Tricks of the Trade, Keeping Your GC System Up Longer for the Analysis of Pesticides (and More!!)

Michelle Misselwitz, Jack Cochran, Julie Kowalski, Christopher Rattray
The Main Problems with GC-MS

- GC inlet
  - Liner
  - Seal

- Dirty samples contaminate liner wool and bottom seal
  - Poor compound transfer
  - Compound degradation

- GC column
  - Guard column
  - Analytical column

- Dirty samples contaminate front of column system
  - Poor compound transfer
  - Compound degradation

Both issues lead to poor data quality and downtime for maintenance...
Splitless injection

27 × Used Motor Oil
50,000 µg/mL

39 × Sediment Extract
(no dilution)
Pentachlorophenol

4,4′-DDT

Endrin

PCB 52

2,4-Dinitrophenol
15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard

<table>
<thead>
<tr>
<th>Compound</th>
<th>Response Factor</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentachlorophenol</td>
<td>1.78</td>
<td>1.3</td>
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<tr>
<td>PCB 52</td>
<td></td>
<td></td>
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<tr>
<td>4-Chloroaniline</td>
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<td></td>
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<tr>
<td>2,4-Dinitrophenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4’-DDT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After 32 splitless injections of used motor oil

<table>
<thead>
<tr>
<th>Compound</th>
<th>Response Factor</th>
<th>Tailing Factor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.816</td>
<td>1.6</td>
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<td>*</td>
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</table>

Inlet liner and seal changed...

<table>
<thead>
<tr>
<th>Compound</th>
<th>Response Factor</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.62</td>
<td>1.1</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What about wool-packed versus non-wool-packed liners?
Standards analyzed after 39 sediment extracts, then liner and seal changes...

Pentachlorophenol

Splitless injections

PCB 52

4,4’-DDD

Endrin

4,4’-DDT

Simple liner change did not restore performance...

Endrin

4,4’-DDD

4,4’-DDT
A Better Way?

• Analyte Protectants
  – GC system active site masking compounds
  – Improve compound transfer from GC inlet
  – Allow better peak shape from GC column

• Split injection (shoot-and-dilute)
  – High GC inlet flow improves compound transfer
  – Less “dirt” on GC column improves peak shapes
  – System stays up longer
Significant peak quality improvements obtained when matrix components are present to fill active sites and reduce analyte interactions.

“Matrix-induced chromatographic response enhancement”.

Addition of “analyte protectants” (e.g. sugars, acids, etc.) to standards and samples.

Provides chromatographic enhancement effect for analytes in a very dirty GC system.
Spermine

H$_3$C – O – CH$_3$

3-Ethoxy-1,2-propanediol

Shikimic acid

H$_2$N – CH$_2$ – CH$_2$ – NH$_2$

D-sorbitol

L-gulonic acid γ-lactone

Analyte protectants
3-Ethoxy-1,2-propanediol

L-gulonic acid γ-lactone
Analyte Protectant Criteria

- Rich in hydroxys/aminos to deactivate systems
- Volatile so they gas chromatograph
- Several may perform better than one
- Volatility range similar to compounds analyzed
- Added in high concentration
- Low m/z ions to avoid quantification bias
- Inexpensive
- Injected with each standard and sample
15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard

Pentachlorophenol

Standard 10 µg/µL AP
Standard 2.5 µg/µL AP
Standard 0.5 µg/µL AP
Standard No AP

Tailing Factor = 1.1

3-Ethoxy-1,2-propanediol

PCB 52

Analyzed after one injection of used motor oil...
Pentachlorophenol

Response Factor = 1.60
Tailing Factor = 2.1

PCB 52

Response Factor = 1.08
Tailing Factor = 2.5

15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard

Already 99 splitless injections of used motor oil on column, but this standard was analyzed after a GC inlet liner and seal change...

After only 3 splitless injections of used motor oil...

4,4’-DDT
Response Factor = 2.51
Tailing Factor = 1.3
Endrin breakdown = 4.5%
DDT breakdown = 10%
With 3-Ethoxy-1,2-propanediol

Endrin breakdown = 4.5%
DDT breakdown = 10%
With 3-Ethoxy-1,2-propanediol

After only 3 splitless injections of used motor oil...

Response Factor = 1.08
Tailing Factor = 2.5
DDT breakdown = 21%
Endrin breakdown = 7.2%

After only 3 splitless injections of used motor oil...

Response Factor = 1.08
Tailing Factor = 2.5
DDT breakdown = 21%
After only 3 splitless injections of used motor oil...

- Endrin
- 4,4'-DDT
- PCB 52
- Pentachlorophenol

With 3-Ethoxy-1,2-propanediol

- 2,4-Dinitrophenol
23 injections of used motor oil at 50,000 ng/µL...

PCP Response Factor w AP

0.5 ng/µL PCP
1µL splitless injection

PCP Response Factor wo AP

Standard Injection Number
After 23 splitless injections of used motor oil at 50,000 ng/µL...
Analyte Protectants Summary for Splitless Injection GC

- Approach shows promise for better GC of active compounds, including pesticides
  - Increased response factors and less peak tailing
- Less successful for reactive compounds
  - Endrin and DDT still subject to degradation
- Additional analyte protectants need testing
  - GC-MS will increase protectant choices
Splitless Injection GC Issues

- Active/polar/thermal sensitive analytes
- Dirty samples
- Matrix enhanced/degraded responses
- Limited transfer from GC inlet to column
- Poor detectability
- Significant quantification bias
- Frequent GC inlet and column maintenance
- Offline cleanup
Pesticide Analysis with GC

- Active/polar/thermal sensitive analytes
- Dirty samples
- Matrix enhanced/degraded responses
- Limited transfer from GC inlet to column
- Poor detectability
- Significant quantification bias
- Frequent GC inlet and column maintenance
- Offline cleanup
Pesticide Analysis with GC

Split injection

Matrix *effect* diluted to the point where it doesn’t negatively impact *GC inlet* efficiency

**Shoot-and-Dilute GC**

Relies on detector sensitivity and selectivity improvements, e.g. MS/MS

- Frequent GC inlet and column maintenance
- Offline cleanup
**Sky® 4mm ID Single Taper Inlet Liner with Quartz Wool Splitless Injections**

- Goal is for part of sample to make it to GC column
  - Based on split ratio
- Wool wipes needle and helps homogenize sample
  - Excellent for repeatability
  - High split ratio reduces wool and “dirt” impact on compounds prone to breakdown or sorption losses

**Sky® 4mm ID Precision® Inlet Liner with Quartz Wool Split Injections**

- Goal is for part of sample to make it to GC column
  - Based on split ratio
Organochlorine
Organonitrogen
Organophosphorus
Carbamate
Pesticides

QuEChERS Performance Standards Kit

- Designed for use in all QuEChERS methods for pesticides in fruits and vegetables, including the original unbuffered method, AOAC 2007.01, and EN15662.
- Kit contains organochlorine, organonitrogen, organophosphorus, and carbamate pesticides commonly used on fruits and vegetables.
- Volatile, polar, active, base-sensitive, and nonvolatile compounds are included to allow comprehensive evaluation of QuEChERS extraction and cleanup efficiencies, and optimization of GC and LC instrumental conditions.
- Ideal for initial method evaluations and ongoing method performance validations.
- Analytes are divided into three ampuls based on compatibility for maximum stability and shelf life.*
- Precise formulations improve data quality and operational efficiency; spend more time running samples and less time sourcing and preparing standards.
- Quantitatively analyzed to confirm the composition and stability of each mixture.
- Produced and tested in accordance with ISO Guide 34 and 17025 accreditation.

Standard C contains difficult pesticides such as Captan, Folpet, Deltamethrin...
QuEChERS Performance Standard C – 200 pg/µL – 1 µL – Split Ratio 10
GC-ECD – 15m x 0.25mm x 0.25µm Rxi-5ms column

Captan
Folpet
Imazalil
Fenhexamid
Iprodione
Bifenthrin
Fenpropathrin
Myclobutanil
Permethrins

Vinclozolin

Deltamethrin

~20 pg on column
Splitless and Split Injections of Strawberry Extracts

- EN QuEChERS organic strawberry extracts
  - dSPE cleaned with 50 mg per mL PSA and C18
  - Spiked to 100 pg/µL each pesticide

- GC-ECD
  - 15m x 0.25mm x 0.25µm Rxi-5ms column

- Splitless injection at 250°C
  - 4mm ID single taper liner with wool

- Split injection at 250°C, split ratio 10
  - 4mm ID Precision split liner with wool
Inlet Ruggedness GC-ECD

- Sample queue
- Two 100 pg/µL solvent-only standards
- Two 100 pg/µL spiked strawberry extracts
- Repeat
- 40 analyses each of standards and extracts
Splitless Injection GC-ECD Method

- **4mm single taper liner with quartz wool**
  - 1 µL, 250°C, **1.4 min splitless valve time**

- **15m x 0.25mm x 0.25µm Rxi-5ms column**
  - Constant flow He, 1.4 mL/min
  - 70°C (1.4 min), 15.2°C/min to 330°C (1.5 min)
  - Total run time: 20 min

- **Electron capture detector**
  - 350°C, 20 Hz
  - Make-up (nitrogen) + column flow 50 mL/min
Spiked Organic Strawberry – Splitless 1

Spiked Organic Strawberry – Splitless 5
Split Injection GC-ECD Method

- 4mm focus-type split liner with quartz wool
  - 1 µL, 250°C, split ratio 10
- 15m x 0.25mm x 0.25µm 5ms column
  - Constant flow He, 1.4 mL/min
  - 70°C (0.1 min), 15.2°C/min to 330°C (0.8 min)
  - Total run time: 18 min
- Electron capture detector
  - 350°C, 20 Hz
  - Make-up (nitrogen) + column flow 50 mL/min
Splitless

Split

RESTEK

Pure Chromatography

www.restek.com
# Comparing Average Response Factor (RF) and % RSD RF for Splitless and Split Injection

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Splitless Avg RF</th>
<th>Split Avg RF</th>
<th>Splitless % RSD RF</th>
<th>Split % RSD RF</th>
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</thead>
<tbody>
<tr>
<td>Captan</td>
<td>2480</td>
<td>4550</td>
<td>31</td>
<td>5.6</td>
</tr>
<tr>
<td>Folpet</td>
<td>2540</td>
<td>4200</td>
<td>26</td>
<td>5.7</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>3240</td>
<td>2810</td>
<td>24</td>
<td>7.4</td>
</tr>
<tr>
<td>Fenhexamid</td>
<td>2460</td>
<td>3820</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Iprodione</td>
<td>2220</td>
<td>3180</td>
<td>6.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>2360</td>
<td>3490</td>
<td>1.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td>2420</td>
<td>4180</td>
<td>3.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>4380</td>
<td>6620</td>
<td>15</td>
<td>2.4</td>
</tr>
</tbody>
</table>

QuEChERS strawberry extracts spiked with 100 pg/µL pesticide standards. Avg RF calculated from 40 analyses of spiked strawberry extracts.
Triplicate analyses at each point from 1 to 200 pg/µL (0.10 to 20 pg on column)

Captan Calibration Curve
GC-ECD, Split Ratio 10

Solvent only standards

$y = 63822.6x + 11851.9$
$r = 0.99916$
Triplicate analyses at each point from 1 to 200 pg/µL (0.10 to 20 pg on column)

Solvent only standards

\[ y = +56475.1x + 27185.9 \]

\[ r = 0.99866 \]
Conclusions
Shoot-and-Dilute GC-ECD

- **Splitless injection** for sensitive pesticides like Captan, Folpet, and Deltamethrin is not robust
  - Response factors quickly fall off when analyzing QuEChERS extracts of strawberries

- **Split injection** for these same pesticides shows better repeatability for response factors
  - Higher flow through inlet leads to decreased pesticide degradation and sorption losses
Shoot-and-Dilute GC-MS/MS
It can’t be as dangerous as this...
Shoot-and-Dilute GC-MS/MS
Fruit and Vegetable

Celery
Low pigment
“Easy”

Orange
Low pH
“Hard”

Green pepper
Medium pigment
“Moderate”

All of the above are high water content...
9 stable mixes containing 203 GC-able pesticides
Organophosphorus (3), organochlorine (1), organonitrogen (3), synthetic pyrethroid (1), herbicide methyl ester (1) compounds
Downloadable transitions table for TSQ 8000
EN QuEChERS Extraction
Homogenize
10 g + 10 mL MeCN
Shake 1 min
Add EN salts
Shake 1 min
Centrifuge 5 min

dSPE Cleanup
Shake 30 sec
Centrifuge 5 min
Formic acid

150 mg MgSO4
50 mg PSA
50 mg C18
7.5 mg GCB
We want it slightly dirty for our ruggedness experiment (and for planar pesticide recovery)...

mL Extract
150 mg MgSO4
50 mg PSA
50 mg C18
Shoot-and-Dilute GC-MS/MS for Multiresidue Pesticide Analysis?

Detectability

Ruggedness
**Splitless injection**
4mm single taper liner with quartz wool
1 µL MeCN, 250°C
Splitless valve, 1.7 min

**GC Conditions**
30m x 0.25mm x 0.25µm 5ms-type column
Constant flow helium, 1.4 mL/min
GC oven program:
90°C (1.7 min), 8.9°C/min to 330°C (hold 5 min)

**Thermo Scientific TSQ 8000 GC-MS/MS**
Transfer line: 290°C
Source temperature: 325°C
Electron ionization: 70 eV
Stored mass range: 2 SRMs per
Minimum Dwell: 26 msec

**Split injection**
4mm focus-type split liner with quartz wool
1 µL MeCN, 250°C
Split ratio 10
Shoot-and-Dilute GC-MS/MS for Multiresidue Pesticide Analysis?

Detectability
Propachlor Matrix-Matched Calibration Curve

Splitless

$R^2 = 0.9987$

Four analyses at each point from 5 to 100 pg/µL (5 to 100 pg on column)

Area Ratio

Concentration (pg/µL)

10 pg
Propachlor Matrix-Matched Calibration Curve

Four analyses at each point from 5 to 100 pg/µL (0.5 to 10 pg on column)

R² = 0.9983

Split

1 pg
Deltamethrin Matrix-Matched Calibration Curve

Four analyses at each point from 5 to 100 pg/µL (5 to 100 pg on column)

Area Ratio

Concentration (pg/µL)

R² = 0.9903

Splitless

10 pg

RESTEK
Pure Chromatography

www.restek.com
Deltamethrin Matrix-Matched Calibration Curve

Four analyses at each point from 5 to 100 pg/µL (0.5 to 10 pg on column)

Area Ratio

Concentration (pg/µL)

$R^2 = 0.9937$

Split

1 pg
Captan Matrix-Matched Calibration Curve

Splitless

Four analyses at each point from 5 to 100 pg/µL (5 to 100 pg on column)

$R^2 = 0.9828$

10 pg
Captan Matrix-Matched Calibration Curve

Area Ratio

Concentration (pg/µL)

Four analyses at each point from 5 to 100 pg/µL (0.5 to 10 pg on column)

R² = 0.9319

Split

1 pg
Captan

Quant SRM, 149 to 105
Qual SRM, 149 to 79
Detectability Summary

• 203 pesticides in celery and orange extracts
  – ~180 pesticides detected < 1 pg on column
  – ~195 pesticides detected 1 pg on column

• Captan and Folpet detected > 1 pg on column
  – Low m/z precursor and product ions

• Isomeric pesticides detected > 1 pg on column
  – Cyfluthrins and cypermethrins, e.g., split response
Calibration Summary
Splitless and Split Injection

• 203 pesticides in celery and orange extracts
  – Matrix-matched standards
  – 5, 7.5, 10, 50, 100 pg/µL – 4 replicates each

• Calibration curve correlation coefficients
  – Large majority of pesticides > 0.99

• Calibration curve Average RF RSD% values
  – Large majority of pesticides < 20%
Calibration Summary
Splitless and Split Injection

- **Splitless**
  - Detectability advantage
  - “Generic” data processing
  - Quicker review

- **Split**
  - Detectability reasonable
  - “Manual” data processing
  - Lengthy review

Does the possibility of longer GC system uptime and more stable response factors from split injection offset some detectability and data processing issues?

**Screening method?**
Does the split injection GC system stay up longer?
4,4′-DDT + 4,4′-DDD + 4,4′-DDE
4,4’-DDD

Splitless

200 pg

Injection 1

Injection 19

4,4’-DDT

Split

20 pg

4,4’-DDD
Comparing Average Response Factor (RF) and % RSD RF for Splitless and Split Injection

<table>
<thead>
<tr>
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<th>Splitless Avg RF</th>
<th>Split Avg RF</th>
<th>Splitless % RSD RF</th>
<th>Split % RSD RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma-BHC</td>
<td>0.131</td>
<td>0.199</td>
<td>7.6</td>
<td>3.2</td>
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<tr>
<td>Chlorothalonil</td>
<td>0.056</td>
<td>0.070</td>
<td>7.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.078</td>
<td>0.129</td>
<td>7.7</td>
<td>2.2</td>
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<tr>
<td>Methiocarb</td>
<td>0.254</td>
<td>0.302</td>
<td>6.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Dichlofluanid</td>
<td>0.381</td>
<td>0.444</td>
<td>4.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Captan</td>
<td>0.004</td>
<td>0.034</td>
<td>25</td>
<td>9.3</td>
</tr>
<tr>
<td>4,4’-DDT</td>
<td>0.117</td>
<td>0.480</td>
<td>30</td>
<td>3.6</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.073</td>
<td>0.051</td>
<td>14</td>
<td>4.9</td>
</tr>
</tbody>
</table>

QuEChERS green pepper extracts spiked with 200 pg/µL pesticide standards. Avg RF calculated from 19 analyses of spiked green pepper extracts.
Methamidophos

Dichlorvos

**Splitless**

- **Methamidophos**
  - 200 pg/µL
  - 1 µL MeCN

- **Dichlorvos**
  - 200 pg/µL
  - 1 µL MeCN

**Split**

- **Methamidophos**
  - 200 pg/µL
  - 1 µL MeCN
  - Split ratio 10
  - 0.1 µL MeCN

- **Dichlorvos**
  - Split ratio 10
  - 20 pg

**Acephate**
Conclusions

Shoot-and-Dilute GC-MS/MS

• Split injection shows promise for GC-MS/MS
  – Ruggedness is superior to splitless injection

• Need better detectability for some pesticides
  – Further optimization of MS/MS parameters

• Data review is somewhat lengthy when LODs and LOQs are approached
  – Better choice of auto integration settings
Other Points

• Food matrix quantification bias (theoretically) the same for splitless and split injections
  – Quantification accuracy defined by split LODs

• Possibility of avoiding dSPE cleanup
  – Split injection keeps the system up longer
  – Better base-sensitive pesticide recoveries (no PSA)
  – Better planar pesticide recoveries (no GCB)

• Possibility of using solvent-only standards instead of matrix-matched standards for split injection
  – High inlet flow encourages good pesticide transfer
Recent Success of Shoot-and-Dilute GC

• Split injection GC-MS (NCI) approach for straightforward analysis of problematic phthalimide fungicides and chlorothalonil
  – Analytical and Bioanalytical Chemistry (submitted)

• QuEChERS extracts of apple, fish, oilseed, feed, kidney fat, and honey

• 3x reduction in GC-MS run time
• 3x increase in GC-MS up time
Thank you!