

PROTOCOL

Study Title:

GLP Method Validation
Determination of Iodomethane (TM-425) in
Fruiting Tomatoes by Gas Chromatography Headspace Analysis

Ricerca, LLC Document Number: 012157-0

Data Requirement:

US EPA
Preliminary Analysis and Enforcement Analytical Method
OPPTS Guidelines – 860.1340 and 860.1380

INTRODUCTION

This method defines the procedure for the determination of iodomethane in tomatoes.

APPARATUS

- Gas Chromatograph (Hewlett Packard Model 5890 or equivalent with an Electron Capture Detector) and an automated headspace sampling system (Hewlett Packard Model 7694, or equivalent)
- Chromatography data system (Perkin Elmer Turbochrom or equivalent)
- J&W Gaspro column, 30 Meter x 0.32mm id (Cat Number 113-4332)
- Analytical balance capable of weighing to 0.00001 g
- Volumetric flasks and Miscellaneous glassware (e.g. 100 mL Class A volumetric flasks, 100 ml graduated cylinders, and disposable pipets)
- 250 mL polyethylene sample bottles (Fisher Cat. 05-562-23)
- Brinkman Polytron Tissue Homogenizer with a 2-cm.-diameter generator (Brinkman Cat. 027-13-066-6)

REAGENTS AND SOLUTIONS

- Water, HPLC grade
- N,N-Dimethyl Formamide, Anhydrous (Acros Cat Number 610320010, or equivalent)
- Iodomethane test material/reference standard (as received from sponsor)

EXTRACTION PROCEDURE

1. Fresh control samples are homogenized using a polytron tissue homogenizer.
2. A 20 gram portion of the chopped homogenized sample is weighed into a 250 mL polypropylene bottle with screw cap.
3. Samples to serve as concurrent recoveries are fortified at this stage.
4. A 10 mL aliquot of dimethyl formamide and 40 mL of HPLC grade water are added to each of the samples. The bottles are capped and shaken briefly and placed in an ice water bath.
5. The samples are centrifuged for 20 minutes at 7000 rpm with the centrifuge temperature set to 5 Celsius.

6. The supernatant is carefully poured-off from the pellet and measured in a 100 ml graduated cylinder. The solution is reconstituted to 100 mL with water and stirred briefly to insure uniform distribution.
7. A 2-mL aliquot of the extracts from each sample is transferred to a 20 mL headspace vial and sealed with a crimp-top cap.
8. Likewise, 2 mL aliquots of the calibration solutions are transferred to a headspace vial for calibration and linearity determinations.

GAS CHROMATOGRAPHIC SYSTEM CONDITIONS

The headspace analyzer (Hewlett Packard Model 7694, or equivalent) will be set with the following operating parameters:

Equilibration of each vial for 15 minutes at 43 °C.

Injection loop of 1 mL.

Loop temperature of 60 °C.

Transfer line temp of 60 °C.

Pressurization time of 0.2 min.

Loop fill time of 0.02 min.

Loop equilibration time of 0.05 min.

Injection time of 1.0 min.

The GC (Hewlett Packard Model 5890 or equivalent with an Electron Capture Detector) will be set with the following operating parameters:

Initial temperature of 180°C for 10 minutes then ramped at 30°C per minute to 260°C and held for 1.0 minute.

Injector temperature of 180°C.

Detector (ECD) set at 300°C.

Column description: J&W Gaspro column, 30 Meter x 0.32mm id.

Column head pressure set at 20 psi.

CALCULATIONS

Quantitation of Iodomethane is made by injecting with the samples a series of calibration standards (0.10, 0.05, 0.025, 0.005 and 0.001 µg/mL). The response of the standards is plotted in area or height versus concentration. The sample concentration in µg/mL is determined from the first order line generated from the calibration standards. The final concentration of the sample in ppb is calculated using the following formula:

$$\frac{\text{sample response from line (} \mu\text{g/mL) } \times \text{ final volume (100 mLs) } \times 1000 \text{ ng/}\mu\text{g}}{\text{weight of sample (20 g)}}$$

INTRODUCTION

This method defines the procedure for the determination of iodide in tomatoes. *The Limit of Determination* (LOD) will be assigned as the lowest visible standard injected in a sequence of standards adjusted for sample weight and volume. *The Limit of Quantitation* (LOQ) will be assigned as the lowest recoverable amount fortified to control tomato samples. It is proposed that the LOQ will be 2x the LOD.

APPARATUS

- Analytical balance capable of weighing to 0.00001 g
- Ion Chromatograph (Dionex Corporation or equivalent)
- Volumetric flasks and Miscellaneous glassware (e.g. 100 mL Class A volumetric flasks, 100 ml graduated cylinders, and disposable pipets)
- Vortex
- 20 x 150 mm culture tubes (Fisher cat. no. 14-961-33)

REAGENTS AND SOLUTIONS

- Water, HPLC grade
- Iodomethane test material/reference standard (as received from sponsor)

EXTRACTION PROCEDURE

9. Fresh tomatoes are homogenized using a polytron tissue homogenizer.
10. A 10 gram portion of the chopped homogenized sample is weighed into a test tube.
11. Samples to serve as concurrent recoveries are fortified at this stage.
12. A 10 mL aliquot of water is added and the samples are mixed with a vortex for approximately one minute.
13. A portion of the sample is passed through a 0.45 μ IC-acrodisc and used for analysis.

ION CHROMATOGRAPHIC SYSTEM CONDITIONS

Instrument:	Dionex 2110i
Column:	Dionex Ionpac AS11
Eluent:	0.05 M HNO ₃
Injection Volume:	50 µL
Detection Mode:	amperometry, Pt electrode, 0.8 V
Flow Rate:	1.5 mL/min
Run Time:	5.5 min
Retention Time:	3.4 min for iodide

CALCULATIONS

A four-point calibration plot using a quadratic fit will be collected with standards made from a stock aqueous solution of potassium iodide (stored refrigerated). Due to the drifting response of iodide peak area counts with time, the responses of the calibration plots at the beginning and end of the sequence will be averaged. From the best fit line represented from the calibration plot the recovery of iodide from the fortified samples will be calculated adjusted as necessary for volume and weight of sample used.