Analysis of herbicides in solid samples (i.e. soils, manure, etc) by microwave-assisted solvent extraction (MASE) and gas chromatography mass spectrometry (GC/MS)

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Introduction

Protocol overview
Two to five grams of wet sediment or soil is weighed into a 10 milliliter (mL) Teflon microwave digestion vessel, spiked with surrogate compounds, and thoroughly mixed with 6 mL of extraction solvent (acetonitrile). Batches of up to 40 samples are subjected to microwave irradiation (400W) for 10 minutes at 90°C using a MARS Xpress microwave digestion system. Sediment is washed a second time with acetonitrile, combined with the first portion, and volume reduced to near 1-2 mL under dry nitrogen. The sample is spiked with internal standard, diluted in 100 mL of water, and extracted onto a C18 solid phase extraction (SPE) cartridge under vacuum. Absorbed compounds are then eluted with ethyl acetate. Sodium sulfate is used to capture water residue. Extracts evaporated to dryness and brought up with 200 µl ethyl acetate before injection onto a gas chromatograph (GC) equipped with a mass selective detector (MSD). Detection limits are in the low nanogram per gram levels depending on compound.

Scope and application
The microwave-assisted solvent method (MASE) is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and solid wastes. This method was developed and validated on commercially-available solvent extraction systems. Its procedure uses microwave energy to produce elevated temperature and pressure conditions in a closed vessel containing the sample and organic solvent(s) to achieve analyte recoveries equivalent to those from a traditional extraction. The benefits of this method are enhanced extraction times, low solvent consumption and improved extraction efficiencies.

Sample sizes
2 – 5 g

Limitations
Only 6 samples can be extracted on the SPE line at one time. Only 8 samples can fit in the rapid vap.
Required Training
Use of the following equipment: Rapid Vap, MARS microwave, vacuum extraction manifold, elution manifold, and nitrogen blowdown manifold

Equipment Required
Consumables – Need to provide item, manufacturer/cat no. if available
- Disposable filter device, 25 mm 0.2 µm PTFE GD/X filter (Cat 6874-2502)
- Laboratory gloves
- Kimwipe tissues
- Auto-sampler vials with crimp cap (National Scientific #C4012-1 & C4011-1A or equivalent)
- Conical vial inserts with spring-type bottom (National Scientific #C4010-S630 or equivalent)
- Disposable Pasteur pipette, 225 mm (Fisher Scientific, 13-678-20D).
- Disposable glass culture tube, 10 x 75mm (Fisher Scientific, 14-961-25).
- Disposable glass culture tube, 15 x 85mm (Fisher Scientific, 14-961-28).
- Compressed nitrogen gas
- Disposable liners for Visiprep DL (Supelco, 57059)

Equipment
- Rapid Vap (LabConco)
- Rapid Vap tubes, 120 mL (LabConco)
- Centrifuge (Beckman Cp)
- 10cc plastic syringes.
- Microwave extraction Teflon vessels, 10 ml (CEM Microwave Technology)
- Microwave solvent extraction apparatus, MARS-Xpress system (CEM Microwave Technology)
- Top-loading analytical balance, precision to 1x10^-2 g (Mettler PJ360 or equivalent).
- Stainless steel spatula.
- Adjustable micro-pipettes with reference tips, 10 mL and 100 µL (Eppendorf).
- Plastic syringe, 20cc (Norm-Ject all-polypropylene design).
- Vortex mixer
- Vacuum elution manifold (Supelco Visiprep, or equivalent) with drying attachment

Reagent Solutions
- Methanol (Optima, Fisher Scientific).
- Acetonitrile (Optima, Fisher Scientific)
- Ethyl acetate (Optima, Fisher Scientific).
- Distilled deionized water (DDW)

Standard Solutions
For the instructions on the preparation of calibrations and spiking solutions, refer to the herbicide stocks and calibrations protocol (or whatever the official name convention will be).

Isotopically labeled internal standards (5.0 ng/uL in MeOH):
- Deethylatrazine-13C
- Deisopropylatrazine-13C
- Atrazine-13C.

Chemically pure unlabeled analytes (1.0 ng/uL in MeOH):
- Acetochlor
- Alachlor
Quality Assurance Solutions

**LFM**
Weigh a 5.0 g replicate of a selected sample in a 10 mL Teflon tube and spike with analyte as described below.

**LFB**
Weigh 5.0 g of quartz sand in a 10 mL Teflon tube and spike with analyte as described below.

**LRB**
Weigh 5.0 g of quartz sand in a 10 mL Teflon tube and spike with surrogate only.

Safety Precautions

Protocol

Extraction procedure:

1. **Weighing samples:**
   a. Remove samples from a freezer and allow them to thaw at room temperature.
   b. Place 10 mL Teflon tube on balance and tare.
   c. Using a spatula, weigh 5.0 g wet sediment into the Teflon tube.
   d. Add 6 mL acetonitrile to each sample.

2. **Surrogate and Analyte Spikes:**
   a. Sonicate spikes for at least 30 min prior to use.
   b. Spike all samples with 100 uL of 2.0 ng/uL surrogate spike for a total mass of 200 ng.
c. Spike LFM and LFB samples with 100 uL of 1.0 ng/uL analyte spike for a total mass of 100 ng. If performing MDLs, spike MDL samples with 15 uL of 1.0 ng/uL analyte spike for a total mass of 15 ng.

3. Vortex and equilibrate for 30 min.

4. Mars Xpress parameters:
   a. Place tubes in the MARS microwave
   b. Extract samples using the following parameters (protocol should be saved under EPA 3546 solids_Xpress)
      
      Ramp time:  10 min  
      Watts:  400 W  
      Power:  100%  
      Temperature: 90°C  
      Hold time:  10 min
   c. After microwave cool down, remove samples from microwave.

5. Following MASE:
   a. Centrifuge samples in Teflon tubes for 10 min at 2500 rpm.
   b. Decent solvent into rapid vap tube.
   c. Rinse sample with 6 mL acetonitrile, vortex, and repeat previous steps.

6. Rapid Vap Parameters:
   a. Concentrate sample to ~1-2 mL in rapid vap using the parameters below. If sample goes to dryness, add 1 mL acetonitrile to dissolve extracted pesticides.
      Temp:  35°C  
      Speed:  50  
      Time:  ~15-20 min
   b. Following rapid vap, spike all samples with 100 uL of 5.0 ng/uL internal standard spike for a total mass of 500 ng.
   c. Fill rapid vap tube with ~100 mL of DDW

7. SPE clean-up of extract:
   a. Place C-18 cartridge on the flow control valve attached to the vacuum flask.
   b. Install a clean, empty 10cc syringe barrel onto the luer fitting of each cartridge.
   c. Pre-condition C-18 SPE cartridges with 6 mL Ethyl Acetate, followed by 6 mL MeOH, and 6 mL DDW. Do not let cartridge run dry between subsequent solvent washes. After the final water wash, do not allow the cartridge to drain completely so that the solid phase remains wet until use.
   d. Insert a pre-combusted 1 µm glass filter into the PFA inline filter holder.
   e. Turn on the vacuum and flush approximately 10 ml of sample through the sample delivery tube.
   f. Insert the SPE cartridge on the sample line and extract the sample through the SPE cartridge.
   g. After the extraction is complete, remove the sample delivery tube from the cartridge. Allow the cartridge to dry under vacuum for 10 min or more.
   h. Store the cartridge in a clean zip-lock bag labeled with the batch number and extraction date in the refrigerator until the elution of the analyte can be performed (up to 3 months).

8. Elution of SPE cartridge:
   a. Place labeled disposable test tubes (15 x 85 mm) in the elution rack.
b. Replace the liner on the underside of the Supelco VisiPrep elution manifold cover with a clean disposable liner. Replace the manifold cover by inserting the Teflon tubing into the test tubes.

c. Place the SPE cartridges in order on the VisiPrep Teflon holder. Install a clean, empty 10cc syringe barrel onto the luer fitting of each cartridge.

d. Measure 5 ml of ethyl acetate into each syringe barrel reservoir. A slight vacuum will be needed to begin the elution process which can then be continued by gravity.

e. After elution, turn on the vacuum and open the valve at full vacuum for 2-3 minutes to ensure all liquid is eluted.

f. Once elution is complete, remove cartridges and place back in storage zip-lock in freezer until the batch is released. Label the bag with the elution date.

9. Removal of water layer:
   a. Blowdown the sample to ~2 mL under nitrogen gas.
   b. Remove the water layer, if visible, from the bottom of the tube with a clean disposable glass Pasteur pipet.
   c. Add a small spatula-full of cleaned and dried anhydrous sodium sulfate. Vortex the test tube for 15 seconds to mix and promote drying. Add more sodium sulfate and repeat vortexing if excess powdered sodium sulfate not visible.
   d. Let the sample settle and then transfer the remaining sample to a clean test tube (10 x 75 mm) via a clean disposable Pasteur pipet to blow-down the sample

10. Blowdown of transfer of extract:
   a. Evaporate the extract to near dryness under a dry stream of Nitrogen gas.
   b. Add 300µL of ethyl acetate and vortex.
   c. Pipet the concentrated eluent into a GC vial fitted with a 300 µl conical spring insert. Cap the vial with the crimping tool.
   d. Include a spike check for analysis of the spiking solutions. Add 100 uL of each spike (analyte, surrogate, and internal standard) into a 300 µl conical spring insert. Mix the solution by pipetting. Cap the vial with a crimping tool.

11. GCMS analysis:
   a. Analyze a 1 µL aliquot on the GC/MS system as described in PESTCAL adjusting calibration concentrations to lower values required for . (See herbicide calibrations protocol – needs updated name).
   b. At the conclusion of data acquisition, use same software settings used for calibration to identify the peaks of interest base on retention time windows of 5-10%. Examine confirmation ion abundances for each compound to determine if the abundances produce approximately the same amount of analyte as the primary quantification ions.

Data Reduction

Complete separation of analyte peaks is not necessary due to high selectivity of tandem mass spectrometry detection. MS/MS can separate co-eluted analytes either by parent ion mass-to-charge ratio or fragment ion mass-to-charge ratio. The calculation of the amount of analyte and surrogate compounds in the extracted solution is by the instrument manufacturer’s software based on the following equation:

\[
\text{Amt (unk)} = \frac{[\text{Area (unk)} \times \text{Amt (Int)}]}{[\text{Area (int)} \times \text{RF}]} \tag{1}
\]
Amount (unk) = amount of an analyte relative to internal standard (ng)
Amount (int) = amount of internal standard added to sample (ng)
Area (unk) = integrated abundance of quantitation ion of analyte
Area (int) = integrated abundance of quantitation ion of internal standard
RF = response factor determined from most recent calibration curve

This amount is converted into a concentration by dividing with the total sample weight (gm), which is done by data entry into the laboratory LIMS system:

\[
\text{Conc (ppb)} = \frac{\text{Amt (unk)}}{\text{Sample wt. (gm)}} \tag{2}
\]

Conc (ppb) = concentration of an analyte in sample in ng/g (=µg/L)
Sample wt. = total sample wt. of spiked sample – wt. of container (gm)

**Hints and Troubleshooting**

**Additional Information**

**References**

**Additional Reading**

**Instrumentation and Technical Support**