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VALIDATION OF HEXAZINONE, METABOLITES A,B,C, A1 AND 1 IN SOIL BY GC/MS USING SELECTED ION MONITORING

PROCEDURE:

A representative soil sample of about 30g was weighed into a centrifuge bottle. 80mL of Acetone/0.1M KH_2PO_4 buffer, pH ~4(1:1) was added, shaken for 20 min. and sonicated for 3 min. The sample was centrifuged at about 5K for about 5 min and the organic layer was decanted into a 500 mL separatory funnel. The soil was re-extracted with another 80mL of extraction solvent as before, centrifuged and the extract added to the separatory funnel. 60mL of a 25% K_2CO_3 in water solution and 150mL of chloroform ($CHCl_3$) was added to the separatory funnel. After shaking, the $CHCl_3$/Acetone layer (bottom layer) was dried through anhydrous Na_2SO_4, into a 500mL pre-silanized boiling flask. The aqueous layer was re-extracted with first 70mL of $CHCl_3/CH_3CN$ (3:1) and then 60mL of ethyl acetate.

The combined organic extracts were concentrated on a rotoevaporator set @ 42°C to near dryness. The residue was transferred to 4mL vials with a Dichloromethane(DCM)/ethyl acetate (EtoAc)/Caffeine/triethylamine (77.5:20:0.5:2) solution.

The final volume was made to 1.0mL and analyzed by GC/MSD using selected ion monitoring.

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REAGENTS AND SOLVENTS:

Silon CT - Supelco Canada (5% Silon CT/Toluene)
Deionized Water - Millipore "Milli Q System"
Acetone - Omni solv (Lot # B90007)
Acetonitrile - B & J Chrompure (Lot # A2577)
Chloroform - B & J Chrompure (Lot #AZ832)
Dichloromethane - B & J Chrompure (Lot # AZ988)
25% Potassium carbonate (K_2CO_3) solution
Sodium Sulfate (Na_2SO_4) (Baked at 400°C)
Ethyl Acetate - B & J High Purity (Lot# AW776)
Caffeine - BDH Lot #101919/12221
Triethylamine - BDH - 99% pure
Toluene - B&J High Purity (Lot# AZ 025)

EQUIPMENT:

250 mL Polypropylene centrifuge bottles
500 mL boiling flasks
Incubator shaker - Psycrotherm (New Brunswick Scientific)
Sonicator - Ultra sonic FS-28 (Fisher Scientific) (Serial No.: 160480)
Centrifuge - Sorvall RC-2 (Serial No: 0031053)
500mL separatory funnels
Rotoevaporator with water bath

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GC/MSD CONDITIONS:

A) INSTRUMENT:

HP 5971 Series Mass Selective Detector (Serial No.: 3050A01647) with 5890 Gas Chromatograph (Serial No.: 3033A32996)

HP 7673 Autosampler (3049A24030) and HP 9133 Computer and controller.

B) CONDITIONS:

Column - DB 1701 (25m x 0.25mm), J&W Scientific

Pre Column - Deactivated Fused Silica (0.5m x 0.53mm) - J&W, Chromatographic Specialties.

Oven Temp. Program -

Initial Temp. -150°C

Hold Time - 0 min.

Rate - 25°C/min.

Final Temp. - 280°C

Hold Time - 20min.

GC to MSD interface - Capillary direct interface

Det. Temp. - 280°C

Injector Temp. - 280°C

EM voltage - + 200 (Relative)

SIM acquisiton -

Hexazinone M/Z 171.0
M/Z 128.0
Metabolite B M/Z 238.0
M/Z 157.0
Metabolite C M/Z 157.0
Metabolite A M/Z 171.0
Metabolite A1 (G3453) M/Z 171.0
Metabolite 1(JS-472) M/Z 171.0

Flow Rate - 1.0mL Helium

Sample Injection Volume - 3µL

Split Valve Closure - 0.50 min.

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STANDARDS:

Standards were supplied by E.I. DuPont de Nemours and Co. Preparation of standards is listed on standard preparation forms in section 3 of the report.

Spiking and working standards were prepared in 20% ethyl acetate/dichloromethane, 0.5% caffeine, 2% triethylamine.

FORTIFICATIONS:

Control soil samples were fortified at levels from about 0.03 to 0.3 ppm for Hexazinone, Metabolites B, A1, A and 1 and from 0.10 to 1.0ppm for Metabolite C.

DETECTION LIMITS:

	MDL	MQL
Hexazinone	0.010	0.030
Metabolite B	0.010	0.030
Metabolite C	0.060	0.20
Metabolite A	0.010	0.030
Metabolite A1	0.010	0.030
Metabolite 1	0.010	0.030

MDL - Minimum Detection Limit

MQL - Minimum Quantifiable Level

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CALCULATIONS:

A) RESPONSE FACTOR (R.F.):

$$R.F. = \frac{\text{CONCENTRATION OF STANDARD}}{\text{PEAK AREA OF STANDARD}}$$

B) AVERAGE RESPONSE FACTOR (AVG.R.F.):

$$AVG.R.F. = \frac{\text{SUM OF RESPONSE FACTORS}}{\text{NO. OF STANDARDS}}$$

C) CONCENTRATION OF ANALYTE (ppm):

$$(ppm)\ CONC. = \frac{(PK.AREA \times AVG.R.F.)\ (F.V.)}{g.EXTRACTED}$$

WHERE:

(ppm) CONC. - Concentration of sample (µg/g)

PK.AREA - Peak Area

AVG.R.F. - Average Response Factor (µg/mL)

F.V. - Final Volume (mL)

g.EXTRACTED - Grams of sample Extracted

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EXAMPLE OF CALCULATION:

$$\frac{(3.0\ E^{-1} \times 5.00\ E^{-4}\ \mu g/mL)\ (1.0mL)}{30.0g} = 0.50ppm$$

D) % RECOVERY:

$$\%\ RECOVERY = \frac{RECOVERY\ LEVEL\ (ppm)}{FORTIFICATION\ LEVEL\ (ppm)} \times 100$$

ERROR CODES:

A: Primary data recorded incorrectly
B: Data transcribed incorrectly
C: Miscalculation
D: Illegible data

Error codes A, B, C, or D are used in the report in case of errors. The error code will be circled beside the error along with the user's initials and date.