

Determination of 3-Monochloropropane-1,2-diol (3-MCPD) in Foodstuff by GC-MS/MS

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Overview

Purpose: A GC-MS/MS method was developed for the determination of 3-Chloro-1,2-Propanediol in foods containing hydrolyzed vegetable protein. In addition a shorter preparation method was used to prepare the samples for analysis.

Methods: The official method for sample preparation as outlined in the Journal of AOAC International is lengthy and requires the use of flash chromatography. A simpler “QuEChERS” type methodology was used in this application with good success.

Results: Two different types of seasoning Soy Sauces and a dried hydrolyzed vegetable protein meat substitute sample were analyzed using this methodology and all three were found to have small amounts of 3-MCPD.

Introduction

3-Chloro-1,2-Propanediol (3-MCPD) is a compound formed as a product of the acid hydrolysis of vegetable proteins during the manufacture of the food seasoning ingredient hydrolyzed vegetable protein (acid- HVP). 3-MCPD is produced by the reaction of hydrochloric acid with the vegetable triglyceride.¹ Studies in laboratory animals have shown that 3-MCPD is carcinogenic therefore the European Community (EC) has set limits on 3-MCPD in acid-HVP and soy sauces at 0.02 mg Kg⁻¹ in dried weight basis.²

The United States currently does not have established levels for 3-MCPD and it is not monitored or enforced by the Food and Drug Administration (FDA). For this reason a study was done using cutting-edge instrumentation to examine a small number of food ingredients to see if 3-MCPD was present and at what level. A TraceGC coupled with a Quantum XLS Triple-quadrupole Mass Spectrometer and a Triplus Autosampler were used in this study.

In addition a sample preparation method was developed based on a Modified “QuEChERS” (Quick, Easy, Cheap, Effective, Rugged and Safe) to prepare the samples.



FIGURE 1. TraceGC Ultra with a PTV (Programmable Temperature Vaporizer Inlet), Quantum XLS Triple-quadrupole Mass Spectrometer, and a Triplus Autosampler.

Materials and Sample Preparation

AOAC Official Method 2000.01

The official method for the determination of 3-MCPD is very involved and laborious as shown in **Procedure 1**. For this reason a method was sought that would be easier and yielded equivalent results. It was thought that a QuEChERS method as practiced for the extraction of pesticides would be much easier and advantageous. QuEChERS was tried but yielded very low recoveries of the internal standard. There fore a modified version of the QuEChERS method was employed which resulted in good recoveries. The modified QuEChERS procedure is delineated in **Procedure 2**.

AOAC Official Method HVP, Soy Sauce, Soups, Stocks, Soup Powders, and Stock Cubes

1. Weigh to the nearest 0.01g 8g portions of HVP or soy sauce, 10g of soup, stock, or Malt extract, or 5g of soup powder or stock cubes.
2. Add 100µL of 3-MCPD-*d5* internal standard working solution (10ug/ml).
3. Add 5M NaCl solution to a total weight of HVP + salt solution of 20g and blend.
4. To the 20g of prepared product, add the content of an Extrelut[™] (EM Science) refill pack and mix. Add this mixture to a flash chromatography tube.
5. Elute nonpolar components with 80ml of diethylether-hexane (1+9).
6. Elute the 3-MCPD with 250ml of diethylether and collect in a 250ml volumetric flask. Add 15g of anhydrous Na₂SO₄ and let stand for 10-15 min.
7. Concentrate the extract to about 5ml by rotary evaporation at 35°C.
8. Transfer the concentrate to a 10ml volumetric and dilute to the mark with diethylether. Add small quantity of Na₂SO₄ and leave for 5-10 min.
9. Using a 1-ml gas-tight syringe, transfer 1ml of extract to a 4ml vial and evaporate to dryness below 30°C under N₂ stream.
10. Add 1.0ml of 2,2,4-trimethylpentane and 0.05ml of heptafluorobutyl-imidazole and seal vial. Heat for 20min. at 70°C and let cool below 40°C.
11. Add 1ml of Distilled water and shake for 30 sec. Let the phases separate.
12. Remove the organic layer to a 2ml vial for GC-MS analysis.

PROCEDURE 1. Official AOAC Sample Preparation Method for 3-MCPD.

Modified QuEChERS Method HVP, Soy Sauce, Soups, Stocks, Soup Powders, and Stock Cubes

1. Weigh to the nearest 0.01g 4g portions of HVP or soy sauce, soup, stock, or Malt extract, soup powder or stock cubes into a 50ml PP centrifuge tube.
2. Add 100µL of 3-MCPD-*d5* internal standard working solution (10ug/ml).
3. Add the content of a clean-up tube (6g MgSO₄, 1.5g NaCOOCH₃) Thermo Fisher p/n 60105-210 and 2.5 g of Celite powder, Thermo Fisher p/n 50-201-464. Mix until it is homogeneous.
4. Add 20 ml of diethylether and cap. Vortex for 30sec and centrifuge for 5 minutes at 3000rpm. Decant the ether extract into a clean 100ml flask.
- Repeat step 4 with another 20ml of diethylether and add to the 100ml flask.
5. Evaporate the diethylether extract under N₂ to about 5ml. Then put concentrate unto a 10ml volumetric and take to the mark with diethylether.
6. Add small amount of anhydrous Mg2SO4 and let sit for 5 min.
7. Remove 1ml of extract and place in a 2ml vial. Dry under N₂. Immediately add 100µl of MSTFA derivatizing reagent and 900µl of 2,2,4-trimethyl-pentane.
8. Heat for 15 min at 70C. Wait for 10 minutes until vial is cool. Analyze by GC-MS.

PROCEDURE 2. Modified QuEChERS Sample Preparation Method for 3-MCPD.

Methods

Gas Chromatography-Mass Spectrometry Conditions

Autosampler:

TriPlus (Thermo Fisher Scientific, Milan, Italy)
Sampling volume : 1.0µL
Syringe : 10µl syringe (Cone needle)

Gas Chromatograph:

TRACE GC ULTRA (Thermo Fisher Scientific, Milan, Italy)
Column : TraceGOLD TG-5MS 15 m × 0.25 mm I.D. df = 0.25µm (Thermo Fisher p/n 26-098-1300)
Injection mode : PTV 2.0mm I.D. Siltek Baffled liner
Injection program : 250°C Splitless (1.0 min.) Split Flow 50ml/min. Cleaning Temp. 300°C for 2.0 min. Solvent Valve Temp. 120°C
Oven Temp : 50°C(1 min)→40°C/min→250°C(1min) Equilibration time 0.5min.
Flow : Constant flow 1.5 ml/min.
Transfer line Temp : 280°C

Mass Spectrometer:

TSQ Quantum XLS (Thermo Fisher Scientific, San Jose, CA)
Ion Source Temp. : 250°C
Emission Current : 50uA
Detector Gain : 3.0 × 10⁵
Ion volume : Closed EI
Ionization mode : EI
Analytical mode : SRM Q1 and Q3 at 1.0u
Filter 1 : Precursor 239.0, Product 147, 0.05sec., 15 Volts
Filter 2 : Precursor 244.0, Product 147, 0.05sec., 15 Volts
Collision Gas Pressure: 1.0mTorr (Ar)
Chrom Filter : 1.5 sec.

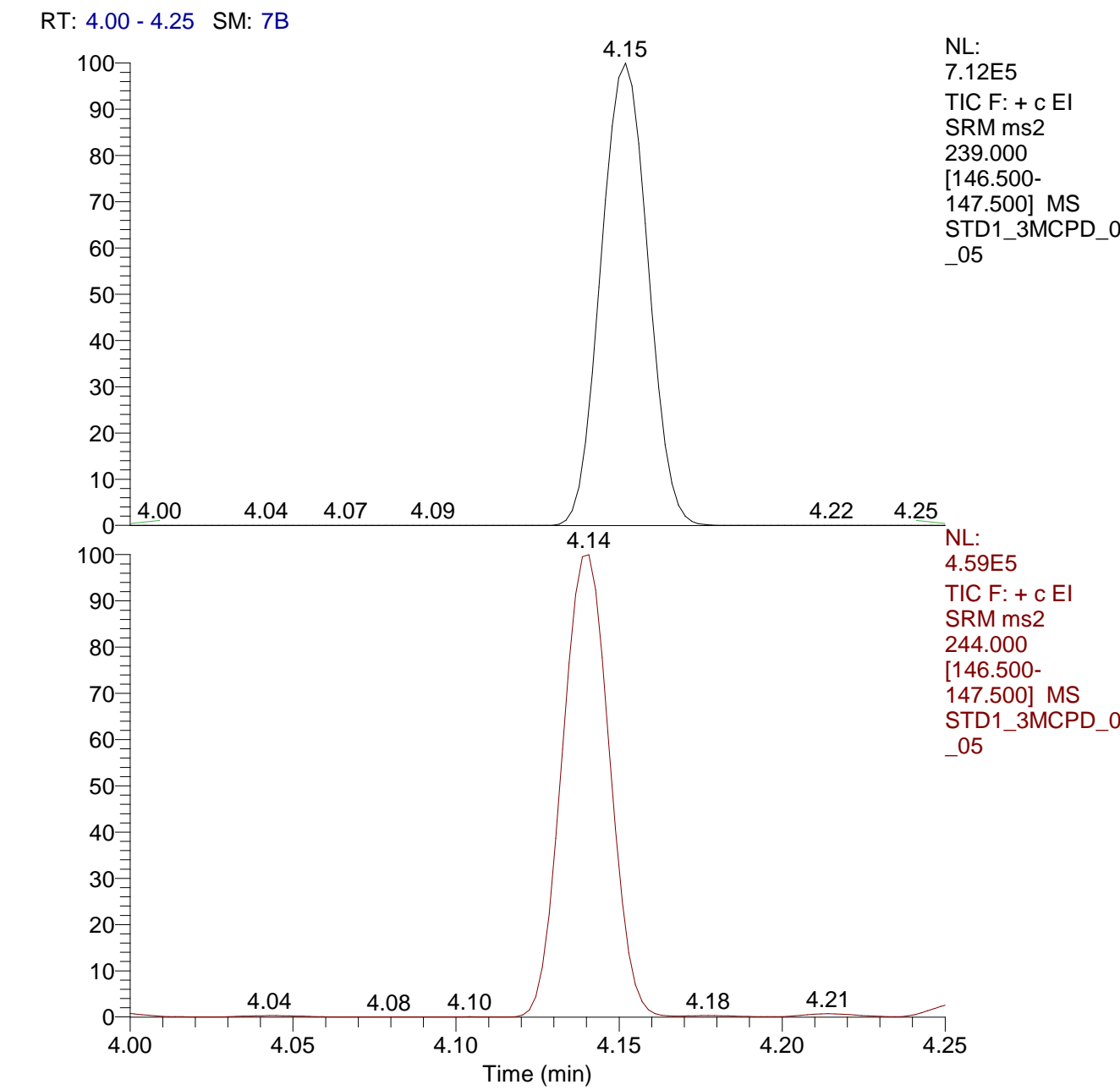


FIGURE 2. Selected Reaction Monitoring (SRM) for Derivatized 3-MCPD and 3-MCPD-*d5*.

Results

Calibration for 3-MCPD

The detection was done with a TSQ Quantum XLS Triple Quadrupole Mass spectrometer. The signal to noise ratio of the Triple Quad is superior to a Single Quadrupole instrument and can be improved even more by using it in H-SRM Mode.

The AOAC method calls for derivatization with Heptafluorobutylrimidazole which results in good abundance of high mass ions at m/z of 253 for 3-MCPD and 257 for 3-MCPD-*d5*. In this method we chose MSTFA (N-methyl-N-trimethylsilyltrifluoro-acetamide) which is the most volatile of the TMS Acetamides. This derivatization process resulted in ions of m/z 101, 116, 147, and 239 for 3-MCPD and 104, 119, 147, and 244 for 3-MCPD-*d5*. After careful consideration the transition of m/z 239 -> 147 was chosen for 3-MCPD and the transition from m/z 244 -> 147 was chosen for 3-MCPD-*d5*. **Figure 2** shows a typical chromatogram obtained using this method.

A five-point calibration curve was constructed using the recommended concentration range as outlined in the official AOAC Method. The concentration range was from 0.00 to 0.205 µg/ml. **Figure 3** shows a typical calibration curve for this procedure.

The amount of 3-MCPD is computed from the formula shown below:

$$\text{3-MCPD, mg/kg} = \frac{(\text{A} \times 10) / (\text{A}' \times \text{C})}{\text{Test portion, g}}$$

Where A = peak area for the 3-MCPD derivative; A' = peak area for the 3-MCPD-*d5* derivative; C = slope of calibration line.

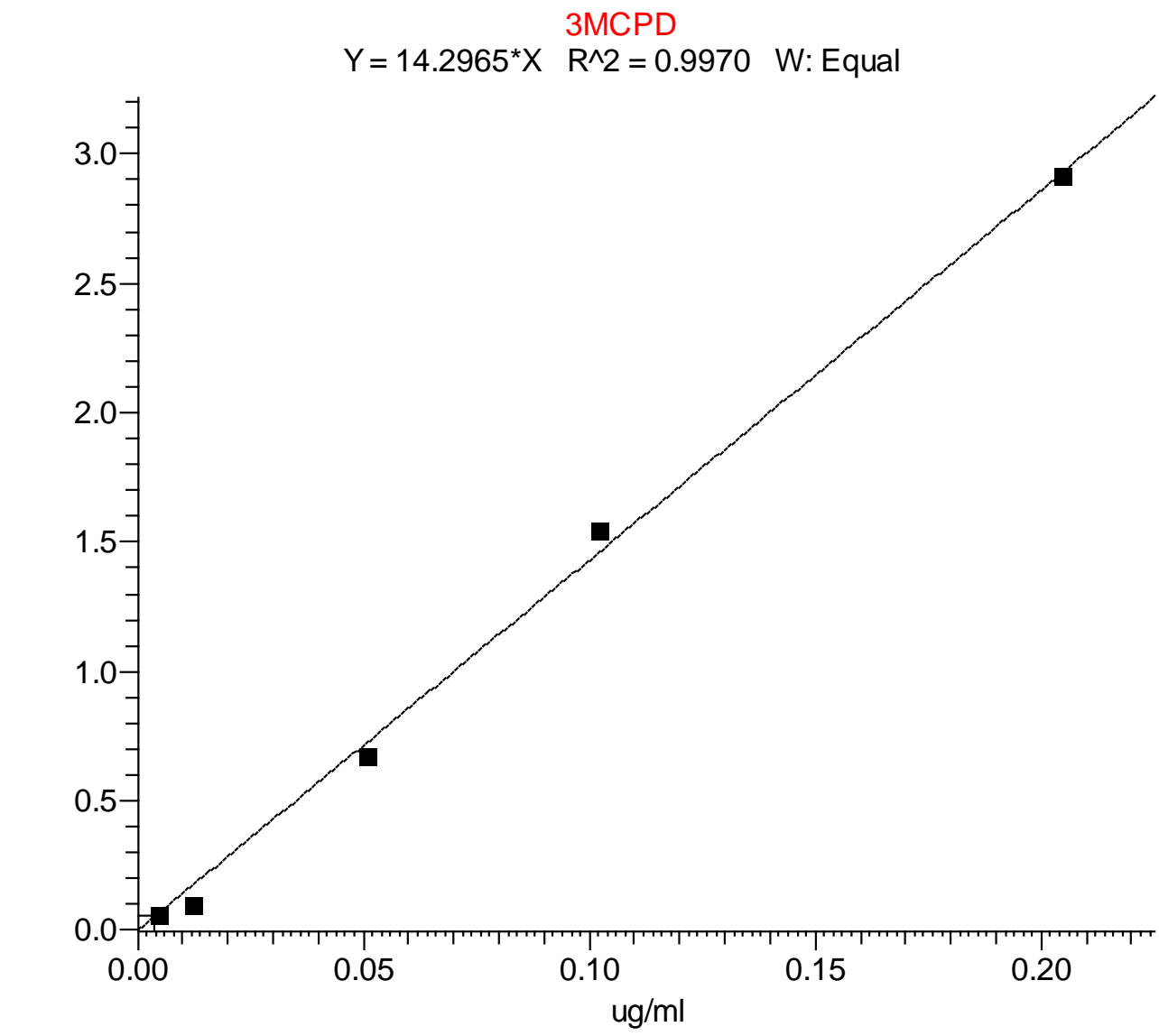


FIGURE 3. Internal Standard Calibration Curve for 3-MCPD

A sample of Soy Sauce was obtained at the local supermarket and a “organic” Soy Sauce and Hydrolyzed Vegetable Protein were purchased at a local health food store. These three samples were analyzed according to the modified method and the results are listed in the Table below.

| Sample ID | Weight of Sample (g) | Area of 3-MCPD | Area of 3-MCPD- <i>d5</i> | Amount of 3-MCPD in Sample (mg/Kg) |
|--------------|----------------------|----------------|---------------------------|------------------------------------|
| Sample A3-01 | 4.0479 | 148717 | 343564 | 0.075 |
| Sample B1-01 | 4.0700 | 113782 | 380689 | 0.051 |
| Sample B1-02 | 4.0162 | 101634 | 367817 | 0.048 |
| Sample C2-01 | 4.2725 | 64301 | 240739 | 0.044 |

Conclusions

A fast GC-MS/MS method was developed for the determination of 3-MCPD (3-Monochloro-1,2-propanediol) in hydrolyzed vegetable protein (HVP) samples. A “QuEChERS” type preparation procedure was developed for the treatment and cleanup of the samples with good recoveries. The detection was done with a TSQ Quantum XLS Triple Quadrupole Mass spectrometer which resulted in excellent linearity and can handle less rigorous sample preparation.

A number of samples were analyzed using the procedure outline in this presentation and found to contain small amounts of 3-MCPD. These amounts are in agreement with results published using the official AOAC method.

References

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3. Paul Breberton, Janet Kelly, Colin Crews, Sarah Honour, and Roger Wood. Journal of AOAC International, Vol. 84, No. 2, 2001. Determination of 3-Chloro-1,2-Propanediol in Foods and Food Ingredients by Gas Chromatography With Mass Spectrometric Detection: Collaborative Study. Pages 455-465
4. Nuria Leon, Vicent Yusa, Olga Pardo, Agustin Pastor. Talanta Vol. 75, 2008, Determination of 3-MCPD by GC-MS/MS with PTV-LV Injector Used for a Survey of Spanish Foodstuffs. Pages 824- 831.

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