

Application Note 127

Solid Phase Extraction Provides Interference-Free Samples for Analyzing Triazine Herbicides

Supelclean solid phase extraction tubes provide high, reproducible recovery of triazines from plant extracts and groundwater.

Key Words

• triazine herbicides • solid phase extraction • groundwater

Although triazine herbicides are widely used to control weeds in food crops, these toxic chemicals generally degrade slowly. They can persist in fields for a year or more after application (1). Therefore, for consumer protection, residual triazines in food plants and groundwater must be accurately monitored.

Chlorophylls, carotenes, and other components of plant extracts frequently interfere with triazine quantification. Sample cleanup through multiple liquid-liquid extractions (2) is time-consuming and frequently fails to yield a pure extract. However, a solid phase extraction (SPE) procedure has been developed for obtaining plant extracts of triazines free of chlorophyll and other interferences. These extracts are suitable for quantifying trace levels of triazines.

Supelclean™ LC-SCX (strong cation exchanger) solid phase extraction tubes isolate simazine, atrazine, and propazine in plant extracts. These compounds are effectively separated from matrix components that would otherwise interfere with the analysis. (For extraction of triazines from fruits and vegetables, see Bulletin 900.)

The procedure is relatively simple. For 20 minutes, shake a mixture of 5g of shredded plant tissue, 4g of anhydrous sodium sulfate, and 20mL of methylene chloride:acetone (4:1). Allow the mixture to stand 1 minute, then aspirate a 2mL aliquot dropwise through a 3mL Supelclean LC-SCX tube previously conditioned with 1mL of methylene chloride. Wash plant pigments and other interferences from the packing with two 2mL aliquots of acetonitrile. Air dry the packing under vacuum for 5 minutes, then pass two 2mL aliquots of water through the tube. Recover the triazines by passing 1.5mL of methanol dropwise through the tube, using a Visiprep™ Solid Phase Extraction Vacuum Manifold, and collecting the eluate in a 2mL volumetric tube.

To improve HPLC peak shapes, dilute the recovered eluate to 2mL with water. Quantify the triazines by HPLC with UV detection at 254nm. For GC analysis, modify this procedure by eliminating the water wash, eluting the herbicides with 1mL of methanol, and diluting the collected eluate to 1mL with methanol.

To study recovery rates for the three herbicides, we added 2ppm standards of each to grass extracts. Then we prepared and analyzed

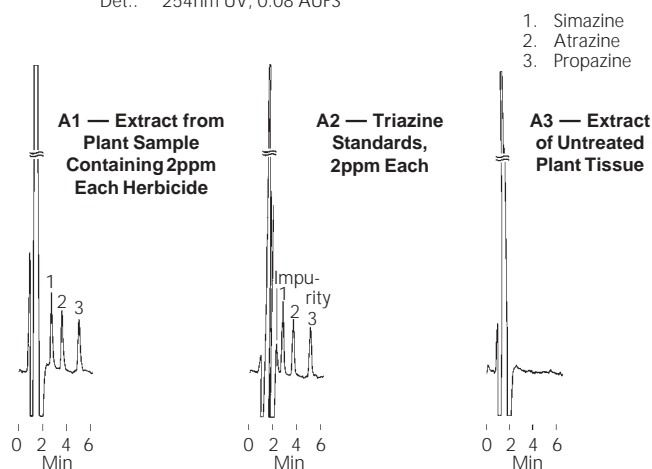
samples by the procedures described. A 100µL aliquot of each final extract was injected onto a SUPELCOSIL™ LC-8-DB HPLC column (Figure A).

Chromatograms from untreated grass showed that samples obtained by this procedure are free of matrix interferences. For quantification, triazine peak heights in the sample chromatograms were compared with those for 2ppm working standards. Triazine recovery was virtually complete (Table 1).

Supelclean LC-SCX tubes, when used to prepare plant extracts for triazines analysis, remove interfering materials at each of three steps. First, when the sample is added to the tube, nonpolar components pass through the packing while the triazines are quantitatively retained. Subsequent washing with acetonitrile

Figure A. Triazine Pesticides Quantitatively Recovered from Plant Tissue

| | |
|------------------|--|
| Sample: | 100µL methanol:water (3:1), extracts containing 50ng (A1, A2), or 0ng (A3) each triazine |
| Extraction Tube: | Supelclean LC-SCX, 3mL tube |
| Cat. No.: | 57018 |
| Conditioning: | 1mL methylene chloride |
| Washing: | 2 x 2mL acetonitrile, air dry for 5 min, 2 x 2mL water |
| Elution: | 1.5mL methanol (dilute recovered sample to 2mL with water) |
| Column: | SUPELCOSIL LC-8-DB², 15cm x 4.6mm, 5µm packing w/ Supelguard™ LC-8-DB, 2cm x 4.6mm |
| Cat. No.: | 58347 |
| Temp.: | 25°C |
| Mobile Phase: | acetonitrile:water, 45:55 |
| Flow Rate: | 1.5mL/min |
| Pressure: | 1392psig |
| Det.: | 254nm UV, 0.08 AUFS |



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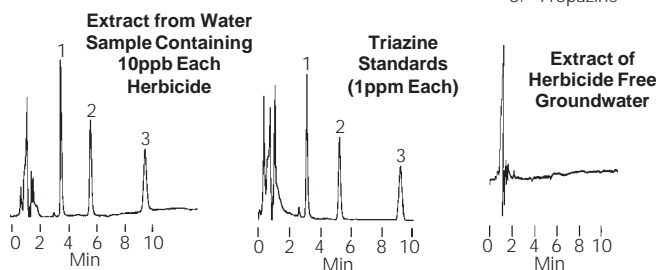
Table 1. Solid Phase Extraction Completely Recovers Triazine Herbicides from Grass Samples

| | % Recovery | | |
|---------|---------------|---------------|---------------|
| | Simazine | Atrazine | Propazine |
| Trial 1 | 102.3 | 102.1 | 99.0 |
| Trial 2 | 104.7 | 104.3 | 102.4 |
| Trial 3 | 99.0 | 101.3 | 100.0 |
| Mean | 102.0 (± 2.9) | 102.2 (± 1.6) | 100.5 (± 1.8) |

Figure B. Triazine Pesticides Recovered from Groundwater

Sample: 20µL methanol:water (1:1) extracts
 Extraction Tube: **Supelclean LC-18, 3mL tube**
 Cat. No.: **57012**
 Conditioning: 2mL methanol, followed by 2mL of deionized water
 Washing: 1mL deionized water:acetonitrile:methanol (4:3:1)
 Elution: 1mL methanol (dilute recovered sample to 2mL with water)
 Column: **SUPEL COSIL LC-8-DB[®], 15cm x 4.6mm, 5µm packing w/ Supelguard™ LC-8-DB, 2cm x 4.6mm**
 Cat. No.: **58347**
 Temp.: 25°C
 Mobile Phase: acetonitrile:water, 45:55
 Flow Rate: 1.5mL/min
 Pressure: 1392psig
 Det.: 254nm UV, 0.005 AUFS

1. Simazine
2. Atrazine
3. Propazine



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removes most moderately polar components that were retained with the herbicides. And finally, when the triazines are selectively eluted from the packing in methanol, very polar materials remain behind. The procedure leaves the final sample virtually interference-free (Figure A).

For extraction of triazine herbicides from groundwater, a 3mL Supelclean LC-18 SPE tube is used. The tube is conditioned with 2mL of methanol, followed by 2mL of deionized water. A 20mL sample reservoir is connected to the tube. The sample, consisting of 20mL of the groundwater sample, is passed through the conditioned tube at a slow dropwise rate, using a Visiprep vacuum manifold. Impurities are removed from the packing by washing with 1mL of a deionized water:acetonitrile:methanol (4:3:1) solution. The triazines are then eluted from the packing with 1mL of methanol and collected in a 2mL volumetric flask. The eluant is diluted to the mark with water prior to HPLC analysis (Figure B). The procedure can be easily adapted for GC analysis by thoroughly drying the packing under nitrogen, using a Visidry™ Vacuum Manifold Drying Attach-

Table 2. Absolute Recovery of Triazine Herbicides from Groundwater

| Herbicide | % Recovery ± S.D. (n = 3) | | |
|-----------|---------------------------|-------------|------------|
| | 10ppb | 100ppb | 1ppm |
| Simazine | 102 ± 1 | 100 ± 4 | 97.2 ± 0.9 |
| Atrazine | 100 ± 0 | 99.0 ± 10.6 | 94.4 ± 0.7 |
| Propazine | 94.0 ± 11.1 | 92.4 ± 7.4 | 92.4 ± 0.8 |

ment, prior to elution and eliminating the water dilution of the methanol eluant. Recovery of atrazine, propazine, and simazine at concentrations of 10ppb to 1ppm in groundwater was greater than 92% by this procedure (Table 2). (For extraction of acidic, as well as base/neutral pesticides and herbicides, see Application Note 105.)

In addition to the extraction tubes and analytical column for this analysis, we offer a kit of neat triazines for preparing standards. The kit consists of ametryn, atrazine, prometryn, propazine, simazine, simetryn, terbutryn (0.5g each), and prometon (0.1g). We recommend protecting the HPLC column with our guard column kit (2cm x 4.6mm column filled with 5µm packing, column holder, and connecting hardware).

Supelclean LC-SCX solid phase extraction tubes reproducibly isolate triazine herbicides from plant extracts, providing excellent recovery. The procedure is rapid and can be adapted to HPLC or GC analyses.

Ordering Information:

| Description | Cat. No. |
|--|----------|
| Supelclean LC-SCX Solid Phase Extraction Tubes, 3mL | 57018 |
| Supelclean LC-18 Solid Phase Extraction Tubes, 3mL | 57012 |
| Reservoirs, 20mL | 57021 |
| Adapters for sample reservoirs | 57020-U |
| SUPEL COSIL LC-8-DB [®] Column, 15cm x 4.6mm | 58347 |
| Supelguard LC-8-DB Guard Column Kit 2cm x 4.6mm cartridge column, column holder, hardware for connecting to 1/16" tubing | 59553 |
| Supelguard LC-8-DB Guard Columns, pk. of 2 | 59563 |
| Neat Triazines Kit | 49092 |
| Visiprep Vacuum Manifold | 57030-U |
| Visidry Vacuum Manifold Drying Attachment | 57100-U |

[®]DB — Deactivated for basic compounds.

References

1. Burnside, O.C., *et al.*, *Weed Sci.*, **19**: 290 (1971).
2. Ramsteiner, K., *et al.*, *JAOAC*, **57**: 192 (1974).

References not available from Supelco.

Trademarks

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