

BROMOXYNIL-01

SOP No. 90020
October 4, 1991
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Rhône-Puolenc Ag Company Standard Operating Procedure

TITLE: BROMOXYNIL
Determination of Residues in Rice Grain and Straw

SCOPE: Bromoxynil residues resulting from applications of BUCTRIL® brand bromoxynil herbicide may consist of parent compound, bromoxynil phenol, or at higher pH levels as bromoxynil ions. Residues are converted to bromoxynil phenol, methylated, measured as the methyl ether and reported as the bromoxynil phenol. The procedure is described in detail herein.

LIMITATIONS: Method Sensitivity = 0.01 ppm

SUCCESSION: Supersedes SOP 90020 (May 9, 1991)

DISTRIBUTION: Food Safety & Residue Programs

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SUMMARY: Samples are hydrolyzed in 1.0 N methanolic potassium hydroxide, cleaned up with anionic exchange resin, methylated with diazomethane, cleaned up by Sep-Pak™ column chromatography if necessary and quantified by electron capture gas chromatography.

SAFETY: Observe all safety precautions for generating or using diazomethane.

DEFINITIONS: N/A

BACKGROUND: The active ingredient in BUCTRIL® brand herbicide is bromoxynil which is present as the octanoate ester. Since the residue compound of interest is bromoxynil phenol, fortifications for recovery determinations are made with the phenol equivalent of the octanoate ester. The equivalent is 1.45 units of the ester per unit of phenol.

EQUIPMENT:

Food Chopper, Hobart food chopper or similar bowl type chopper

Blender, 1 qt. Waring Blender or equivalent

Extraction Flasks, flat bottom, 250 mL, with Standard Taper ground glass joints

Condensers, Friedrich or equivalent to fit extraction flasks

Graduated Cylinders, glass stoppered, 250 mL

Volumetric Flasks, glass stoppered 100 mL

Centrifuge Tubes, screw-capped, 15 mL graduated, with TEFLON® cap liners

Magnetic Stirring Hot Plate

TEFLON®-Coated Stirring Bars, approx. 1-1/2" X 1/4" diam.

Centrifuge for 15 mL centrifuge tubes

Hypodermic Syringe, 5 mL, glass

Gas Chromatograph with ⁶³Ni electron capture detector and provision for wide bore capillary installation

REAGENTS:

Hexane, high purity for use with electron capture detectors

Toluene, high purity for use with electron capture detectors

Methanol, high purity for use with electron capture detectors

Ethyl Ether, high purity for use with electron capture detectors

Potassium Hydroxide, Mallinckrodt 6976 or equivalent

Potassium Chloride, Fisher P-217 or equivalent

Hydrochloric Acid, Mallinckrodt 2612-1 or equivalent

Sodium Chloride, Baker 3624-1 or equivalent

Water, distilled or otherwise purified

15% Ethyl Ether in Hexane

30% Ethyl Ether in Hexane (dry over sodium chloride - Note)

Potassium Hydroxide Solution, 1.0 N in Methanol

Hydrochloric Acid Solution, 1.0 N in Water

Potassium Chloride Solution, 1.0 N in water

Acid "Buffer", mix equal volumes of 1.0 N aqueous HCl and 1.0 N aqueous KCl

QAE Sephadex A-25 Ion Exchange Resin, Pharmacia Fine Chemicals, Piscataway, NJ

Sep-PakTM Silica Cartridges, No. 51900, Waters Associates, Inc., Milford, MA

Diazomethane Solution, - Prepare from N-methyl-N-nitroso-p-toluene sulfonamide according to SOP No. 81330 or equivalent method to obtain high purity reagent. Observe all safety precautions. Store in tightly capped bottle, with TEFLON[®] cap liner, in freezer. Do not store over KOH or other desiccants.

Standard Bromoxynil Octanoate Solution - Accurately weigh 145.6 to 149 mg of bromoxynil octanoate into a 100 mL volumetric flask, dissolve in methanol, dilute to the mark with methanol and then add one mL of additional methanol for each 1.5 mg of compound weighed in excess of 145.6 mg. For example, if 148.3 mg is weighed out, add 1.8 mL of methanol in addition to the 100 mL.

This solution contains 1.45 mg of bromoxynil octanoate per mL which is **equivalent** to 1.0 mg of bromoxynil phenol per mL. Mix the solution well. Make three serial 10 mL to 100 mL dilutions in methanol. The final serial dilution will contain 1.0 µg/mL as bromoxynil phenol equivalent of bromoxynil octanoate. Make a 10 mL to 100 mL dilution in methanol of this last solution to give a solution of 0.1 µg/mL equivalent bromoxynil. The two most dilute solutions may be used for fortification of crop samples for determination of recoveries. Other dilutions may be made as needed.

Standard Bromoxynil Solution - Accurately weigh 100 to 102 mg of bromoxynil into a 100 mL volumetric flask, dissolve in methanol, dilute to the mark with methanol, and then add 1 mL of methanol for each mg of compound weighed in excess of 100 mg. For example, if 101.5 mg of bromoxynil was weighed out, add 1.5 mL of methanol in addition to the 100 mL. The solution will then contain 1.0 mg of bromoxynil per mL. Mix the solution well. Dilute 10 mL of this solution to 100 mL to give a solution of 0.10 mg of bromoxynil per mL. Other dilutions may be made if required.

Standard Bromoxynil Methyl Ether Solution - Transfer 1.0 mL of the 0.10 mg/mL bromoxynil in methanol solution above to a 15 mL screw-capped (with TEFLON[®] cap liner) graduated centrifuge tube. In a good fume hood, add diazomethane solution until a permanent yellow color is obtained (about one mL). Cap the tube and allow to stand at room temperature for about 15 minutes. Remove excess diazomethane by using a gentle stream of nitrogen and a water bath with a temperature up to about 40 °C. The water bath is not necessary, but it shortens the time required for diazomethane removal. Quantitatively transfer the methylated bromoxynil to a 100 mL volumetric flask using 10 to 15 mL of toluene to complete the transfer. Dilute to the mark with hexane and mix well. This solution contains 1.0 µg of bromoxynil, as the methyl ether, per mL. Dilute 10 mL of this solution to 100 mL in hexane to give a solution that contains 0.1 µg of bromoxynil per mL. Dilute 2 mL, 5 mL, 10 mL and 20 mL of the 0.1 mg/mL solution to 100 mL in hexane to give solutions of 0.002 µg/mL, 0.005 µg/mL, 0.01 µg/mL and 0.02 µg/mL. These solutions may be used for gas chromatographic calibration. Other dilutions may be made as circumstances require.

Procedure:

1. Grind 150 to 200 grams (or other amount sufficient to represent the sample to be analyzed) plus an equal quantity of dry ice in a blender until a coarse powder is obtained; or "finely" chop a similar amount of straw along with an equal or greater quantity of dry ice in a bowl-type food chopper. Allow the dry ice to sublime in a freezer - usually overnight.

2. Weigh 5.0 grams of ground sample into a 250 mL extraction flask. Fortification of samples for determination of recoveries should be done at this point. Remember that spiking is done on the basis of bromoxynil phenol. Therefore, a 0.1 ppm spike would require 0.5 mL of a bromoxynil solution containing 1.45 $\mu\text{g}/\text{mL}$ which is equivalent to 1.0 $\mu\text{g}/\text{mL}$ of bromoxynil.
3. Add about 50 mL of 1 N methanolic KOH and a stirring bar. Attach a condenser, and boil under reflux conditions for one hour with constant stirring. Remove flask from hot plate and cool to room temperature. Allow solids to settle. Bromoxynil degradation will occur if the alkaline solution is allowed to stand overnight.
4. Decant supernatant liquid into a 250 mL glass-stoppered graduated cylinder.
5. Rinse residual solids several times with methanol, allowing solids to settle, and adding supernatant washes to graduated cylinder after each rinse.
6. Dilute of 150 mL with methanol and mix well. Allow any fine solids to settle out for a few minutes.
7. Prepare a 6 mL bed of QAE Sephadex A-25 resin as follows:
 - a. Make a slurry of resin in purified water in a beaker.
 - b. Allow resin to "swell" for at least 2 - 3 hours or overnight. If the resin is allowed to stand for an excessively long time, microbial action may begin.
 - c. Transfer a sufficient amount of slurry to yield a 6 mL bed of settled resin into a graduated centrifuge tube. The amount of slurry needed will depend on the consistency of the slurry; some trial and error may be needed to determine the amount of slurry to use. Part of the "swell" time under b above can occur while the resin is in the centrifuge tube.
 - d. Centrifuge for about one minute to settle the bed.
 - e. Add or remove slurry as necessary so that the resin bed volume is 6 mL.

- f. Add or remove supernatant water as necessary so that the final volume of water above the resin bed is about 3 mL.
8. Add 3.0 mL of sample extract to the resin bed, cap the tube and shake it vigorously for five minutes.
9. Centrifuge for about five minutes, or longer if necessary, to settle the resin bed, and discard the supernatant liquid.
10. Add 4 mL of water and 3 mL of 15% ether in hexane. Cap and shake the tube vigorously until the entire resin bed is free from the bottom of the tube, and then shake it for about one additional minute. Centrifuge the tube for about five minutes, and discard the supernatant liquid.
11. Repeat Step 10 one time.
12. Add 1 mL of water, 3 mL of acid "buffer" and 3 mL of 15% ether in hexane. Cap and shake the tube vigorously until the entire resin bed is free from the bottom of the tube, and then shake it for about five additional minutes. Centrifuge the tube for about 10 minutes.
13. Carefully transfer ether-hexane phase to a 15 mL screw-capped graduated centrifuge tube. Do not transfer any of the aqueous phase.
14. Add 3 mL of 15% ether in hexane to the tube containing the resin, cap and shake the tube for about one minute after the entire resin bed is free from the bottom of the tube, centrifuge for about 10 minutes, and then transfer the ether-hexane phase to the tube containing the first ether-hexane extract. Again, do not transfer any of the aqueous phase.
15. Repeat Step 14 two more times, and combine all ether-hexane portions.
16. Using a gentle stream of nitrogen and a water bath at up to 40 °C, concentrate the combined extracts to 1 mL. The water bath is not required, but it speeds the process.
17. In a good fume hood, add one to two mL of diazomethane solution (enough to give a permanent yellow color). Cap tube and allow to stand for 15 minutes.
18. Remove excess diazomethane and concentrate to 1.0 mL using a gentle stream of nitrogen and a water bath up to 35 °C. Each mL of extract now

represents 100 mg of crop. Grain samples are now ready for quantification by gas chromatography as in Step 30. Straw samples require additional cleanup as follows:

19. Attach a Sep-PakTM silica gel cartridge to a 5 mL glass syringe following manufacturer's instructions.
20. Add 5 mL of ethyl ether previously dried over sodium chloride (Note) to the syringe and force solvent through the cartridge over a period of about 30 seconds. The rate is not critical, but it should not be extremely fast so that impurities have no time to dissolve and move. Discard solvent.
21. Add 5 mL of hexane previously dried over sodium chloride (Note) to the syringe and force solvent through the cartridge over a period of about 30 seconds. Again, the rate is not critical. Discard hexane.
22. Repeat Step 21 one time.
23. Place a 15 mL centrifuge tube under the cartridge to receive all subsequent eluents.
24. Add about 0.1 mL of solid sodium chloride to the methylated straw extract from Step 18, mix and allow solids to settle.
25. Using a dropping pipet, transfer the sample to the syringe such that the liquid is placed on the silica gel in the cartridge with no air bubble present. Allow the solvent to flow through the cartridge at its own rate and collect the eluate.
26. Rinse the sample tube with 1 mL of hexane previously dried over sodium chloride (Note), and transfer it to the syringe as above. Allow the solvent to flow through the cartridge at its own rate, and collect it in the same tube as the eluate in Step 25.
27. Repeat Step 26 one more time.
28. Add 3 mL of dry 30% ethyl ether in hexane to the syringe. Force it through the cartridge over a period of about 20 seconds, and collect the eluate in the same tube as above.
23. Concentrate the combined eluates to 1.0 mL using a gentle stream of nitrogen and a water bath up to about 35 °C. The water bath just speeds the process.

30. Analyze a 1 - 2 μ l portion by gas chromatography using electron capture detection. The following conditions were used in our laboratory:

Instrument - Hewlett-Packard 5840

Injector - Hewlett-Packard Capillary Inlet System Model 18835 B

Column - 50 meter x 0,53 mm id with 3 μ OV-1701 film

Carrier Gas - Helium with 1.0 kg/cm² head pressure

Column Temperature - 240 °C

Injector Temperature - 240 °C

Detector Temperature - 300 °C

Detector Make-up Gas - 5% methane in Argon at about 25 mL/min

Bromoxynil methyl ether retention time is about 10 minutes

The analyst should set the chromatographic conditions necessary for the instrument being used. A different column may occasionally be required for the adequate separation of bromoxynil for interfering compounds.

31. Quantify the amount of bromoxynil by comparing peak height to a plotted standards calibration curve or mathematically through least squares regression data.

$$\text{Amount of bromoxynil} = \frac{(\text{Peak Height}) - (\text{Intercept})}{(\text{Slope})}$$

$$\text{Concentration of bromoxynil in ppm} = \frac{\eta\text{g Bromoxynil Found}}{\text{mg Sample Injected}}$$

NOTE: During development and use of the Sep-PakTM clean-up procedure, all solvent solutions were stored over sodium chloride to ensure water removal. This may not be necessary, but it has not been proven.

MAINTENANCE: N/A