Method of Analysis for Iprodione and Its Metabolites in Water

I. INTRODUCTION

A. Scope

This method sets forth the procedure for determining the residues of iprodione and its metabolites RP 30228 and RP 32596 in ground water.

B. Principle

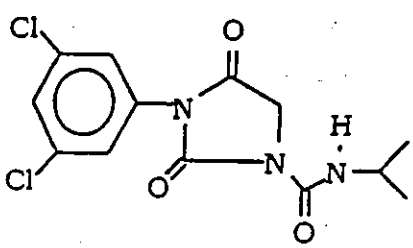
An analytical method is described for the determination of residues of iprodione and its metabolites RP 30228 and RP 32596 in ground water. Residues of iprodione, RP 30228 and RP 32596 are extracted from water using a RP-102 resin cartridge, then removed with acetonitrile.

All residue analysis is accomplished by LC-MS-MS on a C8 column. Quantification of results is based on a comparison of peak areas with those of known standards.

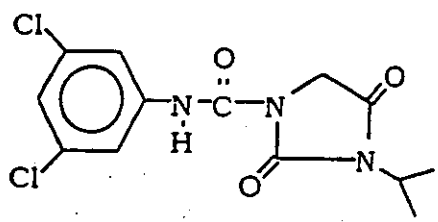
C. Method Limits

The method detection limits (MDL) and limits of quantification (LOQ) for iprodione (RP 26019) and its metabolites RP 30228 and RP 32596 in water have not been determined. This information will be obtained from the subsequent validation study. Target level LOQ is 50 ppt.

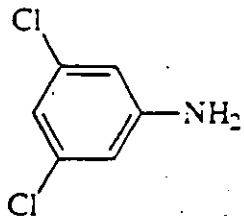
D. Chemical Structures



RP26019 (Iprodione)



RP30228



RP 32596

II. MATERIALS

Unless otherwise noted, equivalent brands and/or suppliers can be used.

A. Reagents/Solvents

Acetonitrile Omni-Solv (EM Science, Cat. No. AX0142-1)

Formic Acid Suprapur (EM Science, Cat. No. 11670-1)

Water Omni-Solv (EM Science, Cat. No. WX0004-1)

B. Equipment and Supplies

Adaptors, 1,3 and 6 mL (Varian, 1213-1001)

Balance :
accuracy ± 0.1 mg (analytical standards) (Mettler AT 201 or equiv)
accuracy ± 0.1 g (samples and chemicals)(Mettler PC 4000 or equiv)

Bottles, amber, 4 oz. (Qorpak)

Cartridges ,Spe-ed™ SPE, RP-102 Resin (100mg/1mL)
(Applied Separations, Cat. No. 4207, *no substitute*)

Cartridge Adaptors, SPE
(University Research Glass, Cat. No. URG-2440-SPECA)

Disposable pipettes

Glass wool

Graduated cylinders

Column. HPLC, Columbus C8, 4.6x100 mm, 5µm, *no substitute*
(Phenomenex, Cat. No. 00D-4187-E0, *no substitute*)

Magnetic stirrer

Pipette bulb

Precolumn HPLC Filter, Ultra Low Dead Volume, 0.5µm frit
(Upchurch, A-318)

Reservoirs, 75 mL volume (Varian, 1213-1012)

Solvent jugs, 4 L brown glass

Stopcocks, Luer Lock (Varian, 1213-1005)

Stoppers, glass, 24/40

Vacuum Gauges

Volumetric flasks

Volumetric pipettes

Vial, clear, 1.5mL; cap, open top; septa, split
(Sun, 200-250; 200-292, 500-870)

C. Solutions

The following is a list of the solutions used in the analyses of ground water. Example procedures for the preparation of each solution are also provided.
Note that the reagent water used in the preparations should be HPLC grade.

1. Solution of ~pH 3 Formic acid in Water
   Pipet formic acid into a stirring volume of HPLC grade water until the pH is 3 ± 0.4. Check the pH with a pH meter. (about 0.5 mL formic acid/liter water)

2. 90:10 Solution of pH 3 Water (formic):Acetonitrile
   Using a graduated cylinder, transfer 900 mL of a solution of pH 3 formic acid in H_2O and 100 mL CH_3CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.

3. Solution of 50:50 Water:Acetonitrile
   Using a graduated cylinder, transfer 500 mL of HPLC grade H_2O and 500 mL CH_3CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.

4. 50:50 Solution of pH 3 Water (formic):Acetonitrile
   Using a graduated cylinder, transfer 500 mL of a solution of pH 3 formic acid in H_2O and 500 mL CH_3CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.

D. Analytical Standards

Common name/alias: Iprodione, RP 26019

Chemical name: *3-(3,5-dichlorophenyl)-N-isopropyl-2,4* - dioxoimidazolidine-1-carboxamide

Solubility [1]:

acetone:	34.2(unit : g/100 ml)
acetonitrile:	16.8
hexane:	0.059
dichloromethane:	45.0
distilled water:	0.00122

Common name/alias: RP 30228

Chemical name: 1-(3,5-dichlorophenyl)carbamoyl-3-isopropyl hydantoin

Common name/alias: 3,5- DCA/ RP 32596

Chemical name: 3,5-dichloroaniline
(CAS No. 626-43-7)

III. FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

A. Preparation

All the standard solutions must be stored in amber glass bottles. Stock solutions will be stored at about -10°C. All other standards solutions will be stored at 4°C ± 3°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example of a procedure to follow in preparing standard solutions. Alternate or additional standards of appropriate weight and volume may be prepared as needed. The "~" symbol indicates approximately.

Stock solutions:

1. Weigh ~0.1000g (corrected for purity) each of iprodione and RP 32596 and ~0.0100g (corrected for purity) of RP 30228 into separate 100-mL volumetric flasks and dilute with ~50 mL acetonitrile. Sonicate for approximately 5 minutes if necessary. Add ~45 mL of pH 3 water and mix by inversion. Allow the solution to reach ambient temperature before diluting to the mark with more pH 3 water. The concentration of these stock standards is ~1000 µg/mL of iprodione and RP 32596 and ~ 100 µg/mL of RP 30228.

Standards solutions:

2. Transfer 10 mL of the ~1000 µg/mL iprodione and RP 32596 standard solutions, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with a 50:50 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this standard is ~100 µg/mL of iprodione and RP 32596.

3. Transfer 20 mL of the ~100 µg/mL RP 30228 standard solution, via a volumetric class "A" pipette, to a 100 mL volumetric flask. Dilute to mark with a 50:50 solution of pH 3 formic acid in H2O:CH3CN. Cap

and mix by inversion. The concentration of this standard is ~20 µg/mL of RP 30228.

4. Transfer 20 mL of the ~100 µg/mL iprodione and RP 32596 standard solutions, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with a 50:50 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~20 µg/mL of iprodione and RP 32596.

Mixed standard solutions:

5. Transfer 10 mL of the ~20 µg/mL iprodione, RP 32596 (III.A.2) and RP 30228 (III.A.3) standard solutions, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~2 µg/mL

6. Transfer 10 mL of the ~2 µg/mL mixed standard solution of iprodione, RP 32596 and RP 30228 via a volumetric class "A" pipette, to one 100 mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~0.2 µg/mL

7. Using a class "A" volumetric pipette, transfer 10 mL of the mixed standard (step III.A.6.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~20 ηg/mL iprodione, RP 30228 and RP 32596.

8. Using a class "A" volumetric pipette, transfer 3 mL of the mixed standard (step III.A.6.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~6 ηg/mL iprodione, RP 30228 and RP 32596.

9. Using a class "A" volumetric pipette, transfer 25 mL of the ~20 ηg/mL mixed standard (step III.A.7.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~5 ηg/mL iprodione, RP 30228 and RP 32596.

10. Using a class "A" volumetric pipette, transfer 2 mL of the mixed standard (step III.A.6.) to a 100-mL volumetric flask. Dilute to mark

with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~4 ηg/mL iprodione, RP 30228 and RP 32596.

11. Using a class "A" volumetric pipette, transfer 3 mL of the mixed standard (step III.A.6.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of pH 3 formic acid in H2O:CH3CN. Cap and mix by inversion. The concentration of this mixed standard is ~2 ηg/mL iprodione, RP 30228 and RP 32596.

B. Stability

1. To evaluate the stability, the following formula has been used :

   percent stability = [1-(old std. soln. / new std. soln.)] x 100

   The old standard solution should give detector responses within 10% of those of the new standard solution in order for the given standard solution to be considered stable under the storage conditions.

2. Stock solutions: Each product prepared in acetonitrile and stored at about -10°C was stable for up to 6 months[2].

3. Standard solutions: Mixed standard solutions of iprodione , RP30228 and RP 32596 prepared in acidified water:acetonitrile and stored at 4°C ± 3°C was stable for up to 6 months [2].

IV. METHOD PROCEDURES

A. General Notes

A1. The "~" symbol indicates 'approximately.'

A2. The pH of samples to be analyzed may be measured with a pH meter or narrow range pH paper. Adjust pH between 2-4 with formic acid.

A3. If the samples are turbid, glass wool can be used in the SPE cartridges to aid filtration. The glass wool must be washed with acetonitrile prior to use. Place ~ 35 g glass wool in a 500 mL Nalgene® bottle, add ~300 mL acetonitrile and shake on a platform

shaker for ~ 15 minutes. Filter through a 9 cm GF/C filter paper on a Buchner funnel, vacuum dry. Rinse with ~ 100mL acetonitrile and vacuum dry on the filter. Store in a wide-mouthed bottle.

A4. Conditioning of the cartridges in step B4 can be started earlier and does not have to be done after the completion of steps B1-B3. However, the cartridges should be used the day of conditioning.

A5. Throughout the conditioning and elution process (unless otherwise specified) cartridges should not be allowed to run dry.

A6. The flow rate for loading the water sample on the cartridges (step B5) is faster than the conditioning and elution flow rate.

B. Ground Waters
(Analysis for Iprodione (RP 26019), RP 30228 and RP 32596)

B1. Weigh ~200 g of sample into a 500 mL Nalgene® bottle. The sample may be stored in a refrigerator until needed.

B2. For recoveries, fortify the sample with the appropriate standard solution. Immediately adjust the pH to 2-4 with formic acid. Cap and mix on a platform shaker for ~5 minutes.

B3. Immediately set-up a RP-102 cartridge (100 mg) on a purification system connected to a vacuum. (If samples are turbid - insert a plug of ~0.1 g of acetonitrile-washed glass wool into the cartridge). Place a reservoir on top of the cartridge.

B4. Condition the cartridge with ~4 ml of acetonitrile followed by ~4 ml of pH3 water. (~1drop/3-4 seconds. Do not allow the cartridge to dry).

B5. Apply prepared sample to the cartridge (~1drop/second). Do not allow the cartridge to dry. *Note that the flow rate for loading the water sample on the cartridges is faster than the conditioning and elution flow rate.*

B6. Add ~2mL of pH3 formic acid in water to the cartridge. Elute and discard the effluent. (~1 drop/3-4 sec. Do not allow the cartridge to dry).

B7. Add ~ 1 mL of a 50:50 solution of water:acetonitrile to the cartridge. Elute and discard the effluent. (~1 drop/2 sec. Do not allow the cartridge to dry).

B8. Air dry the cartridge under high vacuum (~20 inches of mercury) for ~2 minutes.

B9. Add 1 mL of acetonitrile to the cartridge. Apply positive pressure and push ~1/3 of the solvent through the cartridge. *Positive pressure can be applied via a handheld nitrogen line.* Vent the pressure and allow the cartridge to soak for 1-2 minutes. Reapply pressure and elute the solvent (~1drop/second) into an appropriately sized volumetric flask.

B10. Dilute to the mark with pH3 formic acid in H_2O . Mix by inversion. Samples are ready for LC-MS-MS analysis. Suggested final dilution volumes are 2mL for samples fortified at the LOQ level and 25mL for samples fortified at the 10 x LOQ level.

V. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

A. Conditions

Instrument used:	Perkin Elmer Sciex API III+ LC/MS/MS system coupled to a Hitachi L6200 HPLC pump via PE. Hitachi AS2000 autosampler
MS Mode:	MS/MS multiple reaction monitoring (MRM)
Ionization:	Atmospheric Pressure Chemical Ionization (APCI) using Heated Nebulizer Interface
Heated Nebulizer Settings:	Heated air at ~4.00 L/min, 450°C
Nebulizer pressure:	80 psi
Curtain gas flow:	Nitrogen at ~1.2 L/min
Collision gas:	Argon at approximately 275 x 10^{13} atoms/cm^2

Period 1 Positive Mode:	
Orifice voltage:	65 V
Collision energy (R2-R0):	11V - 30V = -19V
Mass Transition:	RP32596: 162.1/127.1
Period 2 NegativeMode:	
Orifice voltage:	-57 V
Collision energy (R2-R0):	-7V - 30V = -37V
Mass Transition:	iprodione(RP 26019): 243.1/42.0
Period 3 Negative Mode:	
Orifice voltage:	-38 V
Collision energy (R2-R0):	-20V - 30V = -50V
Mass Transition:	RP 30228: 328.1/141.0

Column: Phenomenex, Columbus C8, 4.6 x 100mm, 5µm particle size

Column In-Line Filter: Upchurch, Ultra Low Dead Volume, 0.5µm frit

Mobile phase flow rate: 1.0 mL/min

Mobile phase composition: isocratic
60% acetonitrile
40% 0.1% acetic acid in HPLC grade water
~ 8 minutes between injections (set autosampler to 7.5 minutes run time)

Injection volume: 40 µl

Note the indicated LC-MS-MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

APPROXIMATE RETENTION TIMES

RP 32596	3.3 minutes
RP 26019	4.4 minutes
RP 30228	7.0 minutes

Retention times may vary from those presented above.

Example chromatograms are attached (see section X). Note that the retention times may vary from system to system.

B. Performance Criteria

The following criterion should be met before analysis of samples begins. Once the criterion has been met it is not necessay to perform them again.

First criterion:
Run a standard solution corresponding to a level at or below the estimated LOQ and obtain a signal to noise ratio of at least 9:1.

If this criterion cannot be met, optimize instrument operating parameters or change instrument method parameters such as injection size until a signal to noise ratio of 9:1 is obtained.

If this criterion still cannot be met by changing operating parameters, run higher level standards until a signal to noise ratio of 9:1 is obtained. This will require adjusting the method final sample dilution such that this standard level corresponds to the required LOQ.

Second criterion:
Run a set of standards of four or more concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.90 for each analyte. If this criterion is met, the samples may be run with standards interspersed. Do not use any sample run data if the combined regression for standards run immediately before, during and after the samples do not meet this criterion.

Note:
To stabilize the response of the instrument, it has been found useful to run at least one standard and/or a sample or untreated control solutions as "wake up" runs before the actual runs to be used in calculations are commenced.

VI. CALCULATIONS

Linear regression should be used to generate calibration curves for RP 26019 and RP 30228 and RP 32596. After the instrument performance criteria are met, a minimum of four standards over a range of concentration levels should be included with a set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Samples should be diluted such that any peak areas or heights are within the area or height range between the lowest and highest standards injected.

Linear regression coefficients should be calculated on standard concentration (ng/mL) versus peak area or height. The data from the analytical standards should then be fit to the linear model,

$$Y = A + BX.$$

The equation to be used to estimate the residues in the samples is:

$$E = \frac{(Y - A)}{B} \times \frac{C}{D}$$

where: Y = response of analyte of interest (peak area or height)
A = intercept from linear regression analysis (peak area or height)
B = slope from linear regression analysis (response per concentration)
C = final sample volume (mL)
D = starting weight in grams of sample in final volume (g)
E = concentration of analyte in sample in parts per billion (ppb or ng/mL)

VII. SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the study. General laboratory safety precautions should be taken. This method does not present any specific risks.

VIII. REFERENCES

1. "Iprodione technical grade. Solubility at 20°C" Chabassol, Y. & Gomez, J.L. AG/CRLD/AN 9115375, April 9, 1991
2. "Storage Stability of Iprodione (RP-26019), its Isomer (RP 30228), and its Metabolite (RP-32596) in Various Raw Agricultural Commodities and Processing Fractions" RPAC file# 44327 R. S. Plaisance, June 13, 1994.