ANALYTICAL METHOD

SANDOZ CROP PROTECTION CORPORATION

Location: ⊗ 341 E. OHIO ST. CHICAGO, IL 60611

⊗ DEVELOPMENT ○ QUALITY CONTROL

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DETERMINATION OF NORFLURAZON AND DESMETHYL NORFLURAZON RESIDUES IN SOIL AND WATER MATRICES (GC)

1. Summary

1.1 The method has been used for the determination of norflurazon and desmethyl norflurazon in the title substrates.

1.2 A known weight of soil sample (ca 10-25g) is treated with 200 mL of a 1:1 mixture, 1M KOH in methanol and 5% KCl in water, heated at 60°C for 1 hour, mechanically shaken and centrifuged. An aliquot (ca 10 mL) is taken for cleanup and analysis.

1.3 An aliquot (5 mL) of a water sample is added to 5 mL of methanol. The resulting solution is taken for cleanup and analysis.

1.4 An aliquot of either the soil extract or the 1:1 water/methanol solution is washed with 25 mL pentane.

1.5 The pentane is discarded and 50 mL of 5% NaCl in water is added to the aqueous layer.

1.6 The aqueous solution is extracted twice with methylene chloride.

1.7 The combined methylene chloride extracts are concentrated and the sample is brought up to an appropriate volume with toluene.

1.8 The residues are detected by GC/EC using a ^{63}Ni electron capture detector.

3. Safety

3.1 The oral LD_{50} for technical norflurazon in rats is > 8000 mg/kg.

3.2 Personal protective equipment including safety glasses, disposable gloves, and laboratory coats should be used when handling samples.

3.3 Methanol, pentane and toluene are flammable and should be used in well vented laboratories and away from open flame and sources of sparks.

3.4 Solutions of 1M KOH are corrosive and should be handled with care. Alkaline solutions are especially harmful to eyes and protective glasses or shields must be worn when working with these solutions.

3.5 Disposal of samples, extracts, and standards must be done in compliance with on-site policies and procedures.

4. Apparatus

4.1 Bath, temperature controlled, shaking - model 50 from Precision Scientific Group, Chicago, IL, or equivalent.

4.2 Bath, water (65°C) for Kuderna Danish Evaporation.

4.3 Bottles, Glass, 8 oz with Poly-seal screw cap.

4.4 Centrifuge, 3/4 H.P., International Equipment Co., Needham Hts, Mass.

4.5 Cylinders, Graduated, 25, 50 and 100-mL.

4.6 Filtration Columns, Disposable Baker 10 SPE, J. T. Baker Chemical Co., Phillipsburg, N.J.

4.7 Flask, Kuderna Danish, 125-mL with graduated distillation receiver tubes (either 10, 12 or 15-mL size) and Vigreaux column.

4.8 Funnel, filter, 60 mm

4.9 Funnel, separatory with teflon stopcock, 125-mL.

4.10 Gas Chromatograph, equipped with ^{63}Ni electron capture detector.

4.11 Glass Wool.

4.12 Pipet, graduated, 10-mL, disposable, borosilicate glass, by Kimble distributed by American Scientific Products, McGaw Park, IL.

4.13 Platform shaker, Eberbach Co., Ann Arbor, MI.

4.14 Vacuum Manifold, Supelco SPE, Supelco, Inc., Bellefonte, PA.

5. Reagents

5.1 Boiling chip, ten mesh hengar granules, Hengar Co., Philadelphia, PA.

5.2 Deionized water, Millipore System, 12514, Millipore Corp., Bedford, MA.

5.3 Methanol, high purity, Burdick and Jackson, Muskegon, MI.

5.4 Methylene chloride, Baker resi-analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.

5.5 Pentane, Baker resi-analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.

5.6 Potassium chloride, Baker analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.

5.7 Potassium hydroxide, analytical reagent, Mallinckrodt, Inc., Paris, KY.

5.8 Sodium chloride, Baker analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.

5.9 Sodium sulfate, anhydrous granular, Eastman Kodak Company, Rochester, N.Y.

5.10 Toluene, Baker resi-analyzed, J. T. Baker Chemical Co., Phillipsburg.

6. Standards

6.1 Sandoz Analytical Reference Standards

6.1.1 Norflurazon: 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-m-tolyl)-3(2H)-pyridazinone.

6.1.2 Desmethyl norflurazon: 5-amino-4-(chloro)-2-(α,α,α-trifluoro-m-tolyl)-3(2H)-pyridazinone.

7. Procedures

7.1 Soil Extraction

7.1.1 Weigh 10-25 g of sample into an 8 oz bottle.

7.1.2 Add 100 mL of 1M KOH in methanol and 100 mL of 5% aqueous KCl.

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7.1.3 Heat 1 hour at 60°C on a water bath (gentle shaking at ca 120 oscillations per minute is optional). The bottle should be loosely capped during heating.

7.1.4 Let bottle stand at room temperature for 5 minutes. Tighten cap, place bottle horizontally on platform shaker and shake vigorously for 15 minutes.

7.1.5 Loosen cap and cool to room temperature by placing bottle in a cold water bath.

7.1.6 Centrifuge bottle at 510 G (1500 RPM) for 10 minutes. Proceed to section 7.3.

7.2 Water Sample Preparation

7.2.1 Filter ca 10 mL of the water sample through Dual 20 um Frits (Baker 10 SPE Disposable Filtration Columns).

7.2.2 Add 5 mL of methanol to 5 mL of filtered sample water. Proceed to section 7.3.

7.3 Cleanup and Isolation

7.3.1 Transfer 10 mL of sample solution to a 125-mL separatory funnel.

7.3.2 Add 25 mL of pentane and shake vigorously.

7.3.3 Discard pentane layer and combine 50 mL of 5% NaCl in water with the sample solution in either a second or original separatory funnel.

7.3.4 Extract twice with 25 mL of methylene chloride. Shake the separatory funnel gently with each extraction.

7.3.5 Pass the methylene chloride from each extraction into the same Kuderna Danish (KD) set up through anhydrous sodium sulfate. Note: the sodium sulfate must be prewashed with at least 50 mL of methylene chloride if a 5% phenyl methyl (SE-54) Megabore GC column is to be used.

7.3.6 Rinse sodium sulfate with 10 mL of fresh methylene chloride and collect in the KD set up.

7.3.7 Add 1 boiling chip and 1 mL of toluene to KD set up and evaporate methylene chloride in a 65°C water bath to about 1 mL.

7.3.8 Add 25 mL of pentane two times to KD set up in 65°C water bath to drive off methylene chloride. Evaporate the volume to about 1 mL after each addition.

7.3.9 Let graduated distillation receiver tube (see 4.7) cool to room temperature. Bring to appropriate volume (usually 5 mL) with toluene for gas chromatograph analysis.

8. Analysis

8.1 Preparation of Standard Solutions

8.1.1 Weigh 100 mg each of norflurazon and desmethyl norflurazon into separate 100-mL volumetric flasks taking into account the purity of the analytical reference standard. Dilute to the mark with methanol. The concentration of each stock solution is 1×10^{-6} g/µL.

8.1.2 Combine 1 mL of each stock solution in a 100-mL volumetric flask and dilute with methanol to the mark. The concentration of each compound is 1×10^{-8} g/µL. This solution (A) is used for fortifying samples.

8.1.3 Transfer 1 mL of solution A to a 100-mL volumetric flask and dilute with toluene to the mark. The concentration of each compound is 1×10^{-10} g/µL. Further dilutions of this solution are prepared as needed using toluene as diluent.

8.2 Gas Chromatographic (GC) Conditions

8.2.1 Preferred Column

8.2.1.1 Gas Chromatograph: Hewlett-Packard, model 5880 (or equivalent) with Ni-63 electron capture detector.

8.2.1.2 Column: HP-1 crosslinked methyl silicone gum (SE-30) FSOT 30 m x 0.53 mm x 0.88 µm. Hewlett Pakcard catalog no. 19095Z-023.

8.2.1.3 Temperatures, °C:

Oven:	210
Injection Port:	250
Detector:	350

8.2.1.4 Gas, mL/min:

Carrier:	Helium, 22
Makeup:	5% methane/argon, 30

8.2.2 Optional Column

8.2.2.1 Gas Chromatograph: Hewlett-Packard model 5880 (or equivalent) with Ni-63 electron capture detector.

8.2.2.2 Column: HP crosslinked 5% phenyl methyl gum (SE-54) FSOT 30 m x 0.53 mm x 0.88 µm. The Hewlett Packard catalog no. 19095J-023.

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8.2.2.3 Temperatures, °C:

Oven:	220
Injection Port:	250
Detector:	350

8.2.2.4 Gas, mL/min:
Carrier: Helium, 22
Makeup: 5% methane/argon, 30

8.3 Quantitation

8.3.1 Prepare a standard curve for each compound by injecting known amounts of the standards and plotting peak height versus weight of analyte injected on log-log paper or when fixed volumes of the standard solutions are injected plot peak height versus concentration of standard (ng/μL).

8.3.2 Determine the weight or concentration of analyte in an aliquot of injected sample extract from the peak height and interpolation of the standard curve.

8.3.3 Calculate the concentration of the residue in the sample using either of the following expressions:

$$ppm = \frac{ng \times V_s}{V_i \times W}$$

or

$$ppm = \frac{Ce \times V_s}{W}$$

Where

ppm = concentration of analyte in the sample in parts per million (μg/g).

ng = weight of analyte in the injected aliquot in nanograms determined from the standard curve.

V_s = volume of final sample extract in milliliters taking into account all dilutions.

V_i = volume of injected aliquot in microliters.

W = weight of sample taken for analysis, in grams.

Ce = concentration of extract (ng/μL) determined from the standard curve.