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7.0 ANALYTICAL PROCEDURE

7.1 Sample Preparation

The fruits and vegetables are ground using a food processor (Robot-Coupe) or a Retsch mill SM 2000 in the presence of dry ice.

7.2 Extraction

Weigh a representative analytical sample (25 ± 0.1 g) into a 250 mL Teflon centrifuge bottle. Add HCl (1N, 4-6 mL) and methanol (100 mL). Occasionally verify the pH, ensuring that it is acidic, using pH paper. Homogenize the mixture for 1 minute using the Polytron at speed 4. Allow the solution to settle and suction filter the supernatant through a glass fiber filter paper covered with a thin layer of Celite 545, leaving the solid material in the bottle.

Re-extract the solid remaining in the centrifuge bottle by homogenizing for 30 seconds (speed 4) with HCl (1N, 2 mL) and methanol (100 mL). Pass the extract through the same filter paper. Rinse the polytron with approximately 20 mL methanol, which is collected, in the same Teflon bottle. Filter the rinseate through the same filter paper. Collect all filtrates in a 500 mL flat bottom flask. Remove the methanol completely with a rotary evaporator with a water bath set at 45°C leaving only the aqueous extract.

7.3 Liquid/Liquid Partition

Transfer the concentrated aqueous extract to a 45 mL Teflon tube and centrifuge for 5 minutes at 2500 rpm. Transfer the supernatant to a 250 mL separatory funnel. Rinse the 500-mL flask with 30 mL 0.1N HCl which is poured into the same Teflon tube. Shake the tube to mix and centrifuge once more as above. Combine the supernatant with the first portion in the separatory funnel. Partition the acidified extract with dichloromethane (50 mL) and discard the organic phase. Add saturated sodium chloride solution (approximately 30 mL) to the aqueous phase and basify the solution by addition of 10N NaOH (4 mL). Check the pH occasionally to assure that it is greater than 9, using pH indicator paper. Partition the basified extract with dichloromethane (2 x 50 mL) by shaking gently for about 1 minute each time. After the phases have separated (about 10 minutes), collect the organic layer into a 250 mL flat bottom flask containing aqueous HCl (approximately 5 mL of 1N). Combine the second dichloromethane portion with the first extract and discard the aqueous phase after the second
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partition. Cap the flask and shake vigorously to assure that the propamocarb (free base) residues are transferred to the acid phase. Remove the dichloromethane using a rotary evaporator with a water bath set at 35°C.

7.4   RP-18 Clean-up

Condition the RP-18 cartridge, attached to a vacuum box, with methanol (10 mL) followed by water (10 mL) which has been acidified to pH 3 with 0.1N HCl solution. Transfer the concentrated aqueous extract (step 7.3) onto the cartridge. Wash the cartridge with 0.1N HCl (2 x 5 mL). Do not let the column go dry at any point. Elute the active ingredient from the RP-18 cartridge with degassed methanol:1N HCl (1:1) (10 mL) into a glass test tube. Transfer the eluate to a 50 mL round bottom flask with a MeOH rinse (5 mL). Remove the methanol using a rotary evaporator with a water bath set at 45°C.

7.5   ENVI™-Carb Clean-up (optional)

If high matrix effect is found in the sample extract, a second clean up step can be carried out. Attach a long needle (6 inches) to the ENVI™-Carb column, then condition with methanol (10 mL) followed by water (10 mL), which has been acidified to pH 3 with 0.1N HCl solution. Pass the eluate from the RP-18 (after removing methanol) through the ENVI™-Carb and wash the column with 0.1N HCl (2 x 5 mL). Discard both the loading and washing solutions. Elute the active ingredient from the ENVI™-Carb column with degassed methanol:1N HCl (1:1) (10 mL) into a glass test tube. Transfer the eluate to a 50 mL round bottom flask with a MeOH rinse (5 mL). Remove the methanol using a rotary evaporator with a water bath set at 45°C.

7.6   Di-isopropyl Ether Partition

Transfer the aqueous solution from step 7.4 or 7.5 to a test tube containing sodium chloride (2 g). Basify the solution from step by adding 10 N NaOH until a combined volume of approximately 10 mL is obtained. Add di-isopropyl ether (2.5 mL) and extract the mixture by shaking in the tightly capped test tube. After the phases have separated, carefully transfer the upper organic layer to an autosampler vial for quantitation by GC/TSD.
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8.0  QUANTITATION: GC/TSD

8.1  Instrumentation

The gas chromatographic system consists of a Varian 3400 GC equipped with a thermionic specific detector (TSD) and an 8200 autosampler.

8.2  Gas Chromatographic Conditions

| GC Column:  | DB-1, 15 m x 0.53 mm ID, 1.5 or 3.0 μm film |
| Carrier Gas Flow: | 9.0 mL/min |
| Temperatures: Inlet: | 275°C |
| Column: 100°C hold 0.2 min |
| 100-160°C at 4°C/min, hold 2 min |
| 160-260°C at 35°C/min, hold 6 min |
| Detector: | 300°C |
| Injection Volume: | 3.0 μL, on-column |
| Injection Rate: | 1.0 μL/sec |
| Retention Time: | 9.0 min |
| Limit of Quantitation: | 0.020 ppm |
| Limit of Detection: | 0.06 ng (S/N >3) |

8.3  TSD Calibration

The GC/TSD response (peak area) for a series of calibration standards for each analytical set is used to perform a linear regression analysis (Eq. 1). The amount injected (ng) is taken as the X-axis and the detector response (peak area) as the Y-axis gives Equation 2.

\[ y = mx + b \quad \text{[Eq. 1]} \]

Where:
- \( y \) = peak area response for analyte injected in sample/standard
- \( m \) = slope of the regression line
- \( x \) = amount (ng) of analyte found in the sample/standard (Amt)
- \( b \) = intercept of the regression line

Therefore:

\[ \text{Peak area} = (\text{Amt})m + b \quad \text{[Eq. 2]} \]
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8.4 Sample Analysis

The peak area response for Hoe 074189 from the Varian Workstation is used to determine the amount of material from the corresponding calibration plot. For sample extracts of unknown propamocarb content, the amount found (in ng) may be calculated from the observed peak area, using Equation 3.

\[
\text{Amt (ng)} = \frac{\text{peak area} - b}{m} \quad \text{[Eq. 3]}
\]

Both samples and standards must be analyzed under the same GC conditions and within the same analytical sequence.

9.0 CALCULATION OF RESIDUES

9.1 Calculation

The amount of residues in a sample is expressed in parts per million. The residues are calculated using the following equation:

\[
\text{Found (ppm)} = \frac{\text{Amt}}{B} \quad \text{[Eq. 4]}
\]

Where: \( \text{Amt} \) = \( x \) = amount of analyte found from the standard curve (ng)

and \( \text{B} \) = amount of sample injected (mg)

\[
= \frac{\text{Ws (g) \cdot Vinj (\mu L)}}{Vf (mL)}
\]

Where: \( \text{Ws} \) = weight of sample (g)

\( \text{Vinj} \) = sample volume injected (\( \mu \text{L} \))

\( \text{Vf} \) = final volume of extract (mL)

Recovery (\%) = \( \frac{\text{found (ppm) \cdot 100}}{\text{added (ppm)}} \)
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10.0 QUALITY CONTROL PROCEDURES

10.1 Laboratory Fortifications and Control Samples

To assure the quality of the analytical data, laboratory fortified controls are analyzed with each set of samples. These fortified controls should cover the expected range of residues and represent at least 10% of the samples within an analytical set.

10.2 GC Analysis

To verify the stability of the response, a standard plot is drawn for the levels of interest. Within an analytical set, analytical standards are injected after every few samples; this serves as an ongoing quality control for detector sensitivity and analyte retention time. Calibration standards are injected at the beginning and end of an analytical set.

The lowest level analytical standard corresponds to 50 to 70% of the limit of quantitation. Residue results must not be determined by extrapolation outside of the concentration range of the calibration standards (using ±10% tolerance, typically). Samples with residue levels greater than the calibrated range must be diluted and re-injected so that they do fall within the calibrated range.

The recovery of laboratory fortified controls should fall in the range of 70 - 120%. The analyte signal should be > 3 times the background signal. The intra-laboratory reproducibility as indicated by the relative standard deviation (n > 3) obtained from replicated analyses should fall within 20% (rel.) of the averaged result.

10.3 Sample Storage

Field samples are kept frozen until analyzed. After obtaining a representative analytical sample, the remaining material should be promptly refrozen and stored until authorization for disposal is received.