

## **Multiresidue Analysis of Pesticides in Botanical Dietary Supplements, Herbals and Dried Botanicals by GC-MS and LC-MS Techniques**

Jon W. Wong, Feng Shi, Kai Zhang,  
Douglas G. Hayward, Alexander J. Krynitsky

U.S. Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
College Park, MD

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## **Objectives**

- Evaluate simple and efficient procedures to analyze pesticides in botanicals and botanical dietary supplements
- Validate and make methods available for laboratories to use



## Botanical Dietary Supplements

- A botanical is a plant or plant part valued for its medicinal or therapeutic properties, flavor, or scent.
- A botanical dietary supplement is a supplement to the diet, contains botanical products, intended to be taken by mouth as a pill, capsule, or liquid; and labeled as a dietary supplement.
- 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 tolerance levels for many of these products



## Procedures

- **QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe)**  
for GC-FPD and GC-MS/SIM analysis  
(*J. Agric. Food Chem.* **2007**, *55*, 1117-1128)  
  
for LC-MS/MS analysis  
(*J. Agric. Food Chem.* **2010**, *58*, 5897-5903)
- **Solvent extraction, GPC and Solid-phase Extraction Cleanup**  
for GC-MS/SIM and GC-HR-TOF-MS analysis  
(*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  
(*J. Agric. Food Chem.* **2010**, *58*, 5884-5896)



### SPE Procedure for GC-MS Analysis of Pesticides in Ginseng

2 g sample + 10 mL H<sub>2</sub>O + 20 mL ACN + I.S.  
(or acetone/ethyl acetate/cyclohexane 2:1:1)

Add 1.0 g NaCl and 4.0 g MgSO<sub>4</sub>  
Shake on GenoGrinder  
(1000 strokes/min, 1 min)  
and centrifuge (4500 rpm x 5 min)

Reduce to 1 mL

Load extract onto GCB (0.25 g)/PSA (0.5 g)  
SPE cartridge (UCT)

Elute with toluene (3:1 acetone:toluene)

Reduce to ~100 μL, bring to 1.0 mL  
(toluene) + QC standards

GC, GC-MS Analysis



### ACN-salt out extraction and SPE Cleanup for Botanicals

2 g + 10 mL water



20 mL ACN + I.S.  
+ 1 g NaCl + 4 g MgSO<sub>4</sub>

GenoGrinder



Shake 1000 strokes/min  
for 1 min  
Centrifuge 4500 rpm x 5 min



Evaporate 5 mL ACN  
extract to ~1 mL



Solid-phase extraction  
GCB/PSA cartridges (UCT)  
Elute with 12 mL  
25% toluene in acetone



Transfer and  
evaporate to  
~100 μL (for GC)



Add 1.0 mL toluene  
Add QC stds, transfer to vials

GC, GC-MS

## Instrumental Conditions

GC column: HP-5MS (30 m x 0.25 mm x 0.25  $\mu$ ) and 5 m guard column (5 m x 0.25 mm)

### GC conditions:

#### Temperature gradient

Initial Temp:		105 °C	3 min
Ramp 1	10 °C/min	130 °C	0 min
Ramp 2	4 °C/min	200 °C	0 min
Ramp 3	8 °C/min	290 °C	6 min

**Total time = 40 minutes**

### Injector conditions: PTV splitless, 1 $\mu$ L

Inlet temperature	100 °C
Split flow	100 mL/min
Splitless time	3 min

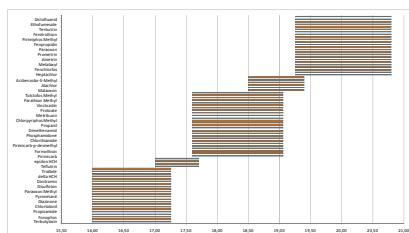
### Injection phases:

	Pressure (kPa)	Rate (°C/sec)	Temp (°C)	Time (min)	Flow (mL/min)
Injection:	70			0.10	50
Evaporation:	140	12.0	120	0.05	
Transfer:	210	14.5	300	3.00	
Cleaning:		14.5	330	5.00	75

## Instrumental Conditions (cont.)

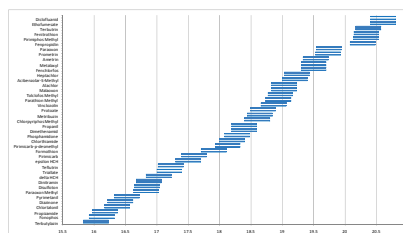
- **GC-MS/MS (triple quadrupole or tandem MS): Quantum GC**  
2 ion transitions (quantitative and confirmatory) per analyte
- **Unique-timed Selected Reaction Monitoring** detects the pesticide at its expected retention time, rather than time segments (i.e., scanning of the analyte's transitions in real time only)

Conventional MRM scanning



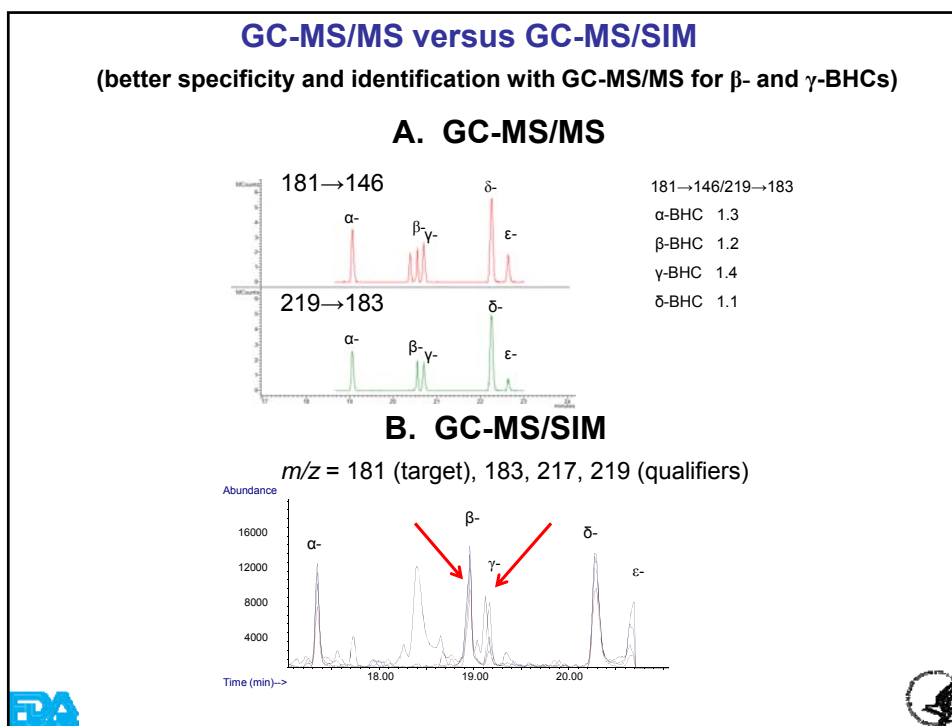
Time-segmented MRM  
(inefficient scanning)

“Unique-timed SRM”



SRM based on analyte's retention time  
and peak width  
(more efficient scanning of analyte)





## Validation Studies for GC-MS/MS Analysis of Pesticides in Botanicals

- **~300 pesticides**  
organohalogenated pesticides  
organophosphorus pesticides  
pyrethroid insecticides
- **Matrix effects (24 different botanicals studied)**

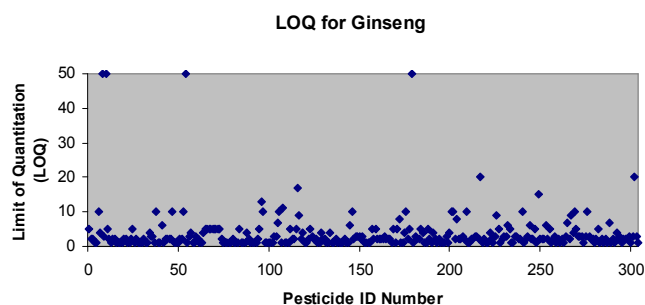
Ginseng	Cinnamon bark	Astragalus root
Ginkgo biloba	Psyllium husk	Hops
St. John's Wort	Jasmine	Comfrey root
Echinacea	Chamomille	Hoodia
Valerian root	Ginger root	Saw palmetto berry
Saw palmetto	Garlic	Fenugreek seed
Milk thistle	Black cohosh root	Bitter orange peel
Dong quai root	Licorice root	Green tea

## Validation (cont.)

- LOD/LOQ**

Method LOD/LOQ – Statistical determination using EPA method  
*Federal Register, U.S. Code of Federal Regulations, Part 136, Appendix B, 49 FR 43430, Oct 26, 1984; 50 FR 694, 696, Jan 4, 1985, as amended at 51 FR 23703, June 30, 1986.*

Geometric mean LOQ: **GC-MS/SIM: 53 ng/g; GC-MS/MS: 2 ng/g (Ginseng)**



## Validation (cont.)

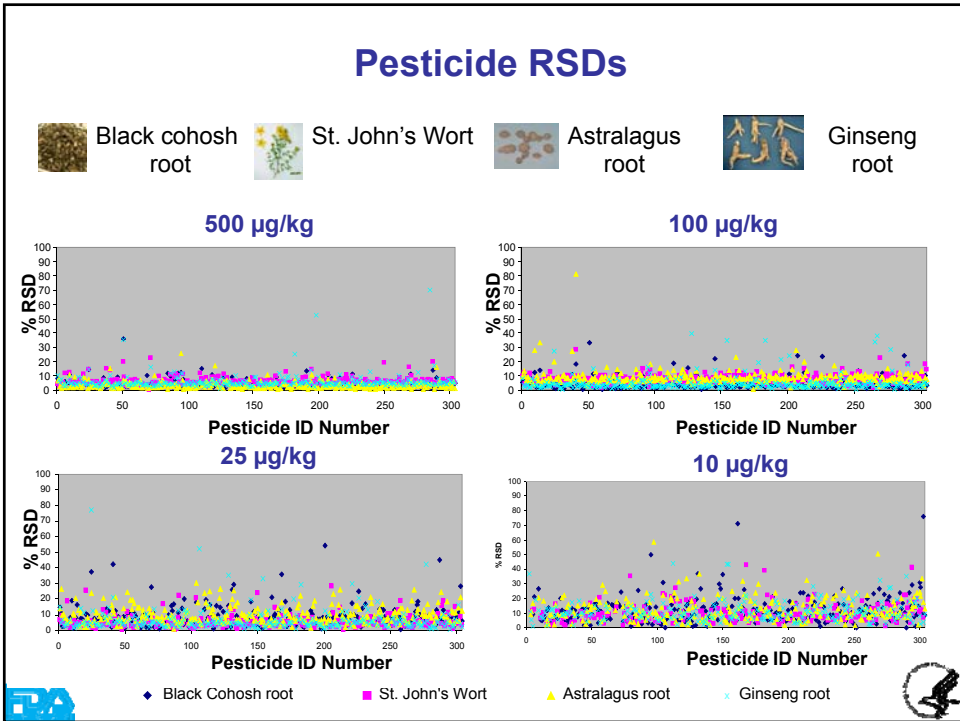
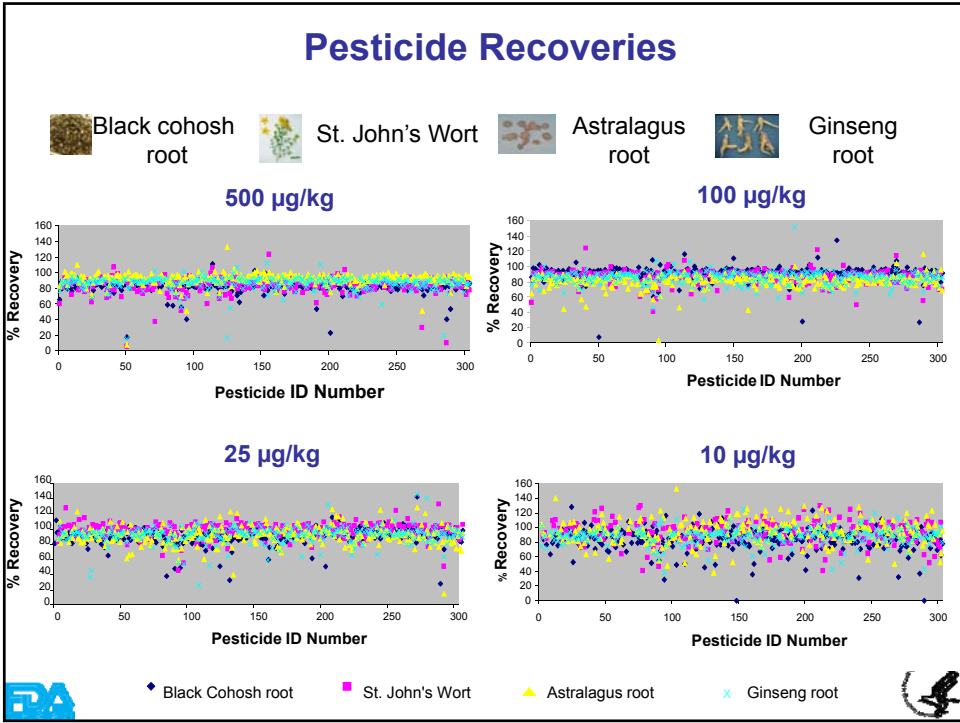
- Linearity**

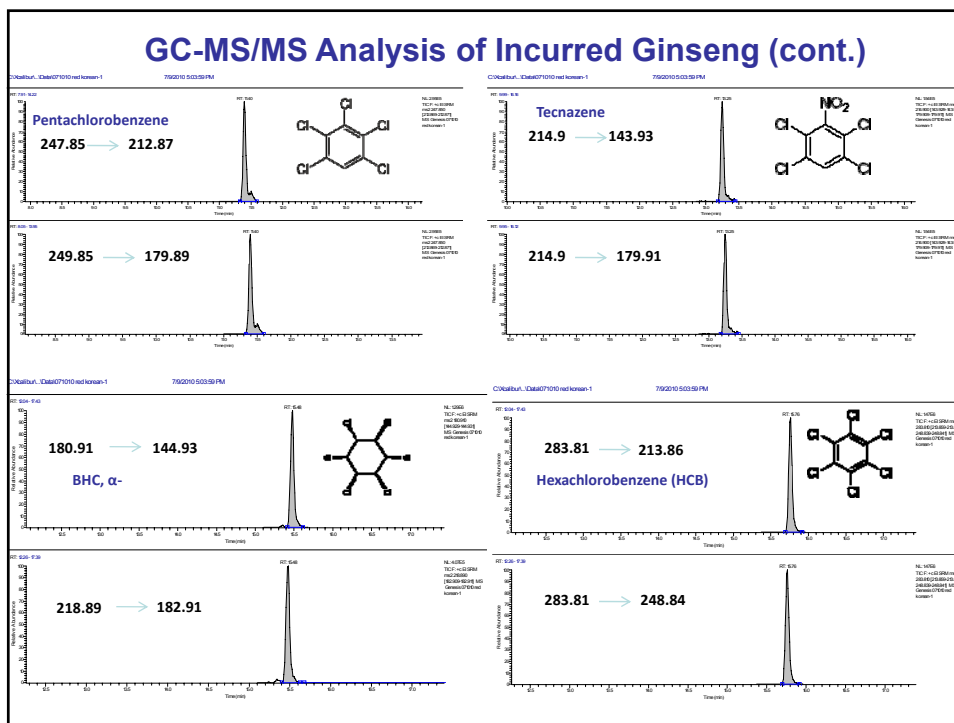
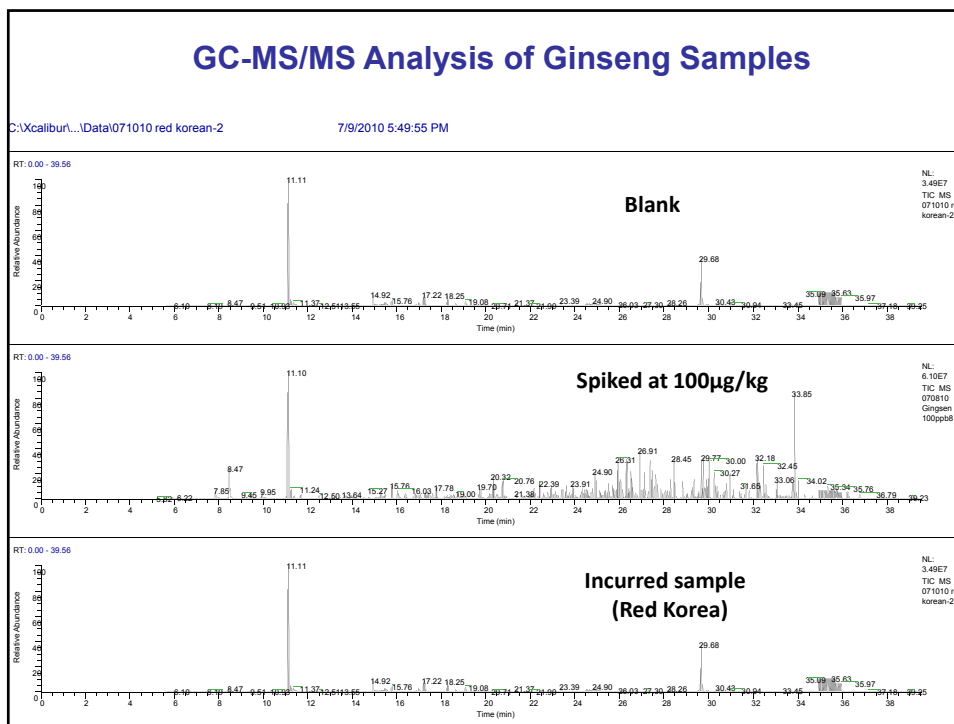
$r^2 \geq 0.99$  or better for most pesticides from 0.5 – 5000 ng/mL

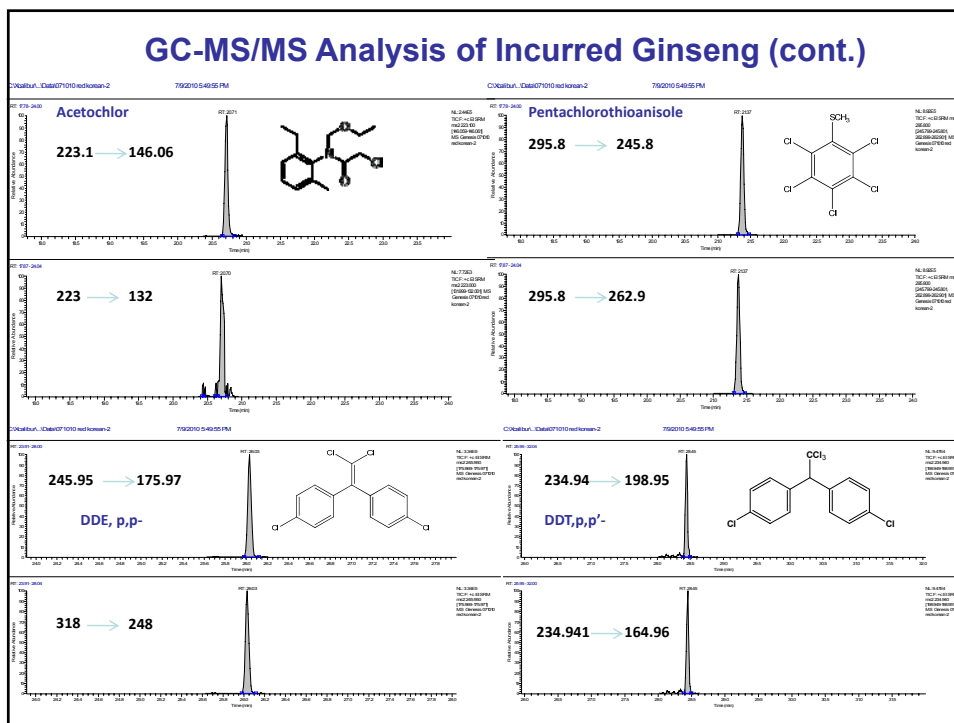
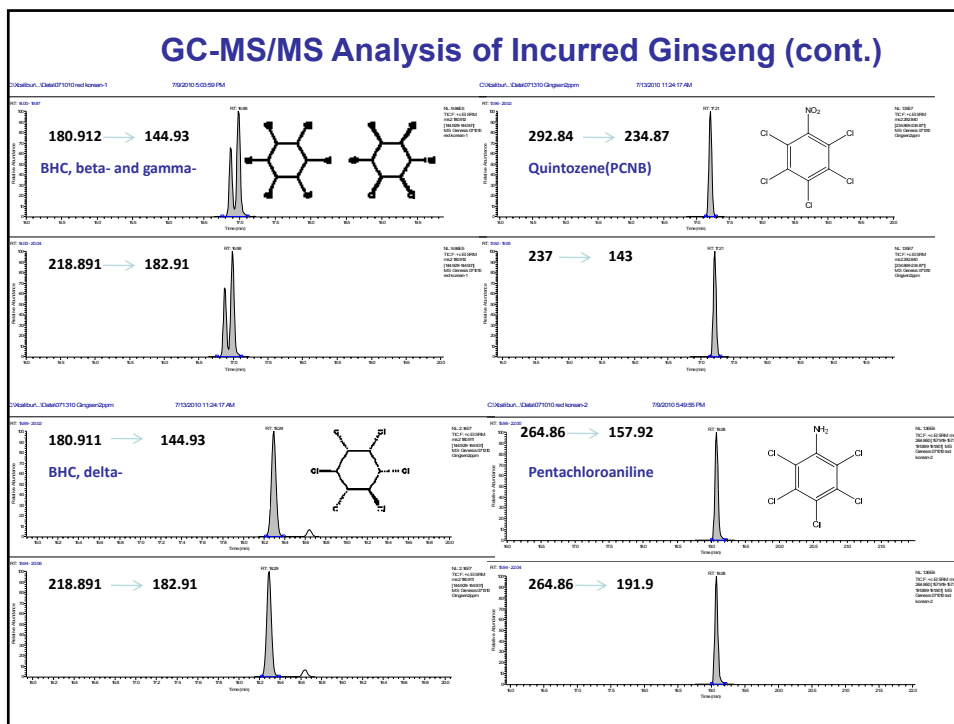
- Fortification studies**

Fortification studies of botanicals to levels of 10, 25, 100 and 500  $\mu\text{g}/\text{kg}$

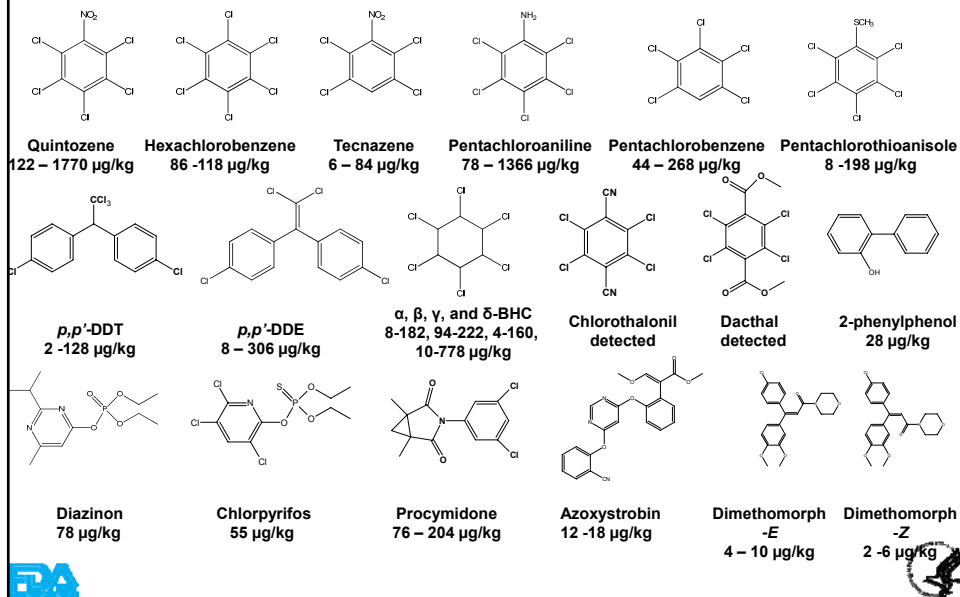
- Recoveries and RSDs**



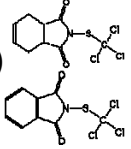
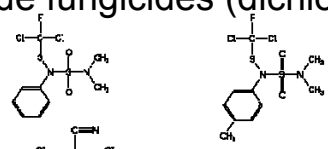
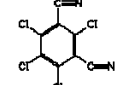
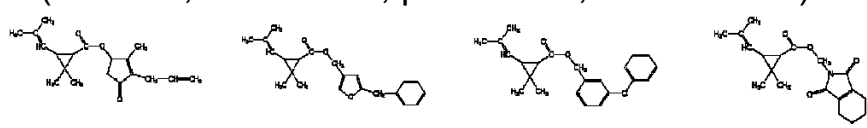




## Pesticides found in Ginseng by GC-MS/MS



## Problematic Pesticides by GC-EI-MS/MS

- Phthalimide fungicides (captan, folpet)
 
- Phenylsulfamide fungicides (dichlofluanid and tolylfluanid)
 
- Chlorothalonil
 
- Some non-halogenated pyrethroid insecticides (allethrin, resmethrin, phenothrin, tetramethrin)
 

## Summary of GC-MS Analysis of Pesticides in Botanicals

- GC-MS methods compared (GC-MS/MS shows better specificity and sensitivity than GC-MS/SIM) for the analysis of organochlorine, organophosphorus and pyrethroid pesticides
- Validated method using GC-MS/MS
- Many ginseng products contain organochlorine pesticides and contaminants (i.e., quintozone)



## Analysis of Pesticides in Botanicals by LC-MS/MS

- Many pesticides (especially newly registered) cannot be analyzed by GC-MS
- More pesticides can be analyzed by LC-MS than GC and GC-MS methods
- Many laboratories have dedicated LC-MS instruments for pesticide screening
- LC-MS/MS requires less clean-up than GC-MS so solid-phase dispersive cleanup (i.e., QuEChERS) is sufficient



### LC-MS/MS Conditions

- HPLC: Shimadzu Prominence/20 series
- Column: Restek Ultra Aqueous, C-18, 100 × 2.1 mm, 3 µm) and guard column (Ultra Aqueous, C-18 cartridges, 10 × 2.1 mm)
- Mobile phase
  - A: 5 mM ammonium formate, 0.1% formic acid in water
  - B: 5 mM ammonium formate, 0.1% formic acid in methanol
  - Gradient: 5% B at 0 min to 70% B at 5 min (1 min hold) to 90% B at 8 min (4 min hold) for a total run time of 15 min
- Injection volume: 20 µL

### LC-MS/MS Conditions (cont.)

MS: AB Sciex 4000 Q Trap mass spectrometer, ESI+, scheduled MRM mode

Curtain, collision, nebulizer, auxiliary gases, and source temperature of the ESI source were set at 15, 6, 35, and 45 psi and 450 °C, respectively

Ion spray voltage: 5200 V

Declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) were optimized by direct infusion, and the two most intense ion pairs of each analyte were chosen for the analysis

## Validation

- **LOD/LOQ**

Geometric mean LOQ:

- 2.5 ng/g Gingko
- 1.6 ng/g Saw palmetto
- 1.2 ng/g Ginseng
- 1.5 ng/g Chamomille

- **Linearity**

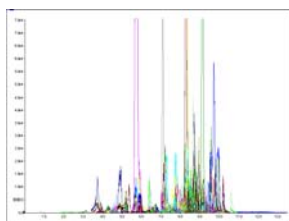
$r^2 = 0.99$  or better for most pesticides from 0.5 – 100 ng/mL

- **Matrices:**

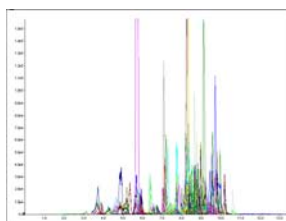
Ginseng, Gingko biloba, Saw palmetto, Chamomile, Hops, Kava kava, Cinnamon bark, Milk thistle, St. John's Wort, Fenugreek



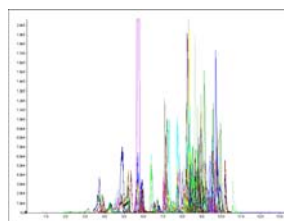
## LC-MS/MS to screen for 200 pesticides in Ginseng extracts



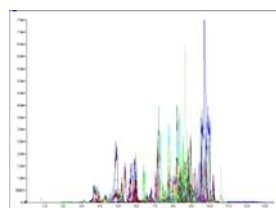
Matrix matched 0.67 ppb



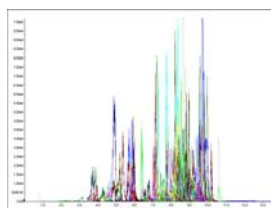
1.67 ppb



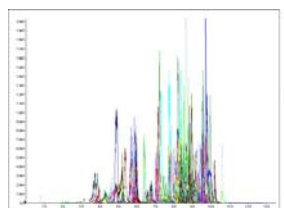
3.33 ppb



Solvent 0.67 ppb

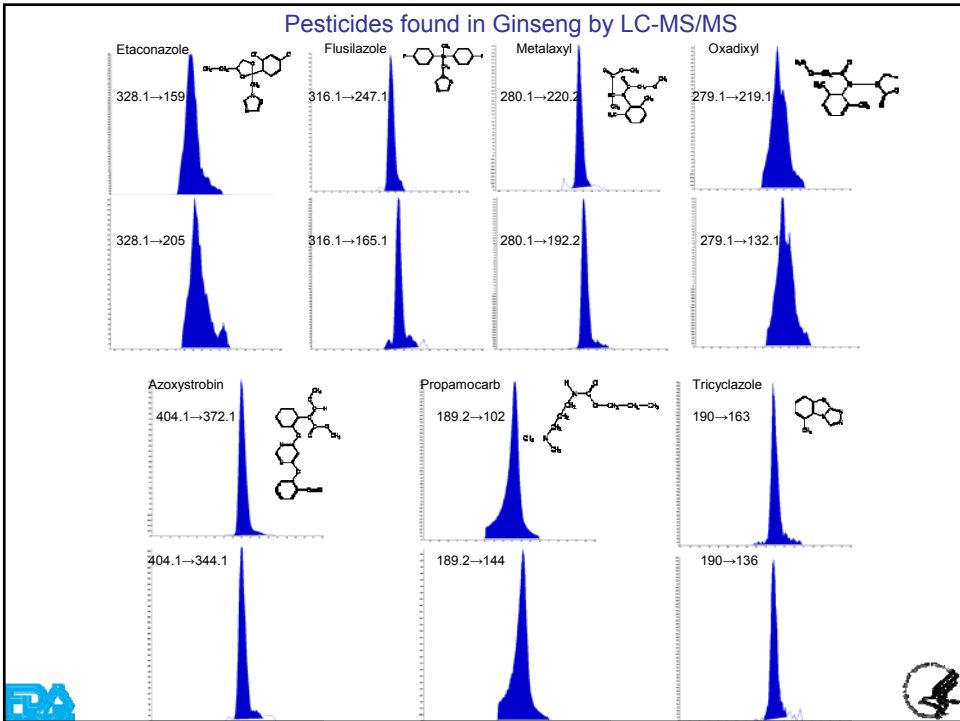
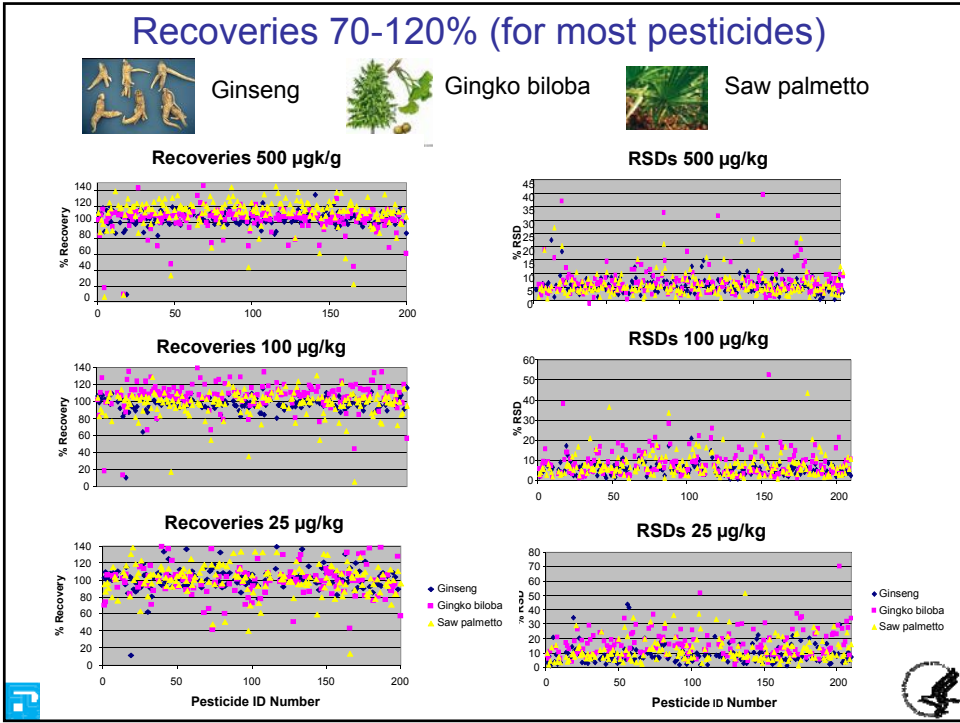


1.67 ppb



3.33 ppb





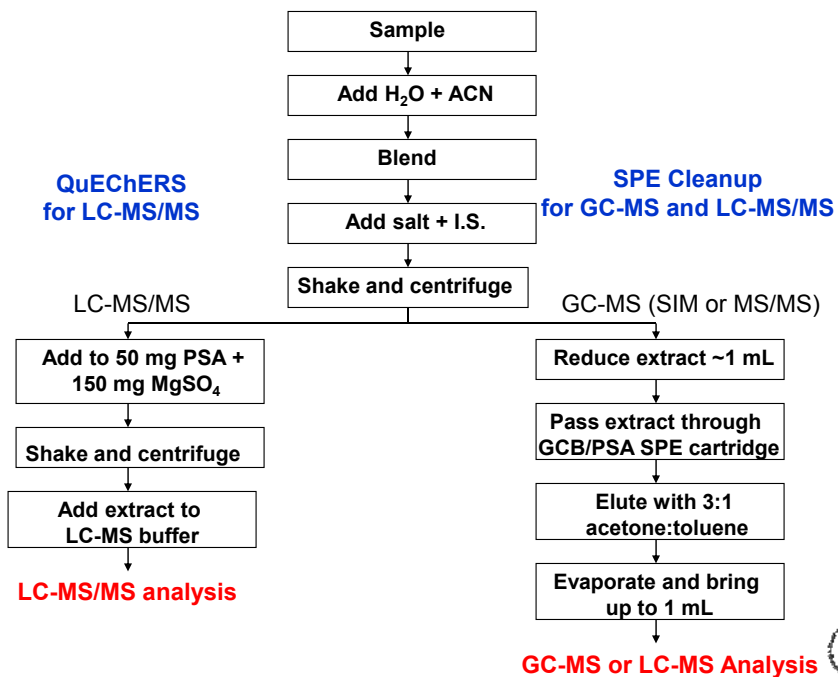
### Pesticides found in ginseng samples

Pesticide	Incurred		Standard	
	Conc (ppb)	Ion ratio	Conc (ppb)	Ion ratio
Azoxystrobin	0.5 ± 0.1	10	0.67	11
Etaconazole	5.0 ± 0.5	1.81	6.67	1.89
Flusilazole	1.0 ± 0.02	1.31	1.67	1.26
Metalaxyl	3.5 ± 0.2	1.43	3.33	1.43
Oxadixyl	40 ± 2	3.24	33.3	2.99
Propamocarb	112 ± 9	3.61	80	3.58
Tricyclazole	0.8 ± 0.04	1.04	0.67	0.97

Pesticides are identified by matching the ion ratios with standards



### General strategy to analyze for pesticides in botanicals



## Summary

- Validated GC-MS and LC-MS methods for analyzing pesticides in botanicals
- Need GC-MS and LC-MS techniques for the analysis of nonpolar pesticides (not ammenable for LC-MS) and for polar and thermally labile pesticides (not ammenable for GC-MS)
- Workflow to screen for pesticides using both GC-MS and LC-MS
- ~400 pesticides screened by GC-MS and LC-MS methods
- Beginning to find new types of fungicides in ginseng



## Future Directions

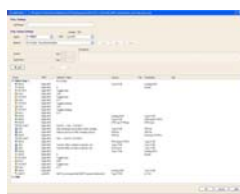
- Beta-testing the methods and multi-laboratory testing
- New sample preparation techniques (DPX) and technologies (GC x GC TOF-MS, LC-MS-IDA-EPI, LC-high resolution MS)
- Automation of methods



## Automated QuEChERS for Multiresidue Pesticide Analysis



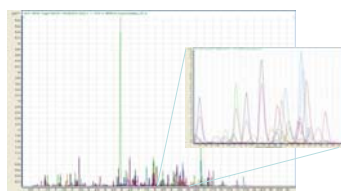
Dual rail Multipurpose sampler on LC-QQQ-MS to perform automated QuEChERS



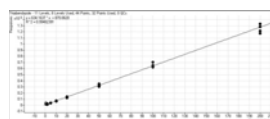
Prep sequence for automation



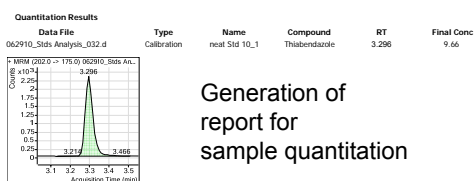
"Prep ahead scheduler" for sample preparation and analysis



Generation of LC-MS/MS chromatogram generated from automated QuEChERS



Generation of calibration curve for all pesticides using automation sequence



Generation of report for sample quantitation

## Many, many people to thank



- **Tim Begley, Steve Capar, Mike Hennessey, Carolyn Makovi, Mark Wirtz, Frank Schenck**
- **Dr. Joseph Betz (ODS, NIH)**
- **Dr. Ed Croom (University of Mississippi)**
- **Butch Weege (Ginseng Board of Wisconsin)**
- **David Steiniger, James Chang, Mike Hauer, Eric Phillips (ThermoFisher Scientific)**
- **Jack Cochran (Restek) and Becky Wittrig (Applied Biosystems)**



# Acknowledgements

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U.S. Food and Drug Administration  
Field laboratories



National Institutes of Health  
Office of Dietary Supplements  
IAG Y1-OD-6004-01



Astragalus



Bitter orange peel



Chamomile



Cinnamon



Echinacea



Fenugreek



Ginkgo biloba



Ginseng



Green tea



Hoodia



Hops



Jasmine

## Thank you for your attention



Kava kava



Milk thistle



Psyllium



Saw palmetto



St John's Wort



Valerian root



Licorice root



Dong quai



Garlic



Ginger



Comfrey root



Black cohosh root

