ANALYTICAL METHOD

Report No. 15, Project 480428

SANDOZ CROP PROTECTION CORPORATION

Location: ⊗ 1300 E. TOUHY AVE.
DES PLAINES, IL 60018

⊗ DEVELOPMENT ○ QUALITY CONTROL

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Approved BAS Date 4-2-84

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DETERMINATION OF PRODIAMINE AND ITS 6-AMINO-IMIDAZOLE METABOLITE IN SOIL

1. SUMMARY

1.1 This method is applicable to the determination of prodiamine and its 6-amino-imidazole metabolite in soil. This method was developed in the residue laboratory at Sandoz Crop Protection Corporation.

1.2 Twenty gram aliquots of sample are extracted by shaking with methanol.

1.3 The samples are centrifuged and aliquots taken from the supernatant.

1.4 Aliquots (5 g equivalent) of the extracts are diluted with 5% NaCl solution and extracted three times with dichloromethane.

1.5 The samples are cleaned up by silica gel column chromatography.

1.6 Prodiamine and the 6-amino-imidazole are quantitated by gas chromatography using an electron capture detector (ECD). The limit of detection for prodiamine and its 6-amino-imidazole metabolite was 0.01 ppm for each compound. Confirmatory analyses are conducted by Gas Chromatography equipped with a Mass Selective Detector (GC/MSD).

2. SAFETY

2.1 The oral LD_{50} of prodiamine in rats is greater than 5000 mg/kg.

2.2 Normal laboratory precautions are adequate for safe handling of prodiamine.

2.3 Pentane, ethyl ether, methanol, and toluene are flammable and should not be used near heat, sparks, or open flames.

2.4 All solvents should be used only in well ventilated laboratories.

2.5 Protective gloves should be worn during extraction and analysis.

2.6 Disposal of samples and standards must be done in compliance with on-site safety policies and procedures.

3. MATERIALS/METHODS

3:1 Apparatus

3.1.1 Water bath; 60°C.

3.1.2 Bottles, screw cap with polyseal® liner; 32 oz.

3.1.3 Bottles, screw cap with polyseal® liner; 8 oz., amber.

3.1.4 Centrifuge, International Equipment Company, Model CS.

3.1.5 Chromatographic columns; 15 mm (I.D.) x 45 cm with teflon stopcock and water jacket, Lab-Crest Scientific Division, 1531 County Line Rd., Warminster, PA 18974.

3.1.6 Concentrator, Kuderna Danish, 125-mL.

3.1.7 Condensers, Vigreaux.

3.1.8 Dish, Pyrex, 190 x 100 mm.

3.1.9 Distillation receivers, 15-mL.

3.1.10 Evaporator, N-Evap with 40°C water bath, (Organomation Assoc.).

3.1.11 Funnels, separatory, 500-mL.

3.1.12 Drying Oven; 150°C.

3.1.13 Pipets, Pasteur, 9", disposable.

3.1.14 Platform Shaker.

3.1.15 Rotary Evaporator, Büchi, RotoVapor-RE

3.1.16 Round Bottom Flask, 250-mL.

3.2 Reagents

3.2.1 Dichloromethane; residue analysis grade.

3.2.2. Ethyl ether containing 2% ethanol preservative; residue analysis grade.

3.2.3 Silica gel 60 (70-200 mesh), EM Reagents, MC/B Manufacturing Chemists, Inc., 2909 Highland Ave., Cincinnati, OH 45212. 3% Deactivated; Spread a layer about 1" deep in a glass dish and activate in a drying oven for 25 hours at 250°C. Cool in a tightly capped bottle. Add 3g deionized water to 97g of silica gel and shake overnight.

3.2.4 Methanol; residue analysis grade.

3.2.5 Pentane; residue analysis grade.

3.2.6 Sodium chloride; reagent grade.

3.2.7 Sodium sulfate; anhydrous, granular, reagent grade.

3.2.8 Toluene; reagent grade.

3.3 Preparation of Standard Solutions

3.3.1 Prodiamine, (N^3, N^3-Dipropyl 2,4-Dinitro-6-Trifluoromethyl-m-Phenylene-diamine) - Sandoz Crop Protection Analytical Reference Standard.

3.3.2 6-Amino-Imidazole, (6-amino-2-ethyl-7-nitro-1-propyl-5-trifluoromethylbenzimidazole) - Sandoz Crop Protection Analytical Reference Standard.

3.3.3 Prodiamine and 6-amino-imidazole are light sensitive. Aliquots of 10^{-10} g/µL prodiamine standard in toluene showed 15-20% loss of prodiamine after 90 hrs. exposure to ambient laboratory light. Standard solutions and sample extracts must be stored in actinic, amber or foil wrapped glassware. Regular glassware may be used during sample extraction and clean-up, but if processing is interrupted, the extracts must be protected from light.

3.3.4 Accurately prepare solutions containing 100.0 mg prodiamine/100 mL toluene and 100.0 mg 6-amino-imidazole/100 mL toluene in actinic or foil wrapped 100-mL volumetric flasks. This gives stock solutions of 10^{-6} g/uL, (1ug/uL).

3.3.5 Transfer 1.0 mL of each stock solutions (10^{-6} g/µL) to separate 100-mL volumetric flasks and bring to the mark with toluene. These solutions (10^{-8} g/µL) are used for fortificaton of recovery samples.

3.3.6 Prepare a range of standards for GC/ECD and GC/MSD quantitation by diluting aliquots of the fortification standards to the mark in 100-mL volumetric flask, with toluene as described below:

Volume of 10^{-8} g/µl Solution	Concentration of Final Solution
10 mL	10^{-9} g/µL
5 mL	5×10^{-10} g/µL
1 mL	10^{-10} g/µL
500 µL	5×10^{-11} g/µL
200 µL	2×10^{-11} g/µL
100 µL	10^{-11} g/µL

A set of standards for prodiamine and a set of standards for 6-amino-imidazole are produced in this way.

3.4 Procedure

3.4.1 Extraction

3.4.1.1 Weigh 20 g of sample in an 8 oz. screw-cap bottle.

3.4.1.2 If fortifying for recovery data, add an appropriate volume of both prodiamine and 6-amino-imidazole fortification standard solutions to the sample, e.g., 200 µL of 10^{-8} g/µL solution to 20 gm of sample = 0.1 ppm fortification. Allow 15 minutes for solvent to evaporate.

3.4.1.3 Add 200 mL of methanol to the 8 oz. bottle. Cap the bottle and shake for 30 minutes on a platform shaker.

3.4.1.4 Centrifuge for 30 minutes at 550 G or until the supernatant is clear and sediments are stable enough to allow supernatant to be decanted.

3.4.1.5 Store extracts in 8-oz. amber bottles in the refrigerator.

3.4.2 Partition I

3.4.2.1 Decant a 50-mL aliquot (5 g equivalent) of sample extract and transfer it to a 500-mL separatory funnel containing 250 mL of 5% NaCl solution and 25 mL of dichloromethane.

3.4.2.2 Shake for 1.0 minute. Allow phases to separate, and drain the dichloromethane through anhydrous sodium sulfate into a 250-mL round bottom flask.

3.4.2.3 Extract the aqueous phase two more times with 25 mL of fresh dichloromethane each time, as described in 3.4.2.2. Combine all three extracts in the 250-mL round bottom flask.

3.4.2.4 Wash the sodium sulfate three times with 5-10 mL of dichloromethane each time, combining all washes with the previous dichloromethane extracts.

3.4.2.5 Evaporate the combined dichloromethane to near dryness on the rotary evaporator using a 40°C water bath.

3.4.2.6 Evaporate the sample from 3.4.2.5 to dryness with a gentle stream of nitrogen. Add 5 mL of 10% ethyl ether in pentane to the 250-mL round bottom flask and thoroughly dissolve the residue. The sample is now ready for silica gel column clean-up.

3.4.3 Silica Gel Column

3.4.3.1 To a 250-mL separatory funnel containing 70 mL of 10% ethyl ether in pentane, slowly add 20 g of 3% water deactivated silica gel. See section 3.2.3 for deactivation procedure.

3.4.3.2 Shake well, and quickly drain into a chromatographic column plugged with glass wool.

3.4.3.3 Rinse the separatory funnel with 10 mL of 10% ethyl ether in pentane and add to the column.

3.4.3.4 When the silica gel is completely settled, add 1.0 cm of granular Na_2SO_4 and drain the solvent to just above the top of the Na_2SO_4 layer.

3.4.3.5 Apply the 5-mL 10% ethyl ether/pentane solution from 3.4.2.6, which contains the extracted residue, to the silica gel column. Allow this solution to drain to the top of the Na_2SO_4 before addition of any other solvent.

3.4.3.6 Rinse the 250-mL round bottom flask twice with 5-mL portions of 10% ethyl ether in pentane and transfer each rinse to the column, allowing the previous rinse to drain to the top of the Na_2SO_4 before adding the next.

3.4.3.7 Pass an additional 70 mL of 10% ethyl ether in pentane through the column. Discard this eluate.

3.4.3.8 Elute the prodiamine with 75 mL of 10% ethyl ether in pentane, collecting the eluate in a KD set-up (15 mL distillation receiver connected to a Kuderna Danish concentrator).

3.4.3.9 Add 1 mL of Hexane to the eluate from 3.4.3.8 (containing prodiamine). Fit a Vigreaux

condenser to the KD set-up and concentrate the sample to about 1 mL using a 60°C water bath. Perform this concentration in an efficent hood.

3.4.3.10 Evaporate the last traces of solvent using a gentle stream of nitrogen. Add 5.0 mL of toluene to the 15-mL distillation receiver and thoroughly dissolve the residue.

3.4.3.11 Cover the distillation receiver with foil to protect the sample from light. Store the sample in the refrigerator if quantitation is not performed immediately.

3.4.3.12 Further elute the column with 100 mL of 50% Ethyl Ether in Pentane. Discard this eluate.

3.4.3.13 Elute the 6-amino-imidazole with 100 mL of ethyl ether collecting this eluate in a 250-mL round bottom flask.

3.4.3.14 Evaporate the 6-amino-imidazole fraction to near dryness on the rotary evaporator using a 40°C water bath.

3.4.3.15 Evaporate the last traces of solvent using a gentle stream of nitrogen. Add 5.0 mL of toluene to the 250-mL round bottom flask and thoroughly dissolve the residue.

3.4.3.16 Protect this sample from light by wrapping the tube with foil. Store the sample in the refrigerator if quantitation is not performed immediately.

3.5 Analysis

3.5.1 Gas Chromatographic Conditions

The following conditions have been shown to be suitable for analysis of Prodiamine and the 6-amino-imidazole metabolite in soil. Other conditions may be acceptable provided that the analytes are separated from sample interferences and the response is linear over the range of interest. Elution time and standard linearity must be checked with an new instrument or any change in operating parameters.

3.5.1.1 Instrument: Hewlett-Packard 5880A with ^{63}Ni ECD

Column: 10M x 0.53 mm (I.D.) Fused silica, HP-17 (crosslinked 50% phenyl/methyl silicone), 2.0 µm film thickness (H-P #19095L-121)

Oven Temperature Profile:

Initial Value: 185°C
Initial Time: 6 minutes
Post Value: 225°C
Post Time: 5 minutes

Detector Temperature: 350°C
Injector Temperature: 250°C
Carrier Gas: He @ 6 PSI (17 mL/min)
Make-up Gas: 5% methane/argon @ 18 mL/min
Injection Mode: Splitless, 0.5 min purge delay, split outlet-28 mL/min
Prodiamine Retention Time: 2.95 min
6-Amino-Imidazole Retention Time: 4.56 min

3.5.1.2 Instrument: Hewlett-Packard 5880A with 5970 MSD
Column: 25M x 0.2 mm (I.D.) fused silica, HP-1 (Crosslinked methyl silicone), 0.11 µm film thickness (H-P part number 190912-002)

Oven Temperature Profile:

Initial Value: 100°C
Initial Time: 0.5 min.

Level 1
Program Rate: 30°C/min.
Final Value: 190°C
Final Time: 5.5 min
Post Value: 250°C
Post Time: 5 minutes

Injector Temperature: 250°C
Interface Temperature: 250°C
Carrier: He @ 5 PSI (0.3 mL/min.)

Injection Mode: Splitless, 0.5 min. purge delay, split outlet - 60 mL/min.

Source vacuum: 8×10^{-6} TORR
Prodiamine Retention Time: 7.25 min.
6-Amino-Imidazole Retention Time: 8.11 min.

3.5.2 Quantitation

3.5.2.1 Prepare a standard curve by injecting 2 µL aliquots of standards of known concentration and plotting peak height versus concentration of injected standard on log-log paper.

3.5.2.2 Determine the concentration of analyte in a 2 µl

1.0

injected aliquot of sample from the peak height and the standard curve from 3.5.2.1..

3.5.2.3 Calculate the concentration of the residue in the sample using the following expression:

$$\text{PPM (ng/mg)} = \frac{Ce(ng/uL) \times Vs(uL)}{Ws(mg)}$$

Where:

PPM = Concentration of analyte in the sample, in parts per million (ng/mg).

V_s = Final volume of sample extract taking into account all dilutions, (in microliters).

Ce = Concentration of Analyte in sample extract, determined from standard curve, (in nanograms per microliter).

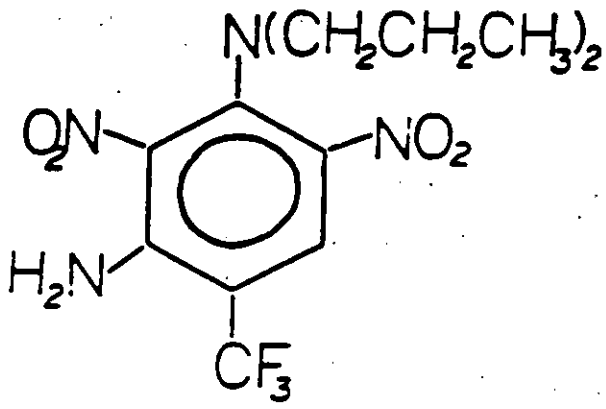
W_s = Weight of sample represented by sample extract, (in milligrams).

3.6 Confirmatory Techniques

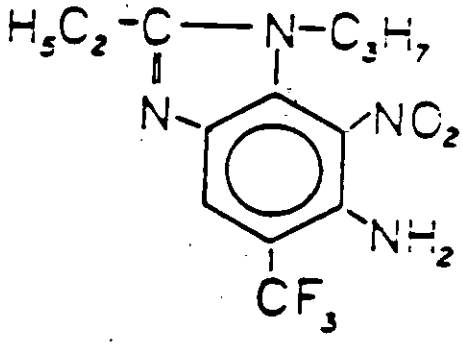
The presence of prodiamine and its 6-amino-imidazole metabolite can be confirmed using a G.C. equipped with a mass selective detector. The conditions described in 3.5.1.2 have been used for GC/MSD quantitation. Autotune values were used for the repeller, lenses, and electron multiplier voltages. Data was acquired in SIM mode with dwell times of 500 μ SEC for each of three ions. Quantitation of prodiamine was based on ion 321 and confirmation of identity was based on the ratios of ions 279 and 333 to 321. Quantitation of the 6-amino-imidazole was based on ion 316 and confirmation of identity was based on the ratios of ions 239 and 228 to 316.

3.7 Time Required for Analysis

A single sample can be extracted and prepared for analysis by gas chromatography in a single eight hour period. A set of six samples required two eight hour days to prepare for gas chromatography. Samples were analyzed by gas chromatography using an autosampler. Using the conditions in 3.5.1.1, each analysis required about 15 minutes.



N^3,N^3-dipropyl-2,4-dinitro-6-(trifluoromethyl)-1,3-benzenediamine (Prodiamine)



6-amino-2-ethyl-7-nitro-1-propyl-5-trifluoromethylbenzimidazole (6-Amino-Imidazole)

Figure 1. Chemical Structures