

# Analysis of Dithiocarbamate Pesticides in Tea Using GC/MS/MS



## Authors

Bappaditya Kanrar,  
Narayan Bhowmick, and  
Ananya Ghosh  
TLabs,  
Tea Research Association,  
Kolkata, India

Soma Dasgupta and  
Vivek Dhyani  
Agilent Technologies, India

## Abstract

Dithiocarbamate pesticides are commonly used to control fungal diseases in ornamental plants and crops, and for control of tea gray blight. The residue definition for dithiocarbamate pesticides is expressed as total dithiocarbamate, determined as mg CS<sub>2</sub>/kg. Methods for analysis of CS<sub>2</sub> use extraction of the sample into an acidified stannous chloride solution with isooctane, where the dithiocarbamate residues are converted to CS<sub>2</sub>, which is captured in isooctane and analyzed by GC/MS. This application note describes the analysis of dithiocarbamate residues in tea using an Agilent GC/MS/MS in both SIM and MRM modes. A limit of quantification of 10 ppb was achieved in tea samples. Linearity was established in matrix-matched standards with R<sup>2</sup> > 0.999 for the range from 5 to 100 ppb, with average recoveries > 85% at the 10 ppb level of fortification.

## Introduction

India is the second largest producer of tea, with total production of 1.374 billion kg in fiscal year 2022 to 23 (approximately 10% of total global exports).<sup>1</sup> However, the climatic conditions of the tea growing regions are conducive to many plant diseases, resulting in close to 15 to 30% crop loss; the fungal disease tea gray blight accounts for 17 to 46% crop loss, and is spreading across the tea gardens.<sup>2</sup> Dithiocarbamate pesticides such as mancozeb and zineb are thus recommended for the control of fungal diseases. There are several dithiocarbamate pesticides, classified as propylene-bis-dithiocarbamates (e.g., propineb), ethylene-bis-dithiocarbamates (e.g., mancozeb, maneb, and zineb), and dimethyl dithiocarbamates (e.g., thiram, ziram, and ferbam) based on their structure. While dithiocarbamates undergo relatively rapid environmental degradation in air, water, and soil through processes such as photolysis and hydrolysis, they remain among the most commonly detected pesticides in tea, frequently exceeding the maximum residue level (MRL). The European Commission established MRL for dithiocarbamates is 0.05 to 25 ppm in various plants and products of vegetable or animal origin, and 0.1 in tea (expressed as  $CS_2$ , including maneb, mancozeb, metiram, propineb, thiram, and ziram).<sup>3</sup> The FSSAI (Food Safety Standard Authority of India) has set the MRL for ferbam as low as 0.01 mg/kg.<sup>4</sup>

The EURL method for the analysis of dithiocarbamate pesticides involves cleavage of the pesticides into  $CS_2$ , partitioning into isooctane, and determination by GC-ECD or GC/MS/MS.<sup>5,6</sup> During sample preparation for dry matrices, the sample is often diluted 5 to 10x. To achieve the MRL of 0.01 ppm in tea, the instrument should be able to attain detection levels 5 to 10x lower in matrix, reaching up to 0.001 to 0.002 ppm.

With GC-electron capture detector (ECD)-based analysis, for complex matrices such as tea, attaining low detection levels is difficult due to elevated baseline and several other interferences from the matrix. Thus, a method for GC/MS and GC/MS/MS analysis of dithiocarbamate pesticides in tea matrix was developed.

## Experimental

### Stock solution

NIST-traceable certified reference standards of carbon disulfide were procured from Dr. Ehrenstorfer. Isooctane (HPLC grade) and hydrochloric acid (37%, ACS grade) were procured from Merck, India. Stannous chloride was procured from Sigma-Aldrich. Type 1 water was obtained from Milli-Q (Millipore, Bedford, MA, resistivity 18.2 M $\Omega$ cm at 25 °C).

### Calibration standards and sample preparation

Matrix-matched calibration standards in the range of 5 to 100 ppb and recovery samples fortified at 10 ppb were prepared as per the sample preparation protocol in Figure 1.

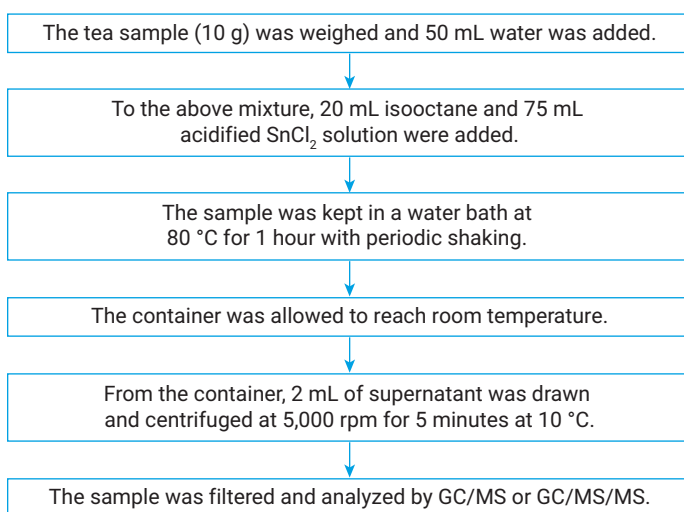
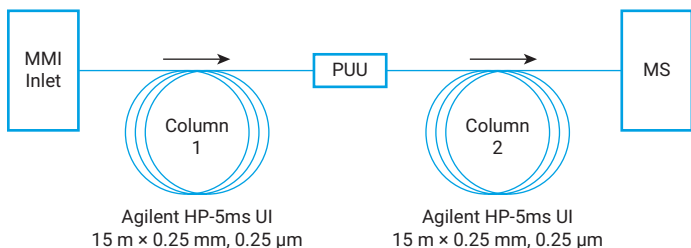


Figure 1. Sample preparation workflow.

## Instrumentation and analytical conditions

An Agilent 7890/8890 GC with Agilent 7000D/E or 7010C MS/MS equipped with a multimode inlet was used for analysis. The instrument setup is shown in Figure 2. GC and MS conditions are shown in Table 1. Data acquisition and processing was performed with MassHunter software.



**Figure 2.** System configuration schematic for the analysis of dithiocarbamate pesticides as  $\text{CS}_2$ .

The GC setup for this analysis is like the mid-point backflush setup for pesticide analysis. Hardware changes are therefore not required, and the samples can be appended to a regular pesticide batch by updating the acquisition method.

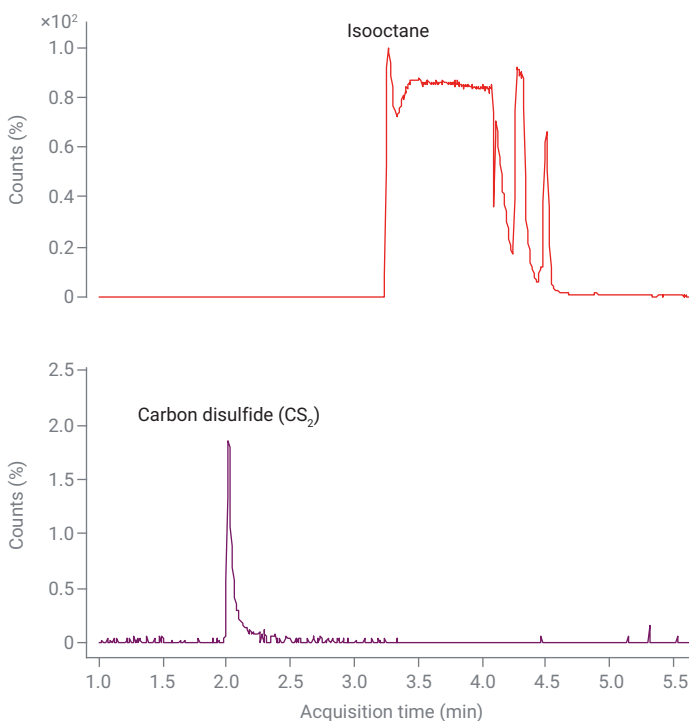
**Table 1.** Analytical conditions for the Agilent 7890/8890 GC with 7000D/E and 7010C MS/MS.

Parameter	Value
<b>Agilent 7890/8890 GC</b>	
Injection Volume	2 $\mu\text{L}$
Liner	Agilent inlet liner, UI, splitless, dimpled (p/n 5190-2297)
Carrier Gas	Helium
Injection Mode	Split
Split Ratio	15:1
Injector Program	70 °C (Hold time 0.1 min) 280 °C (Ramp at 100 °C/min)
Oven Program	40 °C (Hold for 5 min) 200 °C (Ramp at 40 °C/min, hold for 2 min) Total run time: 11 min Backflush at 300 °C for 5 min
Column 1 and 2	HP-5ms Ultra Inert (15 m x 250 $\mu\text{m}$ , 0.25 $\mu\text{m}$ )
Column Flow 1 and 2	1 and 1.2 mL/min
<b>Agilent 7000D/E and 7010C MS/MS</b>	
Source Temperature	230 °C
Quadrupole Temperature	Q1 = Q2 = 150 °C
Collision Cell Gas Flows	$\text{N}_2$ : 1.5 mL/min He: 2.25 mL/min (for 7000D/E) and 4 mL/min (for 7010C)
SIM Ions	76, 78; Dwell 150 ms each
MRM Parameters	76.0 $\rightarrow$ 44.0 CE 44, dwell 75 ms 76.0 $\rightarrow$ 46.0 CE 58, dwell 75 ms 78.0 $\rightarrow$ 44.0 CE 45, dwell 75 ms 78.0 $\rightarrow$ 46.0 CE 50, dwell 75 ms

## Results and discussion

### Optimization of injection parameters

A basic functional requirement of GC inlets is that they introduce the sample into the column as a narrow band having a composition that is identical to the original sample. The inlet should not produce peaks that are wider than the peak width that will result from the column band broadening process.<sup>7</sup> For  $\text{CS}_2$  analysis, the sample preparation solvent used is isooctane. The boiling point of isooctane (99 °C) is higher than that of  $\text{CS}_2$  (46.3 °C). This results in poorly controlled solvent effects due to the combination of a relatively high-boiling solvent with low boiling analytes. Hot spitless injections result in poor peak shapes for  $\text{CS}_2$ . Better peak shapes are obtained with cold injections in split mode (Figure 3). Further improvement in peak shapes is obtained by using a multibaffled liner (part number 5190-2297). As shown in Figure 3, the isooctane peak (RT = 3.2 minutes onwards) elutes after the  $\text{CS}_2$  peak (RT = 2.013 minutes); therefore, the detector was switched off after  $\text{CS}_2$  elution at 2.9 minutes.



**Figure 3.** Separation of  $\text{CS}_2$  and isooctane.

### Matrix-matched calibration

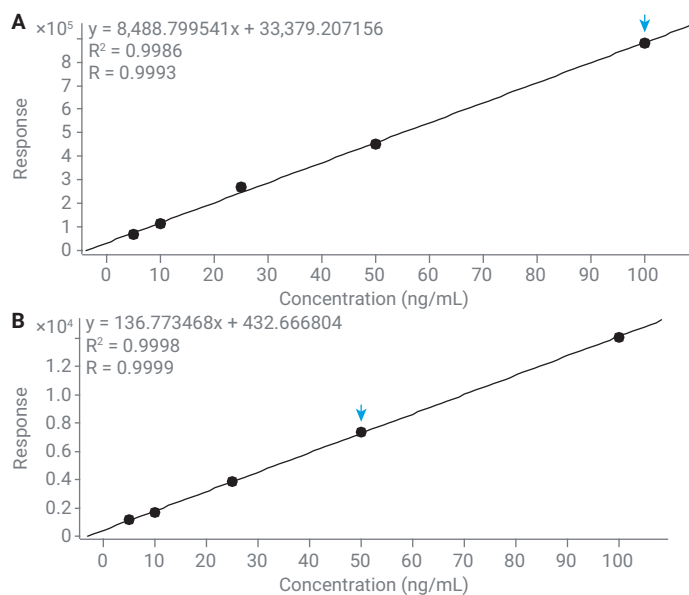
The calibration performance was assessed using a set of matrix-matched calibration standards ranging from 5 to 100 ppb (w/v), specifically 5, 10, 25, 50, and 100 ppb.

These concentrations, expressed in ppb (w/v), represent the pesticide concentration in the injected sample.

Due to the sample preparation process, a dilution factor of 2 was applied. Therefore, the reported concentration in ppb in the sample corresponds to  $\mu\text{g}/\text{kg}$  in the original commodity. The calibration performance of the method was validated using 7000D, 7000E, and 7010C GC/MS/MS, following the analytical method validation and performance criteria specified in SANTE 11312/2021v2.<sup>8</sup> Linearity was established with  $R^2 > 0.999$  in both SIM and MS/MS modes.  $\text{CS}_2$  peaks could be detected up to 5 ppb in 7000D, 7000E, and 7010C GC/MS/MS.

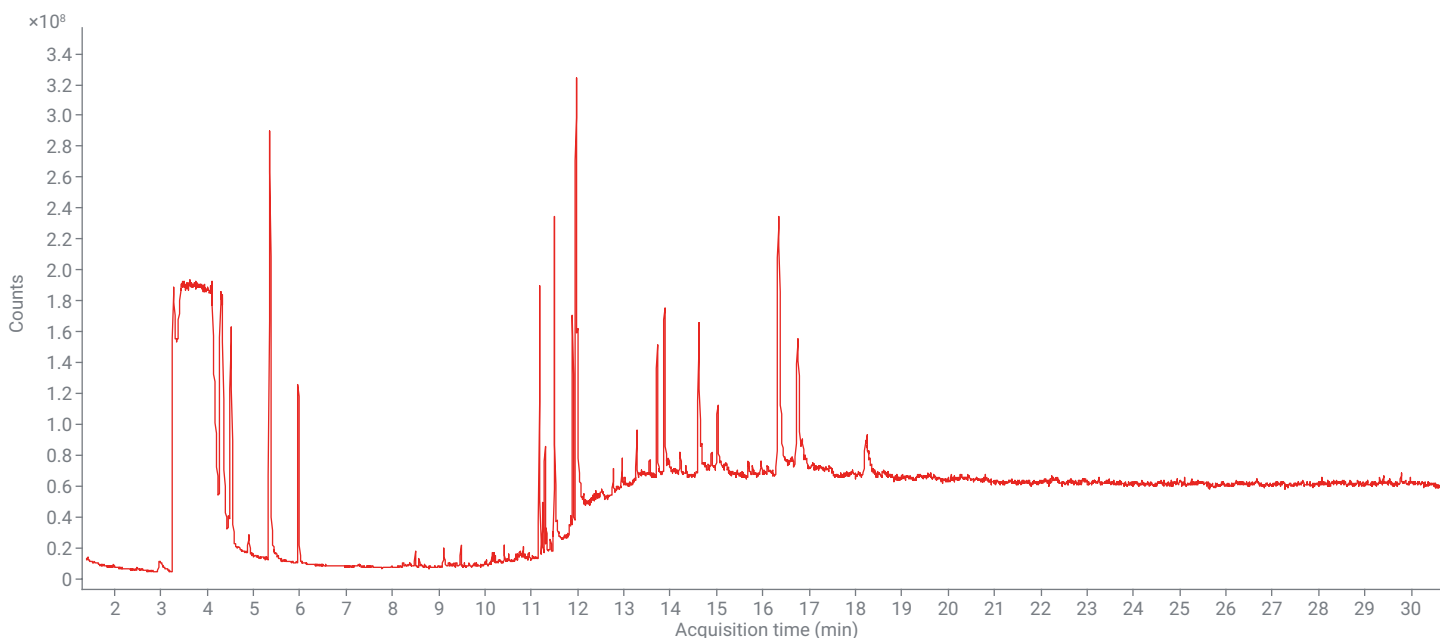
### Advantages of backflush

The tea matrix is known to be rich in co-extractive compounds, many of which have high boiling points. These compounds are also injected into the GC column along with the target compound (Figure 5). However, due to their high boiling points, these compounds tend to elute late in the chromatographic run. As a result, a minimum run time of 19 minutes is typically required to ensure these high boilers are adequately removed from the column, even though the target analyte of interest elutes much earlier at 2.013 minutes.



**Figure 4.** Matrix-matched calibration curves in SIM (A) and MS/MS (B) for the Agilent 7010C GC/MS/MS.

To address this issue, an effective backflush method can be used. Backflushing involves reversing the flow of the carrier gas in the column, pushing the high-boiling compounds back towards the injection port, where they are vented out of the system. By doing so, the backflush method not only helps in removing the unwanted matrix components, but also significantly reduces the total run time of the analysis.



**Figure 5.** The total ion chromatogram of the tea sample shows a high concentration of matrix components, which persist up to 19 minutes.

Implementing a backflush method in the GC/MS analysis of tea samples can lead to several advantages. Firstly, it allows for a more efficient separation of the target analytes from the complex matrix, improving the overall sensitivity and accuracy of the analysis. Secondly, by reducing the run time, the backflush method enhances the throughput of the analysis, allowing for more samples to be analyzed in a given time period. Overall, the use of an effective backflush method is instrumental in achieving optimal results in the analysis of tea samples by GC/MS.

### Repeatability and recovery

Repeatability was assessed by injecting six consecutive samples at a concentration of 10 ppb. The calculated relative standard deviations (RSDs) for these measurements were found to be consistently less than 4.2%, indicating a high level of precision and repeatability. The chromatograms from these injections show average recoveries ranging from 81.62% to 99.83% at 10 ppb level of fortification (Figure 6).

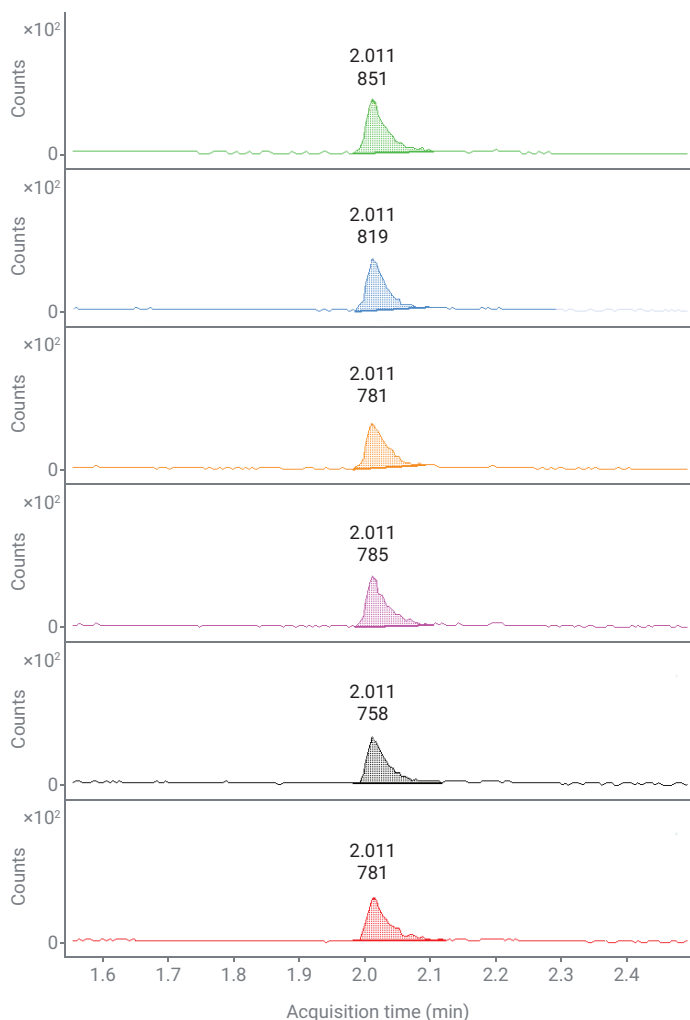


Figure 6. Repeatability of injections of CS<sub>2</sub> samples at 10 ppb.

## Conclusion

Analysis of dithiocarbamate pesticides as CS<sub>2</sub>, using isooctane as a sample preparation solvent, can lead to the combination of a relatively high boiling solvent with low boiling analytes. Hot spitless injections result in poor peak shapes for CS<sub>2</sub>; better peak shapes can be obtained with cold injection in split mode. Further improvement in peak shapes can be achieved by using a multibaffled liner. Matrix-matched calibration standards ranging from 5 to 100 ppb were used to assess calibration performance, with linearity established at  $R^2 > 0.999$  in both SIM and MS/MS modes. The method was validated following the criteria specified in SANTE 11312/2021v2, with the ability to detect CS<sub>2</sub> peaks down to 5 ppb using Agilent 7000D, 7000E, and 7010C GC/MS/MS instruments. Implementing a backflush method in the GC/MS analysis of tea samples can significantly improve the separation of target analytes from complex matrix components, enhancing sensitivity and throughput. Repeatability tests showed RSDs consistently below 4.2% for injections at 10 ppb concentration, indicating high precision and repeatability.

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