INTRODUCTION

Fosamine Ammonium is the active ingredient in Krenite® Brush Control Agent. This is a product applied as a foliar spray for control and/or growth suppression of many woody species.

This document describes a sensitive and selective method for determining fosamine ammonium in soil down to 0.05 ppm using high performance liquid chromatography (HPLC) with detection by thermospray mass spectrometry (MS).

In the procedure, the fosamine ammonium is extracted from the soil into 50% methanolic ammonium carbonate. The supernatant is passed through a C-18 solid phase extraction (SPE) cartridge to remove most hydrophobic contaminants and is reduced to a small volume by rotovapping. The concentrated extract is passed through an additional C-18 cartridge in tandem with a strong anion exchange (SAX) cartridge. The effluent from the SAX cartridge is evaporated to dryness, dissolved in water and chromatographed using a 25 cm SAX analytical HPLC column. Just prior to the elution of fosamine ammonium, the effluent from the column is directed into the thermospray mass spectrometer. Fosamine ammonium is detected at the M+1 ion, 171 a.m.u., using single ion monitoring (SIM). See Figure 1 for a flowchart.

APPARATUS AND REAGENTS

The ammonium carbonate used in the extracting solvent is HPLC grade (J. T. Baker Chemical Co., Phillipsburg, New Jersey.) The water used throughout this method is purified though a Milli-Q Water Purification System (Millipore Corp., Bedford, Massachusetts). The methanol used is HPLC grade (Fisher Scientific, Pittsburgh, Pennsylvania). The ammonium acetate used in the mobile phase is 99+% pure available from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin as a Gold Label Reagent.

The rotary evaporation system is a Buchi Rotovapor® (Brinkmann Instruments, Inc., Westbury, New York) with a water bath operating at temperatures up to 70°C.

The solid phase extraction (SPE) cartridges used are Bond Elut® C18 and SAX cartridges, 6 cc capacity, catalog #607306 and 3 cc capacity, #618313, respectively (Analytichem, International Harbor City, California). The charging solutions and the eluents are poured into a 75 mL capacity reservoir, (catalog #607500, Analytichem, as above). The reservoir is connected to the cartridge by an adaptor (catalog #636001, Analytichem, as above). When necessary, two cartridges are connected to each other through the same type of adaptor. The supernatants are decanted into the reservoirs and through the cartridges by applying an indirect vacuum. This may be accomplished by placing them on a SPE manifold (Supelco, Inc., Bellefonte, Pennsylvania) if the effluent is to be collected in a 50-mL capacity tube. If the effluent is to be

collected in a round-bottom flask, the reservoir and cartridge are connected to a wide bore Luer lock needle which is fitted into a rubber stopper. The effluent exits through the needle into a glass adaptor which is connected to the flask via an additional glass adaptor with a side-arm to vacuum. See Figure 2 for a diagram of the apparatus. All cartridges are preconditioned by passing 3 to 5 mL of methanol through the cartridge followed by 3 to 5 mL of water before use.

The effluents are collected in 50-mL capacity Kimble tubes and concentrated to dryness using a Meyer "N-Evap" Nitrogen Analytical Evaporator (Organomation, Northborough, Massachusetts).

The filters used are 0.45 u Millex HV filter units (Millipore Corp., Bedford, Massachusetts.)

The HPLC column is a 4.6 mm i.d. x 25 cm Zorbax® SAX column (available from Mac-Mod Analytical, Chadds Ford, Pennsylvania). The HPLC pumps used for through column and post-column addition are Spectroflow 400 solvent delivery systems available from ABI Analytical, Kratos Division, Ramsey, New Jersey. The thermospray LC/MS unit is the Vestec 201 from Vestec Corporation, Houston, Texas which is fitted with a dry ice cold trap (catalog #00107-10601, #40062-98-004, and # 40062-98-005 from Finnegan MAT Corp., Cincinnati, Ohio) before the roughing pump for the source. The data system used is the MS Chemstation, HP 59970 from Hewlett Packard, King of Prussia, Pennsylvania.

EXPERIMENTAL PROCEDURE

Sample Preparation

The samples are received and maintained frozen unless being prepared or analyzed. The pre-processing is done as quickly as possible to minimize degradation of fosamine ammonium. The samples are prepared by placing the entire contents of a sample into a heavy cloth or plastic bag. The bag is pounded with a wooden mallet until the contents are broken into small pieces of about 1 cm or less in diameter. The bag is shaken to mix the pieces. The entire sample, except for stones and plant material, is passed through a mesh sieve of about 5 mm (Size 4) or less mesh size 5 by rubbing the thawing soil against the sieve mesh.

About 30-g of the wet sample is immediately and accurately weighed into a 250-mL centrifuge tube. The weight is recorded and the first portion of extracting solvent is added as described below. Moisture content of the soil is determined on a separate position of sample so that the final calculations can be done on a dry-weight basis.

EXTRACTION

The sample is extracted on a wrist action shaker for 15 minutes with 3 x 100 mL portions of a 1:1 mixture of 4% w/v ammonium carbonate : methanol. After each extraction, the sample is

centrifuged until the soil particles form a cake (about 5 to 10 minutes at 1000 rpm). The liquid is decanted into a reservoir and passed through a C_{18} SPE cartridge into a round-bottom flask as described in "Apparatus and Reagents".

The combined extracts are rotovapped to about 5 mL. The concentrated extract is then passed through a C_{18} SPE cartridge connected to a SAX SPE cartridge as described under "Apparatus and Reagents" including the preconditioning procedure. The C_{18} cartridge is the uppermost cartridge and all charges and rinses are collected in a 50-mL capacity Kimble tube. The round-bottom flask is rinsed with 2 to 3 small volumes of water and the rinses are passed through the cartridges. The C_{18} cartridge is removed. Any residual fosamine ammonium remaining on the SAX cartridge is eluted by rinsing the cartridge with 4 mL of 0.2 M ammonium acetate. The combined rinses are evaporated to dryness on a N-Evap under a stream of nitrogen at temperatures up to 60°C. The dry tubes have been held in the refrigerator for three weeks with no sign of degradation.

The residue is dissolved in 4 mL of water using sonication followed by vortexing to aid in dissolution. The solution is passed through a 0.45 u Millex HV filter.

CHROMATOGRAPHY AND DETECTION

The thermospray MS system must be properly tuned in accordance with the manufacturer's instructions.

The chromatographic system is allowed to equilibrate under the following conditions with the thermospray in "standby" condition.

Column:	4.6 mm x 25 cm Zorbax* SAX analytical column
Mobile Phase:	0.05 M ammonium acetate plus 6.0 g of glacial acetic acid per liter.
Flow rate:	1.0 mL/minute
Temperature:	40°C
Injection Volume:	100 µL

The system is designed to mix the column effluent with a solvent of high ionic strength before entering the thermospray interface to improve ionization.

Post-column additive:	0.5 M ammonium acetate
Flow rate:	0.4 mL/minute

The optimal temperature settings on the thermospray interface will vary depending on the probe used. The settings may also vary with the condition of the probe and the vacuum system. The following temperature settings are given as a reference.

T1 (Stem):	130°C
T2 (Tip):	220°C
Vapor:	230°C
Block:	310°C
Tip Heater:	300°C

After the system is equilibrated, the interface is kept in a standby position and the injection is made. The retention time of fosamine ammonium is about 13 to 15 minutes and can be found on the day of analysis from a standard injection. The instrument remains in the standby mode until 1 to 2 minutes before the elution of fosamine ammonium. The timing should be kept as consistent as possible from injection to injection. When the elapsed time is within about two minutes of the elution of fosamine ammonium, the thermospray unit should be switched to the operate mode, the T1 temperature brought to the predetermined optimal value, and data collection begun.

Data is acquired by SIM at m/e of 171 with low resolution and a typical SIM dwell of 500.

CALCULATIONS

The calculation for the amount of fosamine ammonium in a sample or fortified control is made with the use of a response factor based on the area the peak at the retention time of fosamine ammonium. The response factor is determined from the average of all standard injections or from the standards bracketing the sample being calculated.

Calculate the response factor from a standard injection using 1. Calculate the ppm of fosamine ammonium in a sample using equation 2.

$$\text{Response Factor} = \frac{\text{Peak Area}}{\mu\text{g/mL of fosamine ammonium in the standard}} \quad (1)$$

$$\text{ppm of fosamine ammonium in the sample} = \frac{\text{Peak Area x Final Volume of Sample}}{\text{Response Factor x Sample Weight (g, dry-weight basis)}} \quad (2)$$

FIGURE 1: Flow Diagram of the Method