

# The Examination and Automation of GPC, SPE and QuEChERS for Pesticides in Olive Oil

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## Abstract

Several techniques for the separation of pesticides from edible oils are used today to investigate the amount of pesticides in these oils. GPC, SPE and QuEChERS can be used to isolate the pesticides that are found in edible oils. Each technique provides strengths for its separation of pesticides. GPC has the ability to process large amounts of sample, SPE provides disposable cartridges with numerous sorbents to provide separation of the analyte from the matrix, and QuEChERS involves uncomplicated sample cleanup of pesticides in aqueous matrices. This application investigates each of these separation techniques in separating pesticides from oil matrices (olive oil) and presents detailed information on the automation of each separation system.

## GPC

### Benefits to GPC post-extraction clean-up:

- Improve method efficiency
  - Sample repeat reduction lowers cost
  - Simple data interpretation
  - Improve accuracy and linearity
  - Lower detection limits
- Decrease damage to analytical instrumentation and columns
  - Extend column life
  - Reduce maintenance costs and downtime
- Amenable to automation
  - Increase reproducibility of results
  - Reduce operator errors
  - Reduce test costs
  - Reduce time requirements of lab personnel
- Continuous and stable process
  - Remove interferences that cause poor analytical results
  - Separate analytes from interferences by size
  - Effective for both polar and non-polar analytes
  - No alteration of isomer ratios

## GPC Cleanup Instrumentation

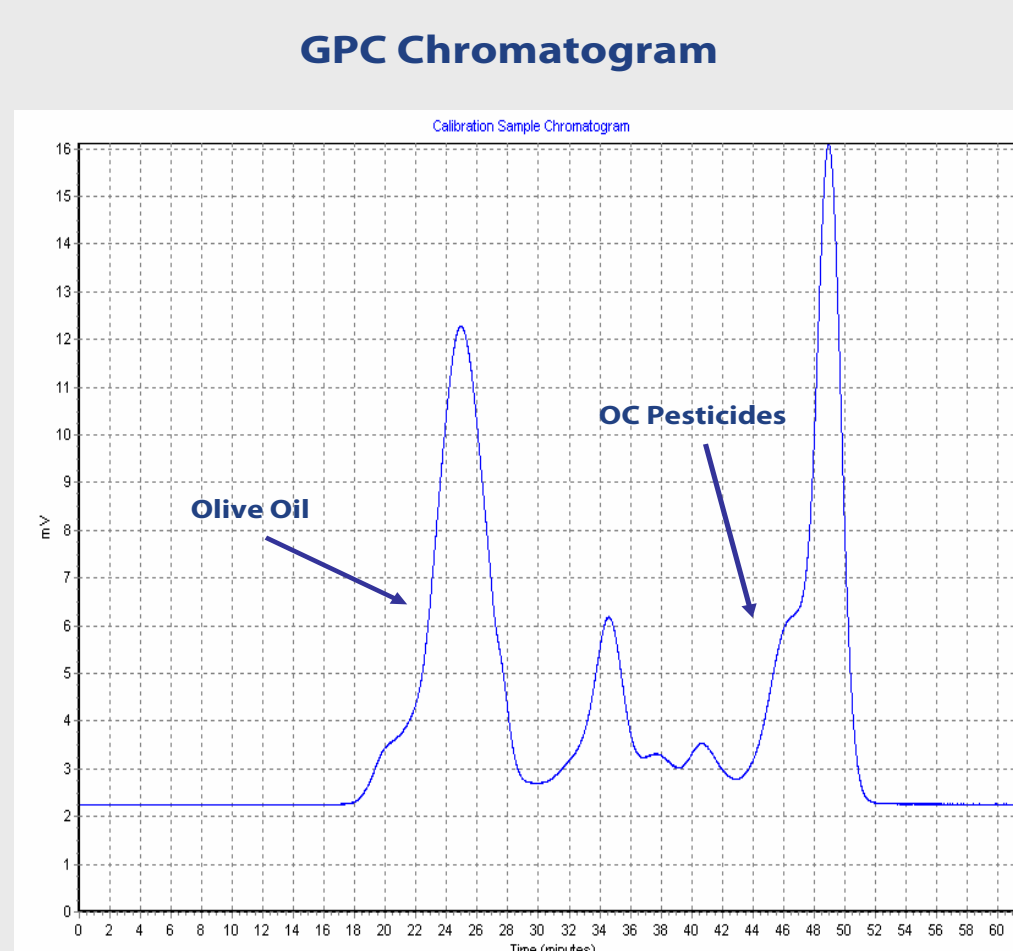


- Gilson 307 Pump
- Quad Z-215 with 4 probes, 849 injector
- 112 UV Detector
- OI-Analytical column, Bio-Beads® 5-X3 Optima column, 70 gram, 50 cm.

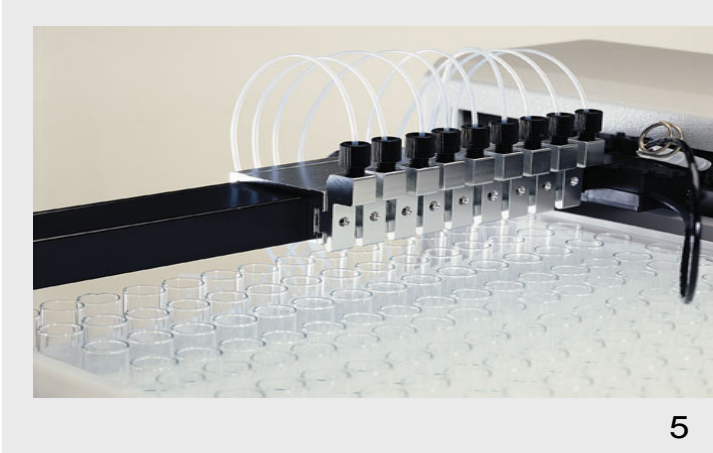
## GPC Method

- 5 mL of diluted olive oil (50 gm/L) sample injected onto GPC.
- Mobile phase: Dichloromethane 5 mL/min.
- Collect fraction from end of oil peak through the pesticide peak
- Dry down eluent
- Add 200  $\mu$ L ethyl acetate
- Inject 1  $\mu$ L sample onto GC outfitted with Rtx®-CLPesticides Column
- Starting temp 150C and ramp 3 degrees per minute until 300C is reached then hold for five minutes.

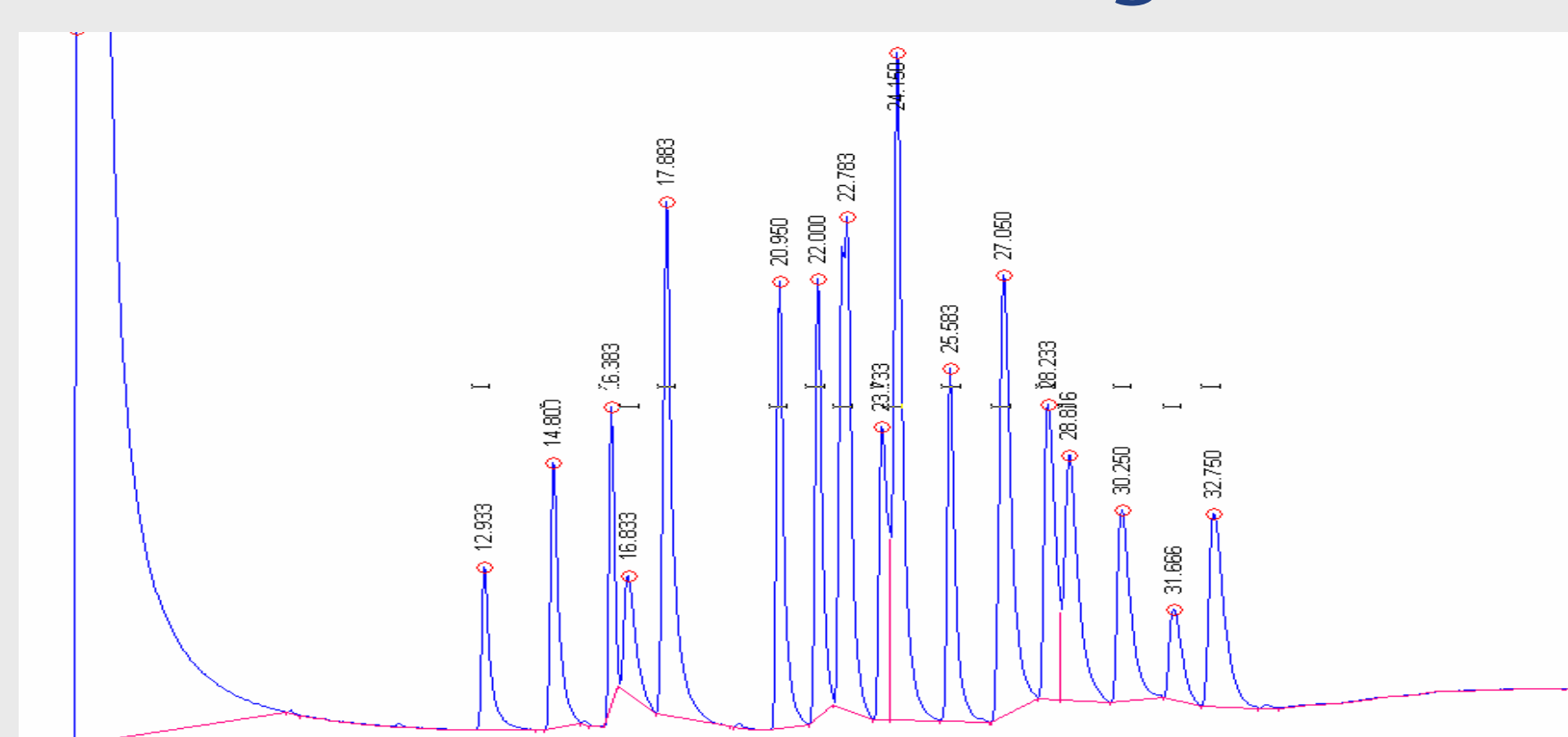
## Results



The first peak is the olive oil coming through. The second peak which is the pesticides was collected via a FC 204 equipped with multi-column adapter, capable of collecting from 4 separate columns at once. The pesticides were found in the 39 minute to 52 minute time frame and collected into 1 tube.



## GPC: GC Chromatogram



## SPE

### Benefits to Solid Phase Extraction:

- Improve method efficiency
  - Disposable columns eliminate carry over
  - Less solvent used compared to other extraction techniques
  - Low cost per sample
  - Improve accuracy and linearity
  - Lower detection limits
- Decrease damage to analytical instrumentation and columns
  - Extend column life
  - Reduce maintenance costs and downtime
  - Remove interferences that cause poor analytical results
- Amenable to automation
  - Increase reproducibility of results
  - Reduce operator errors
  - Reduce test costs
  - Reduce time requirements of lab personnel
- Flexibility
  - Multiple extraction techniques available
  - Effective for numerous analytes
  - Availability of many extraction methods
  - Several sizes and apparatus variations

## SPE Instrumentation

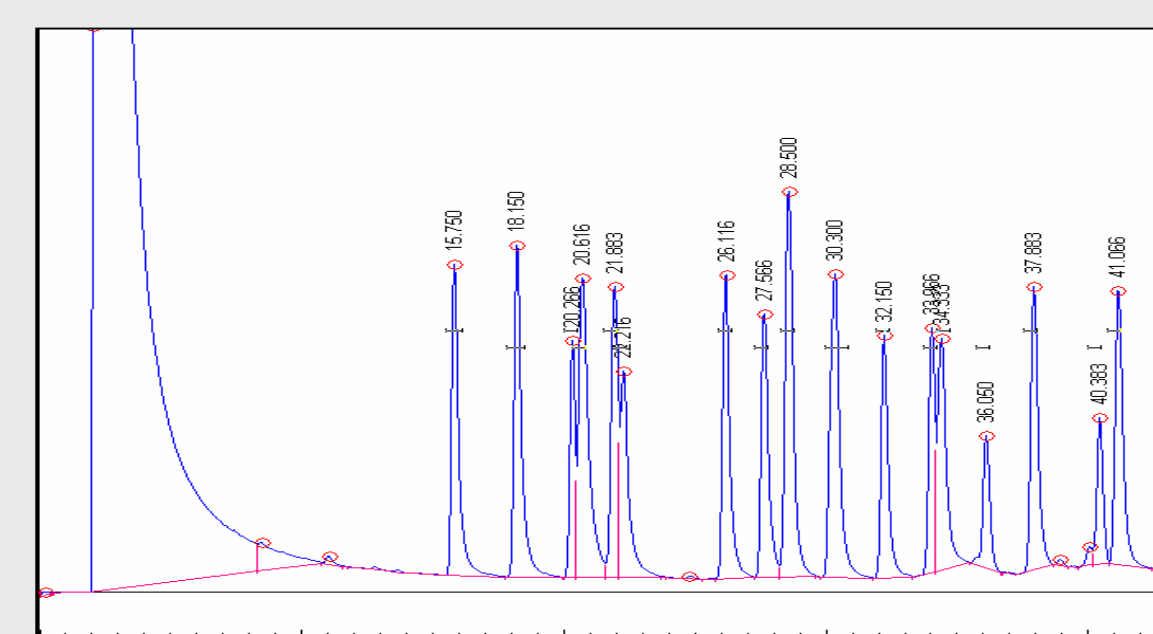


- Gilson GX-271 ASPEC with 406 dilutor and injection
- Gilson Orbital Shaker

## SPE Method

- 1.5 mL of olive oil sample added to 1.5 mL of Hexane.
- 3 mL mixture is placed in test tube and 6 mL ACN is added.
- Sample is mixed on an Orbital shaker at 720 rpm for 30 minutes
- Wait 20 minutes for sample to separate.
- Condition SPE with 5 mL ACN.
- Extract 3 mL of ACN mixture from the top layer and place in SPE cartridge.
- Elute with 6 mL ACN.
- Collect eluent from sample load and Elute.
- Repeat ACN rinse and collect
- Dry down eluent.
- Add 200  $\mu$ L of hexane
- Inject 1  $\mu$ L sample onto GC outfitted with Rtx®-CLPesticides Column
- Starting temp 150C and ramp 3 degrees per minute until 300C is reached then hold for five minutes.

## SPE Chromatogram



Method development of the solid phase extraction procedure allowed optimization of the recovery. Sample was loaded to determine breakthrough of pesticides and several elution solvents were tested to determine the highest recovery percentage. 3 mL could be loaded before breakthrough of the pesticides was observed. The SPE should not be over saturated with sample to prevent the eluting of interfering compounds. The elution with 10 mL of ACN provided highest recoveries.

## QuEChERS

### Benefits to QuEChERS:

- Improve method efficiency
  - Less solvent used compared to other extraction techniques
  - Ease of use
  - Low cost per sample
  - Improve accuracy and linearity
  - Lower detection limits
- Decrease damage to analytical instrumentation and columns
  - Extend column life
  - Reduce maintenance costs and downtime
  - Remove interferences that cause poor analytical results
- Amenable to automation
  - Increase reproducibility of results
  - Reduce operator errors
  - Reduce test costs
  - Reduce time requirements of lab personnel
- Easily separates hydrophobic analytes from matrices
  - Separate analytes from interferences by using different sorbents
  - Effective for numerous hydrophobic analytes

## QuEChERS Instrumentation

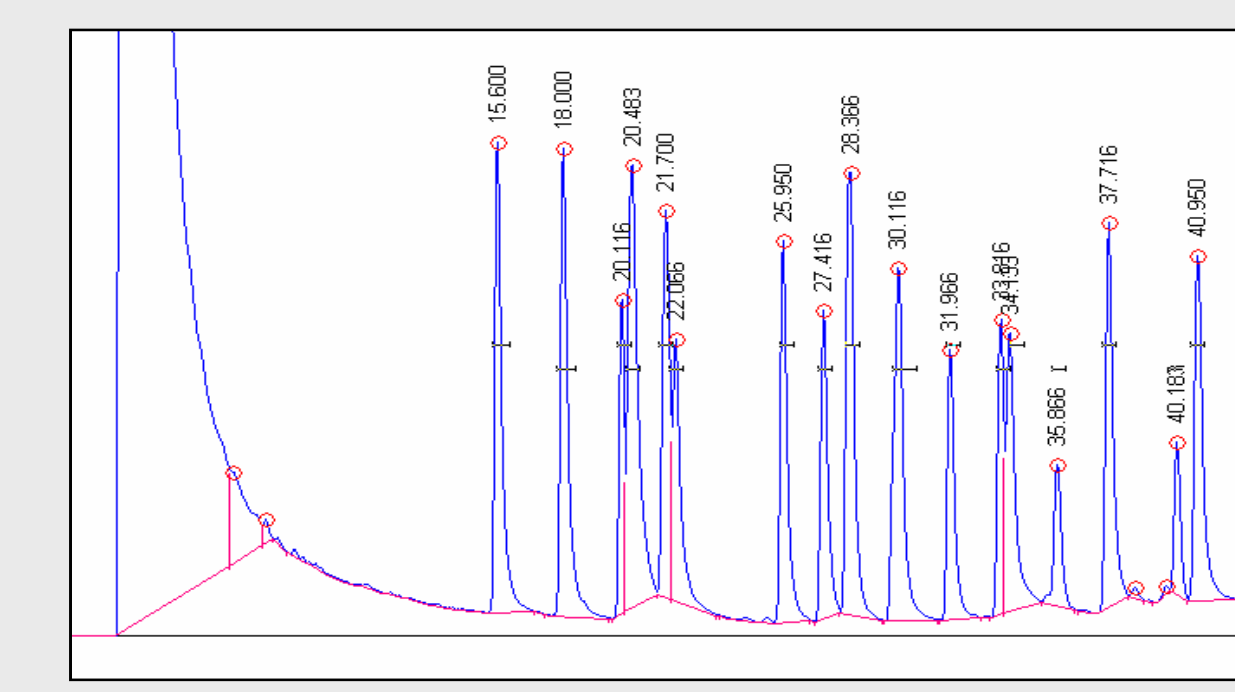


- Gilson GX-271 Prep Liquid Handler with solvent selection system and injection
- Gilson Orbital Shaker
- The instruments automate the QuEChERS method by processing the samples in cartridges similar to SPE.
- A special rack and probe are used to process each sample in the QuEChERS automated method.

## QuEChERS Method

- 1.5 mL of olive oil sample added to 1.5 mL of Hexane.
- 3 mL mixture is placed in test tube and 6 mL ACN is added.
- Sample is mixed on an Orbital shaker at 720 rpm for 30 minutes
- Wait 20 minutes for sample to separate.
- Extract 1 mL of ACN mixture from the top layer and place in QuEChERS tube. Shake on Orbital shaker at 650 rpm for 2 minutes.
- Push through filter tube and collect eluent.
- Rinse with 2 mL ACN and mix for 5 minutes.
- Push through filter tube and collect eluent.
- Repeat ACN rinse and collect
- Dry down eluent.
- Add 200  $\mu$ L of ethyl acetate
- Inject 1  $\mu$ L sample onto GC outfitted with Rtx®-CLPesticides Column
- Starting temp 150C and ramp 3 degrees per minute until 300C is reached then hold for five minutes.

## QuEChERS Chromatograms

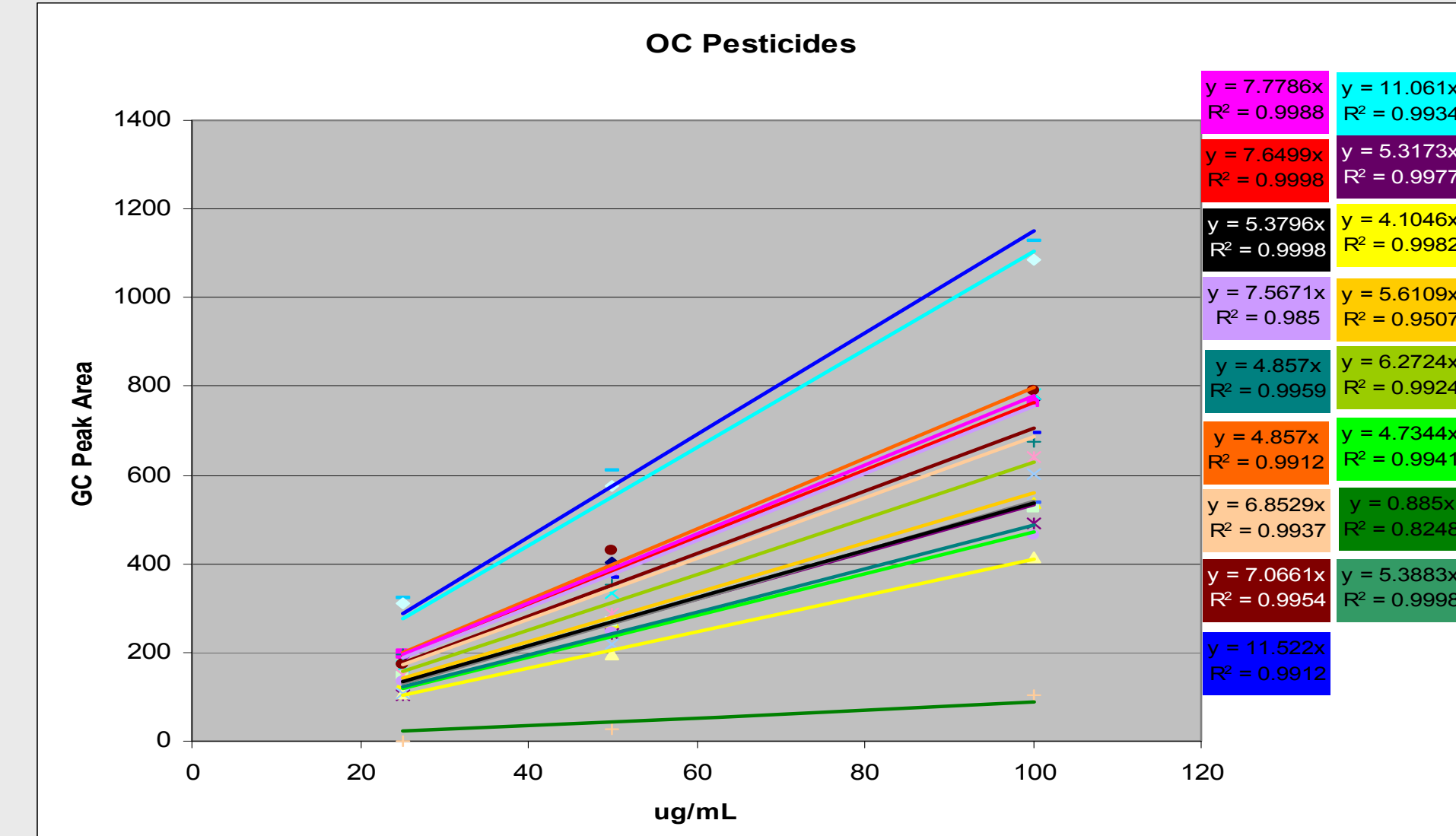


Optimization of the QuEChERS method was evaluated. The load of 1 mL was determined by the size of the cartridge and the amount of QuEChERS sorbent and magnesium sulfate within the cartridge. Several solvent amounts and types were tested rinsing the cartridge. ACN provided the highest recovery yield and a rise of 2 mL followed by another rise of the same amount optimized this yield.

## Pesticide Standards

- Pesticide standards for linear concentration determination were provided by Restek and diluted to 25, 50 and 100  $\mu$ g/mL.
- Each was injected onto the Restek Rtx®-CLPesticides Column for detection via DELCD.
- Linear curves were constructed for each peak.
- 100  $\mu$ g/mL concentration in Hexane or Ethyl Acetate was used to perform an LLE as used in the SPE and QuEChERS methods. This was used to determine the recovery of the LLE within the methods.
- 100  $\mu$ g/mL concentration in Oil was used to compare the LLE to the standards. Both extractions produced similar results.
- The GC peak area for each method, GPC, SPE and QuEChERS was used to determine the concentration via the pesticide standards plot.

## Pesticide Standards Linear Plots



## MS Analysis

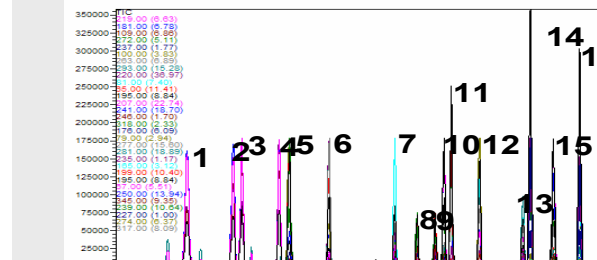
- All analyses were performed on Rtx-CLPesticide2, 30m x 0.25mm x 0.2  $\mu$ m, Cat # 11323, Serial # 882698
- Standard used was Organochlorine Pesticide Mix AB # 3, Cat # 32415, Lot # A054449
- The standard and each extract was analyzed in both scan and SIM mode.
- The TIC data obtained from the standard was used to compile the SIM table used for the SIM acquisition mode.

## Standard

Compound	CAS	RT	Quant	Ion 1	Ion 2
1. alpha-BHC	319-84-6	5.342	219	181	109
2. gamma-BHC	58-89-9	5.733	219	181	109
3. beta-BHC	319-85-7	5.808	219	181	109
4. delta-BHC	319-86-8	6.125	219	181	109
5. heptachlor	76-44-8	6.217	272	237	100
6. aldrin	309-00-2	6.558	263	293	230
7. heptachlor epoxide	1024-07-3	7.177	263	237	81
8. gamma-chlordane	12789-03-8	7.308	272	237	65
9. alpha-chlordane	5103-71-9	7.487	272	237	65
10. endosulfan II	960-08-8	7.542	188	207	241
11. 4'-DDE	72-85-9	7.600	246	318	176
12. diazinon	60-57-1	7.850	70	263	277
13. imidacloprid	72-20-8	8.277	263	281	81
14. 4'-DDD	72-84-8	8.275	230	195	199
15. endosulfan II	33213-85-9	8.475	195	207	169
16. 4'-DDD'	50-29-3	8.700	230	195	169
17. azinphos methyl	7421-93-4	8.935	67	290	345
18. endosulfan sulfate	1031-07-8	9.342	272	239	239
19. methoxychlor	72-43-5	9.725	227	274	274
20. imidacloprid	92484-79-5	10.290	67	317	281

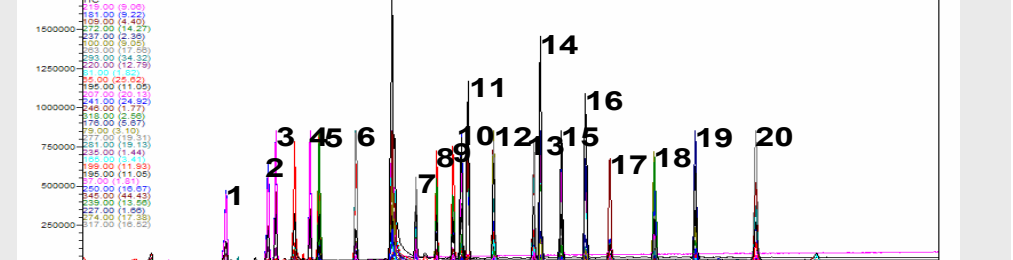
## Standard

Injection Temp: 225°C  
Injection Mode: splitless @ 20 min hold  
Column Flow: 1.00 mL/min  
Oven Temp: 150 min ramp 10°C/min to 200°C @ 20°C/min to 280°C @ 2°C/min to 320°C @ 2°C/min to 320°C/min



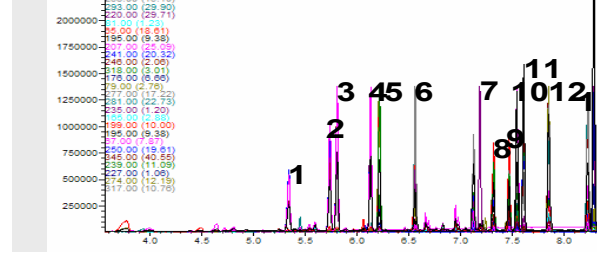
## GPC Extract

Injection Temp: 225°C  
Injection Mode: splitless @ 20 min hold  
Column Flow: 1.00 mL/min  
Oven Temp: 150 min ramp 10°C/min to 200°C @ 20°C/min to 280°C @ 2°C/min to 320°C @ 2°C/min to 320°C/min



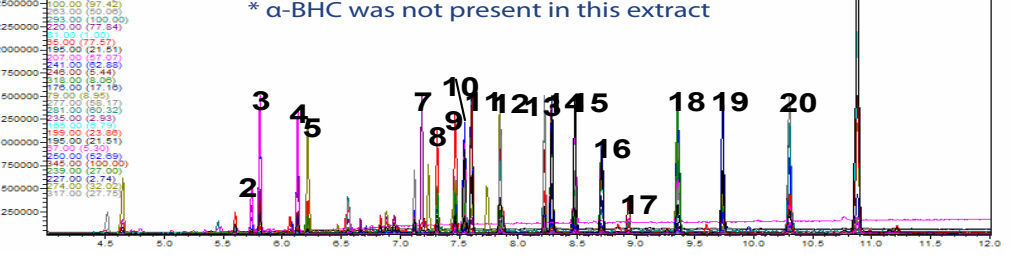
## SPE Extract

Injection Temp: 225°C  
Injection Mode: splitless @ 20 min hold  
Column Flow: 1.00 mL/min  
Oven Temp: 150 min ramp 10°C/min to 200°C @ 20°C/min to 280°C @ 2°C/min to 320°C @ 2°C/min to 320°C/min



## QuEChERS Extract

Injection Temp: 225°C  
Injection Mode: splitless @ 20 min hold  
Column Flow: 1.00 mL/min  
Oven Temp: 150 min ramp 10°C/min to 200°C @ 20°C/min to 280°C @ 2°C/min to 320°C @ 2°C/min to 320°C/min



## GPC, SPE, QuEChERS

- The GPC sample used was olive oil, 50 gm/L in dichloromethane. This was injected onto the GPC column and the fraction collected, and dried down.
- The SPE sample used was olive oil in hexane 50:50. This sample was extracted using LLE with ACN. The ACN was placed on the SPE and eluted with ACN then dried down.
- The QuEChERS sample used was olive oil in hexane 50:50. This sample was extracted using LLE with ACN. The ACN was placed on the SPE and eluted with ACN then dried down.
- All dried down samples were brought up in 200  $\mu$ L of hexane or ethyl acetate for injection on the GC.

## Results

LLE was the determining factor in the recoveries of pesticides as utilized in the SPE and QuEChERS methods. With recoveries of 50% to 70% at the lower spectrum and an increase in recoveries of 25% when the LLE was optimized it is evident that optimization of the liquid/liquid extraction is essential to provide good recoveries.

The GPC system can be accomplished with a basic HPLC instrument or advanced via a parallel system for increased throughput, since GPC runs are usually lengthy. GPC is a well understood methodology that doesn't require method development or optimization, unlike SPE and QuEChERS.

All methods attained good recoveries. GPC attained recoveries of >95% for all the pesticide analytes. The QuEChERS and SPE methods attained a recovery of 70% to 80%. Each of these methods used organic solvents to elicit recovery, whereas the GPC column is reusable, the SPE and QuEChERS are disposable columns.

The throughput of each method varied as expected. The GPC method took approx 50 minutes to complete with about an hour dry down time since 65 mL fraction was collected. The GPC single probe system could process 1 sample per 1.5 hours, whereas the 4 probe parallel GPC system increased throughput 4 fold. SPE and QuEChERS methods both had similar processing times with LLE, sample loading, eluting, and dry down. Both the dry down and LLE were the determining factors for throughput each at 30 minutes.

The GPC method has the greatest ability for lowest detection level by using a larger amount of sample versus the SPE or QuEChERS. 5 mL of sample could be processed and dried down to 200  $\mu$ L, an increase of 12.5 times in detection. The SPE would be the next lowest detectable limits. 1.5 mL of sample processed and brought up to 200  $\mu$ L in hexane, an increase of 3.75 in detectable limits. The QuEChERS provided an increase of 1.25 times detection with the processing of 0.5 mL of sample dried down and brought back up to 200  $\mu$ L in hexane. However, this QuEChERS method did not elute  $\alpha$ -BHC with the rest of the pesticides. Assuming it was not retained at all the method will need to be adjusted in order to retain and extract  $\alpha$ -BHC.

## Conclusion

Each of these methods could be used to attain good results for the detection of pesticides in olive oil. For laboratories with enough capital to purchase a GPC system and a high quantity of sample to process this system would provide a good return in their initial investment. For labs smaller labs that are looking for a more cost effective process of fewer samples the SPE and QuEChERS method provide the ability to process samples in a short amount of time. GPC in this method attains the lowest detectable limits at 3.3 times that of SPE and 10 times that of the QuEChERS extraction method. All methods attained levels of 25  $\mu$ g/mL of each pesticide and extrapolated a possible low detectable limit of 5  $\mu$ g/mL for QuEChERS. SPE attaining 1.7  $\mu$ g/mL and GPC at 400 ng/mL.