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Development of Analytical Methods for Escort Herbicide in Forest Environment Samples

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INTRODUCTION

The USDA Forest Service laboratory in Auburn, Alabama, is engaged in research to determine the environmental fate and ecosystem impacts of forestry herbicides. Much of the effort is associated with the conduct of field dissipation studies in which herbicides are applied to forest sites and then monitored over time in a variety of environmental matrices (soil, water, and plant tissue). While some methodology does exist for both sampling and analytical procedures, the available methods have often been developed in an ideal laboratory setting and are not directly suitable for forest environmental samples. Often, new methods must be developed to solve site-specific problems. This paper presents work completed to date on soil and water method development for a relatively new forestry herbicide, Escort.

Escort is the DuPont trade name for a formulation of metsulfuron methyl, a herbicide of the sulfonyl urea family, whose chemical name is methyl 2-[[[[(4-methyoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl] benzoic acid. Sulfonyl urea herbicides are much less stable chemically than other classes (like the phenoxy acids 2,4-D and 2,4,5-T) and break down rapidly following application. This makes them environmentally preferable, but it also presents challenges for storage and handling of field samples collected for environmental fate studies. Analytical methods for these herbicides must incorporate measures to minimize their decomposition between the time they are collected in the field and the time they are analyzed in the laboratory.

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DEVELOPMENT OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) SEPARATION FOR METSULFURON METHYL

Metsulfuron methyl is similar chemically to sulfometuron methyl (trade name Oust), another sulfonyl urea herbicide previously studied in our laboratory. DuPont's published method for sulfometuron methyl (Zahnow 1985) utilized the Tracor 965 photoconductivity detector together with normal-phase HPLC on a bare silica column. We found this detector to be extremely temperamental and unsuitable for routine use. The hydrocarbon-based mobile phase proved to be incompatible with our Waters WISP autoinjectors, necessitating manual injections on round-the-clock shifts. The chromatographic system was itself unstable, requiring constant recalibration for changing retention times and responses,and periodic column rejuvenation whenever the system "crashed". We developed a more dependable autosampler-compatible reversed-phase HPLC separation for sulfometuron methyl using the ultraviolet absorbance detector, which proved highly successful (Wells and Michael 1987). These conditions were therefore used as the starting point for development of a metsulfuron methyl separation. Successive refinements of these conditions produced the following mobile phase/column combination, which is optimized for peak shape and retention time:

Column:	Supelco LC8-DB, 150 mm x 4.6 mm I.D., 5 um diameter spherical porous particles
Mobile phase:	30:70 (v/v) Acetonitrile/H_2O (0.1 M H_3PO_4, pH 2.0)
Flow rate:	1.0 ml/min at 25 °C
Detection:	U.V. Absorbance at 224 nm wavelength

The optimum wavelength for detection was determined from the ultraviolet spectrum of metsulfuron methyl in the mobile phase. Under these conditions, metsulfuron methyl eluted at 6.5 minutes (fig. 1). The detection limit was 0.8 ng injected on-column, based on a minimum peak height five times the baseline noise level. Detector response was linear up to 2,000 ng injected.

DEVELOPMENT OF AN ANALYTICAL METHOD FOR METSULFURON METHYL IN WATER

Our method for analysis of metsulfuron methyl in water samples is derived from the method developed in our laboratory for sulfometuron methyl (Wells and Michael 1987). It is based on the principle of solid phase extraction (SPE). In SPE, a dilute solution of the analyte is passed through a column of adsorbent particles whose surface has a higher affinity for the analyte than does the solvent. The analyte is immobilized on the adsorbent, then released again by elution with a small volume of a more powerful solvent. In reversed-phase SPE, the analyte is non-polar, the solvent is polar (usually water), and the adsorbent is non-polar (usually silica gel derivatized with long-chain alkyl silane groups of up to 18 carbon atoms). Elution of the analyte is achieved with a less polar solvent, such as methanol.

Since metsulfuron methyl is a weak acid (pKa = 3.3), its ionization had to be suppressed in order to make it sufficiently non-polar for reversed-phase SPE. This was accomplished by acidifying the water sample to pH 2.0 with 85 percent phosphoric acid. Elution from the SPE column was effected by acetonitrile or methanol, but the resulting concentrates were unstable, probably due to traces of phosphoric acid coeluted with the metsulfuron methyl. A better elution buffer was found to be 30:70 (v/v) acetonitrile/H_2O (pH 7.0/w 0.1 M potassium phosphate), which stabilized the extracts and was compatible with the HPLC mobile phase. Due to the extreme sensitivity reported for one aquatic species to metsulfuron methyl, the lowest possible detection limit was desired. This was done by

maximizing the size of the water samples (to 500 ml) and minimizing the volume of the final eluted extract (to 5 ml).

Sulfonyl urea herbicides are known to be hydrolytically unstable in acid solution (E. I. Dupont de Nemours and Co., Inc. 1984). Samples of surface water taken from a treatment site in Florida had a pH value of 4.2. In order to determine the stability of metsulfuron methyl in field water samples of varying pH, a set of distilled water samples was buffered with 0.1 M potassium phosphate to pH 4.0, 5.0, and 6.0, then spiked with known amounts of metsulfuron methyl and stored at 35 °C. Five replicate samples at each pH level were analyzed every few days to monitor the percent of the original spike remaining. The results are plotted in figure 2. Hydrolysis was rapid at pH 4.0 and pH 5.0 and appreciable even at pH 6.0. It was therefore deemed necessary to add buffer to the sample containers in the field to stabilize the water samples as they were collected. Accordingly, a mixture of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_4, which produces a pH of 7.0, was added in dry form to each 1 liter field container. The containers were frozen as soon as possible and kept frozen during transport and storage.

Since the water samples are adjusted to pH 2.0 to suppress their ionization prior to SPE, a recovery study was done to determine the effect of time at this pH on metsulfuron methyl recovery. A set of spiked water samples was adjusted to pH 2.0 and allowed to stand on the lab bench at 25 °C for periods of 1, 2, and 3 hours before being adsorbed on SPE columns and eluted with pH 7.0 buffer. Significant loss was observed even after 1 hour (fig. 3) reaching 15 percent by 3 hours. This loss was minimized by performing the SPE adsorption and elution steps as quickly as possible after acidification (less than 1 hour if possible) and by recording the time at pH 2 so that a correction factor could be applied if necessary. Prompt elution gave a recovery of 98.7 percent.

A test was conducted to determine if acidification to pH 2.0 was really necessary to get maximal adsorption of metsulfuron methyl on the C_{18}-SPE columns. A set of 500 ml water samples was buffered to pH 7.0 with 0.1 M potassium phosphate, then spiked with 40 ppb each of metsulfuron methyl. The pH of each spiked solution was then adjusted with 85 percent H_3PO_4 to values ranging from pH 2.0 to pH 7.0. These pH-adjusted water samples were then adsorbed and eluted from the SPE columns. Some of the columns were prewashed with methanol followed by pH 2.0 water and postwashed with pH 2.0 water. Some were prewashed with methanol and pH 2.0 water but not postwashed. Some were prewashed with methanol and pH 7.0 buffered water with no postwash. The results are graphed in figure 4. In all cases but one, the recoveries were in the range of 95--99 percent. Apparently, metsulfuron methyl is sufficiently insoluble in water that it adsorbs to the C_{18} SPE columns even without ion-suppression by acid. The 0.1 M potassium phosphate buffer in the water samples may also contribute to suppression of ionization of metsulfuron methyl by the "salting-out" effect.

The final method is outlined as follows:

1) Collect the water sample in the field from a well or an automatic streamflow sampler (ISCO), add 0.1 M potassium phosphate buffer to adjust the pH to 7.0, and freeze as soon as possible.

2) Thaw the sample just prior to analysis. Measure the sample volume with a graduated cylinder.

3) Precondition an SPE column containing 1000 mg of C_{18}-derivatized silica gel adsorbent (Baker #7020-7) by washing with 5 ml Methanol followed by 10 ml of 0.1 M Potassium phosphate buffer (pH 7.0).

4) Pass the sample (500 ml) through an SPE column at a rate of 4 drops per second or less, controlled by adjusting the vacuum applied to the column.

5) Elute the column with 4.5 ml 30:70 (v/v) acetonitrile/H_2O) (ph 7.0/w 0.1 M potassium phosphate buffer). Make up to exactly 5.0 ml with buffer. Mix thoroughly.

6) Transfer the sample to an autosampler vial, filtering through a Millipore Millex-SR membrane filter if cloudy.

7) Inject 10 μl on the HPLC. Quantitate against external standards injected before and after every fourth sample.

Many samples of water from ponds and shallow wells at treatment sites are not clear, but instead tinted amber with dissolved organic matter. This complex polymeric material, principally humic and tannic acids, co-adsorbs and co-elutes with metsulfuron methyl from the solid phase adsorbent, competing for adsorption sites and reducing recovery. It also interferes with the HPLC separation, reducing the signal-to-noise ratio and lengthening equilibration time between injections. At the Florida treatment site, this has limited the overall concentration factor we can reliably use to 100:1 (500 ml water sample concentrated to 5 ml final extract) and the amount of extract we can safely inject on the HPLC system to 10 μl. This has made our practical detection limit in water about 1 ppb.

We have experimented with adding small amounts of divalent metal salts to "humified" water samples to precipitate the organic material prior to SPE. Lead (II) acetate (0.01 M) was very effective in precipitating organic material from unbuffered water, producing a clear supernatant and a flocculent brown precipitate that could be removed by suction filtration through glass fibre paper. Unfortunately, when this treatment was applied to water buffered with 0.1 M potassium phosphate, a grey precipitate of lead phosphate formed that interfered with filtration and left the organic material in solution. This treatment may prove more effective when applied to herbicides that do not require buffering for field stabilization.

DEVELOPMENT OF AN ANALYTICAL METHOD FOR METSULFURON METHYL IN SOILS

In environmental fate studies, it is necessary to sample soil at different depths to detect movement of the herbicide underground. In our laboratory, a method has evolved for collecting and processing soil samples that has proven effective for other herbicides (Michael and Neary 1988). A 2-inch I.D. PVC pipe is driven into the ground to a prescribed depth, then removed and frozen for transport to the laboratory. The frozen pipe is cut into 4-inch (10 cm) sections with a band saw. Each section is thawed, extruded, and spread out to dry on aluminum foil. When dry, each soil sample is screened to remove roots and pebbles, then refrozen in a zip-lock bag. The dry soil is passed through a 14-channel soil splitter several times until a representative sample of suitable size for analysis is obtained. The extraction of metsulfuron methyl proceeds as follows:

1) A 50.0-g sample of the soil is weighed into a 250 ml polypropylene wide-mouth, screw-capped jar.

2) A 100-ml portion of extracting buffer solution [50:50 (v/v) methanol/H_2O (pH 7.0/w 0.1 M potassium phosphate)] is added, and the mixture is shaken for 10 minutes on a table shaker.

3) The mixture is centrifuged at 3,000 rpm for 10 minutes, and the supernatant is decanted.

4) Steps 2 and 3 are repeated, and the supernatants are combined and suction-filtered through glass-fibre paper (1 micron, GF/B, Whatman).

5) The filtrate is rotary-evaporated under reduced pressure on a warm water bath (45-50 °C) to remove the methanol component.

6) The aqueous residue is extracted and eluted by SPE in the same manner as the water samples.

Unfortunately, polymeric materials also co-extract with metsulfuron methyl from soils, and present serious problems in surface soils with high organic matter content. Soils low in organic matter give metsulfuron methyl recoveries of about 90 percent, whereas, high organic soils can give recoveries as low as 70-80 percent. The detection limit is about 20 ppb for high organic soils.

Soils cannot be buffered as can water samples, and many forest soils have pH values around 4-5. We therefore ran an experiment to test the effect of soil drying time at room temperature on metsulfuron methyl recovery. A large sample of surface soil (pH 4.3) was tested to determine its field water capacity (43 percent w/w) and then was air-dried and subdivided into test samples using a soil splitter. These samples were individually treated with water to bring them to 25 percent, 50 percent, and 75 percent of their field capacity, then spiked with metsulfuron methyl and allowed to air-dry for 1, 2, and 4 days before being extracted and analyzed (fig. 4). We observed an appreciable drop in metsulfuron methyl recovery over the first 2 days of drying with a less rapid decline thereafter. This corresponds to visual observations that drying was complete by 2 days and suggests that hydrolysis is indeed taking place while the soil is damp. We are presently trying to modify our soil workup to eliminate the drying step and obtain a representative soil sample without splitting.

CONCLUSIONS

We have developed workable methods for analysis of metsulfuron methyl (Escort) herbicide in environmental samples of water and soil from forestry test sites. Detection limits are about 1 ppb in water and 20 ppb in soil. Measures for minimizing hydrolysis prior to analysis have been established for water and are under development for soil. Co-extraction of dissolved organic matter remains a problem, especially for high organic soils.

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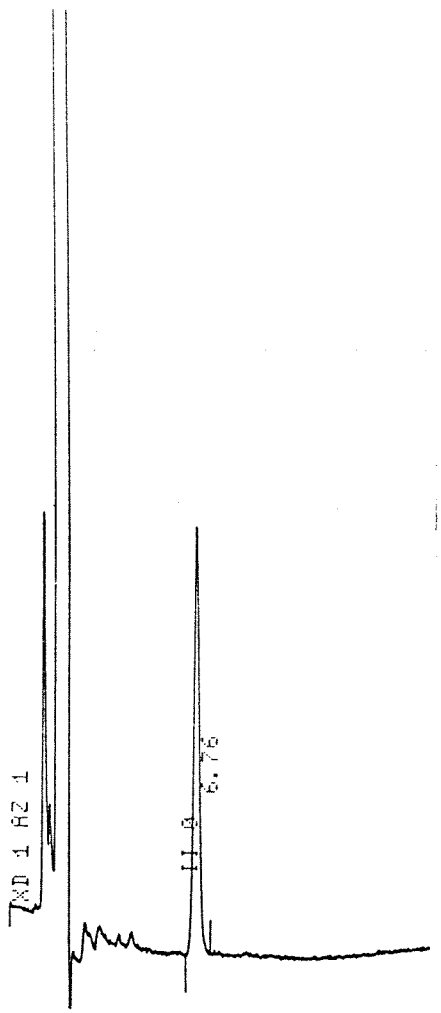
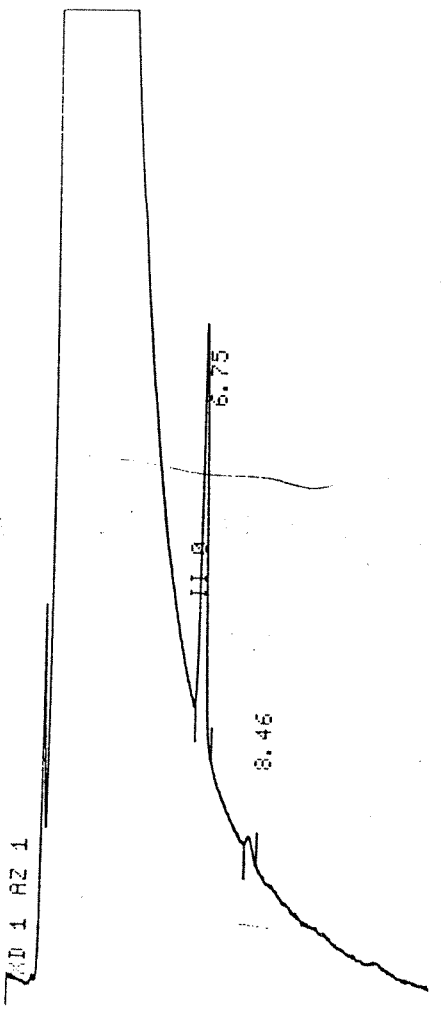


Metsulfuron Methyl Standard
20 ng injection



Spiked Well Water Extract
20 ppb Metsulfuron Methyl

Figure 1. HPLC chromatograms of metsulfuron methyl.

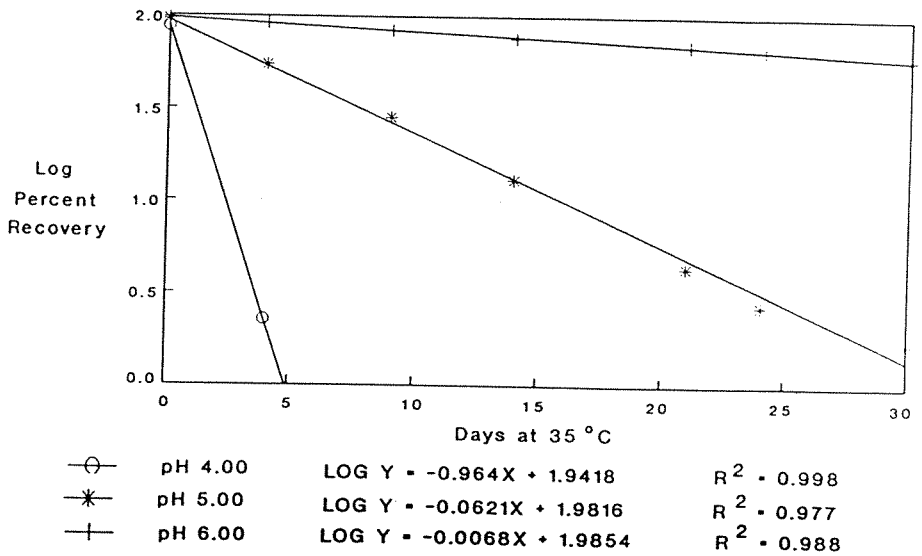


Figure 2. Effect of water sample pH on metsulfuron methyl recovery.

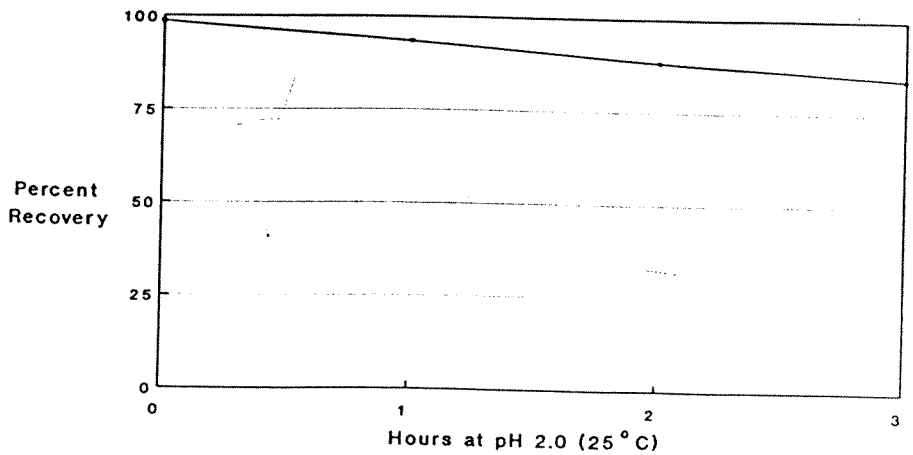


Figure 3. Effect of time at pH 2.0 on metsulfuron methyl recovery.

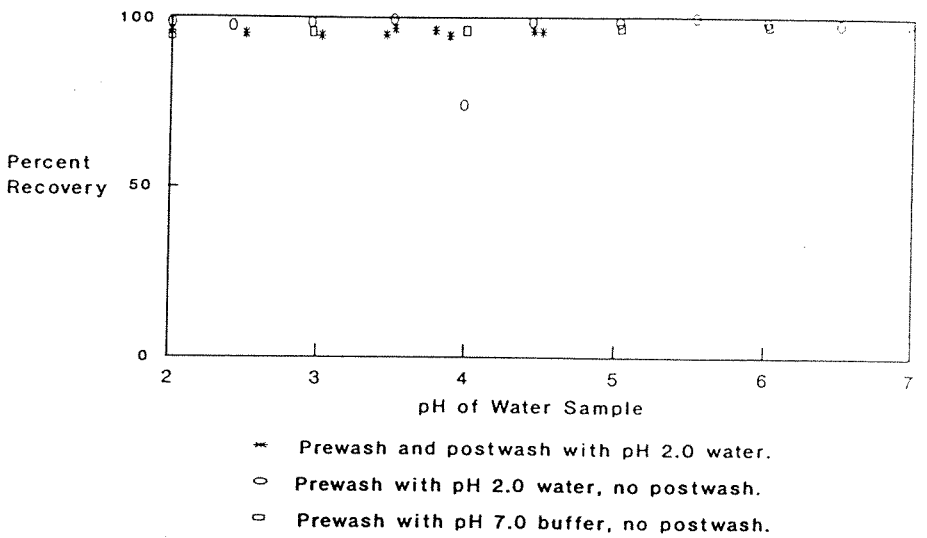


Figure 4. Metsulfuron methyl SPE recovery vs. pH of water extracted.

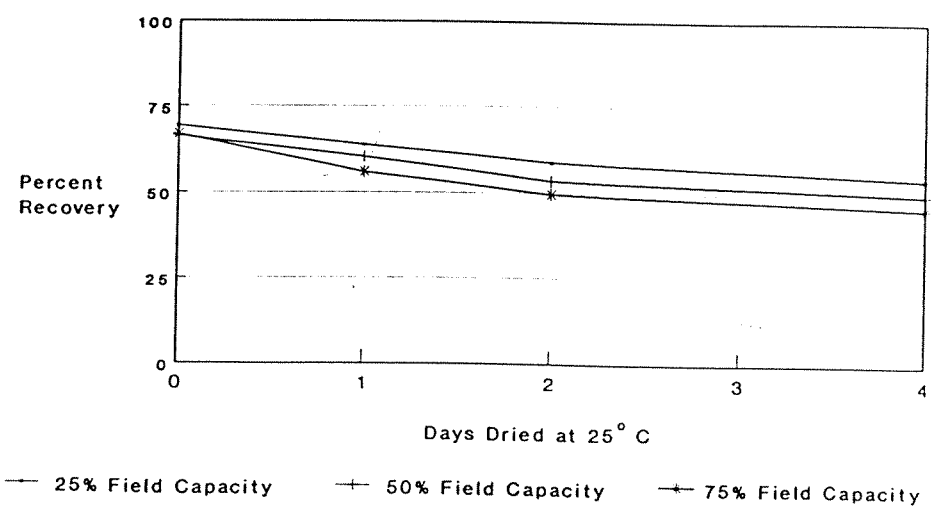


Figure 5. Effect of soil drying time on metsulfuron methyl recovery.