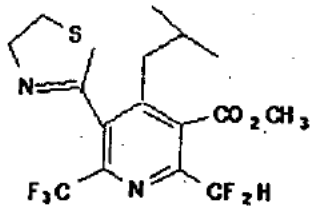
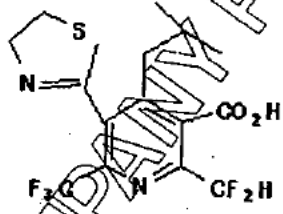
1. Introduction

Thiazopyr (RH-123652) is a herbicide developed for citrus, grapes, tree nuts and other crops. The CAS registry number of thiazopyr is 117718-60-2 and its chemical name is 3-Pyridinecarboxylic acid, 2-(difluoromethyl)-5-(4, 5-dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-, methyl ester. Its molecular structure is shown below. This analytical method describes the analysis of thiazopyr and its metabolite, monoacid, in water and soil.



Thiazopyr



Monoacid

2. Summary

Thiazopyr and monoacid are quantitated separately in this method. The two analytes are first separated by a hexane partition step (after extraction step for soil samples). Thiazopyr, which is extracted into the hexane phase, is quantitated by gas chromatography with an electron capture detector (GC/ECD). The monoacid in the aqueous phase is then acidified and partitioned into ethyl acetate. Derivitization is performed to convert monoacid to its methyl ester, thiazopyr. After a Florisil column clean-up, the monoacid, now in the form of thiazopyr, is quantitated by GC/ECD. The effectiveness of the analytical method is evaluated based on the recoveries of known concentrations of thiazopyr and monoacid fortified into the untreated water and soil samples which are then carried through the analytical procedure. The limit of quantitation (LOQ) is 0.1 ppb in water and 0.01 ppm in soil for both thiazopyr and monoacid. A full set of soil or water samples, including control and fortified control samples, is expected to be extracted and prepared for quantitation within eight hours.

An enforcement analytical method will be issued after more fortification recovery data are available from the study sample analysis.

3. Chemicals and Supplies

Diazald kit	Aldrich, Z10,025-0, AL-131
Diazomethane	Lab made from the diazald kit.
Ethyl acetate (EtOAc), OPTIMA™ Grade	Fisher
Ethanol (EtOH), 200 proof	Midwest Grain, #6810-00-242-3645
Florisil	Fisher
Hexane, HPLC Reagent	Baker
Hydrochloric acid (HCl), 37% (12 N), Reagent Grade	Fisher
Isooctane (IO)	Fisher
Methanol (MeOH), OPTIMA™ Grade	Fisher
Sodium Chloride (NaCl)	Fisher
Sodium Sulfate (Na_2SO_4)	Fisher
Sodium hydroxide (NaOH)	Fisher
Thiazopyr standard	Rohm & Haas Lot PIT-9001-1445-A
Thiazopyr monoacid standard	Rohm & Haas Lot HET-8912-1256-A
Trimethylsilyldiazomethane (TMS)	Aldrich, #36283-2
Water, Milli-Q	Laboratory

The following solutions may be prepared in advance:

0.4 N HCl, 0.24 N HCl, 0.5 N NaOH, 0.01 N NaOH, 10% NaCl solution by weight, 1:1 ethyl acetate/isooctane (EtOAC/IO) by volume, 70% methanol/0.4 N HCl by volume, 10% ethyl acetate/hexane by volume, and 20% ethyl acetate/hexane by volume.

Any equivalent suppliers may be used after demonstrating suitability.

4. Equipment

Büchner Funnel	Waring
Centrifuge Tubes (50 mL)	Pyrex
Chromatographic Column (14.5 cm id)	Pyrex
Glass Fiber Filter Paper	Whatman, #934-AH
Filter Flasks (500 ml)	Pyrex
Round Bottom Flasks, 24/40 (100, 250 and 300 ml)	Pyrex
Rotary Evaporator	Büchi
Separatory Funnels (500 ml)	Pyrex
Standard Laboratory Equipment: (Balances, Beakers, Vortex, and etc.)	Pyrex, Kimax, Mettler
Teflon FEP Round Bottles, 250mL	VWR
Wrist-Action Shaker	Burrell

Any equivalent equipment may be used after demonstrating suitability.

5. Analytical Procedure

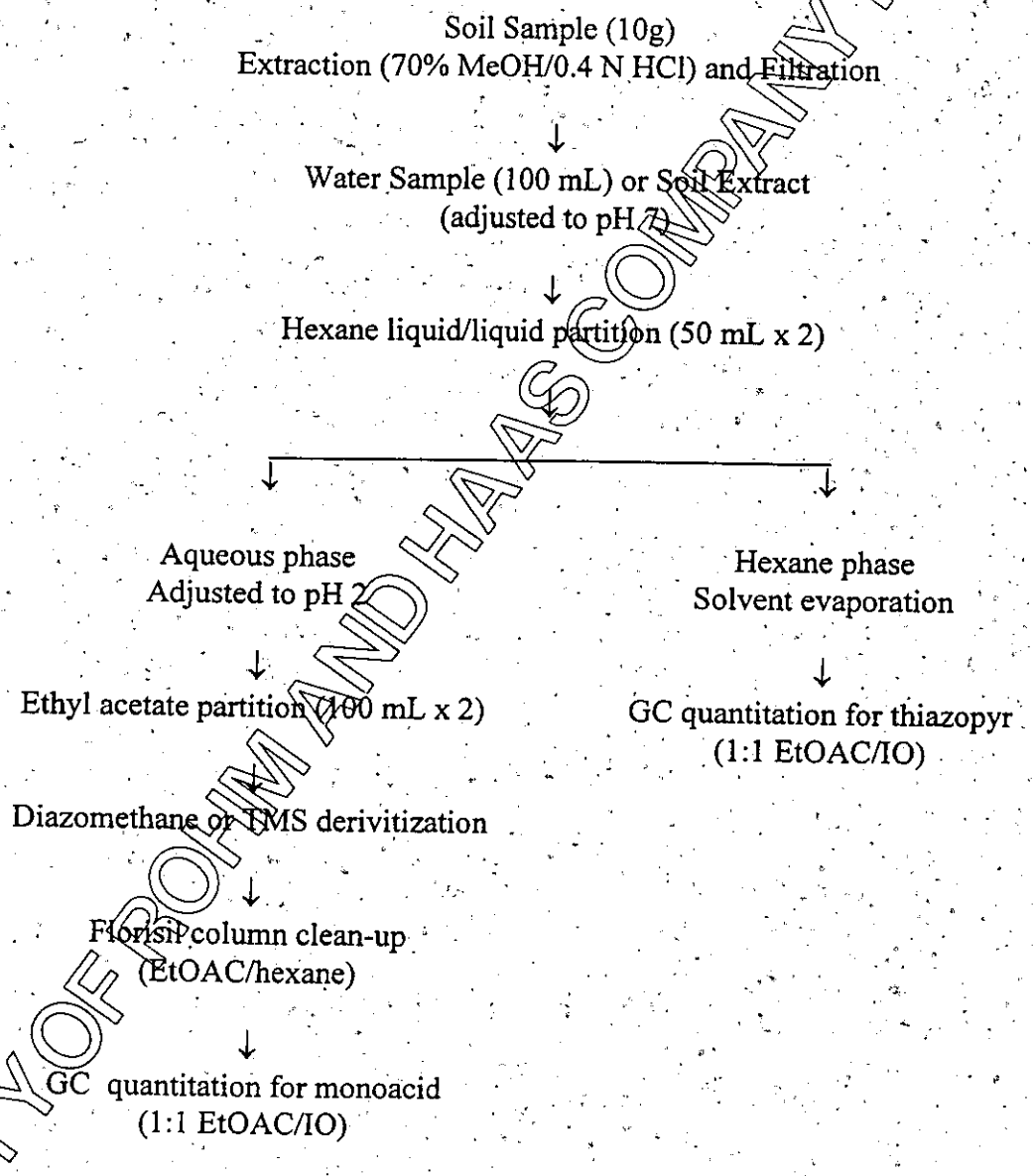
This analytical method describes the analysis of thiazopyr and its metabolite, monoacid, in water and soil. The recommended sample sizes are shown below. However, sample sizes and final volume can be varied depending on the expected analyte concentrations and the amount of sample available. The concentrations

and volumes of some of the chemicals, such as NaOH, HCl, diazomethane, and etc., may also be modified to achieve the best performance of the method.

A full set of soil or water samples, including control and fortified control samples, is expected to be extracted and prepared for quantitation within eight hours.

5.1 Flow Diagram

A flow diagram of the analytical procedure is shown below.



5.2 Extraction of Soil Samples

Weigh 10 grams of soil into a 250 mL Teflon round bottle, if required, spike the sample with the analyte(s) of interest, and add 50 mL of 70% methanol/0.4 N HCl. Shake for 15 minutes on a wrist-action shaker set at maximum speed. Filter the sample and rinse the flask with 20 mL of the same extraction solution twice and pass through the filter cake with vacuum. The extract is then rotary evaporated to less than 25 mL. Adjust volume to about 100 mL with Milli Q water.

5.3 Hexane Partition of Soil Extracts and Water Samples

Measure 100 mL of water sample or transfer the soil extract sample from 5.2 into a beaker. If required, spike the water sample with the analyte(s) of interest. Adjust the sample to a pH of 7 with 0.01 N NaOH for water samples and 0.5 N NaOH for soil extracts, then transfer to a 500-mL separatory funnel. Rinse the original water or soil extract container with 50 mL of hexane and transfer to the separatory funnel. Partition twice with 50 mL of hexane each time. Reserving the aqueous layer. Combine the hexane layers into a round bottom flask and rotovap to dryness. Dissolve the sample in an appropriate final volume of 1:1 ethyl acetate/isooctane. The final volume is usually 50 to 200 mL for soil samples and 5 to 20 mL for water samples. The sample is now ready for GC quantitation of thiazopyr.

Samples at this stage are stable at room temperature. If they are not expected to be injected right away, they should be sealed well to prevent solvent from evaporating.

5.4 Ethyl Acetate Partition

Transfer the aqueous phase from the previous step to a 250 mL beaker and adjust to pH 2 with 0.24 N HCl solution. Transfer to a 500 mL separatory funnel and extract twice with 100 mL ethyl acetate each time. Discard the aqueous layer. Combine the ethyl acetate layers in a 250 or 300 mL round bottom flask and evaporate to approximately 5 mL, then transfer to a 50 mL centrifuge tube. Rinse the round bottom twice with 2 mL of ethyl acetate and add to the centrifuge tube.

5.5 Derivitization

Evaporate the ethyl acetate solution to about 1 mL by nitrogen. Add about 100 µL of MeOH and 1 mL of diazomethane or 0.5 mL trimethylsilyl-diazomethane (TMS) until the solution turns yellow. Cap and vortex to mix. Allow to stand for at least 30 minutes and transfer to a separatory

funnel containing 40 mL of MeOH and 60 mL of 10% NaCl. Partition twice with 50 mL hexane each time. Discard the aqueous layer. Combine hexane layers in a 250 mL round bottom flask and evaporate to approximately 5 mL.

Note: Special care needs to be taken when making and storing diazomethane. Instructions from the diazald kit should be closely followed.

5.6. Florisil Column Clean-up

Pre-activate Florisil, 60-100 mesh, by heating in oven for 24 hours at 200°C. Insert a small glass wool plug into a 14.5 mm ID chromatographic column and slurry pack the column with 15 cc of activated Florisil in hexane. Top the column with approximately 5 cc of anhydrous sodium sulfate. Rinse the column with 20 mL of hexane. Apply the hexane layer from step 5.5. Then rinse the round bottom flask with 30 mL of hexane which is then applied to the column. Wash the column again with 30 mL of 10% ethyl acetate/hexane. Discard the washes. Elute the column with 40 mL of 20% ethyl acetate/hexane and collect the eluent in a 300 mL round bottom flask. Concentrate eluent to dryness under vacuum at ~45°C using a rotary evaporator. Dissolve sample in an appropriate final volume of 1:1 isooctane/ethyl acetate. The final volume is usually 50 to 200 mL for soil samples and 5 to 20 mL for water samples. The sample is now ready for GC quantitation of the monoacid (converted to thiazopyr).

Samples at this stage are stable at room temperature. If they are not expected to be injected right away, they should be sealed well to prevent solvent from evaporating.

6. Instrumentation

Gas chromatography (GC) with an electron capture detector (ECD) is used for quantitation. The conditions on an HP 5890 GC/ECD are shown below.

Column:	RTX-5, 30 meters x 0.53 mm ID x 1.5 µm film
Carrier gas:	Helium
Flow Rate:	10 ml/min
Injection Volume:	5 µl
Injection Mode:	Splitless
Injection Temp:	250°C
ECD Temp:	300°C
Column Temp:	160°C for 1.0 minutes, 160 - 260°C at 6°/minute, 260°C for 5 minutes

Under these conditions, the typical retention time for the thiazopyr is about 7.4 minutes. Control (untreated) samples are run concurrently with the analytical samples to determine the presence of matrix interference. A solvent blank may be injected with the samples as part of an analytical set to confirm the cleanliness of the solvent used.

Similar conditions may be used after demonstrating suitability.

7. Analytical Standards

Analytical standard solutions are prepared for fortifying control matrices to determine analytical recoveries and for calibrating the response of the analyte in the gas chromatographic system.

7.1 Standard Stock Solutions

100 µg/ml thiazopyr solution

Weigh 0.0100 grams (weight adjusted for purity) of analytical grade thiazopyr into a 100 ml volumetric flask, dilute to volume with ethanol and mix well to insure complete dissolution. This solutions contains 100 µg/ml of thiazopyr.

100 µg/ml monoacid solution

Weigh 0.0100 grams (weight adjusted for purity) of analytical grade thiazopyr monoacid into a 100 ml volumetric flask, dilute to volume with ethanol and mix well to insure complete dissolution. This solutions contains 100 µg/ml of monoacid.

7.2 Fortification Solutions

Samples will be fortified at different analyte levels. The solutions used to fortify control samples are prepared in the following manner.

1.0 µg/ml solution

Pipet 1.0 ml the 100 µg/ml thiazopyr and/or monoacid solution into a 100 ml volumetric flask, dilute to volume with ethanol and mix well. This standard contains 1.0 µg/ml of thiazopyr and/or monoacid.

0.10 µg/ml solution

Pipet 10.0 ml the 1.0 µg/ml thiazopyr and/or monoacid solution into a 100 ml volumetric flask, dilute to volume with ethanol and mix well. This standard contains 0.10 µg/ml of thiazopyr and/or monoacid.

7.3 GC Calibration Standard Solutions

The GC calibration standard solutions are made at convenient concentrations of the analyte, thiazopyr, in a 1:1 EtOAC/IO solution. These standards are used to construct a calibration curve which is then used for quantitation of an analyte. Concentrations of thiazopyr in soil and water can be directly calculated based on the calibration curve.

When the standard solutions used for quantitation of monoacid, however, the thiazopyr concentrations of the standard solutions are multiplied by a conversion factor. Therefore, concentrations of monoacid in soil and water can be calculated directly from the reconstructed calibration curve for monoacid.

$$\text{Conversion factor} = \frac{\text{MW monoacid } 382.4}{\text{MW thiazopyr } 396.4} = 0.9647$$

Where MW is the molecular weight of monoacid (382.4) and thiazopyr (396.4), respectively.

1.00 µg/ml solution

Pipet 1.00 ml of the 100 µg/ml thiazopyr stock solution into a 100 ml volumetric flask, dilute to volume with 1:1 EtOAC/IO and mix well. This solution contains 1.00 µg/ml of thiazopyr. When used for the quantitation of monoacid, the monoacid equivalent concentration of this solution is 1.00 x 382.4/396.4 = 0.965 ppm.

0.10 µg/ml solution

Pipet 10.0 ml of the 1.00 µg/ml thiazopyr stock solution into a 100 ml volumetric flask, dilute to volume with 1:1 EtOAC/IO and mix well. This solution contains 0.10 µg/ml of thiazopyr. This is the working solution from which calibration standard solutions are made. When used for the quantitation of monoacid, the monoacid equivalent concentration of this solution is 0.10 x 382.4/396.4 = 0.0965 ppm.

The following is an example of calibration standard levels.. Concentrations other than the ones shown below also may be prepared and used. The range of concentrations used in the method development is from 0.0010 to 0.010 µg/ml. When the standard solutions used for thiazopyr, the concentrations are listed in the column of Final Conc. Thiazopyr (ppm). When the standard solutions used for monoacid, the concentrations are listed in the column of Final Conc. Monoacid (ppm).

Vol. of 0.100 ppm Std. Solution (mL)	Final Volume (mL)	Final Conc. Thiazopyr (ppm)	Final Conc. Monoacid (ppm)
1.00	100	0.0010	0.00096
2.00	100	0.0020	0.00193
5.00	100	0.0050	0.00482
10.00	100	0.0100	0.00945

Dilute each of the detector calibration standards to a final volume of 100 ml with 1:1 EtOAc/IO.

8. Calculations

Standard solutions are prepared in the concentration range of 0.0010 µg/mL to 0.010 ppm. If necessary, sample final volumes should be adjusted to give a response within the standard curve range.

Standards and samples should be quantified using peak areas or heights. Construct a calibration curve with every set of samples. A minimum of four standards should be used for every curve.

8.1 Residue Concentration

Equation 1,

$$\text{ppm} = \frac{(\mu\text{g/mL detected})(\text{final volume, mL})}{\text{sample weight, g}}$$

In the case of the metabolite, monoacid is converted to thiazopyr for quantitation. Since the calibration standards used for monoacid quantitation are already translated to monoacid equivalent concentrations (Section 7), equation 1 can be used directly for calculating ppm of monoacid in the original samples.

8.2 Fortification Recovery

The analytical recovery for an individual fortification is calculated as below.

Equation 2,

$$\%\ \text{recovery} = \frac{[(\mu\text{g/mL detected} \times \text{final volume, mL}) - \mu\text{g detected in control}] \times 100}{\mu\text{g fortified}}$$

8.3 Total Thiazopyr Concentration

When this method is used for residue analysis, total residue may be reported as thiazopyr equivalent by use of equation 3.

Equation 3,

Total ppm of Thiazopyr = ppm of Thiazopyr + (ppm of Monacid x 1.04)

Where,

Conversion factor = $\frac{\text{MW thiazopyr } 396.4}{\text{MW monoacid } 382.4} = 1.04$

10. Confirmatory Procedure

A conformation procedure is developed to ensure the nature of a detected peak, if necessary. This is done by preparing the soil or water samples as described in section 5. The final volume is usually 10 to 20 mL for soil samples and 1 to 2 mL for water samples. However, the samples are injected to a gas chromatographic system (GC) with a mass spectrometer detector (MSD). The conditions on a Hewlett Packard 6890 GC with a 5973 MSD are shown below.

Under these conditions, the typical retention time for the thiazopyr is about 8.7 minutes. Control (untreated) samples are run concurrently with the analytical samples to determine the presence of matrix interference. A solvent blank may be injected with the samples as

part of an analytical set to confirm the cleanliness of the solvent used. Similar conditions may be used after demonstrating suitability.

Column:	HP-5MS, 30 meters x 0.25 mm ID x 0.25 µm film
Carrier gas:	Helium
Flow Rate:	2 ml/min for 5 min, decrease to 1 ml/min
Injection Volume:	2 µl
Injection Mode:	Pulsed Splitless, 25 psi for 1 min
Injection Liner:	2 mm ID cyclo double gooseneck - splitless
Purge Flow to Split Vent:	25 ml/min
Injector Purge Delay:	1 min
Injection Temp:	250°C
Dwell Time:	100 msec
Transfer Line Temperature:	300°C
Ions Monitored:	363 and 396
Column Temp:	120°C for 1.0 minutes 120 - 280°C at 15°/minute 280°C for 4 minutes

Tables 3 and 4 show the fortification recoveries from the same sets of soil and water samples on GC/ECD and GC/MS. Results are satisfactory. Representative standard chromatograms and the calibration curve on GC/MSD are shown in Figures 22-26. Control and fortified sample chromatograms can be found in Figures 27-34 for soil and Figures 35-42 for water.