

Analysis of Dithiocarbamate Pesticides by GC-MS

Soma Dasgupta^{1,2}, Sumaiyya Mujawar¹, Kaushik Banerjee¹, Hans-Joachim Huebschmann³

¹National Research Center for Grapes, Pune, India, ²Thermo Fisher Scientific, Mumbai, India,

³ Thermo Fisher Scientific, Singapore

Keywords: Food safety, Dithiocarbamate fungicides, DTCs, Hydrolysis, Thiram, GC-MS, SIM

Introduction

The class of dithiocarbamate fungicides (DTCs) is widely used in agriculture. They are non-systemic and both the formulation and their break-down products typically remain at the site of application. DTCs are characterized by a broad spectrum of activity against various plant pathogens, low acute mammal toxicity, and low production costs ^[1]. The dithiocarbamate moiety is highly reactive: it readily chelates most heavy metals, reacts with sulfhydryl groups of proteins, rendering itself neurotoxic, teratogenic, and cytotoxic.

DTCs are not stable and cannot be extracted or analyzed directly. Contact with acidic plant juices degrades DTCs rapidly and they decompose into carbon disulfide (CS₂) and the respective amine ^[1]. It is not possible to homogenize plant samples and extract DTCs by organic solvents, as it is, for instance, with the QuEChERS standard procedure in pesticide-residue analyses. Maximum residue levels (MRLs) of DTCs are generally expressed as mg CS₂/kg food.

Dithiocarbamates can be quantitatively converted to carbon disulphide by reaction with tin(II)chloride in

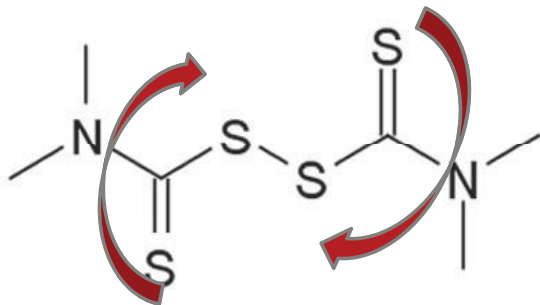


Figure 1. Thiram - 1 mole of Thiram generates 2 mole of CS₂.



aqueous HCl (1:1) in a closed bottle at 80 °C. The CS₂ gas produced is absorbed into iso-octane and measured by GC-MS. The analysis of DTCs for this application follows the acid-hydrolysis method using SnCl₂/HCl ^[2]. For method validation of the DTC pesticides, Thiram (99.5% purity) was used as representative bis (dithiocarbamate) compound considering its simple structure (1 mole of Thiram = 2 mole of CS₂ =>1 mg of Thiram theoretically generates 0.6333 mg CS₂, 1 mL of 100 ppm Thiram in 25 g of grapes = 2.5 ppm of CS₂); see Figure 1. The total DTC residues were estimated by analysing CS₂ as the DTC hydrolysis products by GC-MS. This is a non-specific DTC sum method that does not distinguish between the different species of DTCs in the sample. Interferences are known from natural precursors e.g. from crops or brassica, that can produce CS₂ as well during the hydrolysis ^[1,2].

Sample Preparation

A previously reported SnCl₂/HCl acid-hydrolysis method was employed for sample preparation ^[3]. The described method follows the established methods applied in the EU reference laboratories and European commercial testing laboratories for CS₂ analysis. From the homogenized sample, 25 g are taken in a 250 mL glass bottle, 75 mL of the reaction mixture is added, followed by 25 mL iso-

octane. The bottle is closed immediately (gas-tight) and placed in a water bath at 80 °C for 1 h with intermittent shaking and inverting the bottle every 20 min. After cooling the bottle to < 20 °C by ice water, a 1-2 mL aliquot of the upper isooctane layer is transferred into a micro centrifuge tube, and centrifuged at 5000 rpm for 5 min at 10 °C. The supernatant is then transferred into GC vials, and the residues of DTCs are estimated by determining the CS₂ concentration by GC-MS. The sample preparation procedure depending on the type of food used takes approx. 1-2 hrs.

Preparation of Standard Solutions and Reaction Mixture

For method validation, Thiram (99.5% purity) was used as representative DTC compound considering its simple structure (1 mole of Thiram = 2 mole of CS₂).

Carbon disulphide standard solution

A stock solution of CS₂ (2000 µg/mL) was prepared by accurately pipetting out 79.0 µL of CS₂ into a volumetric flask (certified A class, 50 mL) containing approximately 45 mL of iso-octane and made up to 50 mL with iso-octane. The CS₂ stock solution was kept in a refrigerator at -20 °C and used within two days of preparation. The CS₂ working standard solutions of 200 and 20 µg/mL concentrations (10 mL each) were prepared by serial dilution of stock solution with iso-octane.

Standard Solution of Thiram

10 mg (± 0.05) of Thiram was weighed into a 10 mL volumetric flask (certified A class) and dissolved in ethyl acetate up to the mark to get a stock solution of 1000 µg/mL. A 100 µg/mL Thiram working standard was prepared from stock solution by 10-times dilution.

Preparation of Reaction Mixture

An amount of 30 g of tin (II) chloride was accurately weighed in the 1000 mL volumetric flask (certified A

class) to which 1000 mL of concentrated HCL (35%) was added. The solution was then gradually added to 1000 mL water with continuous stirring until a clear solution was obtained.

Calibration Standards

Calibration standard solutions of CS₂ at six different concentration levels (0.04, 0.08, 0.16, 0.32, 0.64, and 1.3 µg/mL) were prepared by appropriate dilutions of 20 µg/mL CS₂ working standard in iso-octane.

Matrix matched standards at the same concentrations were prepared by spiking the iso-octane extract of fresh control grapes, potato, tomato, green chili, and eggplant (all organically grown) using the following formula derived from above conversion of Thiram to CS₂:

$$\text{Spike quantity} = \frac{\text{Concentration to be achieved} \times \text{weight of the sample}}{0.6333 \times \text{concentration of the stock solution}}$$

Before the preparation of matrix matched standards, the control samples were carefully monitored for absence of DTCs (in terms of CS₂).

Experimental Conditions

A Thermo Scientific™ TRACE GC Ultra™ gas chromatograph equipped with Thermo Scientific™ Triplus™ RSH liquid autosampler and coupled to a Thermo Scientific™ ITQ™ 900 ion trap mass spectrometer was used for analysis. See Tables 1 and 2 for instrument parameters.

Two GC columns of different polarity, stationary phase, and film thickness have been evaluated. The first column was a medium polarity cyanopropylphenyl phase (6% cyanopropylphenyl/94% dimethyl polysiloxane, 30 m x 0.32 mm ID, 1.8 µm film thickness, e.g. Thermo Scientific™ TraceGOLD™ TG-624, p/n 26085-3390) and as a second column a low polarity 5%-phenyl stationary

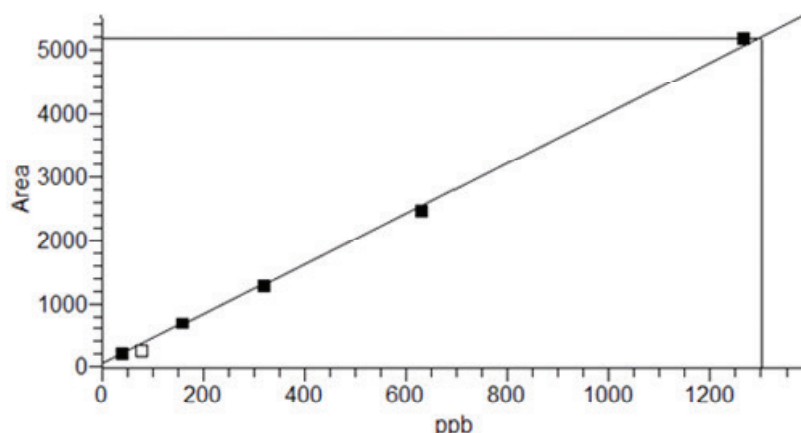


Figure 2. Calibration curve, range 0.04 - 1.300 µg/mL Thiram matrix spike, R₂ = 0.9990.

phase (5% diphenyl/95% dimethylpolysiloxane, 30 m x 0.25 mm ID, 0.25 µm film thickness, e.g. Thermo Scientific™ TraceGOLD™ TG-5MS p/n 26098-1420). The TG-624 column type is a mid-polarity column ideally suited for the analysis of volatile analytes, whereas

the TG-5MS column is more commonly used especially for pesticide analysis and is commonly available in all laboratories. Both columns were thus tested for the applicability of the method. Either column can be used for the DTC analysis.

Table 1. GC Conditions

Injector, temperature prog.	PTV-LVI
	40 °C, 0.1 min (injection phase, @ 100 kPa)
	10 °C/min to 80 °C, 0.3 min (@ 200 kPa)
	10 °C/min to 110 °C (transfer phase)
	14.5 °C/min to 290 °C (cleaning phase)
Split flow	20 mL/min
Solvent vent	open until 0.17 min
	closed until 4.17 min
	open until end of run
Injection mode, volume	split, 4 µL
Carrier gas, flow	Helium, constant flow 1 mL/min
Oven program	40 °C, 5 min
	40 °C/min to 200 °C
	200 °C, 5 min
Transfer line temperature	205 °C

Table 2. MS Conditions

Ionization	El, 70 eV
Scan mode, range	SIM, m/z 76, 78
Acquisition rate	2 scans/s
Ion source temperature	200 °C (optimized for CS ₂ S/N ratio)

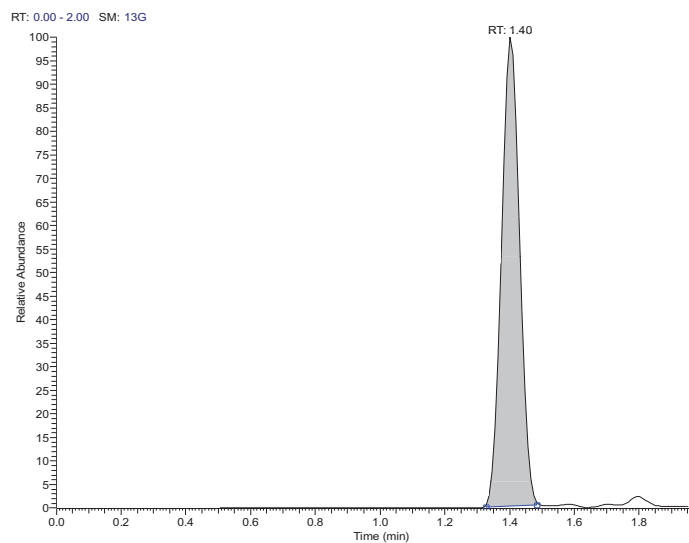


Figure 3. CS₂ chromatogram, 5 ppb matrix spike calibration.

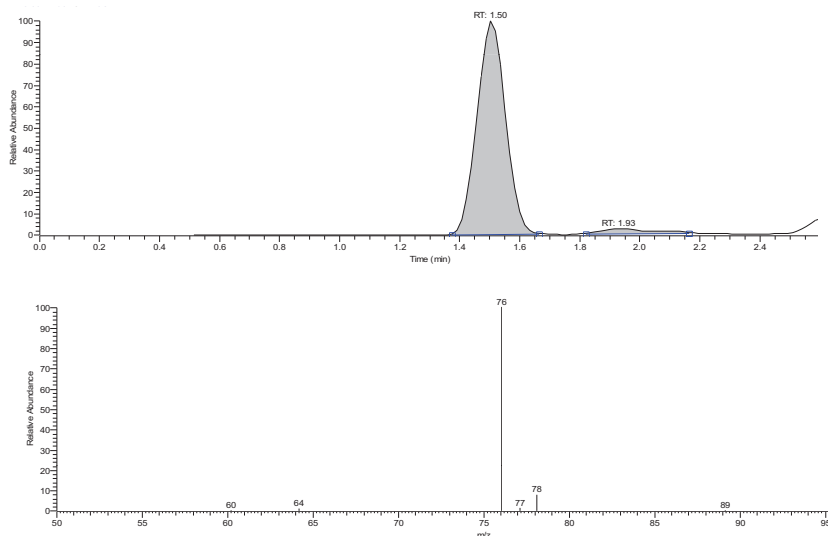


Figure 4. Chili sample analysis with confirming CS₂ ion ratio 100:10 for *m/z* 76:78.

Sample Measurements

A typical GC-MS batch consisted of matrix-matched calibration standards, samples, one matrix blank and one recovery sample for performance check after a set of every six samples.

The data acquisition was carried out in Full Scan mode using the compound-specific ions *m/z* 76 and 78 (the 34S isotope, ion ratio 10:1) as extracted chromatograms for a selective identification of CS₂.

Results

Sensitivity

The sensitivity of the method was evaluated in terms of the limit of detection (LOD) and limit of quantification (LOQ) which were respectively 0.005 and 0.04 µg/mL. The LOD is the concentration at which the signal to noise ratio (S/N) for the quantifier ion is > 3, whereas LOQ is the concentration for which the S/N is > 10.

Recovery

The recovery experiments were carried out on fresh untreated potato, tomato, eggplant, green chili, and grapes by fortifying 25 g of the samples with Thiram solution at 0.04, 0.16, and 1.30 µg/g levels in six replicates. The control samples of each of the tested commodities were obtained from an organic farm near Pune, India, and screened for absence of DTC residues before spiking. The spiked the samples were extracted using the sample preparation method described above. The quantitation of the residues was performed using matrix matched standards.

Table 3. Recoveries from different foods:

Spike level [ppb]	Grapes [%]	Chili [%]	Potato [%]	Egg plant [%]	Tomato [%]
1300	96 (±4)	81 (±10)	90 (±9)	90(±5)	81 (±4)
160	94 (±10)	80 (±13)	94 (±10)	92 (±8)	85 (±10)
40	104 (±15)	79 (±9)	104 (±15)	86 (±10)	96(±15)

Precision

The precision of repeatability was determined by three analysts preparing six samples each on a single day. The intermediate precision was determined by the same analysts with six samples each on six different days. The method precision was determined with 0.04 mg/kg.

General Guidelines for DTC Analysis

The analysis of cruciferous crops, including brassica samples, may not be unequivocal, because they contain naturally occurring compounds that may generate carbon disulfide.

It is necessary to avoid the use of rubber material (natural/synthetic) e.g. gloves, when performing DTC analyses as they contain dithiocarbamates, and this could lead to contamination problems. Silicone rubber and polyethylene do not contain dithiocarbamate.

Samples, other than fresh foodstuffs, will be comminuted by cryogenic milling. Fresh samples should be sub-sampled prior to extraction by removing segments from fresh samples following current Codex Alimentarius guidelines.

The samples should be analyzed within 4 weeks of cryogenic milling. If the storage of fresh produce is necessary it should be in a cool place (<-10°C) keeping condensation at minimum ^[4].

Conclusions

A reliable routine method for the analysis of dithiocarbamates with high precision in different vegetable and fruits has been developed. The method allows a wide calibration range of 0.04 – 1.300 µg/mL Thiram. The LOQ has been determined as 0.04 µg/mL.

The extraction uses a SnCl₂/HCl acid-hydrolysis with iso-octane as solvent to form CS₂ which finally gets quantified by GC-MS. The recovery from different food commodities has been shown to be very high with 79 to 104%.

The GC injection method and column separation has been optimized for the injection of 4 µL of extract, using GC columns of standard film and dimensions, typically used for other types of residue analysis as well, so that a column change to a specific column for CS₂ determination only is not required.

The mass spectrometer ion source conditions had been optimized for best sensitivity and S/N ratio. The analysis in SIM mode is preferred providing a high selectivity with easy to integrate chromatograms.

This method has been developed initially for the ITQ ion trap mass spectrometer, but the same parameter setup is suitable for the Thermo Scientific™ ISQ™ series single quadrupole or Thermo Scientific™ TSQ™ Quantum XLS Ultra or Thermo Scientific™ TSQ 8000™ triple quadrupole mass spectrometers, as well.

References

- [1] Crnogorac, G., Schwack, W., Residue analysis of dithiocarbamatefungicides, Trends in Analytical Chemistry, Vol. 28, No. 1, 2009.
- [2] Reynolds, S., Analysis of Dithiocarbamates, presented at the SELAMAT Workshop, Bangkok 2006.
- [3] Analysis of Dithiocarbamate Residues in Foods of Plant Origin involving Cleavage into Carbon Disulfide, Partitioning into Isooctane and Determinative Analysis by GC-ECD. http://www.crl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCS2_EurlSrm.PDF
- [4] Amvrazi, Elpiniki G., Fate of Pesticide Residues on Raw Agricultural Crops after Postharvest Storage and Food Processing to Edible Portions, Pesticides - Formulations, Effects, Fate, Prof. Margarita Stoytcheva(Ed.), ISBN: 978-953-307-532-7.

www.thermoscientific.com

©2012 Thermo Fisher Scientific Inc. All rights reserved. ISO is a trademark of the International Standards Organization. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



Thermo Fisher Scientific,
Austin, TX USA is ISO 9001:2008 Certified.

Africa-Other +27 11 570 1840
Australia +61 3 9757 4300
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 10 8419 3588
Denmark +45 70 23 62 60

Europe-Other +43 1 333 50 34 0
Finland/Norway/Sweden
+46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9434
Italy +39 02 950 591

Japan +81 45 453 9100
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Russia/CIS +43 1 333 50 34 0
South Africa +27 11 570 1840

Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo
SCIENTIFIC

Part of Thermo Fisher Scientific

AN10333_E 07/13S