A Rapid and Universal Extraction Method for Determination of Pesticide Residues in Environmental and Food Samples

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Overview

• What is the issue?

• What are the objectives of the project?

• What are our new approaches to the challenges?

• What has been accomplished so far?

• What is the take-home message?
The issue

- Pesticides are a major concern in emergency preparedness. Some pesticides have a similar toxicity to that of chemical weapon agents and are often odourless and colourless and commercially available in large quantities.

- Each year it is estimated that over half a million samples are analyzed for pesticide residues. Current sample preparation methods use huge amounts of solvents which are detrimental to the environment and to human health.

- With the advent of new instrumentation and fast separation techniques, sample preparation has become the bottleneck of the sample analysis process. In an emergency situation, rapid sample preparation is especially critical.

- There is a need to develop a so-called “universal” sample extraction method to deal with emergency situations.
Project Objectives

To develop an extraction method that satisfies the following requirements:

• Rapid: up to 36 samples per day per chemist.

• Small solvent usage: must be less than 50 mL per sample.

• Good recovery: 40 to 120%.

• Robust: RSD of the recoveries less than 20% at LOQ.

• Wide application: five sample types targeted:

  Drinking water  sewage water  soil  cucumber  beef
Literature Review

- **Soxhlet extraction:**
  Gold standard of recovery, but time consuming and large volume solvent usage.

- **QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe):**
  Very quick, small solvent usage, but not for liquid samples and small sample size (0.5 g only).

- **PESC standard method:**
  Good recovery, wide applications, but laborious, time consuming and large volume of solvent usage.
Approaches

• Regard all sample matrices as aqueous (water laden with particular materials, either lightly such as drinking water or heavily such as suspended soil).

• Perform a binary solvent liquid-liquid extraction. Firstly taking advantage of the superior extraction efficiency of dichloromethane (DCM) to extract targeted compounds into an organic solvent, then the DCM is “extracted” into hexane, which in turn forms the up layer in an aqueous solution.

• Collect the extracts by decanting the upper organic layer into another centrifuge tube once the aqueous solution is frozen.

• Replace nitrogen blow-down units with a centrifugal vacuum solvent removal apparatus (e.g. SpeedVac or EZ-2) to reduce and concentrate the samples.
# Targeted Pesticide Compounds

<table>
<thead>
<tr>
<th>Dichlorvos</th>
<th>Metalaxyl</th>
<th>Trifluralin</th>
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<tbody>
<tr>
<td>Mevinphos</td>
<td>Malathion</td>
<td>Desethylatrazine</td>
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<tr>
<td>Demeton-O</td>
<td>Chlorpyrifos</td>
<td>Quintozene</td>
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<td>Demeton-S</td>
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<td>Napropamide</td>
<td>Myclobutanol</td>
<td>Phosmet</td>
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<td>Phosalone</td>
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Extraction procedure: liquid samples

• To 400 mL of water sample add 80 mL of DCM:Hex mixture (40:60; v:v). Shake vigorously for 30 sec. Repeat after 5 min.

• Wait for 20 min until two layers formed. Freeze the sample at – 40 ºC for 80 min or until the aqueous layer is frozen.

• Decant the organic layer into pre-labeled glass centrifuge tubes.

• Evaporate the sample to dryness using EZ-2 with settings of low boiling solvent, 55 ºC, 55 min.

• Reconstitute the sample with 2 mL of hexane, Vortex, add to GC sample vial, GC/MS analysis.
Extraction procedure: non-tissue solid material

- To 5 g of solid sample add 15 mL of de-ionized water. Homogenize the sample either by Polytron or agitation. Add 20 mL of DCM/Hex mixture (40:60; v:v). Vortex or shake vigorously for 30 sec. Repeat after 5 min.

- Centrifuge at 5000 xg for 6 min to settle the particulates and form two layers. Freeze the sample at – 40 ºC for 30 min or until the aqueous layer is frozen.

- Decant the organic layer into pre-labeled glass centrifuge tubes.

- Evaporate the sample to dryness using EZ-2 with settings of low boiling solvent, 55 ºC, 25 min.

- Reconstitute the sample with 2 mL of hexane, Vortex, add to GC sample vial, GC/MS.
Extraction procedure: fatty tissue solid material

- To 5 g of solid sample add 15 mL of d.i. water. Homogenize the sample either by Polytron or agitation. Add 20 mL of hexane. Vortex vigorously for 30 sec. Wait for 5 min, withdraw the organic layer with a disposable pipette. Discard lipid/hexane mixture.

- Add DCM/Hex mixture (40:60; v:v). Vortex or shake vigorously for 30 sec. Repeat after 5 min.

- Centrifuge at 5000 g for 6 min to settle the particulates and form two layers. Freeze the sample at – 40 ºC for 30 min or until the aqueous layer is frozen.

- Decant the organic layer into pre-labeled glass centrifuge tubes.

- Evaporate the sample to dryness with EZ-2 with settings of low boiling solvent, 55 ºC, 25 min.

- Reconstitute the sample with 2 mL of hexane, Vortex, add to GC sample vial, GC/MS.
Binary Solvent Extraction
Freeze and Decant
EZ-2 Solvent Removal and Concentration
GC/MS Operating Conditions

- Instrumentation: Agilent 6890/5973

- GC/MS Column: ResTek Rxi-5ms, 30 m, 0.25 mm ID, 0.25 μm film thickness.

- GC/MS Parameters
  - Injection Volume: 1 μL
  - Injection Type: Splitless
  - Injector Liner: 4 mm ID, Glass-wool packed
  - Injector Temperature: 280 ºC
  - Transfer Temperature: 300 ºC
  - Carrier Gas: Helium, 1.0 mL/min
  - Oven Program: Initial hold 60 ºC for 3 min, then 12 ºC to 300 ºC, hold time 8.5 min. Total run time is 35 minutes.
QA and QC Consideration

- Surrogate standards: Malathion D-6 and Atrazine-D5
- Internal standard: Anthracene D-10
- Triplicate samples were prepared spiked at 100 ppb for water samples and 200 ppb for solid samples.
- One in five samples was re-injected to determine instrument repeatability

- GC/MS Acquisition sequence: Solvent blank
  Standards
  Reagent blank
  Samples
  Standards
  Solvent blank
Results and Discussion

Average Recovery of OPNP Pesticides Spiked into Various Sample Matrices

- Average Recovery%
- Pesticides: Mevinphos, Demelon-O, Naled, Demelon-S, Dimethoate, Simazine, Atrazine, Propazine, Diazinon, Carbaryl, Metalaxyl, Malathion, Chlorpyrifos, Parathion, Amlazine, Methidathion
- Matrices: Drinking Water, Soil, Cucumber, Beef, Sewage Water
Results and Discussion

• With a few exceptions, the recovery of most spiked compounds is within the range specified in the project objective.

• The repeatability of extraction method is good as a screening method with RSD less than 15% for compounds from triplicate samples within the same batch.

• Relatively poor recovery is found from the beef and sewage extracts. In the case of beef extract, some loss may be attributed to the loss during the hexane extraction step, and matrix effects. Matrix matching standards may be used to address the problem.

• In the case of sewage water, matrix effects is the main concern. Standard-addition method may have to be used since it is difficult to obtain materials for preparing matrix matching standards.
Summary

• The preliminary results indicated that this binary L-L extraction method has the following advantages:

  Rapid (up to 36 samples per chemists per day)
  Simple (no laborious steps, minimal sources of errors)
  Cheap (no SPE cartridge)
  Low Solvent Consumption (less than 80 mL)
  Wide Pesticide Range (NP, OP, neutral)
  Applicable to many sample matrices (water, vegetation, soil and animal tissue)

• A small number of pesticides demonstrated poor recovery, presumably due to either hexane extraction or exposure to air during the concentration step.

• Combine traditional L-L extraction and modern centrifugal vacuum solvent removal unit may be the key to solving many sample preparation problems.
Future Work

• Expand the current list of pesticide compounds from 40 to 200 by adjusting pH, ion strength (salting out) and adding ion pairing agents.

• Develop LC/MS method to analyze the polar compounds left in aqueous phase and those not amenable to GC/MS.

• Build a searchable LC/MS spectral library to cover the whole range of pesticides.

• Modify the method and make it practical in a mobile laboratory to be used in an emergency situation.
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