# A Novel Method for Total Sulfur Determination for Garlic-derived Sulfide Compounds

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## Abstract

Garlic-derived sulfide compounds are well known natural nematicides, especially after European Union approved the use of ECOspray. As sulfur is a component element of these compounds, and elemental sulfur is typically used in the synthesis and remains in the final products, accurate sulfur content determination becomes critical in product purity evaluation. Although sulfur determination on ICP-OES has been challenging, an efficient approach has been developed and successfully utilized at EPL for agricultural products.[1] However, the garlic-derived sulfides pose a new challenge as they are insoluble in water. When tested on the ICP with the assistance of a hydrotrope, inconsistent and erratic signals were observed. In addition, when an average recovery of 180% was obtained for sulfides, and a recovery of 60% for widely used organic sulfur standard MSA (methanesulfonic acid), it was evident that the signal distortion became so detrimental that it invalidated all the preliminary test results. To improve the method an optimized oxidation reaction was performed to transform the sulfides. After the transformation the samples were dissolved in aqueous dimethylformamide (DMF) solution. The ICP conditions were also modified with several critical changes in order to minimize the signal enhancement of the sulfide, the signal suppression of the MSA, and to eliminate sample carry-over. Using the standard diallyl sulfide as a representative of the garlic derived sulfides we demonstrated the efficiency of this method in both accuracy (98 and 102 % recovery) and precision (<3% CV).


## Conclusion

An analytical method for the determination of sulfur in garlic derived sulfide compounds was developed and validated with satisfactory accuracy and precision.

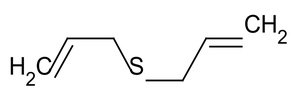
## Objective

Develop and validate an analytical method for the determination of total sulfur for garlic-derived sulfides.

**Materials and Methods**

Chemicals and Reagents
- Sulfur as methane sulfonic acid (MSA) standard
- Diallyl sulfide standard (Fig. 1)
- Yttrium standard
- Cesium standard
- Dimethylformamide (DMF)
- Peracetic acid (or hydrogen peroxide and acetic acid)
- DI water

**Figure 1.** Diallyl Sulfide




**Test Matrices**

The method was validated using MSA as a major standard, diallyl sulfide as an additional standard, and a sample of garlic derived sulfide mixture.

**Equipment**

Inductively Coupled Plasma Optical Emission Spectrometer Perkin Elmer Optima 7300 with WinLab32 software or equivalent. (Waltham, MA, USA)

**Sample Preparation**

Weigh approximately 0.0500 g (0.0450-0.0550 g) of sample into a tared scintillation vial.
Add 5 mL peracetic acid solution.
After vortexing, heat for 3 hours at 40°C (39°C-41°C) in a water bath.
Allow the extracts to cool for about 1- 1.5 hour to ambient temperature.
Pipette 0.5 mL into a 50 mL centrifuge tube, dilute with 8% DMF and mix well.
Fortifications: multiple samples were spiked with 0.2 mL of MSA or 0.05 mL of diallyl sulfide

**ICP-OES Analysis**

Analytes: Sulfur (S) at 181.980 nm
Calibration Parameters: Linear, Minimum Correlation Coefficient 0.99
Calibration standards: 10 - 200 µg/mL of sulfur
Internal standard: Yttrium (Y) at 371.029 nm

**Table 1.** ICP-OES parameters

| | Parameters | Settings |
|---|---|---|
| Gas flow (L/min) | Plasma Flow | 13 |
| | Auxiliary Flow | 0.2 |
| | Nebulizer Flow | 0.8 |
| RF | RF Power (kW) | 1.4 |
| Plasma | View | Radial |
| | View Distance (mm) | 15.0 |
| Sample Introduction | Flow Rate (mL/min) | 1.5 |
| | Rinse (sec) | 80 |
| | Delay Time (s) | 100 |

## Results & Discussion

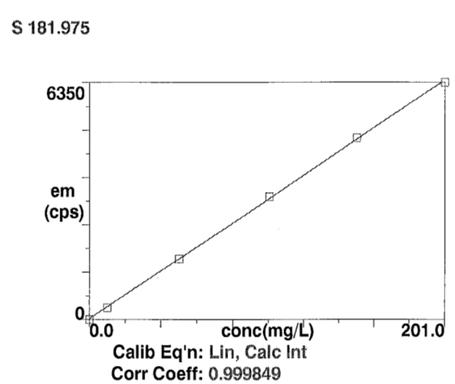
Preliminary Test Results

Total sulfur recovery tests of both organic sulfur standards (Table. 2)
- MSA: a common organic sulfur standard
- Diallyl sulfide (Fig. 1): a representative sulfide component of garlic origin
- MSA used to build calibration curve (Fig. 2)
- Both spiked into samples of garlic derived sulfide mixture
- Both standards: spiked at 1x level with respect to the sample concentrations
- Both standards: inconsistent recoveries obtained among the three spikes
- Diallyl sulfide: severe signal enhancement observed
- MSA: severe signal suppression observed

**Table 2.** Preliminary Test Results.
Samples were dissolved in aqueous DMF solution and tested on ICP-OES.

| Sample | Spike Standard | Spike Level | Spike Recovery | Average Recovery | %CV |
|---|---|---|---|---|---|
| 1 | Diallyl Sulfide | 1x | 176% | 181% | 18.2 |
| 2 | | | 217% | | |
| 3 | | | 152% | | |
| 1 | MSA | 1x | 5.65% | 58.6% | 78.8 |
| 2 | | | 79.7% | | |
| 3 | | | 90.3% | | |

**Figure 2.** Calibration Curve of Sulfur.




## Challenges in Sample Preparation and ICP Analysis

Typical Garlic Sulfur Agents (Fig. 3)[2], most insoluble in water

Sample Digestion
- Microwave assisted acid digestion facilitated elemental analysis including total sulfur analysis[1]
- Most garlic sulfides are insoluble in water and highly volatile, hence subject to low digestion efficiency and severe sample loss, unsuitable for acid digestion

Direct Sample Test on ICP
- Requires Peltier cooled spray chamber, and oxygen addition to Ar to avoid carbon deposition, therefore inconvenient for normal ICP operations

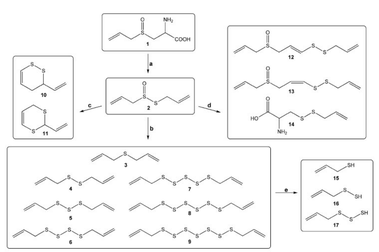
Hydrotrope Options
- Including detergent and organic solvents
- Detergent (Triton X-100) was successfully used in sulfur determination[3]
- Low sulfur content (< ~5,000 mg/Kg), non-aqueous samples including gasoline, kerosene and diesel fuel successfully tested[3]
- Oxygen needed in ICP operation in the Triton X-100 method[3]
- Detergents may not be applicable for the garlic sulfides due to their high sulfur content (e.g. 28% in diallyl sulfide)
- A broad range solubility test for sulfide was conducted at EPL
- DMF identified as the most efficient hydrotrope with zero sulfur content
- Non proficient recoveries received from the preliminary tests when dissolved in aqueous DMF solution (Table 2)
- Using a sulfide as calibration standard appeared infeasible due to the poor precision of the sulfide recoveries (Table 2)

Oxidation and Transformation
- Erratic results from the preliminary tests (Table 2) indicated that high volatility and severe sample carry-over should be controlled or limited
- A chemical reaction could transform the sulfides to a less volatile form
- Mild reaction conditions preferred
- Peracetic acid was tested on diallyl sulfide and proved to be a sufficient oxidation reagent
- Oxidation under strictly controlled temperature for precision and safety purposes
- Signal enhancement was greatly reduced by the transformation

Ionization Buffer
- Signal suppression observed for MSA standard (Table 2)
- Several schemes of improvement proposed and tested to reduce signal suppression
- Ionization buffer of cesium proved to be the most efficient option

Eliminate Sample Carry-over
- Aqueous DMF flush of the ICP sample injection system reduced carry-over
- Adding acid to the flush solution further helped in the carry-over elimination

**Figure 3.** Selection of biologically important sulfur agents found in garlic.[2]



## Final Test Results

Total sulfur tests of samples composed of garlic derived sulfides (Table. 3)
- Sets of 10 samples were prepared and tested on three different days
- All sample sets achieved good precision (<3% CV) and accuracy (98 and 102% in average recoveries)
- MSA recovery improved to 97.8% with good precision (2.49% CV)
- Diallyl sulfide improved to 102% in recovery with good precision (1.55% CV)

**Table 3.** Final Test Results
Samples were prepared following the procedure in the sample preparation section. Samples: three sets of 10 prepared and tested on three days; representative sample spikes: three 1x spikes performed in one set for each sulfur standard respectively.

| Sample | Spike Standard | Spike Level | Spike Recovery | Average Recovery | %CV |
|---|---|---|---|---|---|
| 1-10 | - | - | - | - | 2.65 |
| 1-10 | - | - | - | - | 2.29 |
| 1-10 | - | - | - | - | 2.72 |
| 1 | Diallyl Sulfide | 1x | 102% | 102% | 1.55 |
| 2 | | | 104% | | |
| 3 | | | 101% | | |
| 1 | MSA | 1x | 100% | 97.8% | 2.49 |
| 2 | | | 95.6% | | |
| 3 | | | 97.3% | | |

## References


1. Sheng L. Determination of sulfur in canola seed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Poster session presented at: AOAC Annual Meeting; 2013 Aug 25-28; Chicago, IL.

2. Munchberg, u.; Anwar,A.; Mecklenburg, S.; Jacob, C. (2007). Polysulfides as biologically active ingredients of garlic. Org. Biomol. Chem., 5, 1505–1518.

3. Santelli, R.; Oliveira, E.; Carvalho, M.; Bezerra, M.; Freire, A. (2008). Total sulfur determination in gasoline, kerosene and diesel fuel using inductively coupled plasma optical emission spectrometry after direct sample introduction as detergent emulsions. Spectrochimica Acta Part B, 63, 800–804.