Analysis of Botanical Dietary Supplement Ingredients for Pesticides using QuEChERS Combined with Capillary Gas Chromatography-Triple Quadrupole Mass Spectrometry:

Collaborative Study of Pesticides in Dietary Supplements using GC/MS/MS

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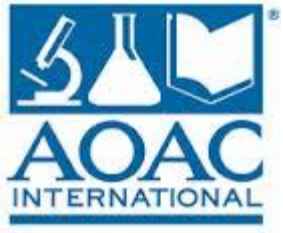

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Botanical Dietary Supplements

- Diverse class of products (root, leaves, fruits, flowers, seeds, bark, husks, whole plant)

- Botanicals are difficult to analyze because they are dried and concentrated (pigments, lipids, tannins, polyphenols, lignin)

- No pesticide tolerance levels for many of these products

Procedures Investigated

- *QuEChERS* (*Qu*ick, *E*asy, *Ch*eap, *E*ffective, *R*ugged and *S*afe)
  for GC-FPD and GC-MS/SIM analysis of ginseng
  (*J. Agric. Food Chem.* **2007**, *55,* 1117-1128)

- Solvent extraction, GPC and Solid-phase Extraction Cleanup
  for GC-MS/SIM and GC-HR-TOF-MS analysis of ginseng
  (*Anal. Chem.* **2009**, *81*, 5716-5723)

- Salt-out Organic Solvent Extraction with Solid-phase Extraction Cleanup
  for GC-MS/SIM and GC-MS/MS analysis of ginseng
  (*J. Agric. Food Chem.* **2010***, 58*, 5884-5896*)*

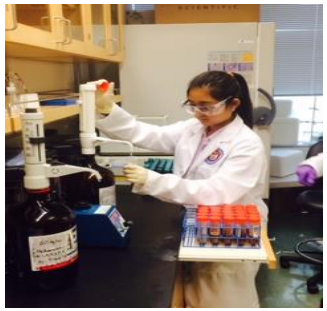
- Salt-out Acetonitrile extraction with solid-phase extraction clean-up on SPE column for 24 Botanical Dietary Supplements (*Anal. Chem.* **2013**, *85*, 4686-4693).

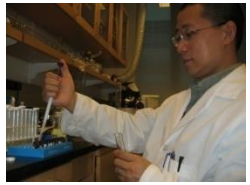
Single Laboratory Validation

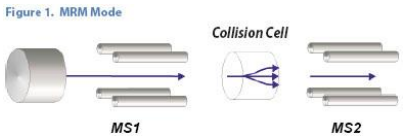
Anal. Chem. **2013**, *85*, 4686-4693

- Validation based on salt-out acetonitrile extraction, solid-phase extraction cleanup followed by capillary gas chromatography-triple quadrupole mass spectrometry (GC-QqQ-MS/MS)
- 24 botanical dietary supplements evaluated
- Recovery studies involved fortification of all botanicals at 10, 25, 100, 500 µg/kg (ppb) in quadruplicates with 355 pesticides
- Linearity studies conducted based on 14 point matrix matched calibration standards (0.5 – 10,000 ng/mL)
- LOQs estimated statistically for all 24 botanicals using matrix matched standards
- Pesticides determined from a variety of incurred samples

Procedure for GC-MS Analysis of Pesticides in Botanicals







Anal. Chem. **2013**, *85*, 4686-4693

1 g dry + 10 mL H_2O (soak) + 10 mL ACN + I.S.

↓

Add 1.0 g NaCl and 4.0 g $MgSO_4$
Shake and centrifuge (4500 rpm x 5 min)

↓

Load 1.25mL extract on conditioned SPE cartridges GCB/PSA (0.25/0.5 g)

↓

Elute /w 12 mL (3:1 acetone:toluene)

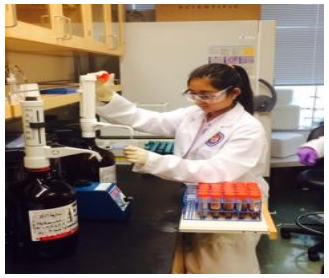
↓

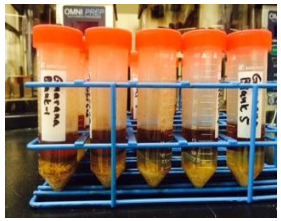
Reduce to ~100 µL, bring to 0.5 mL (toluene) + QC standards

↓

GC-MS/MS Analysis

Modifications of Procedure





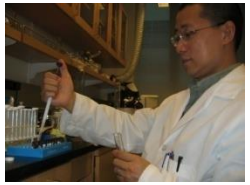
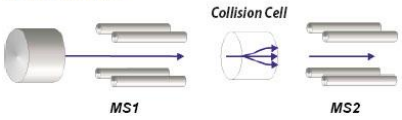


Figure 1. MRM Mode



1 g dry + 10 mL H_2O + 10 mL ACN + I.S.

↓

Add 1.0 g NaCl and 4.0 g $MgSO_4$
Shake and centrifuge (4500 rpm x 5 min)

↓

Load 1.25mL extract on conditioned SPE
Carbon X CCS/PSA (0.5/0.5 g)

↓

Elute /w 12 mL ethyl acetate or acetone ← No Toluene required

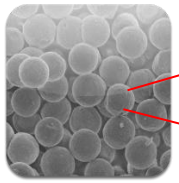
↓

Reduce to ~100 µL, bring to 0.5 mL
(toluene) + QC standards

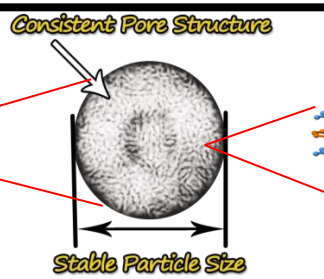
↓

GC-MS/MS Analysis

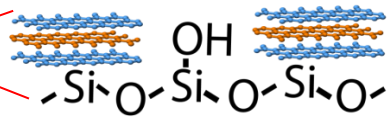
Carbon-coated silica



Carbon X



3-5 Carbon layers



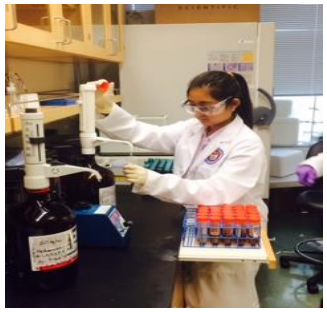
Method Validation

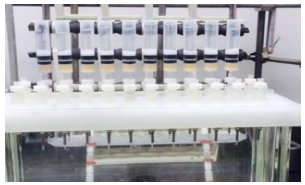
Comparison of GCB/PSA and CCS/PSA SPE Clean-up for Analysis of 168 Pesticides in Green and Black Teas

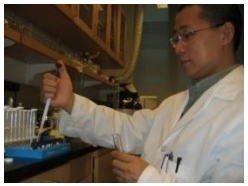
Green tea recovery ranges					Mean recoveries		SPE (Elution solvent)	Black tea recovery ranges					Mean recoveries	
< 50%	50-70%	70-120%	> 120%	*n*	%	RSD		< 50%	50-70%	70-120%	> 120%	*n*	%	RSD
							GCB/PSA (3:1 Acetone:Toluene)							
4	11	80	12	107	96	13	10 µg/kg	1	12	59	7	79	90	16
4	15	106	6	131	88	10	25	0	10	90	13	113	95	14
12	29	119	3	163	76	6	100	0	6	148	6	160	90	7
1	56	106	1	164	74	5	500	1	8	152	7	168	94	9
							CCS/PSA (Ethyl acetate)							
4	13	88	12	117	96	13	10 µg/kg	2	23	90	9	124	85	14
3	30	100	1	134	82	9	25	9	85	45	1	140	68	10
9	41	111	2	163	76	6	100	31	90	39	1	161	61	10
6	17	143	1	167	83	6	500	15	21	129	2	167	82	7
							CCS/PSA (Acetone)							
3	5	73	36	117	109	18	10 µg/kg	4	20	73	11	108	92	14
8	2	100	6	141	83	12	25	3	27	107	4	141	81	10
9	12	137	4	162	89	7	100	5	19	134	2	160	90	6
11	7	144	5	167	94	6	500	5	45	118	0	168	80	6

GCB/PSA: Graphitized Carbon Black/Primary-Secondary Amine dual layer sorbent
CCS/PSA: Carbon Coated Silica/Primary-Secondary Amine dual layer sorbent

Procedure for GC-MS Analysis of Pesticides in Black Pepper







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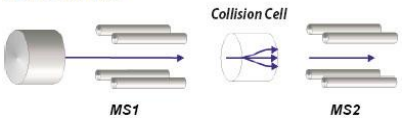

1 g dry + 10 mL H_2O + 10 mL ACN (hexane saturated) + 5mL Hexane + I.S.

↓

Add 1.0 g NaCl and 4.0 g $MgSO_4$
Shake and centrifuge (4500 rpm x 5 min)

↓

Discard hexane and Load 1.25mL ACN extract on SPE Carbon X COA/PSA/Lipid X (0.5/0.5/0/5 g)

↓

Elute /w 12 mL Acetone

↓

Reduce to ~100 µL, bring to 0.5 mL (toluene) + QC standards

↓

GC-MS/MS Analysis

Method Validation for Black Pepper

Method uses Hexane partitioning and Lipid-X/Carbon Coated Silica/primary-secondary amine SPE cleanup

		Black Pepper					Mean recoveries	
Lipid X/ CCS/ PSA (Acetone)	No detects	< 50%	50-70%	70-120%	> 120%	*n*	%	RSD
10 µg/kg	100	16	28	94	3	241	79	12
25	79	25	69	67	1	241	66	7
100	68	12	39	122	0	241	75	6
500	57	14	46	124	0	241	72	5

Still requires some better cleanup but not bad for black pepper (difficult matrix)

Botanicals studied so far…

Astralagus

Bitter orange peel

Chamomile

Cinnamon

Echinacea

Fenugreek

Gingko biloba

Ginseng

Green tea

Hoodia

Hops

Jasmine

Kava kava

Milk thistle

Psyllium

Saw palmetto

St John’s Wort

Valerian root

Licorice root

Dong quai

Garlic

Ginger

Comfrey root

Black cohosh root

Study Overview

- Botanicals for Collaborative Study – Black tea, green tea, chamomile, gingko biloba, ginseng, saw palmetto (all ground and powdered form).
- Three matrices will be fortified at 50 and 250 ppb using standard mixture with COA required under ISO 17025 procedures.
- 35 pesticides (not including pesticide isomers) are to be evaluated. Labs who wish to expand validation to ~200 pesticides will be able to do so.
- Quantitation will be based on the use of matrix-matched standards.
- Identification using retention time and presence of the precursor ion and product ions.
- Triplicate determinations will be performed on 4-5 incurred ginseng (2 samples), green (2 samples) and black tea (2 samples).

Target pesticides

(ginseng, green tea, black tea and ginseng)

Bifenthrin

BHC, α, β, γ, and δ- isomers

Chlorbenzilate

Chlorothalonil

Chlorpyrifos

Cyhalothrin

DDE, p, p'-

DDT, p, p'-

Deltamethrin

Diazinon

Endosulfan, α– and β- isomers

Endosulfan sulfate

Endrin

Fenvalerate

Fluquinconazole

Flutalonil

Heptachlor

Hexachlorobenzene

Iprodione

Methoxychor, p,p'-

Mirex

Oxyfluorfen

Pentachloroaniline

Permethrin, cis- and trans- isomers

Phosmet

Pirimphos

Pyrimethanil

Quintozene

Tebufenpyrad

Tecnazene

Terbufos

Tetrachlorvinphos

Tolclofos-methyl

Triazophos

Vinclozolin

Study Participation

- Laboratories will be provided with botanicals and consumables (vendors indicate they will be able to contribute)

- Certified standards will be provided if participant agree to complete study (or participant may receive a discount or be refunded upon completion and results submitted)

- Participant must have GC-QQQ-MS or equivalent GC-MS. GC conditions, pesticide transitions and energies for GC-MS/MS systems (if vendors will provide) will be available

- Phase one will involve a preliminary test to ensure all laboratories are able to complete the study with minimum of difficulty (e.g. might involve analysis of an incurred botanical).

Timeline
(to begin in 2015)

- **Collaborative study will be advertised and interested laboratories are encouraged to participate and recruited**

- **Study protocol will be made available for AOAC committees and communities and participating laboratories for input**

- **Laboratories will be provided time to acquire necessary consumables and materials and to optimize equipment and laboratory conditions. Instrument and science vendors are strongly encouraged to support participating labs that use their instruments or materials.**

- **Laboratories shall self-evaluate themselves to determine if they are qualified or have the means and materials to participate**

- **Samples and protocol will be shipped to the participating laboratory to begin collaborative study. Time table shall try to be flexible and accommodating to all participating laboratories but target date to begin study: January 2015**

- **Participating laboratories are to provide results, including raw data, for evaluation**

Acknowledgements

U.S. Environmental Protection Agency

National Pesticide Standard Repository

U.S. Food and Drug Administration

Field laboratories

National Institutes of Health

Office of Dietary Supplements

Institute for Food Safety and Health

Thank you for your attention.

Questions?

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LC/MS-MS Study on Fruits & Vegetables Procedure

Update

By Joe Konschnik, RESTEK Corporation

LC/MS-MS Study on Fruits & Vegetables Procedure

- All current study lab data submitted to FDA
- Expect to finalize data by January
- Will publish/present at AOAC meeting in Sept. 2015
- Next study is expected to launch next Fall

LC/MS-MS Study on Fruits & Vegetables Procedure

- Looking for 10 to 15 food labs with a variety of LC-MS/MS systems to participate
- Open to ILI running this laboratory study for FDA
- Schedule visit with CFSAN early 2015 bring list of volunteer labs for study