Bromoxynil in Soil
Modified by McKenzie Laboratories
04/89

**Reagents**

Hexane, Burdick & Jackson Distilled-in-Glass
Ethyl Ether, Burdick & Jackson Distilled-in Glass
Methanol, Burdick & Jackson Distilled-in -Glass
15% Ethyl ether in hexane
Deionized Water
Sodium Bicarbonate 0.05N in D.I. Water ( 4.2 g/L)

QAE Sehpadex A-25 ion exchange resin, Pharmacia Fine Chemicals, Piscataway, NJ #17-D190-01

Diazomethane solution - prepare from N-methyl-N-nitroso-p-toluene sulfonamide (Diazald, Aldrich Chemical Co. #D2800-0; or Eastman #7066) according to manufacturer's instructions. Observe all safety precautions. Store in tightly capped bottle, with Teflon cap liner, in Freezer. Do not store over KOH or other desiccants. Note: Rinse outside of collection flask with B&J Methanol before transferring Diazomethane solution to storage container.

Standard bromoxynil solution - weigh 10 mg into a 100 ml volumetric for concentrate. Make to volume with methanol. Dilutions can be made for spiking.

Standard bromoxynil methyl ether solution - transfer 1.0 ml of 1.0 mcg bromoxynil per ml of methanol solution to a 15 ml screw-capped graduated centrifuge tube with Teflon cap liner. In a good fume hood, add diazomethane solution until a permanent yellow color is obtained. Cap and allow to stand at room temperature for 15 minutes. Remove excess diazomethane by using a gentle stream of nitrogen and a 35 - 40 deg. C water bath. Quantitatively transfer solution to a 50 ml volumetric flask, using 15 - 30 ml toluene to rinse. Adjust volume to 50 ml with hexane. This solution may be used for gas chromatographic calibration.

**Equipment** (Note: Rinse all glassware with B&J methanol then let dry before using.)

Food Chopper, Scharfen "Senator" or other bowl type chopper
Blender, 1 qt. Waring Blender or equivalent
Extraction flasks, flat bottom, 250 ml, with Standard Taper ground glass joint.
Friedrich or equivalent condensers to fit extraction Flasks
Graduated Cylinders, glass stoppered, 100 ml and 250 ml
Volumetric flasks, glass stoppered, 50 ml
Centrifuge tubes, screw-capped, 15 ml, graduated, with Teflon cap liners.
Magnetic stirring hotplate

Teflon-coated stirring bars, approx. 1-1/2" x 1/4' diam.
Centrifuge capable of maintaining 2000 rpm
Gas chromatograph with 63Ni electron capture detector

**Procedure**

1. Transfer the preweighed (25 g) sample to a MeOH pre-rinsed 500 ml RB flask.

2. Fortify at this point. Immediately go to step 3.

3. Add 250 ml 0.05 N Bicarbonate solution and a stirring bar. Attach condenser and boil under reflux conditions for one hour with constant stirring. Remove flask from hot plate and cool to room temperature, letting solids settle at the same time.

4. Decant supernatant liquid into a 500 ml glass-stoppered graduated cylinder.

5. Rinse residual solids several times with methanol, allowing solids to settle and adding supernatant to graduated cylinder after each rinse.

6. Dilute to 500 ml with methanol and mix well.

7. Prepare a 6 ml bed of QAE Sephadex A-25 resin as follows:

   a. Make a slurry of resin in distilled water in a beaker.

   b. Allow resin to swell for 2-3 hours or overnight in a refrigerator.

   c. Transfer a sufficient amount of slurry to yield 6 ml 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 how much slurry to use.

   d. Centrifuge for 5 minutes to settle the bed.

   e. Add or remove supernatant water, as necessary, so the final volume of water above the bed is about 3 ml.

8. Add 4 mls of sample extract (0.2 gm aliquot) to the resin bed and shake vigorously for five minutes.

9. Centrifuge for five minutes to settle the bed, and discard

the supernatant liquid.

10. Add four ml of water and three ml of 15% ether in hexane, shake for one minute, centrifuge for five minutes and discard the supernatant liquid.

11. Repeat step 10.

12. Add one ml of water, three ml of acid buffer and three ml of 15% ether in hexane. Shake vigorously for five minutes and then centrifuge for ten minutes.

13. Transfer ether-hexane phase to a 15 ml screw-capped graduated centrifuge tube.

14. Add three ml of 15% ether in hexane to the resin bed, shake for one minute, centrifuge for ten minutes and then transfer ether-hexane phase to the tube containing the first hexane extract.

15. Repeat step 15 two more times, combining all hexane portions.

16. Using a gentle stream of N2 and a 35 - 40 deg. C water bath, concentrate the combined hexane extract to one ml.

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 one ml using a gentle stream of nitrogen and a 35 - 40 deg. C water bath. Each ml of extract now represents 200 mg crop.

19. Analyze a 1 ul portion gas chromatographically, with 63Ni electron capture detection. The operating parameters used in our laboratory is as follows:

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| Instrument: | Tracor Model MT-220 |
| Detector: | Tracor ECD Linearized |
| Column: | 50 m x 0.53 mm ID 3 micron film of OV-1701 |
| Carrier: | Nitrogen at 9.8 psi |
| Make Up: | Nitrogen 37 mls/min @ 40 psi gauge at the tank |
| Oven Temp: | 245 deg. C |
| Detector Temp: | 290 deg. C |

| | |
|---|---|
| Injector Temp: | 245 deg. C |
| Attenuation: | 2 Linearized |
| Chart speed: | 0.25 in/min |

20. Calculate the amount of bromoxynil present in treated or fortified samples by comparing peak height with that obtained for 0.02 - 0.10 ng of bromoxynil standard injected as the methyl ether. Any convenient measurement units (mm, scale divisions. etc.) may be used. A linearity curve should be run occasionally , to insure that the ECGC system is linear to at least 0.10 ng.

Cleanup Column

Column Preparation:

1. Plug the bottom of a champagne column with a piece of glass wool.
2. Add 0.5 cm of anhydrous Na2SO4.
3. Add 7 cm of Florisil. Note: Florisil must be heated in a 145 oC oven overnight before use.
4. Add 1 cm of anhydrous Na2SO4.

Column Elution:

1. Prewet the column with 6 ml of hexane.
2. The sample is in one ml of hexane after derivatization. To the sample add 1 ml hexane and 2 ml of toluene. Mix well.
3. Apply this 4 ml sample to the column. Wash the column with 6 additional ml of 1:1 hexane:toluene.
4. Wash the column with 8 ml toluene. Drain to the top of the Na2SO4. Repeat the toluene wash.
5. Elute the bromoxynil methyl ether with 8 ml of 5% acetone in toluene.
6. Dry down to < 1 ml but not to dryness and make to 1.0 ml.
7. Submit sample to G.C. for quantitation.