

Determination of Pesticide Residues in Honey by Modified QuEChERS Extraction and a Variety of Instrumental Method

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Introduction

- Samples: **Honeys**
- Analytes: 11 pesticides
- Methods: QuEChERS
- Instruments:
 - GC/XSD
 - GC/PFPD
 - GC/MSD
 - LC/TOF

Sample Type: **Honeys**



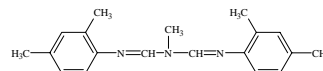
Analytes: 10 pesticides

Organochlorines	Molecular Structure	Type
Chlorpyrifos Methyl		Insecticides
Endosulfans		Insecticides
Endosulfan sulfate		Insecticides
Permethrins		Insecticides (Pyrethroid)
Cypermethrins		Insecticides (Pyrethroid)
Coumaphos		Insecticides

Organophosphates

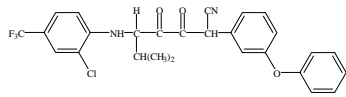
Acephate		Insecticides
Phosmet		Insecticides
Chlorpyrifos Methyl		Insecticides
Coumaphos		Insecticides
Azinphos Methyl		Insecticides

- Organonitrogen Pesticide:
 - Amitraz



• The New Challenge of LOD –

Fluvalinate:



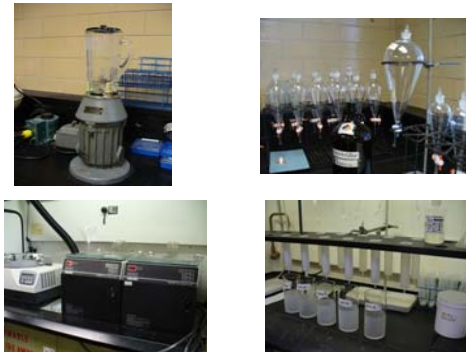
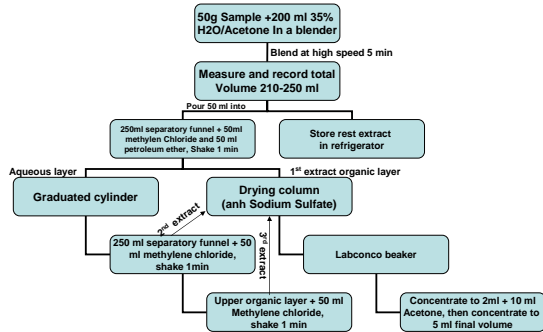
Tolerance Level in Honey:

Fluvalinate	0.05 ppm
Coumaphos	0.1 ppm
Amitraz	1.0 ppm

Extraction Methods

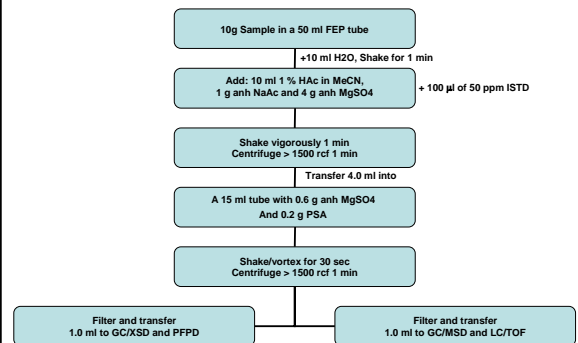
- The old “Luke” methods
- New QuEChERS method
- The history of our first small scale Luke method
- Our modified QuEChERS method

The Old Luke method for Honey Extraction



QuEChERS Method Catches Pesticide Residues

The new QuEChERS method for honey extraction

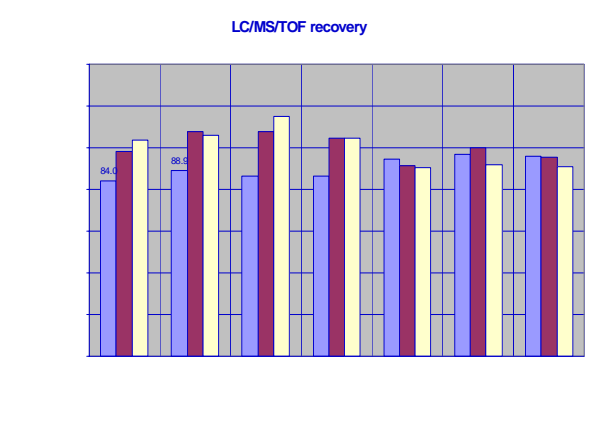
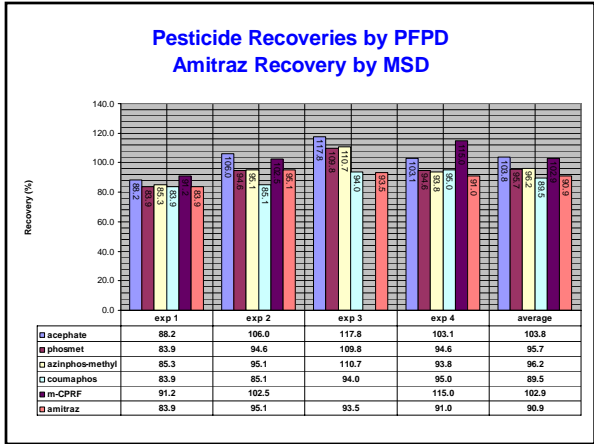
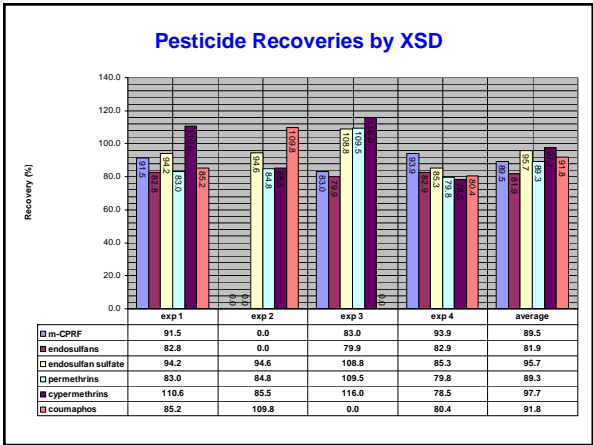




Condition experiment

Pesticides	5 ml H ₂ O	10 ml H ₂ O	15 ml H ₂ O	20 ml H ₂ O
M-CPRF	54.3	116.7	106.7	141.6
Endosulfans	49.7	115.0	100.0	115.5
Endosulfan Sulfate	52.0	117.5	101.1	129.7
Permethrins	43.4	109.6	99.3	128.0
Coumaphos	53.4	123.5	110.7	141.0
Cypermethrins	44.8	113.6	103.1	117.1
Avg	49.6	114.5	103.5	131.5
Dev%	50.4	-14.5	-3.5	-31.5

- ### The recoveries
- Experiment Design:
 - Extract each sample in triplicate
 - Inject each sample extract in triplicate
 - Spike level:
 - 100 µl of 50 ppm ISTD in 10 gram sample:
 - 0.5 ppm

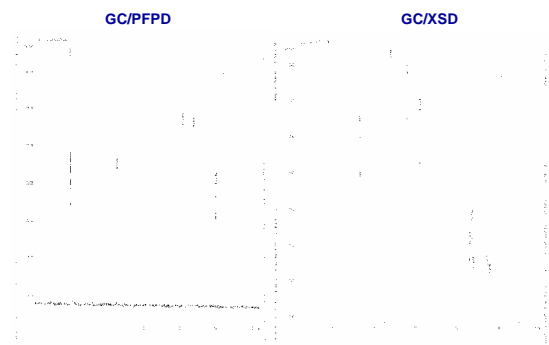


Instrumentation

- GC/XSD and GC/PFPD:



GC chromatogram



- **Instrument:**

- Agilent 6890 Gas Chromatography
- OI Analytical 5380: Pulsed Flame Photometric Detector (PFPD)
- OI Analytical 5360: Halogen Specific Detector (XSD)
- Agilent 7683 Autosampler

- **Temperature Program**

Oven Ramp (65c)	Rate (c/min)	Next temp (c)	Holding time (min)	Run time (min)
1	25	200	0	5.9
2	10	285	2	16.4

- **Column Information**

- PFPD: ZB-1 (30m x 0.32mm x 0.25µm)
- XSD: ZB-5 (30m x 0.32mm x 0.25µm)

GC/MSD:



- **Instrument:**

- Agilent 5890 Gas Chromatography
- Agilent 5972 Mass Detector
- Agilent 7683 Autosampler

- **Temperature Program**

Oven ramp	Rate (c/min)	Next temp (c)	Holding time	Run time (min)
70c (Ini 2 min)	25	180	0	6.4
2	3	200	0	13.1
3	8	280	12	35.07

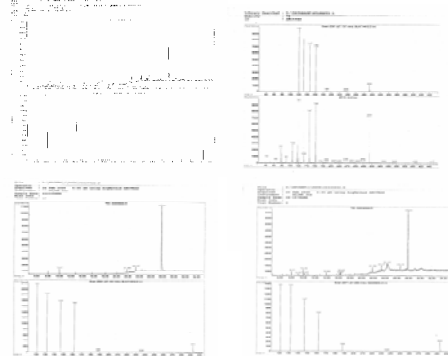
- **Column Information**

- DB-5MS (30m x 0.25mm x 1 μm)

- **SIM: m/z**

- 293, 121, 132, 147, 162

GC/MS chromatogram (in SIM)



LC/TOF Premier



- **Instrument:**

- Waters Acquity UPLC System

- Binary solvent manager
- Sample manager
- Column manager
- A specialized column

- Waters LCT Premier oa-TOF mass Spectrometer

- (orthogonal acceleration Time-of-Flight)

- **Gradient**

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.45	100	0	
8.50	0.45	0	100	6
11.00	0.45	0	100	6
11.10	0.45	100	0	6
13.50	0.45	100	0	1

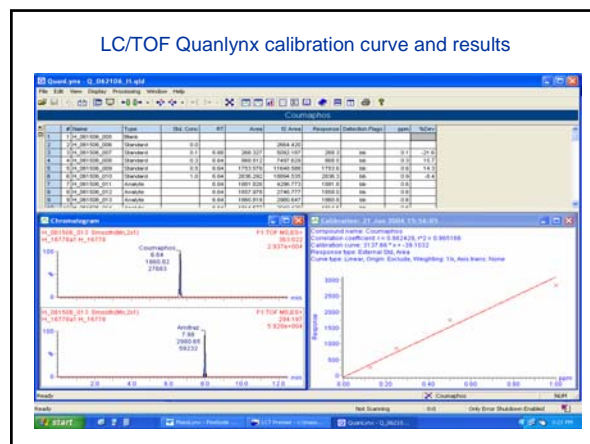
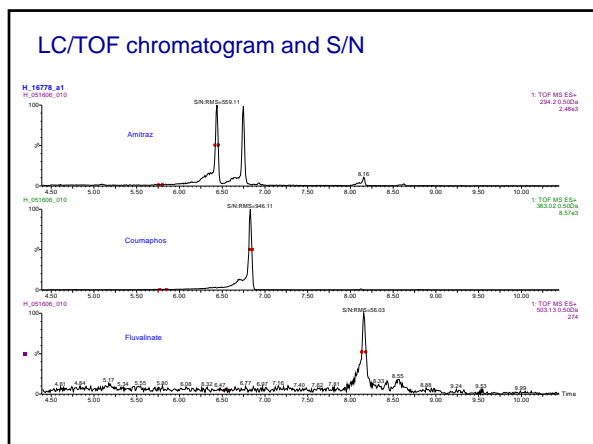
- Mobile ph A: 5% aqueous MeOH + 2mM CH₃CO₂NH₄
- Mobile ph B: 95% aqueous MeOH + 2mM CH₃CO₂NH₄

- **Column Information**

- Acquity UPLC BEH C18, 1.7 μM, 2.1 x 50mm

- **MS Conditions**

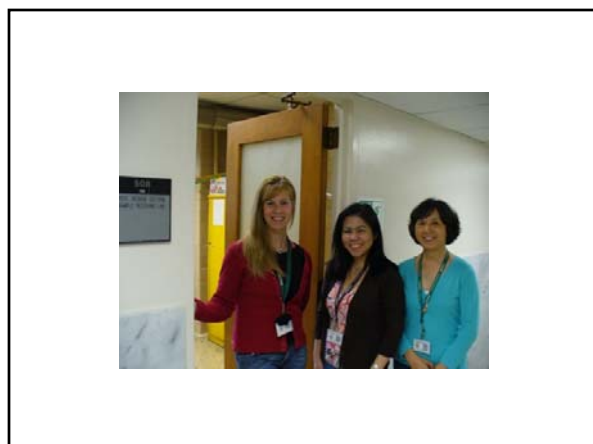
- Ion Mode: ES / W+
- Capillary V: 250V
- Source T: 120 c
- Desolvation T: 350 c
- Gas Flow: 600 L/hr
- Mass Range: 50 – 1000
- Acq. Time: 0.25 s



Conclusion

Method	Sample (gram)	Solvent (ml)	Experiment time	Equipment	Toxicity	Others
Old Luke	50	400	4 hrs	250 ml Glass separate funnels, Drying columns	Methylene Chloride	A lot of Dish washings And Waste
QuEChERS	10	10	1 hr	Tubes	MeCN	Most tubes disposable, and less waste
Advantages	Save 80% sample	Save 97% solvent	Save 75% time	Save money	Less toxic	Good for environment

- ### References
- Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues. *By Steven J Lehotay at the 2004 FIFRA Fall Pesticide Analytical Workshop*
 - Validation of a Fast and Easy Method for the Determination of 229 pesticide Residues in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detectors *By Steven J Lehotay, A. DE Kok, M. Hiemstra, P. Van Bodegraven, Journal of AOAC International* V. 88, P595-614, 2005
 - UPLC 0a-TOF MS For Rapid Screening of Multiple Pesticide Residues *Daniel McMillan and Maria Ibanez Martinez, Waters Corp. Application NOTE* 2006



- ### Acknowledgement
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Everybody say: Cheese

