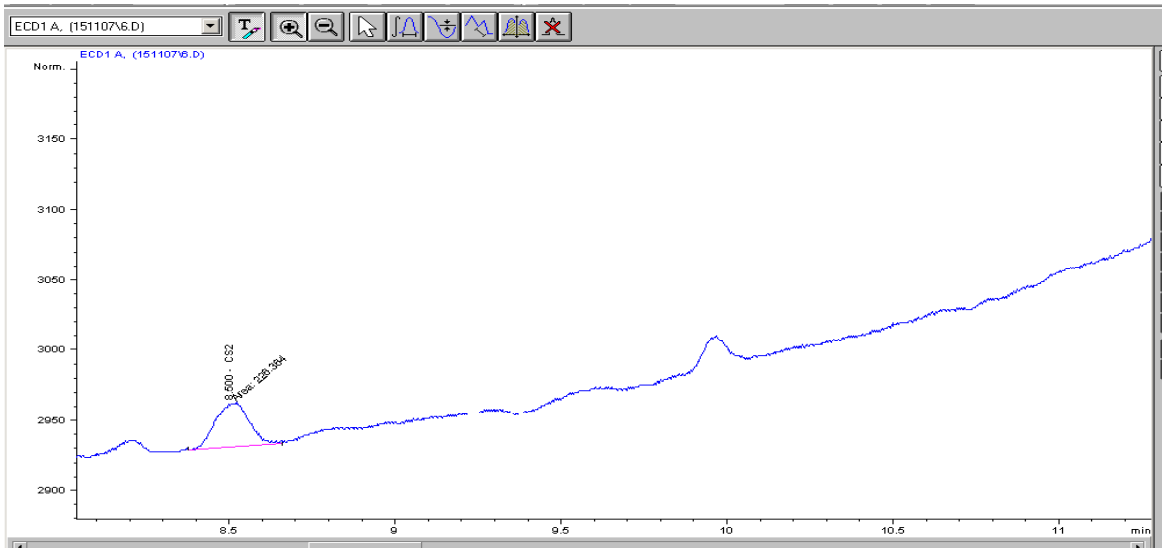
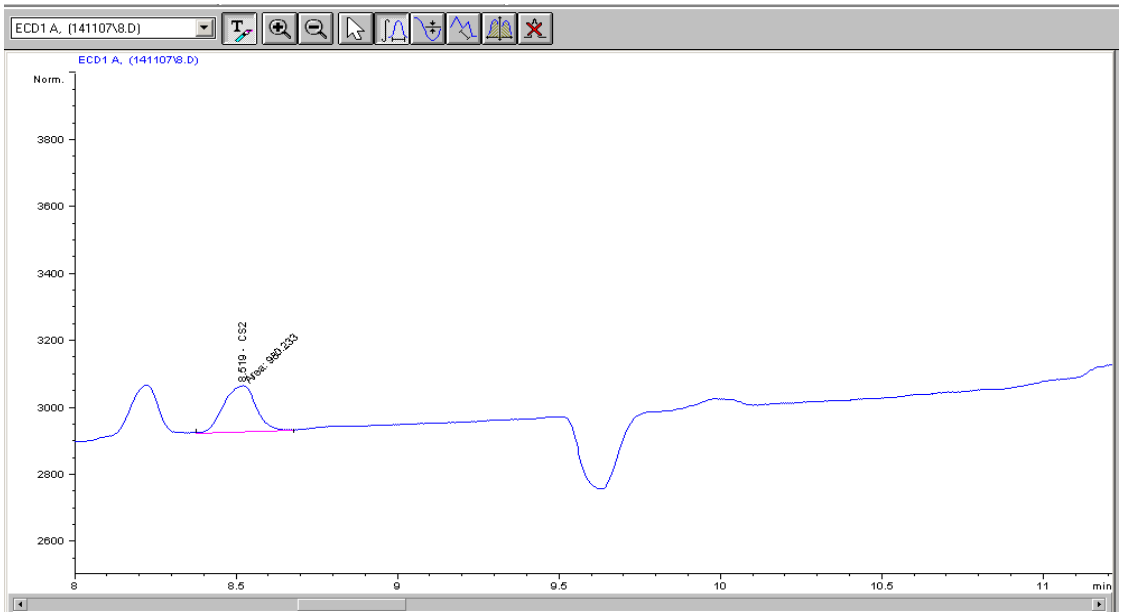


Fig.3: cucumber sample with 0.20 mg/kg CS_2



11. References

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12. Document History

Action	When?	Details on changes introduced	Version
Elaboration the method	2008		
Drafting of document	2008-2009		**V1**
Placing of document in CRL-Website	Mar 2009		**V1**
Updating of document	Dec 2009	- Addition of exemplary chromatograms - Improvement of sample preparation part - removal of text errors	**V2**