

6. DITHIOCARBAMATE ANALYSIS: THE CS₂ PROBLEM

THE MYSTERY OF THE UNTREATED CABBAGE



Imagine a farmer growing cabbages without using any fungicides.

The soil is fertile, the plants grow normally and, at harvest time, some samples are sent to a laboratory to check for any pesticide residues.

The result might come as a surprise.

The analysis detects carbon disulfide, a substance used as an indicator in the determination of dithiocarbamates.

Yet the farmer has not used those fungicides.

How is this possible?

One might suspect accidental contamination, a laboratory error or an undeclared treatment.

But there is another possible explanation, linked to the natural chemistry of plants.

Some vegetables contain substances that, under the conditions used in the analytical method, can release carbon disulfide even when no fungicide residues are present.

A laboratory can therefore obtain a positive result even though a dithiocarbamate has not necessarily been used.

To understand this apparent paradox, we need to explore the world of chemical analysis.

HOW DO WE MEASURE A SUBSTANCE THAT IS DIFFICULT TO IDENTIFY?

When we want to know whether a food contains a particular pesticide, we generally imagine that the laboratory looks directly for that molecule.

For many substances, this is indeed the case.

Modern analytical techniques, such as liquid or gas chromatography coupled with mass spectrometry, make it possible to separate and identify numerous compounds in a food sample.

Dithiocarbamates, however, are a special case.

Some of these fungicides are poorly soluble in commonly used solvents, may have polymeric structures and may degrade during sample preparation.

These characteristics make their direct determination more difficult.

To overcome this difficulty, a different approach was developed and has been widely used.

Rather than looking directly for each fungicide, the laboratory chemically converts dithiocarbamates into a substance that is easier to measure: carbon disulfide, with the formula CS_2 .

It is an ingenious method.

But, as we shall see, it has a fundamental limitation.

CARBON DISULFIDE: A COMMON INDICATOR

Carbon disulfide is a small molecule consisting of one carbon atom and two sulfur atoms.

Its chemical formula is CS_2 .

Some dithiocarbamates release this substance when subjected to particular chemical treatments.

The laboratory can therefore use this reaction to determine the amount of CS_2 formed and obtain an analytical result reported as dithiocarbamates.

It is rather like trying to determine how much wood is in a pile by measuring the amount of gas produced during a controlled reaction.

The system can work, provided we know how the transformation occurs and which other substances might produce the same gas.

And this is precisely where the difficulty arises.

Different dithiocarbamates can release the same CS_2 molecule.

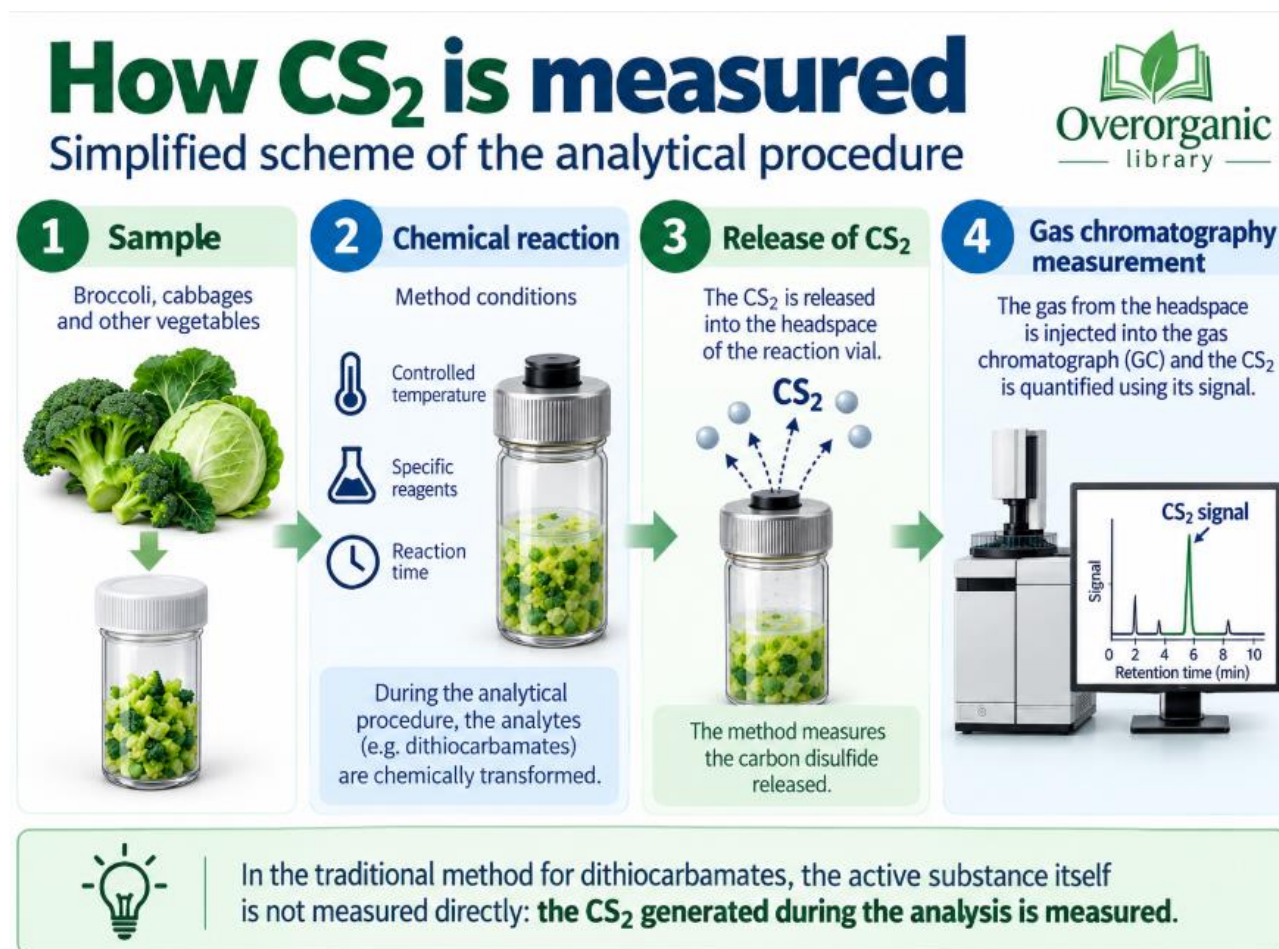
The result can therefore express the amount of carbon disulfide generated during analysis, but it does not necessarily identify the fungicide from which it came.

A value of 0.05 mg/kg of CS₂, for example, does not automatically tell us whether the residue comes from mancozeb, metiram, ziram or another member of the family.

This distinction matters, because these compounds may have different toxicological properties.

WHAT HAPPENS IN THE LABORATORY?

Let us look, in simplified form, at how one of the methods works.



The laboratory takes a representative portion of the food and places it in a reaction system.

The sample is treated with chemical reagents, generally under acidic and controlled conditions.

Some methods also use stannous chloride, a substance that promotes the conversion of dithiocarbamates into carbon disulfide.

The released CS₂ is then separated from the rest of the sample.

It may be collected in the gaseous phase above the sample, known as the headspace, or transferred into an organic solvent.

At this point, a highly sensitive instrument comes into play: the gas chromatograph.

Gas chromatography allows the various volatile substances in the sample to be separated.

When coupled with mass spectrometry, it can identify the target compound and determine its amount.

The result is generally expressed as milligrams of CS₂ per kilogram of food.

A concentration of 0.05 mg/kg corresponds to 50 micrograms of CS₂ per kilogram of product.

These are very small quantities.

The crucial point, however, is that the precision with which CS₂ is measured does not automatically resolve the question of its origin.

The method can correctly identify the final product of the reaction without knowing with certainty which substance originally present in the sample produced it.

CS₂ THAT OCCURS NATURALLY IN PLANTS

Let us return to our cabbage.

Plants belonging to the Brassicaceae family, including cabbages, broccoli, cauliflower, turnips and other species, naturally produce numerous sulfur-containing compounds.

These include glucosinolates and other substances involved in the plant's defence mechanisms.

These compounds also contribute to certain sensory characteristics of vegetables, such as their pungent taste and the smell released during preparation and cooking.

Under particular analytical conditions, some naturally occurring sulfur compounds in vegetables, or their transformation products, can contribute to the formation of CS₂.

Consequently, a method based on measuring carbon disulfide can produce a positive result even for a product that has not been treated with dithiocarbamates.

This phenomenon has been demonstrated experimentally.

In a study published in 2000 in the Journal of Agricultural and Food Chemistry, researchers analysed various Brassicaceae crops grown without pesticide applications.

Under certain sample-treatment conditions, non-anthropogenic CS₂ values of up to approximately 4 mg/kg were detected.

This is a surprising figure.

Two origins, the same analytical signal

Why the CS₂ signal does not always prove a dithiocarbamate treatment

1 Origin 1: dithiocarbamates

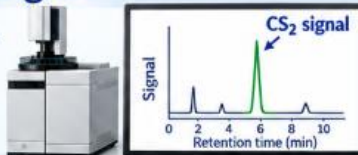


2 Origin 2: natural compounds in Brassicaceae



3 Same CS₂ signal

The gas from the headspace is injected into the gas chromatograph (GC) and the CS₂ is quantified by means of its signal.



A CS₂ signal indicates that the method detected carbon disulfide, **but by itself it does not identify the origin of the signal with certainty.**

To appreciate its significance, remember that laboratory methods can quantify CS₂ at much lower concentrations.

A relatively high analytical result, therefore, does not in itself demonstrate that a fungicide was used.

Of course, this does not mean that all CS₂ found in vegetables is of natural origin.

It means that, in certain plant matrices, natural compounds can significantly interfere with the interpretation of the result.

THE PROBLEM IS NOT LIMITED TO CABBAGES

Brassicaceae are not the only plant group that can show this characteristic.

Some plants belonging to the Alliaceae family, such as garlic, onions and leeks, also naturally contain sulfur-rich organic compounds.

Under certain conditions, these substances may contribute to the formation of the compound measured by CS₂-based analytical methods.

The issue became so important that EFSA addressed it in its 2023 review of maximum residue levels for dithiocarbamates.

Thousands of analytical results from numerous plant products were used to investigate the phenomenon, with particular attention paid to samples from organic farming.

The idea was simple: examining products for which the use of these fungicides is not permitted could help estimate the natural contribution to CS₂ formation.

Overall, the assessment used 9,870 analytical results covering 173 unprocessed plant products.

These data helped characterise background levels of CS₂ and develop proposals for maximum residue levels.

However, it is important to remember that an organic farming origin is not, by itself, absolute proof of the absence of contamination or unauthorised uses.

The data are therefore a useful tool for assessing background levels, not definitive proof of the origin of every individual result.

WHEN AN ANALYSIS IS PRECISE BUT NOT SPECIFIC

Here we encounter two fundamental concepts in analytical chemistry: precision and specificity.

In everyday language they may seem similar, but they are not.

Precision describes how closely repeated measurement results agree under the same conditions.

Specificity, by contrast, concerns the ability of a method to identify a particular substance and distinguish it from others.

Imagine a highly precise scale.

If we weigh the same object ten times, we might obtain ten virtually identical results.

The scale is precise.

But if we place a closed box on the scale, we cannot tell how much each object inside weighs.

The overall measurement may be perfectly correct, but it does not provide all the information we want.

Something similar happens with the CS₂ method.

The laboratory may accurately measure the amount of carbon disulfide present at the end of the reaction, but the result does not necessarily distinguish the different dithiocarbamates that could have generated it.

Moreover, some of the CS₂ may come from natural substances in the plant matrix.

The problem, therefore, is not necessarily the quality of the measurement.

It is the method's ability to attribute the result to a particular original substance.

CAN WE IDENTIFY INDIVIDUAL DITHIOCARBAMATES?

The answer is yes, at least for some substances and under certain conditions.

In recent years, alternative methods have been developed that allow more selective detection of specific fungicides or subgroups of dithiocarbamates.

Some use chemical reactions different from those used to determine CS₂.

Others use chromatographic and spectrometric techniques capable of identifying derivatisation products characteristic of particular chemical structures.

However, there is no universal solution.

More selective methods are available for some dithiocarbamates, while identifying others individually remains complex.

Furthermore, some substances may transform during analysis or share chemical structures that make them difficult to distinguish.

In its 2023 review, EFSA noted that specific methods were available for some substances, including propineb, while difficulties remained for ziram and thiram, partly because they may interconvert during determination.

For the ethylenebisdithiocarbamate group, including mancozeb, maneb and metiram, some methods can distinguish the subgroup but not necessarily each individual active substance.

Analytical research is therefore working to overcome the limitations of traditional methods, but the problem cannot yet be regarded as fully resolved.

WHAT DOES THE LIMIT OF QUANTIFICATION MEAN?

When reading an analytical certificate, we often come across the abbreviation LOQ.

It stands for Limit of Quantification.

It is the lowest concentration at which a method, under the conditions for which it has been validated, can quantitatively determine a substance with performance considered acceptable.

Imagine that a laboratory uses a method with an LOQ of 0.01 mg/kg.

This means that the method has been validated to quantify the target parameter from that concentration upwards.

A result below the LOQ does not necessarily mean that the substance is completely absent.

It may be present at a concentration too low to be reliably quantified by that method.

But in the case of dithiocarbamates, there is an additional complication.

Suppose we obtain a result above the LOQ, expressed as CS₂.

We know that carbon disulfide has been quantified, but not necessarily which fungicide generated it.

Analytical sensitivity and the ability to identify the origin of a residue are therefore two different characteristics.

Lowering the limit of quantification does not, by itself, solve the specificity problem.

Indeed, in certain plant matrices, a more sensitive method might also reveal the contribution of natural substances more frequently.

WHAT HAPPENS IF THE RESULT EXCEEDS THE LEGAL LIMIT?

Imagine that a food sample has a CS₂ value above the applicable maximum residue level.

Can we immediately conclude that a prohibited fungicide was used?

Not necessarily.

The first step is to verify that sampling and analysis were carried out correctly and that the result is compared with the regulatory limit actually applicable.

Measurement uncertainty must also be considered, in accordance with the rules used in official controls.

If the exceedance is confirmed under the relevant criteria, the product may be found non-compliant with residue legislation.

But establishing non-compliance and proving the origin of the residue are two different matters.

Where a result is expressed as CS₂, particularly for foods in which natural formation of this compound is documented, further investigation may be needed to identify the substance responsible.

EFSA itself has recognised that the natural contribution to CS₂ must be considered when setting maximum residue levels.

A positive result therefore does not, on its own, prove that a particular fungicide was used.

At the same time, it would be incorrect to automatically attribute every exceedance to natural causes.

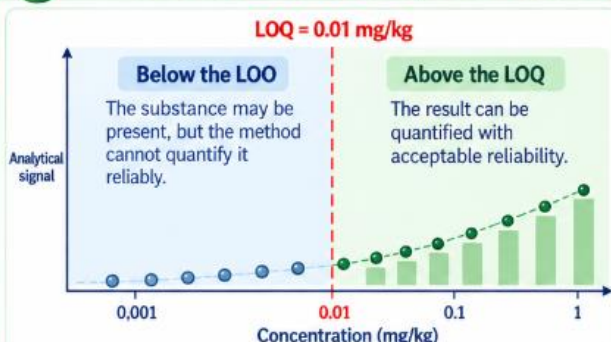
Interpretation must be based on all the available evidence.

LOQ, legal limit and interpretation of the result

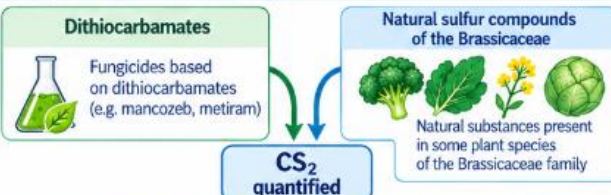
How to correctly read an analytical result expressed as CS₂



1 What does the limit of quantification (LOQ) mean?



CS₂ can have different origins



Analytical sensitivity ≠ identification of the origin

Lowering the LOQ improves sensitivity, but it does not solve the problem of specificity.

In some plant matrices, a more sensitive method can more often detect the contribution of natural substances.

2 What happens if the result exceeds the legal limit?



LOQ, legal limit and origin of the residue are three different levels:
quantification, regulatory compliance and scientific interpretation.

AN IMPORTANT LIMITATION FOR TOXICOLOGICAL ASSESSMENT TOO

So far, we have mainly discussed chemical identification.

But the problem becomes even more significant when we try to assess health risks.

As we saw in the previous chapter, different dithiocarbamates do not necessarily have the same toxicological properties.

Mancozeb, metiram and ziram, for example, have characteristics that must be assessed individually.

If an analysis provides only an overall value expressed as CS₂, we do not necessarily know which substance contributed to the result.

It therefore becomes harder to link that measurement directly to the toxicological profile of a particular active substance.

To address this issue, scientific assessments may use different exposure scenarios, conservatively attributing measured residues to specific substances.

This is a useful approach for estimating potential risks, but it inevitably introduces uncertainties.

EFSA has explicitly discussed these difficulties in its assessment of dithiocarbamate residues.

The laboratory result remains important information, but risk assessment must also consider the actual composition of the residue, relevant metabolites and available toxicological knowledge.

WHAT DOES THE CASE OF CS₂ TEACH US?

We began with a cabbage grown without fungicides and an apparently inexplicable analytical result.

We discovered that certain substances naturally present in plants can interfere with methods used to determine dithiocarbamate residues.

We also saw that different fungicides can produce the same compound during analysis, making it difficult to identify them individually.

This does not diminish the value of laboratory testing.

On the contrary, it demonstrates the importance of understanding analytical methods, their validation and the correct interpretation of results.

Because a chemical analysis is not merely a number printed on a certificate.

It is the outcome of a scientific procedure whose capabilities, limitations and uncertainties must be understood.

The quality of analytical information depends not only on how sensitively a substance is measured, but also on our ability to understand what that measurement actually represents.

This lesson goes far beyond dithiocarbamates.

It applies to many other contaminants for which measuring a chemical indicator does not always allow all the substances that generated it to be identified with certainty.

In the next chapter, we shall temporarily leave dithiocarbamates behind and examine another group of fungicides: fosetyl-aluminium and phosphonates.

Here too, we shall encounter a problem of analytical interpretation, but of a different kind.

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