Elanco

STANDARD OPERATING PROCEDURE

Subject or Title:
Determination of Prometryn, GS-11354 and GS-11526 in Soil by LC/MS/MS

SOP No.:	Revision No.:	Effective Date:
LM-CAL-3045	Original	April 23, 1990

Supersedes: None

1. PRINCIPLE

Prometryn and metabolites (GS-11354 and GS-11526) are extracted from soil using a 80:20 (v/v) acetonitrile-water solution. Sample extracts are centrifuged, reduced under nitrogen, and then analyzed by TSP-LC/MS/MS. The detection limit for this method is 10 ug/kg.

2. STANDARDS

2.1 Standards

2.1.1 Prepare a 1 mg/mL individual stock solutions of each analyte in HPLC grade methanol. Replace stock solution every four months or sooner.

2.1.2 Using the 1 mg/mL individual stock solutions from 2.1.1 prepare a 10 ug/mL mixed standard in methanol. Replace mixed analyte solution every four months or sooner.

2.1.3 Using the 10 ug/mL mixed analytical standard solution from 2.1.2 prepare 0.10 ug/mL, 1.0 ug/mL and 10 ug/mL fortification standards in methanol. Replace spiking standards every four months or sooner.

2.1.4 Using the 10 ug/mL mixed standard solution from 2.1.2 prepare calibration standards at 5 ng/mL, 25 ng/mL, 100 ng/mL, 250 ng/mL, and 500 ng/mL in 50/50 Meoh/H_2O. Replace calibration standards every four months or sooner.

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Management Approval: Calvin K. Hazel	Date: April 24, 1990
QA Officer Approval:	Date:

3. PROCEDURE

3.1 Extraction

3.1.1 Weigh 10.0 grams of soil into a 40-mL vial.

3.1.2 If applicable, prepare fortification samples by adding fortification standard.

3.1.2.1 Add 1.0 mL of the 0.10 ug/mL fortification standard to yield 10.0 ug/kg (ppb).

3.1.2.2 Add 1.0 mL of the 1.0 ug/mL fortification standard to yield 100.0 ug/kg (ppb).

3.1.2.3 Add 1.0 mL of the 10.0 ug/mL fortification standard to yield 1,000 ug/kg (ppb).

3.1.3 Add the acetonitrile-water solution (80:20 v/v).

3.1.3.1 Add 20.0 mL to the sample and method blank.

3.1.3.2 Add 19.0 mL to the fortified samples to give a total volume of 20.0 mL of the extracting solution.

3.1.4 Adjust the "pH" of the extracting solution to 7.2 - 7.8 with acetic acidic or NH_4OH.

3.1.5 Shake the extracts on the orbital shake for 3 hours at ca 240 rpm with the vials positioned horizontally.

3.1.6 Centrifuge the extracts up to 1000 rpm for about 5 minutes to obtain a clear supernatant.

3.1.7 Aliquot 5.0 mL of the supernatant and filter it through a 0.45 um syringe filter into an 8-mL test tube. Filter an additional 0.5 mL of 80:20 acetonitrile-water through the filter into the test tube.

3.1.8 Reduce under nitrogen to less than 1 mL to remove the acetonitrile.

3.1.9 Adjust the final volume to 1.0 mL with methanol.

3.1.10 Transfer the extract to a 4 mL vial. Final concentration of the sample is 2.5 g/1.0 mL.

3.1.11 Organize the sample vials into the vial box, and store it in the refrigerator at 2-6° C until analysis.

4. LIQUID CHROMATOGRAPH OPERATING PARAMETERS

4.1 The LC chromatography system parameters have been optimized for these analytes as follows:

Column: 1) HP Hypersil ODS 6 cm x 4.6 mm ID (3um)
No Guard or equivalent

Flow rate: 0.8 mL/min. + 0.4 mL/min.
Post column addition of Buffer

Mobile Phase: ACN/Water
(Water contains 0.1M ammonium acetate)

Gradient: 30/70 ACN/H_2O
Gradient to 65/35 at 2.5 minutes
to 100% ACN at 4 min.
Return to 30/70 at 6.5 minutes
or equivalent conditions which provide
baseline resolution of all analytes.

Retention Times: Prometryn 7:20 minutes (approx.)
GS-11354 2:40 minutes (approx.)
GS-11526 1:50 minutes (approx.)

5. TSP - MASS SPECTROMETER OPERATING PROCEDURES

5.1 Instrument Tuning. - The instrument must be capable of detecting 100 pg of each analyte, (20 uL of 5 ng/mL) with a signal/noise (S/N) ratio of 5:1.

5.1.1 If desired, a mixture of prometryn and the metabolites (1 mg/L) may be used as a tuning solution. This provides fine tuning of the TSP interface parameters.

5.2 TSP-LCMS Operation Parameters.

5.2.1 The following TSP-LCMS parameters must be optimized in order to achieve an optimum performance.

Vaporizer Control Point	- 75 °C - 120 °C
Aerosol Temp	- 250 °C - 320 °C
Repeller Voltage	- 40 V - 75 V

5.2.2 It has been determined that optimal sensitivity is achieved with filament on ionization mode, however, either mode is acceptable.

5.3 Selected Ion Monitoring.

5.3.1 Samples and standards products are analyzed by selected reaction monitoring (SRM) of products derived from the protonated molecular ions $(MH)^+$. DS - Atrazine is included as an internal standard for retention time verification and possible future quantitation.

5.3.2 The appropriate masses to be monitored are listed below:

Analyte	Precursor (MH^+)	Product Ion	Dwell Time
Prometryn	m/z 242	m/z 158 ± .3 amu	0.3 seconds/window
GS-11354	m/z 200	m/z 158 ± .3 amu	0.3 seconds/window
GS-11526	m/z 212	m/z 128 ± .3 amu	0.3 seconds/window
DS-Atrazine	m/z 221	m/z 179 ± .3 amu	0.3 seconds/window

The seconds/window time may be changed providing that at least 6 scans are acquired per peak.

5.4 Calibration and Sample Analysis.

5.4.1 During the course of analysis of a batch of samples each of the calibration standards (see Section 2.1.4) is run once. Between 1 and 6 samples is analyzed between these standards.

5.4.2 Calculate the calibration factor (CF) for each standard, defined as the ratio of the standard concentration to the response for each analyte at each standard concentration.

5.4.3 If the percent relative standard deviation is less than 20% over the working range, linearity can be assumed and the average calibration factor can be used in place of a calibration curve.

5.4.3.1 Average Calibration Factor = $\left|\frac{\text{Concentration (ng/mL)}}{\text{Total Area Counts}}\right|_n$

Where n = number of calibration injections.

5.4.4 Inject 20-50 uL of extract into the TSP-LCMS system for analysis.

6. CALCULATIONS

6.1 The concentration of each analyte in a sample is calculated as follows:

$$\text{Concentration (ug/Kg, dry weight)} = \frac{(CF)\,(Vx)\,(Ax)}{(Ws)\,(Rw)\,(1000)}$$

CF = Average Calibration factor (ng/mL)
Vx = Sample extract final volume (mL)
Rw = Dry Weight/Wet Weight Ratio
Ax = Sample Total Area Counts
Ws = Sample Wet Weight (Kg)