Analysis of Multi-Pesticide Residues in Vegetables, Food, and Fruits by SPE/GC-MS

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Introduction

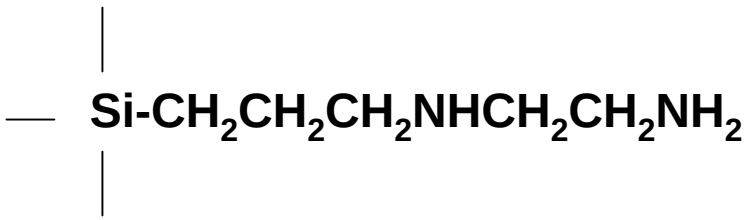
Cleanup of matrices is the first, the most important, and the most critical step for both broad-spectrum screening and accurate determination of pesticides and their metabolites in vegetables, meats, drinks and fruits. In most cases, solid phase extraction (SPE) methods are used to clean up the extracts before the GC-MS analysis. The ideal SPE material(s) should remove the greatest number of food matrix interferences and offer truly high recoveries for a broad-spectrum of pesticides.

Glass beads, polymeric sorbents, florisil, alumina, silica, C18 and charcoal, although useful for particular classes of pesticides, were found to be inadequate for trapping diverse pesticides.

Luke method, using strong and weak anion-exchange SPE materials, requires the use of a large amount of chlorinated organic solvents, and has inability for the samples for high amount of fatty acids. ENVI-Carb/NH_2 dual layer SPE, although useful for extensive pesticide screening, were found to be also inadequate for removal of fatty acids.

Introduction (contd.)

Primary Secondary Amine (PSA) has been found as the most effective sorbent for removal of various matrices and significantly reducing matrix- enhancement effect. On the other hand, graphitized carbon black (GCB) is very useful for removal of coloring substances (i.e. pigments) and sterols. Therefore, PSA itself and/or combination of GCB/PSA dual layer should be most effective for sample cleanup.



Two pKa: at 10.1 and 10.9

Problems with Current PSA and Carbon/PSA SPE Products

- Low capacity and phase bleeding.
- Poor lot-to-lot reproducibility for removal of color.
- Low recoveries on difficult pesticide, such as acephate.
- Several different elution protocols, particularly by acetone:hexane (1:1) or acetonitrile:toluene (3:1), has been extensively used. But their merits and disadvantages have been not revealed clearly.

Experiments and Results

Properties of Improved PSA and ENVI-Carbon

PSA

- PSA bonded on spherical silica (70Å, 60 µm)
- Ion exchange capacity: 0.96 mmole/g
- PSA bleed: 0 – 1.2 µg/g
- Removal of oleic acid: 224 mg/g

ENVI-Carbon

- Non porous
- Granular graphitized carbon 125 µm
- Surface area: 100 m^2/g
- Density: 1.8 g/mL

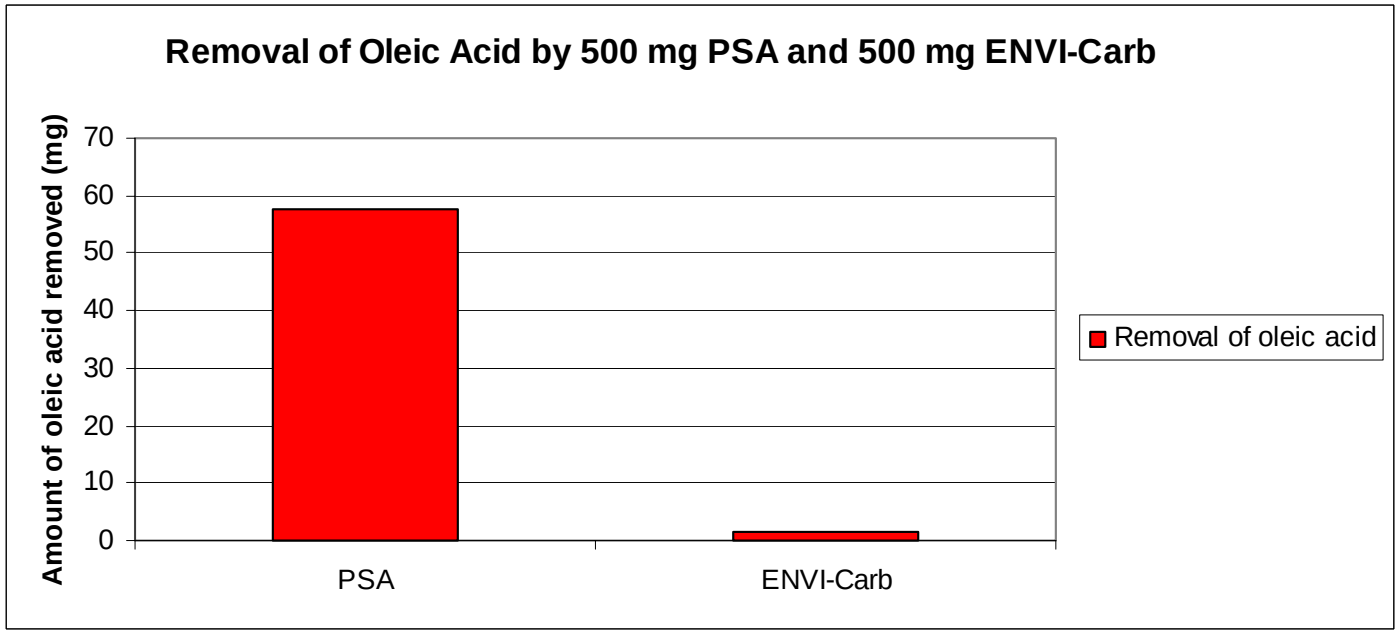
Comparison of PSA from Several Vendors

Items	Vendor A	Vendor B	Vendor C	Supelclean™
Ion-exchange capacity (mmol/g)	0.65	0.92	0.72	0.96
PSA bleed (µg/g) (1)	2.7 - 5.8	0 - 5.4	2.4 - 7.2	0 - 1.2
Removal of oleic acid (mg/g)	148	198	202	224

(1) Salicylaldehyde was used as a probe to detect the PSA silane bleeding from SPE cartridges.

PSA Plays a Vital Role for Removal of Fatty Acids

Using acetonitrile:toluene (3:1) as elution solvent, PSA (500 mg) removes 57.8 mg oleic acid while the same amount of ENVI-Carb removes only 1.6 mg oleic acid.



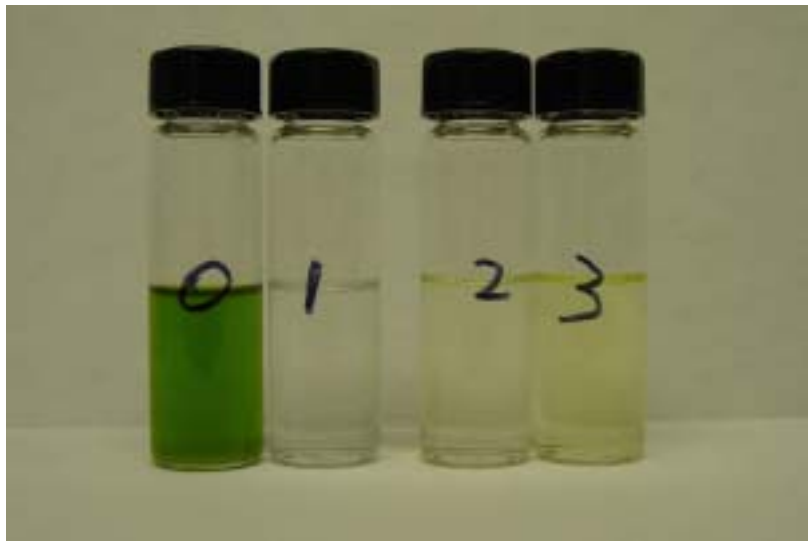
ENVI-Carb Plays a Vital Role in Color Removal

0: Spinach extracted by acetonitrile without SPE cleanup

1: Typical results with ENVI-Carb/PSA (500 mg/400 mg) cleanup

2: Cleaned by one lot of carbon/PSA from one vendor

3: Cleaned by another lot of carbon/PSA from the competitor

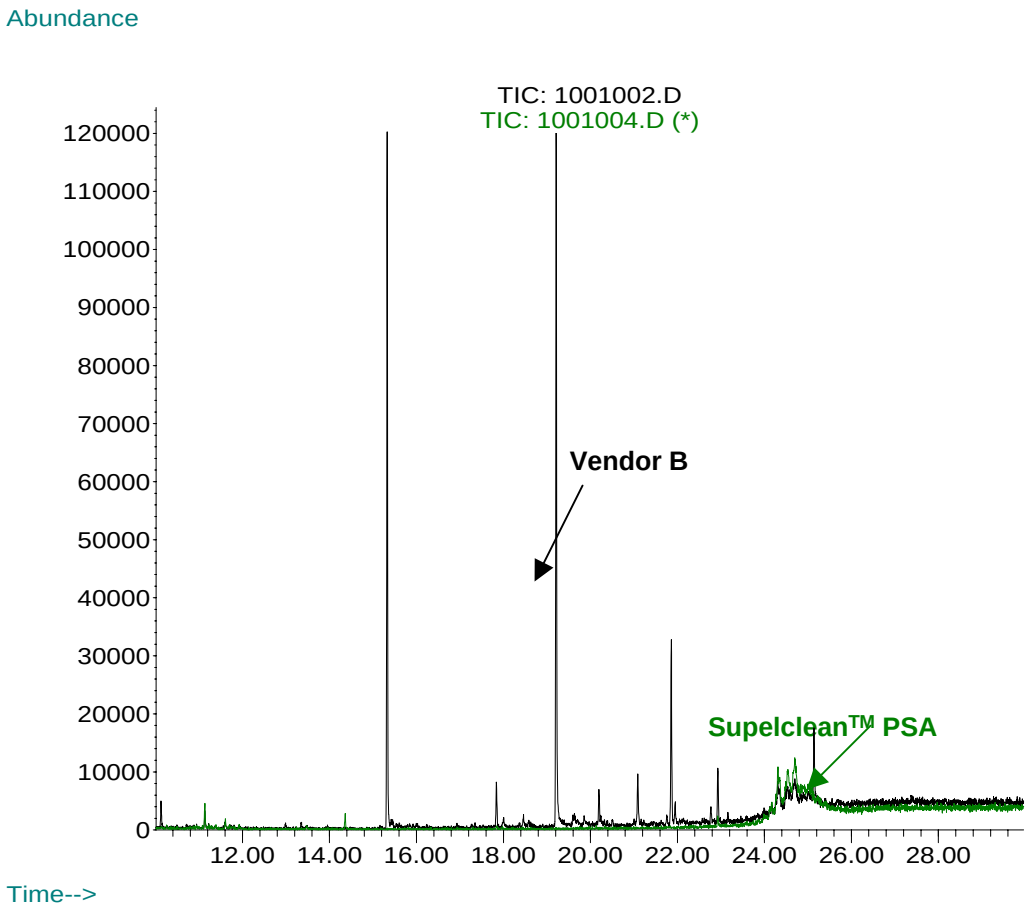


GC-MS Background of PSA SPE Cartridges

An ideal SPE cartridge should not bring additional impurities in the final elution.

Experimental:

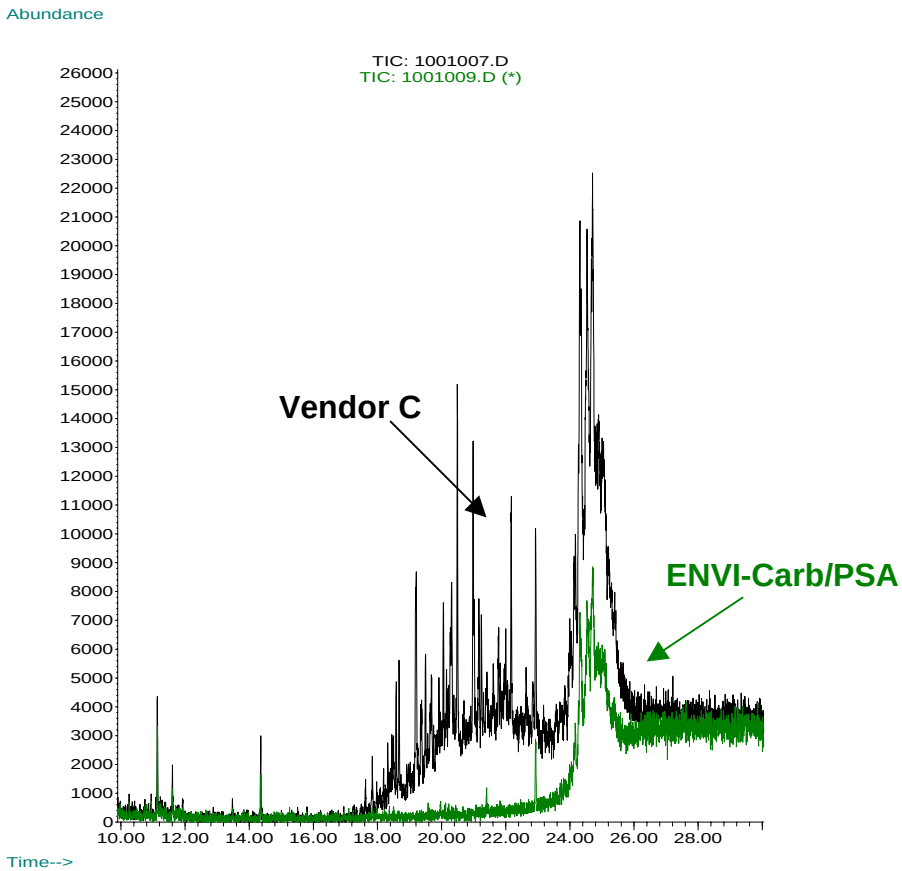
500 mg/6 cc PSA tube was conditioned with 5 mL acetonitrile and then eluted with 14 mL acetonitrile. The elution was collected, concentrated and re-constituted into 1.0 mL acetone:hexane for GC-MS total ion scanning.



GC-MS Background of Carbon/PSA Dual Layer SPE Products

Experimental:

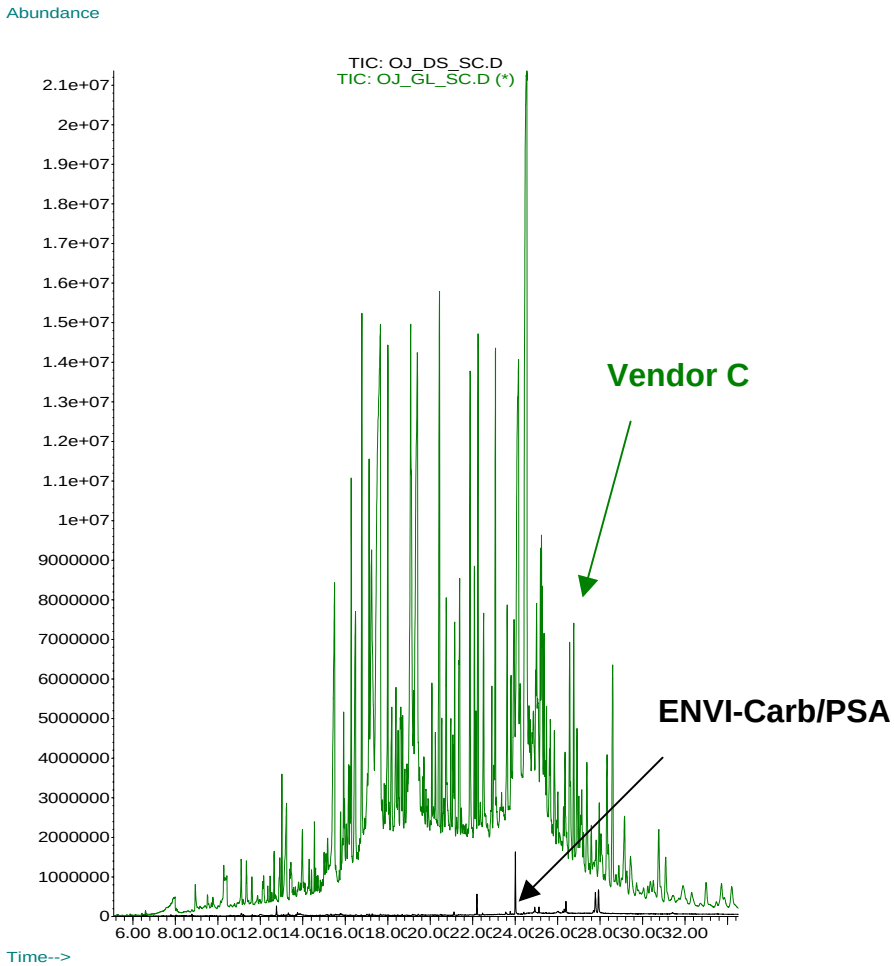
Carbon/PSA dual-layer SPE cartridges were conditioned with 5mL acetontrile:toluene (3:1) and eluted with 20mL of the same solvent. The eluate was dried and re-constituted into1.0 mL acetone:hexane (1:1) for GC-MS total ion scanning. The bump between 24 min and 26 min may be from column bleeding rather than SPE materials themselves.



ENVI-Carb/PSA on Orange Juice Matrices Clean-Up

Extraction and Cleanup of Florida Orange Juice:

1. 50 mL of the orange juice was extracted with 100 mL acetonitrile by PMRA method.
2. Carbon/PSA cartridges were first conditioned with 5 mL acetonitrile:toluene (3:1). Then 10 mL acetonitrile extract was loaded, followed by 20mL acetone:hexane elution (1:1). The elution was concentrated and re-constituted into 1.0 mL acetone:hexane (1:1) for GC-MS analysis.



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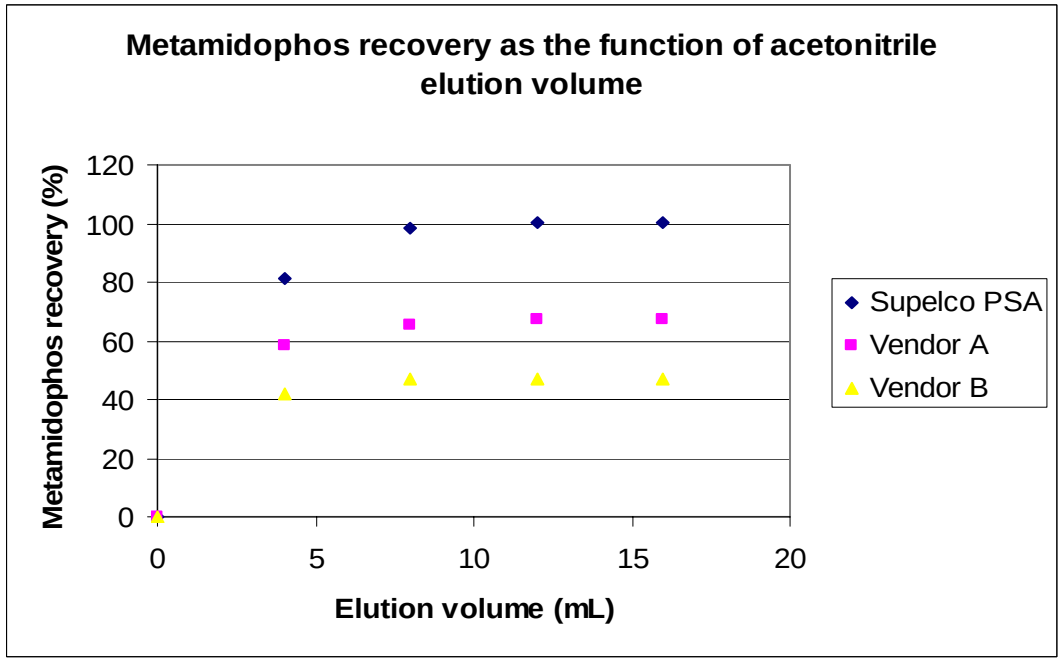
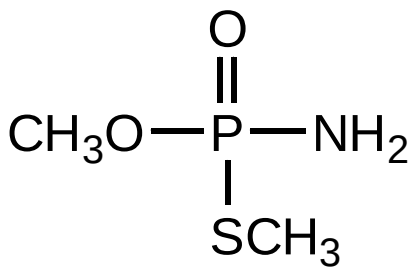
Recoveries of Metamidophos from PSA

Experimental:

Supelclean PSA SPE tube: 500 mg, 6 mL; condition and elution with acetonitrile; sample: 1 mL (1 ppm) in acetonitrile.

Metamidophos has a full recovery from Supelclean PSA. On the other hand, its recovery is only 67.2% on Vendor A, and 46.2% on Vendor B.





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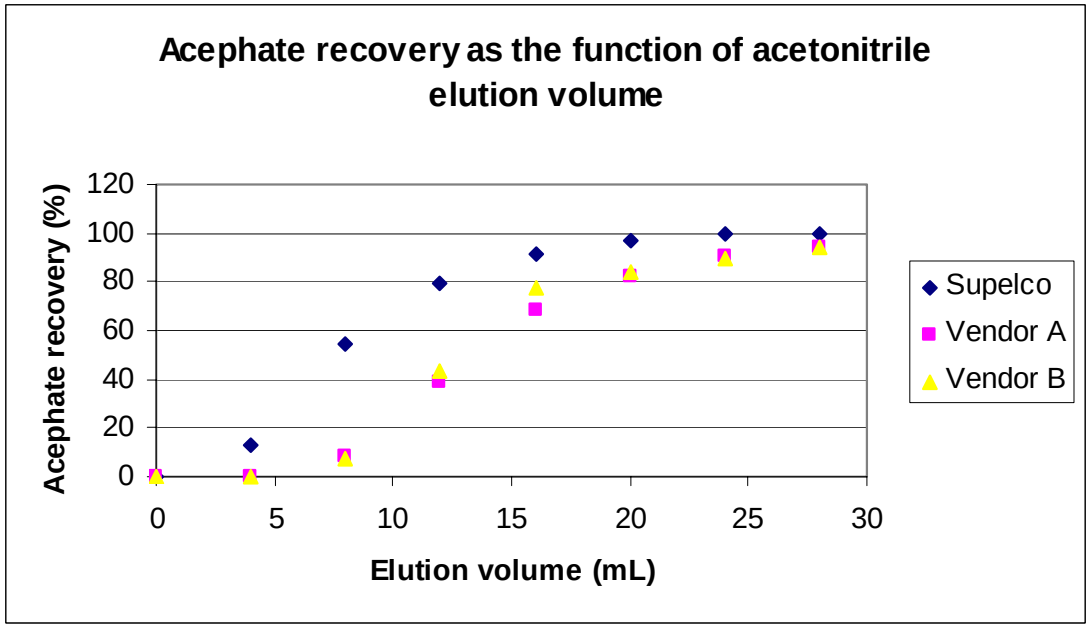
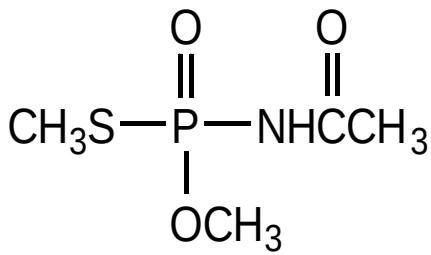
Recoveries of Acephate from PSA

Experimental:

SPE tube: 500 mg, 6 mL cartridge; condition and elution with acetonitrile; sample: 1 mL (1 ppm) in acetonitrile.

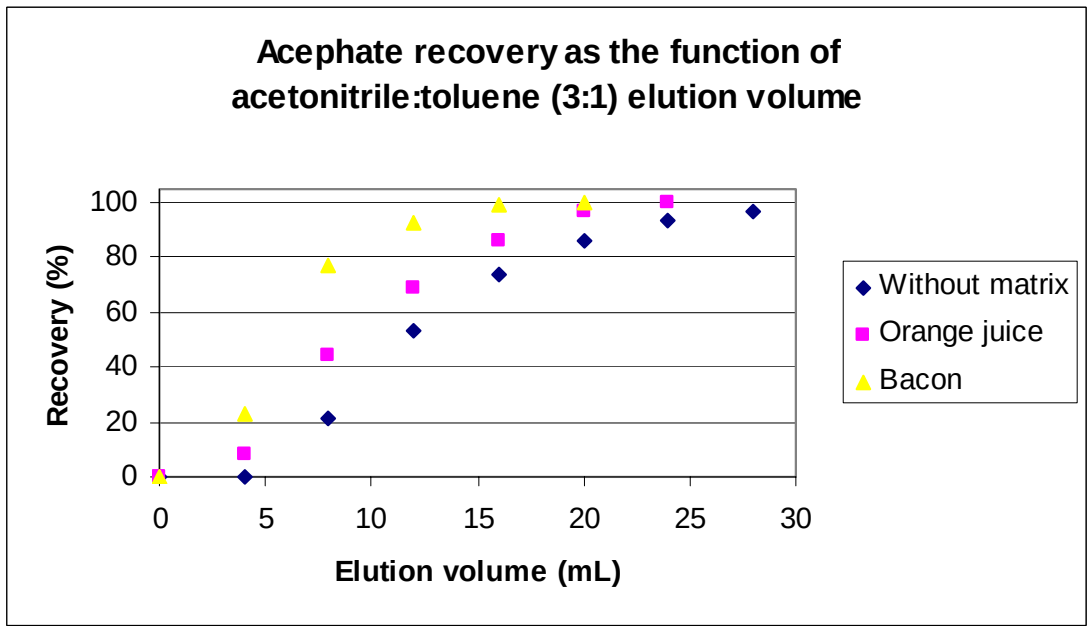
Less volume of acetonitrile is required to fully desorb acephate from Supelclean PSA.





Matrix-Induced Effect on the Desorption of Acephate from ENVI-Carb/PSA

Although it is recommended to collect 25 mL acetonitrile:toluene (3:1) elution, matrices compete with acephate for active sites on PSA and ENVI-Carb and therefore generally enhance the desorption of acephate and other pesticides.



Generic Elution Protocol with ENVI-Carb/PSA Dual Layer SPE Cartridges

1. Pre-condition the cartridge 5 mL acetonitrile:toluene (3:1)
2. Load the sample
3. Elute with 25 mL acetontrile:toluene (3:1)
4. Collect the eluant
5. Concentrate the eluant down to 1 mL

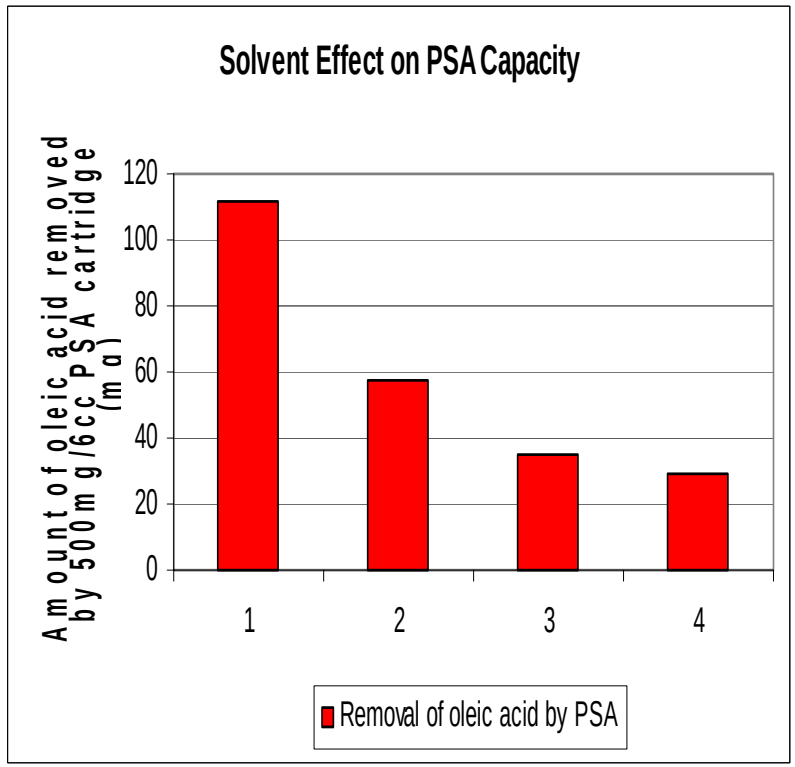
Why Not to Choose Acetone:Hexane?

1. **Acetone:hexane eluate has much higher GC-MS background. Acetone may react with PSA.**
2. **PSA's capacity for removal of fatty acids is severely damaged.**
3. **Dichlorvos, quintozene, chlorothalonil, tolyfluanid and captafol have lower recoveries.**

Solvent Effect on PSA Capacity of Fatty Acids

Supelclean PSA 500 mg/6 cc

1. Conditioned and eluted with acetonitrile.
2. Conditioned and eluted with acetonitrile:toluene (3:1).
3. Conditioned and eluted with acetone:hexane (1:1).
4. Preconditioned by acetonitrile: toluene:acetic acid (74:25:1), then conditioned and eluted by acetonitrile:toluene (3:1).



Recoveries of Sixteen Pesticides from Milk

Compound	Pesticide Recovery from ENVI-Carb/PSA (%)	Pesticide Recovery from Competitor (%)
Metamidophos	98	82
Dichlorvos	83	75
Acephate	94	64
Trifluralin	91	80
Diazinon	99	83
Chlorothalonil	79	33
Dimethipin	99	77
Vinclozoline	107	72
Methyl parathion	100	88
Methyl primophos	107	91
Triadimenol-1	104	148
DDE	104	93
Cypermethrin-3	103	132
Difenoconazole-1	114	132
Difenoconazole-2	107	160
Imibenconazole	104	142

A Typical GC-MS Selective Ion Chromatogram

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Operator      : CP
Acquired      : 12 Mar 2004    9:04 am using AcqMethod PESTSUPI
Instrument    :    GCMS #5
Sample Name: L2067-1; C0028603
Misc Info     : P700
Vial Number: 1
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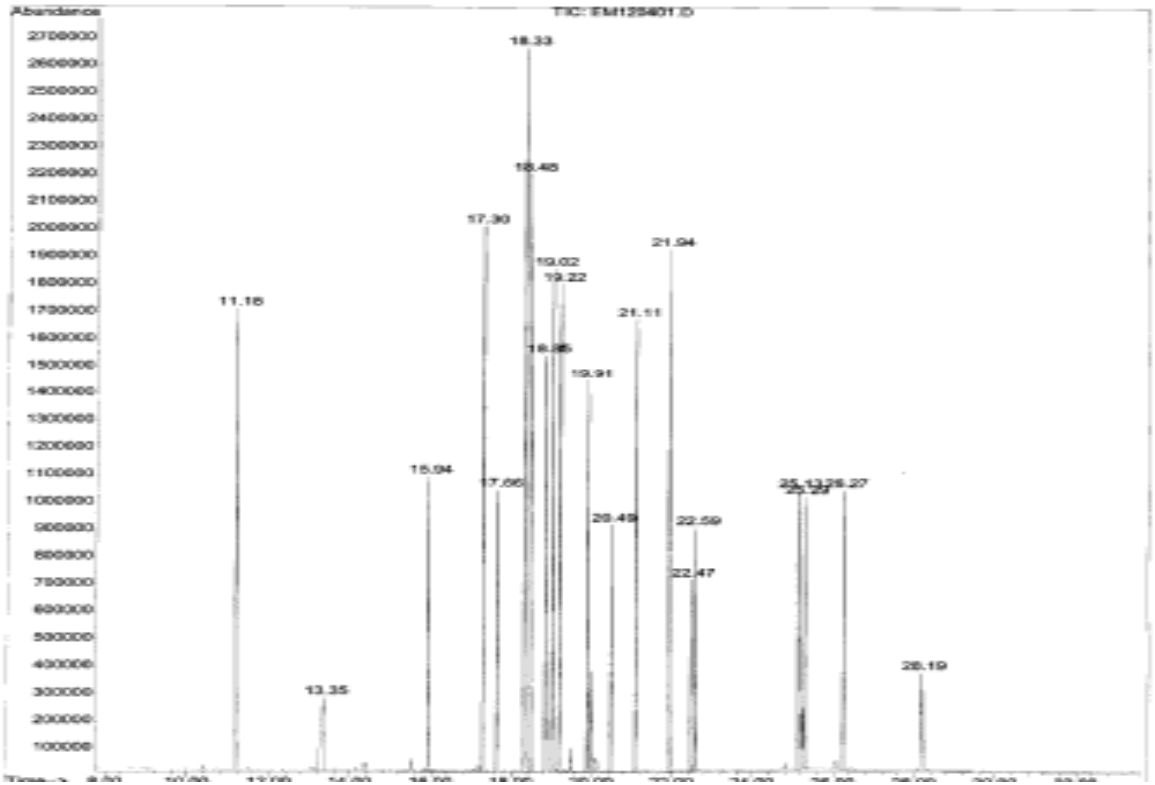

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Conclusions

- The improved PSA showed clean GC-MS background, low bleeding, high capacity for fatty acids, and powerful for removal of food matrices.
- The PSA ensures full recoveries for almost all the difficult pesticides reported in the literatures.
- The ENVI-Carb/PSA dual-layer SPE cartridges can be operated under gravity flow.
- Acetonitrile:toluene (3:1) is recommended as the generic protocol
- The improved ENVI-Carb/PSA dual-layer cartridges are a reliable SPE product for cleanup of food matrices and determination of multi-residue pesticides.

Acknowledgement

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