

Determination of Chloroacetanilide Metabolites in Drinking Water by
Solid Phase Extraction and Liquid Chromatography / Mass Spectrometry

New York State Food Laboratory
Debra Oglesby, Robert Sheridan and Roger Pollman
3/25/2002

A method was developed based on a United States Geological Survey (USGS) method (1) to determine Chloroacetanilide Metabolites in drinking water. An octadecylsilane (C-18) solid phase extraction (SPE) cartridge is used to isolate these pesticides from drinking water. Pesticides are eluted from the cartridge with methanol. A high pressure liquid chromatograph (HPLC) coupled with a triple quadrupole mass spectrometer (MS) with multiple reaction monitoring (MRM) is used to identify, quantitate and confirm the pesticides isolated by this procedure.

Reagents

- Methanol (MeOH), J. T. Baker, "Baker Analyzed", HPLC grade
- Nitrogen gas, 99.9% Pure
- Distilled De-ionized water (DDI)
- Sodium Thiosulfate, J. T. Baker, "Baker analyzed"
- O-tolidine reagent, commercially available in swimming pool test kits

Solutions

- 25% Methanol/Distilled De-ionized Water (MeOH/DDI), Add 250ml MeOH to a 1000ml volumetric. Add DDI water to base of neck of flask and mix.
CAUTION. Mixing produces an exothermic reaction and solution will expand. Allow solution to return to room temperature (unstoppered) and adjust volume with DDI.

Apparatus

- Supelclean LC-18, SPE cartridge, 6 ml x 500 mg. Supelco part # 57054
- Supelco Visidry attached to nitrogen source.
- Zymark TurboVap LV attached to nitrogen source.
- Zymark Autotrace workstation
- Kuderna Danish (KD) receiving tubes, 10ml. Tubes are graduated to 1ml in 0.1 ml increments.
- Vortex genie.

Precautions

Fire all glassware used in the procedure at 450° C for 2 hrs.

Analytical Instrument

LC/MS/MS – Waters 2690 HPLC with a Micromass Ultima Mass Spectrometer.
Water's XTerra MS C-18 column, 2.1 x 150mm. Flow: 0.2 ml/min.

<u>Time</u>	<u>Solvent Gradient</u>	
	<u>% A (0.15% Acetic Acid)</u>	<u>%B (MeOH)</u>
0.00	50.0	50.0
0.50	50.0	50.0
30.00	15.0	85.0
31.00	15.0	85.0
32.00	50.0	50.0
40.00	50.0	50.0

Sample Pickup and Transport

Samples are obtained by personnel at selected municipal water plants. Each sample is collected in a 1000ml amber glass bottle containing 1 gm Sodium Thiosulfate and fitted with a Teflon-lined screw cap. The sample is refrigerated and transported to the lab within 24 hours of pickup.

Sample Reception

1. Samples are to be kept refrigerated until the time of analysis.
2. Remove 5ml water and test for residual chlorine with 2 drops O-tolidine reagent.
3. A yellow color indicates the presence of residual chlorine.
4. Add Sodium Thiosulfate in 1gm increments until residual chlorine is absent.

Analytical Procedure

1. Samples are to be analyzed within three days of sample receipt or four days of sample pickup.
2. Weigh a 6ml x 500mg LC-18 SPE cartridge and record the weight on the side of the cartridge.
3. Condition the SPE cartridge with 2ml MeOH followed by 2ml DDI water. DO NOT ALLOW THE CARTRIDGE TO GO DRY.
4. Samples may be extracted either by using the Zymark Autotrace or manually.

Zymark Autotrace

- a. Attach the conditioned cartridge to the Zymark Autotrace.
- b. Remove 100ml of sample and place in a clean container.
- c. Add process controls and/or spiking solutions at this step and mix well.
- d. Using the Zymark Autotrace, pump water samples thru the conditioned cartridge at a flow rate of 10ml/min.

Manually

- a. Attach the conditioned cartridge to a 70 ml reservoir using an appropriate adapter.
- b. Remove 100ml of sample and place in a clean glass container.

- c. Add process controls and/or spiking solutions at this step and mix well.
 - d. Transfer sample to reservoir and push it thru conditioned cartridge using nitrogen. Set the nitrogen flow so that the sample elutes from the cartridge at a rate of 10ml/min. Sample should elute in drops rather than a steady flow.
5. When the sample has passed thru the cartridge, attach it to the Supelco Visidry. Dry cartridge with nitrogen until original weight is attained.
 6. Elute cartridge by gravity into a 10ml KD receiving tube with 5ml MeOH.
 7. Apply pressure to ensure that all MeOH is removed from the cartridge.
 8. Concentrate the eluant to near dryness (1-2 drops) using TurboVap LV.
 9. Add 25% MeOH/DDI to bring volume to 0.2ml and mix using Vortex mixer.
 10. Transfer to auto sampler vial for LC/MS/MS analysis.

Zymark Autotrace Cleanup

After each sample has been pumped thru the Zymark Autotrace, clean the components in the following manner

1. Pump 30 ml of a mixture of 10% Toluene, 20% Methylene Chloride and 70% Isopropanol thru the various lines and fittings of the Autotrace at a flow rate of 10ml / min.
2. Pump 30 ml MeOH thru the Autotrace at a flow of 10 ml / min.
3. Pump DDI thru the Autotrace at a flow of 10 ml / min.

Process Control

Propoxur is to be used as the process control for this extraction. 100ul of a 0.1 ppm solution of propoxur when added to each 100ml sample will result in a concentration of 0.1 ppt (100 ppt) .

Spike Sample

Add 1gm Sodium Thiosulfate to 1000 ml water known to be free of the pesticides under study. Place 100ml of the water in a clean container. Add 100ul, 200ul, 500ul or 1 ml of a prepared solution containing the pesticides under study so that the resulting levels of pesticides correspond to 1, 2, 5, or 10 x LOQ respectively.

Standards

Standards are prepared in 25% MeOH/water to encompass the linear range from 1 x LOQ to 10 X LOQ. Standard curves are prepared for each group of samples run. For each standard prepared, pump 100ml dechlorinated DDI water thru a weighed and conditioned 6 x 500mg LC-18 SPE cartridge. Continue with Analytical Procedure thru Step #10. Concentrate to dryness and reconstitute with the appropriate level of ESA standard.

Quantitation

The level of pesticide in each 0.2 ml assay solution is determined from standard curves or by direct comparison of standards that fall within +/- 30% of the response of the sample.

$$\text{Sample (ppt)} = \frac{\text{Assay (ppm)} \times 0.2\text{ml} \times 10^6}{100\text{g}}$$

References

1. Method of Analysis by the U. S. Geological Survey Organic Chemistry Research Group- Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry. Open-File Report 01-10.