

# ***LIMITS OF THE CURRENT MULTI-RESIDUE METHODS APPLIED BY THE EU OFFICIAL LABORATORIES FOR FRUITS AND VEGETABLES***

**Amadeo R. Fernández-Alba**

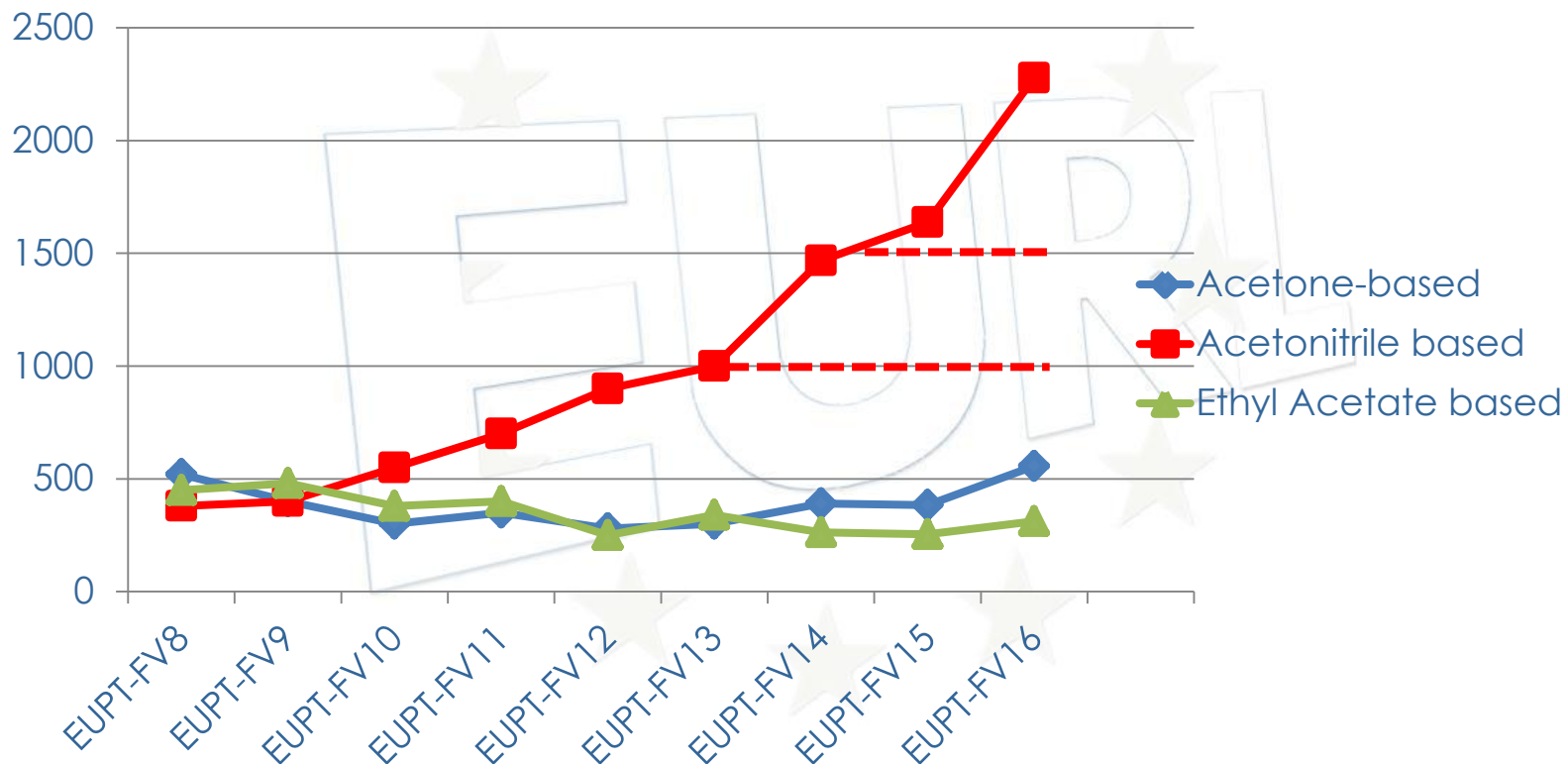


**EURL-FV**



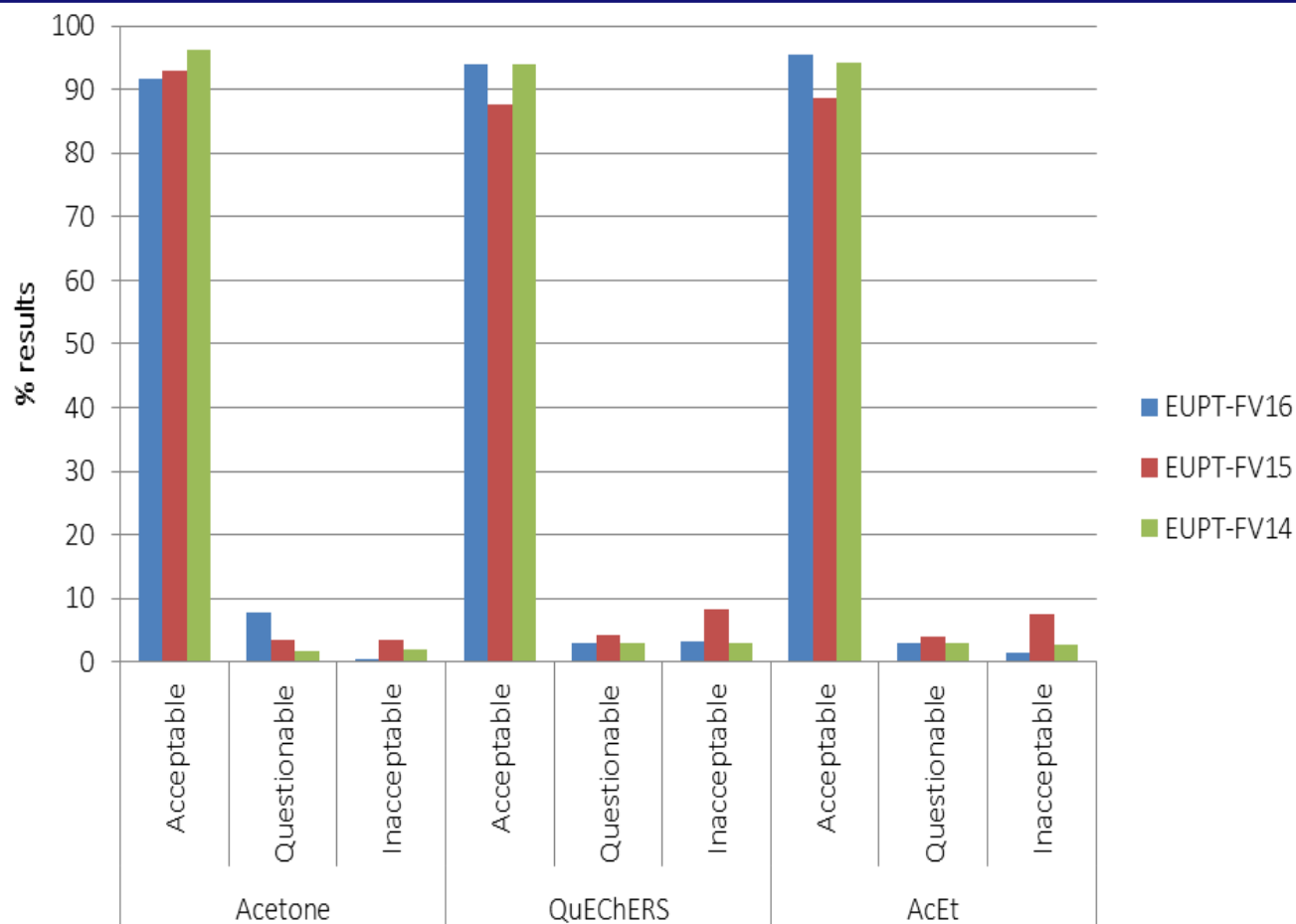
## Extraction methods

Number of results reported by extraction method





|           | Acetone (Luke-NL Method) |              |              | Acetonitrile (QuEChERS) |              |              | Ethyl Acetate (SweEt) |              |              |
|-----------|--------------------------|--------------|--------------|-------------------------|--------------|--------------|-----------------------|--------------|--------------|
|           | Acceptable               | Questionable | Inacceptable | Acceptable              | Questionable | Inacceptable | Acceptable            | Questionable | Inacceptable |
| EUPT-FV16 | 91,7                     | 7,7          | 0,6          | 93,9                    | 2,9          | 3,2          | 95,5                  | 2,9          | 1,6          |
| EUPT-FV15 | 93,0                     | 3,4          | 3,6          | 87,7                    | 4,2          | 8,2          | 88,6                  | 3,9          | 7,5          |
| EUPT-FV14 | 96,2                     | 1,8          | 2            | 93,9                    | 3,1          | 3            | 94,3                  | 3            | 2,7          |





|                  | Compounds Evaluated | % F- | % F+ |
|------------------|---------------------|------|------|
| <b>EUPT-FV14</b> | 17                  | 1,8  | 0,9  |
| <b>EUPT-FV15</b> | 18                  | 1,4  | 0,3  |
| <b>EUPT-FV16</b> | 22                  | 1,3  | 0,1  |
| <b>EUPT-FV17</b> | 11                  | 0,8  | 0,5  |

**Similar number/percentage with AcN, EtAc, Acetone based methods**



## Solubility of solvents in water

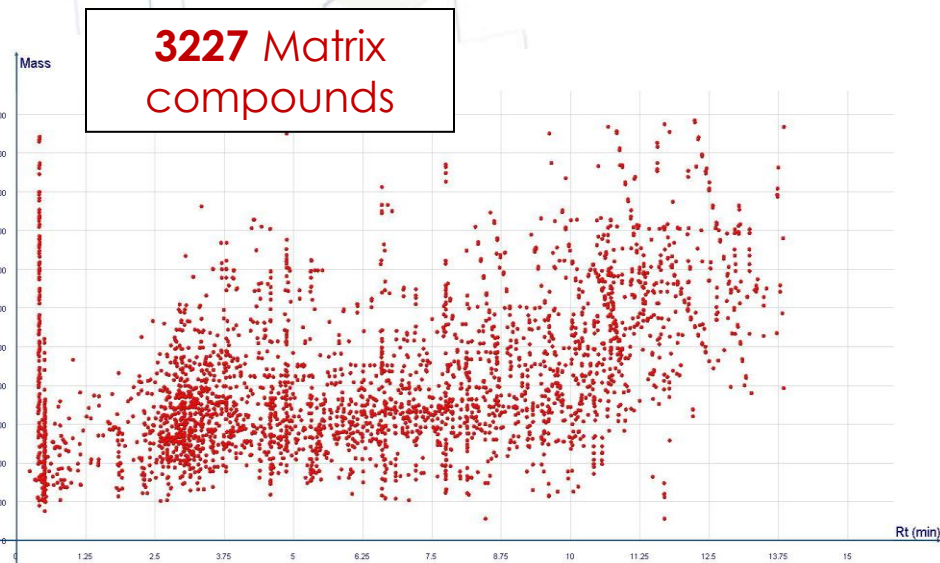
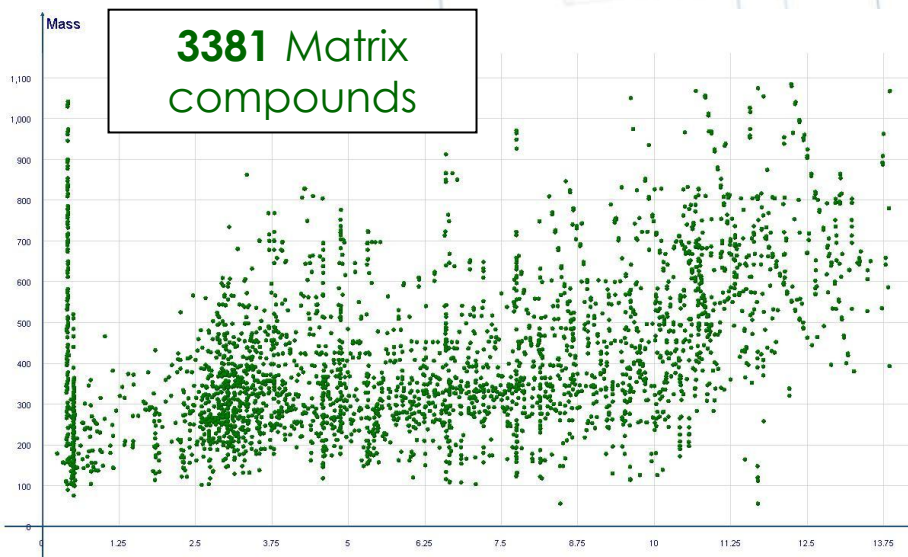
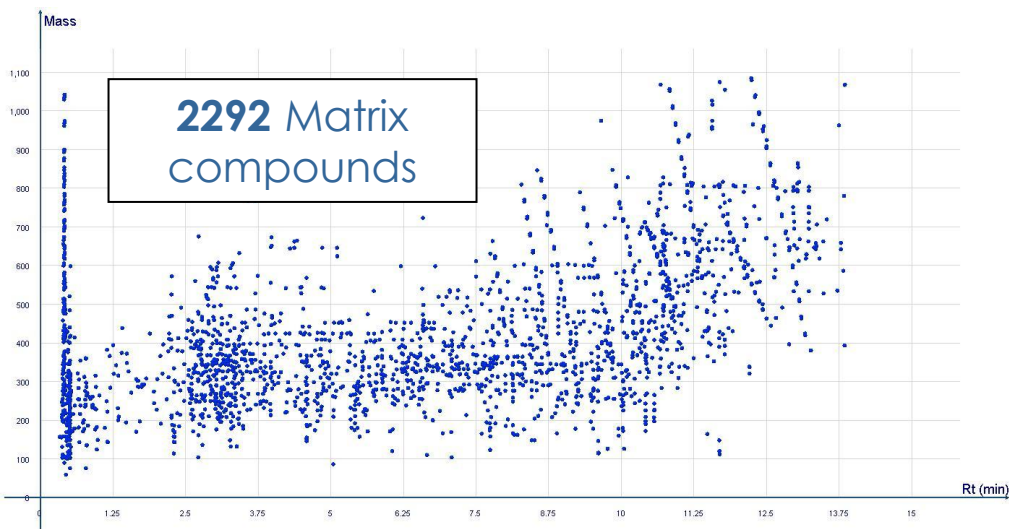
| Solubility in water | PubChem                        | Handbook of Chemistry and Physics. 94 <sup>th</sup> |
|---------------------|--------------------------------|-----------------------------------------------------|
| Acetonitrile        | 800 (g/L) to 25 <sup>a</sup> C | miscible                                            |
| Acetone             | 100%                           | miscible                                            |
| Dichloromethane     | 13,2 (mg/L) to 25°C            | slightly soluble                                    |
| Petroleum Ether     |                                | insoluble                                           |
| Ethyl Acetate       | 80 (g/L) to 25° C              | soluble                                             |

## Solubility of fatty acids with various organic solvents

| No of C atoms | Solubilities at 20° C |         |              |
|---------------|-----------------------|---------|--------------|
|               | Ethyl acetate         | Acetone | Acetonitrile |
| 10            | 289                   | 407     | 66           |
| 11            | 425                   | 706     | 185          |
| 14            | 15,3                  | 15,9    | 1,8          |
| 15            | 15,4                  | 13,8    | 1,1          |
| 18            | 0,5                   | 1,54    | <0,1         |



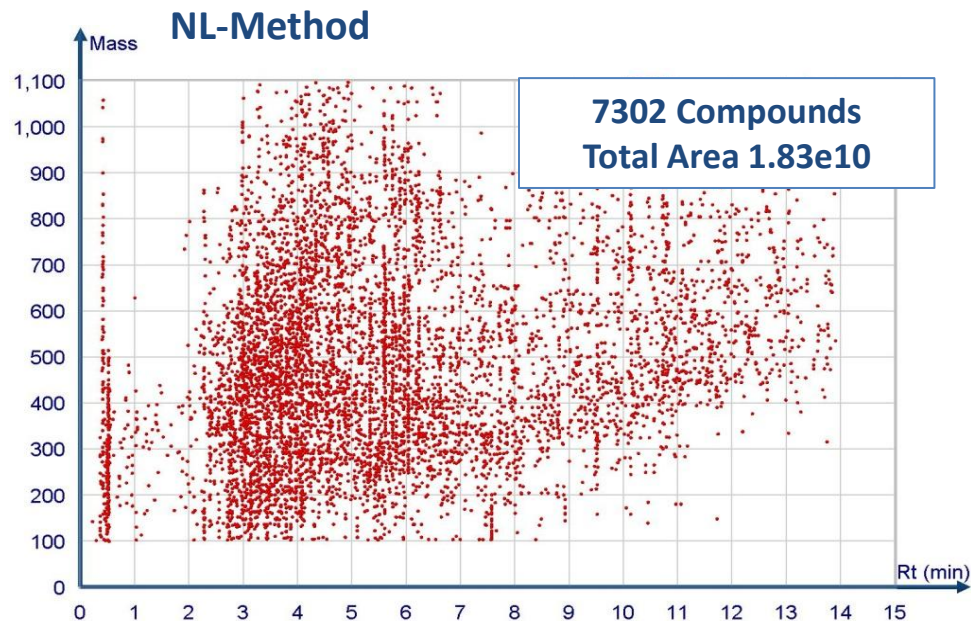
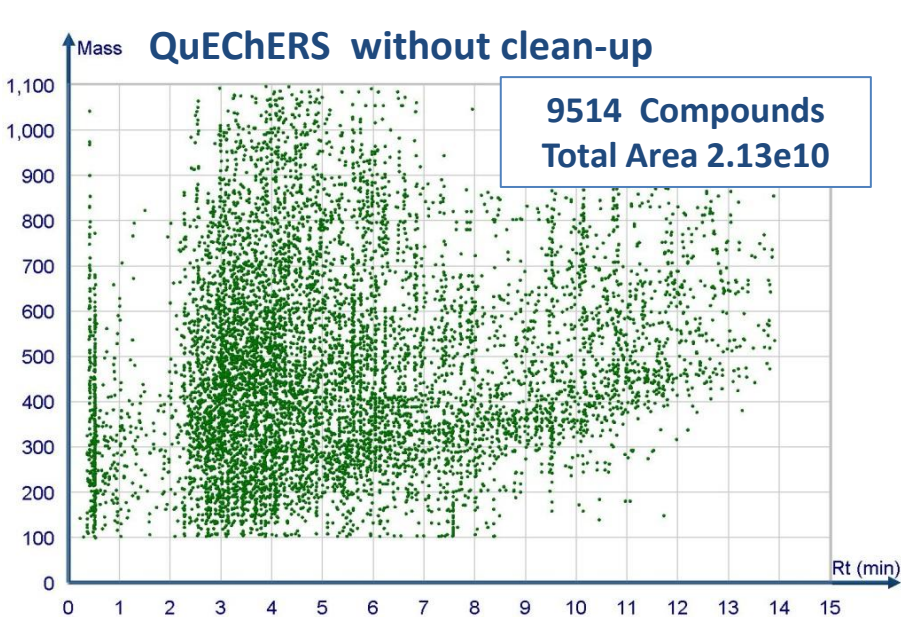
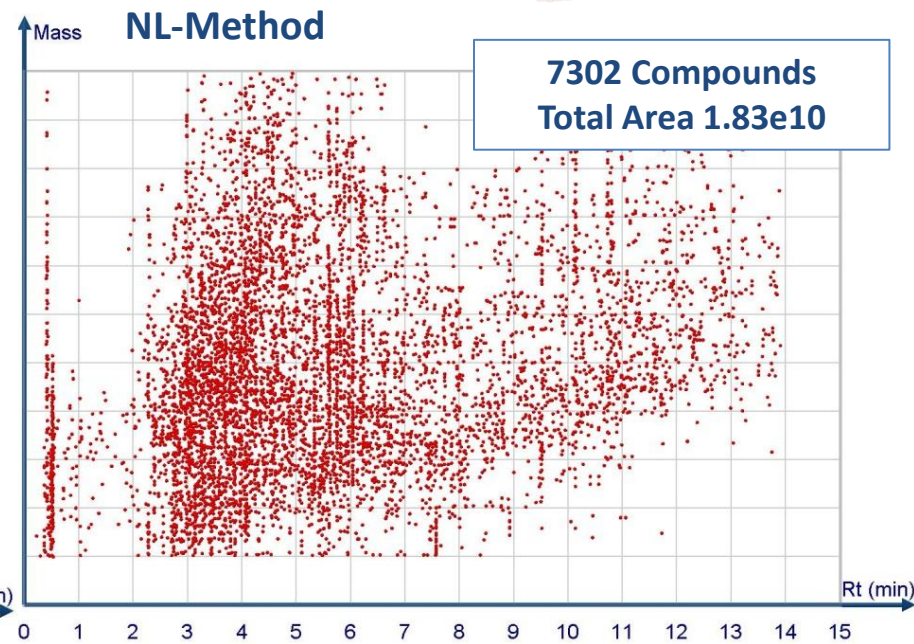
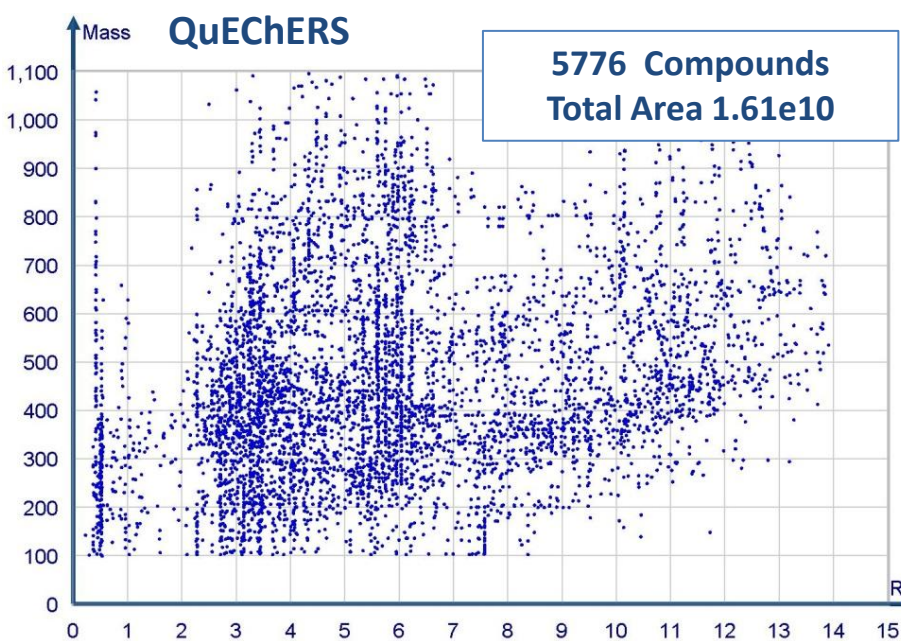
Citrate QuEChERS  
Mini-Luke-Method  
Ethyl Acetate method





# Co-extracted matrix components of orange Dilution 1/5

Absolute height > 10000 counts LC-TOF-MS



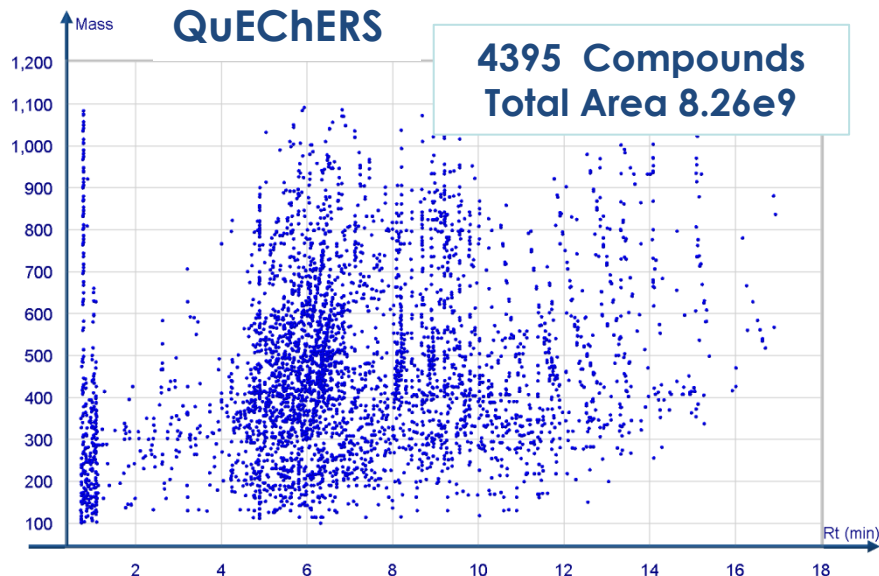
# Co-extracted matrix components of orange Dilution 1/10

Absolute height > 10000 counts LC-TOF-MS

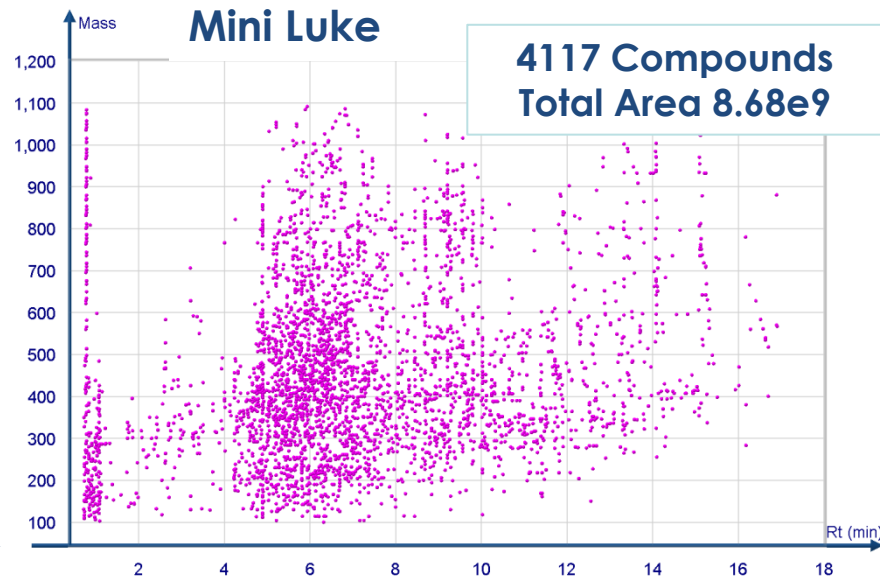


0.1 g sample/mL

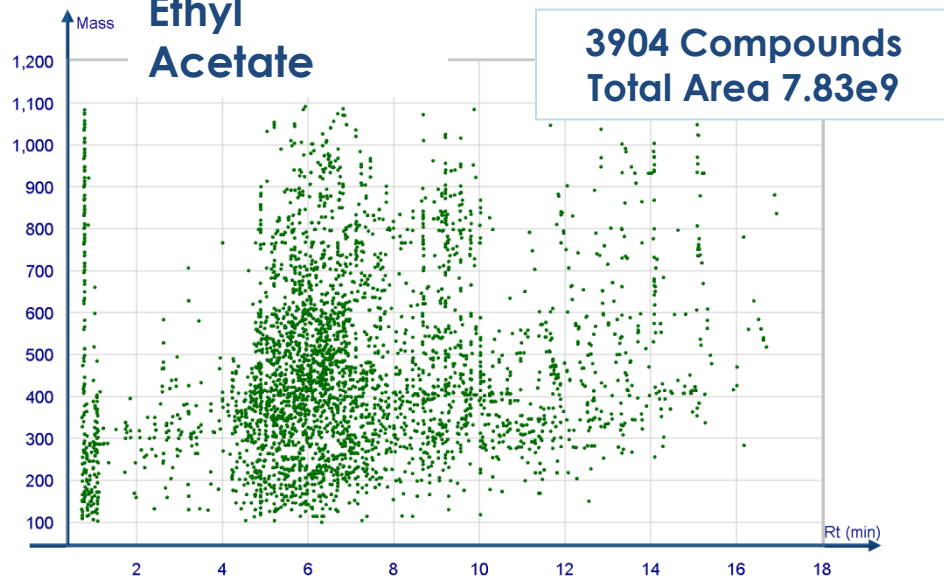
**QuEChERS**



**Mini Luke**



**Ethyl  
Acetate**







## 25 Commodites



Pear



Cucumber



Potato



Orange



Green bean



Melon



Lemon



Apple



Lettuce



Strawberry



Cauliflower



Cherry



Onion



Pumpkin



Broccoli



Aubergine



Celery



Avocado



Leek



Banana



Pepper



Green tea



Parsley



Tomato



Carrot



# Methods

- **Citrate QuEChERS with clean-up**
  - Sample weigh: 10 g
  - Extraction solvent: Acetonitrile
- **Citrate QuEChERS without clean-up**
  - Sample weigh: 10 g
  - Extraction solvent: Acetonitrile
- **Ethyl acetate method**
  - Sample weigh: 10 g
  - Extraction solvent: Ethyl Acetate
- **NL method**
  - Sample weigh: 15 g
  - Extraction solvent: Acetone

**Final extract:**

- GC: 1 g sample/mL extract
- LC: 0.2 g sample/mL extract

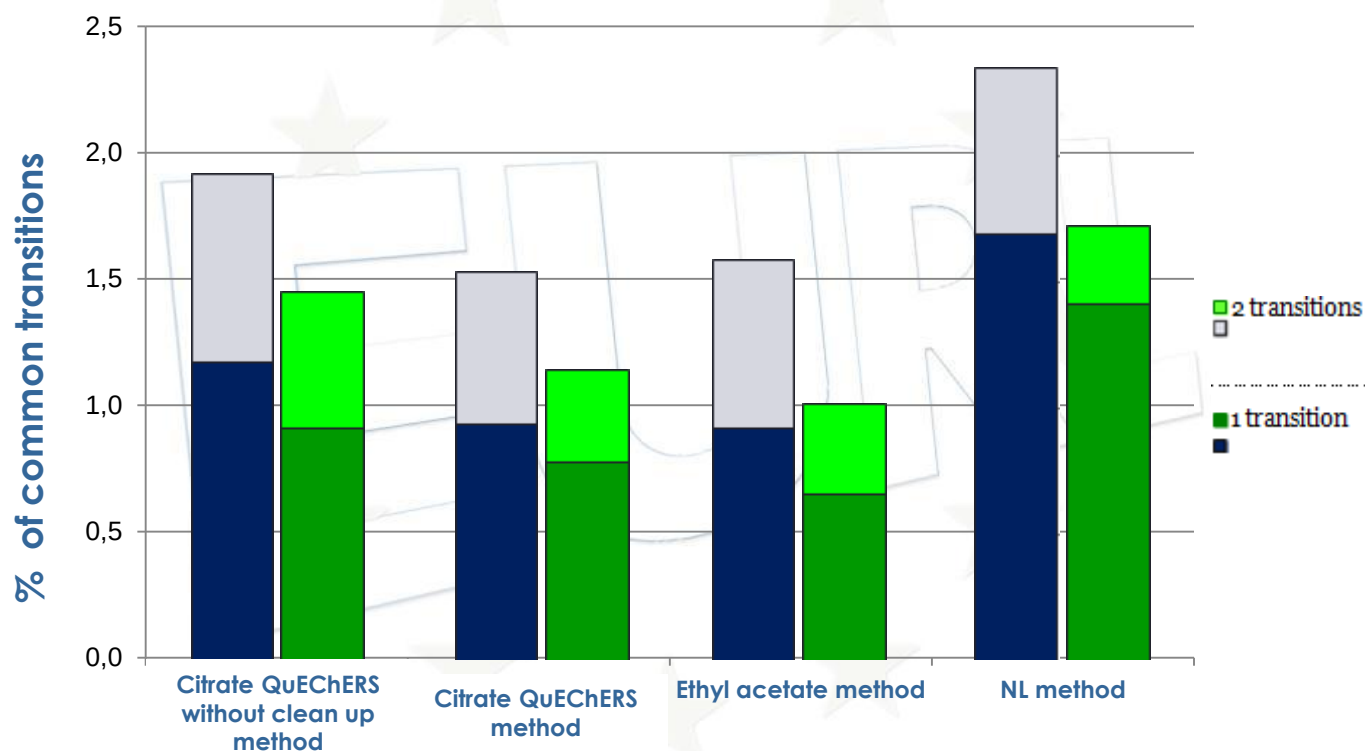
Total injected amount  
2 mg for GC  
1 mg for LC

**Total number of results= 225 compounds · 25 commodities = 5625 results/comm**



# GC-QqQ-MS/MS

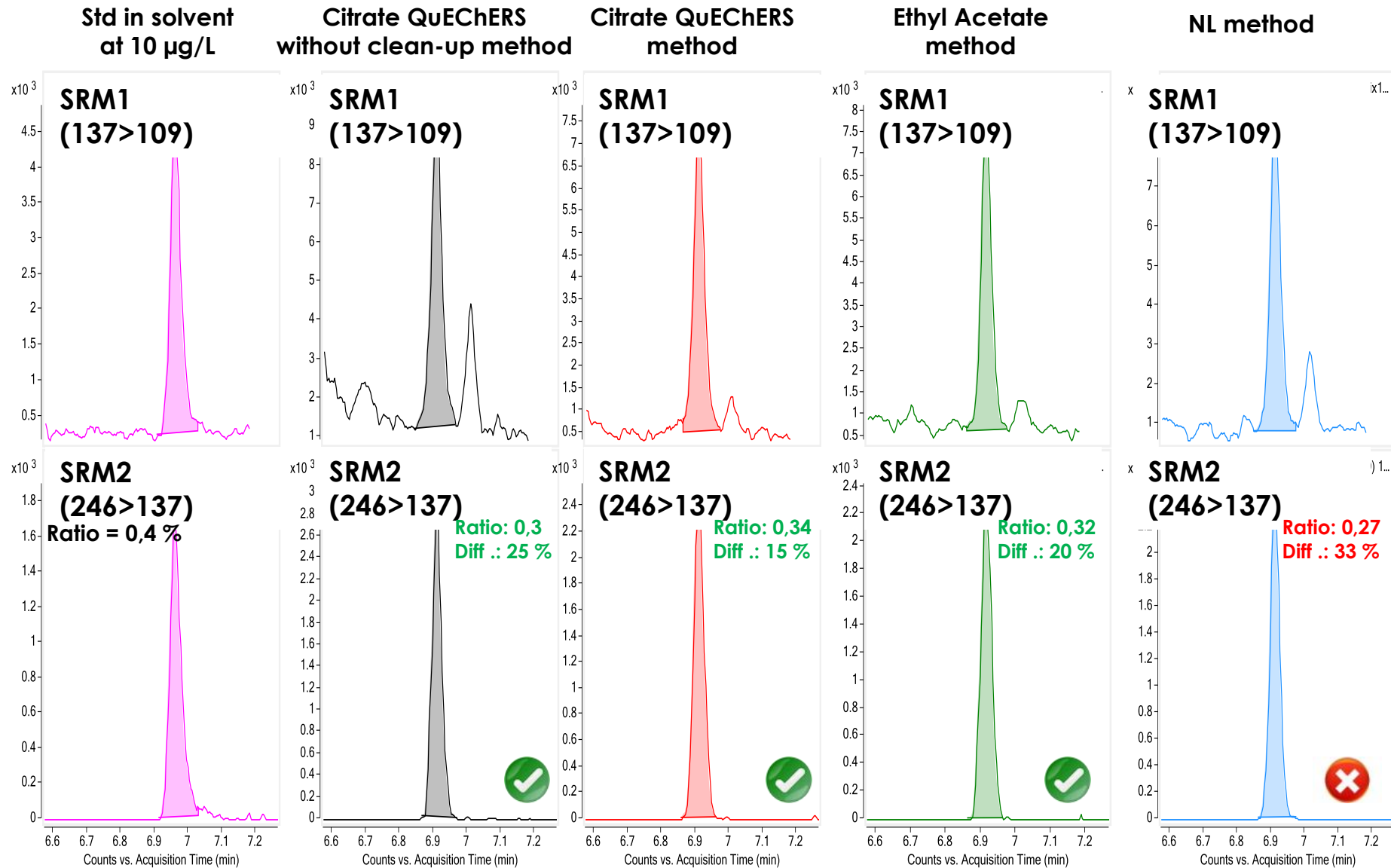
Percentage of Common Transitions by method  
(same transition(s) at different retention time windows)



Blue 0.2 min. Green 0.1 min.

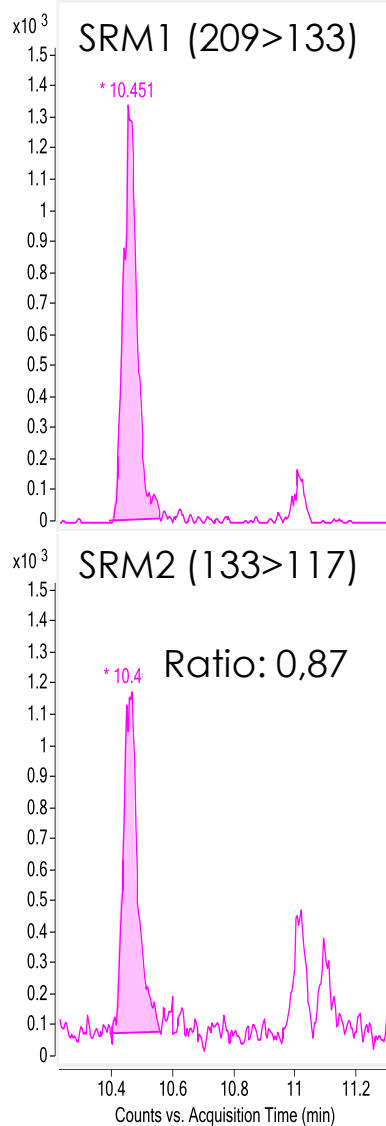
1g sample /mL extract

# Fonofos at 10 µg/kg in Avocado. GC-MS/MS

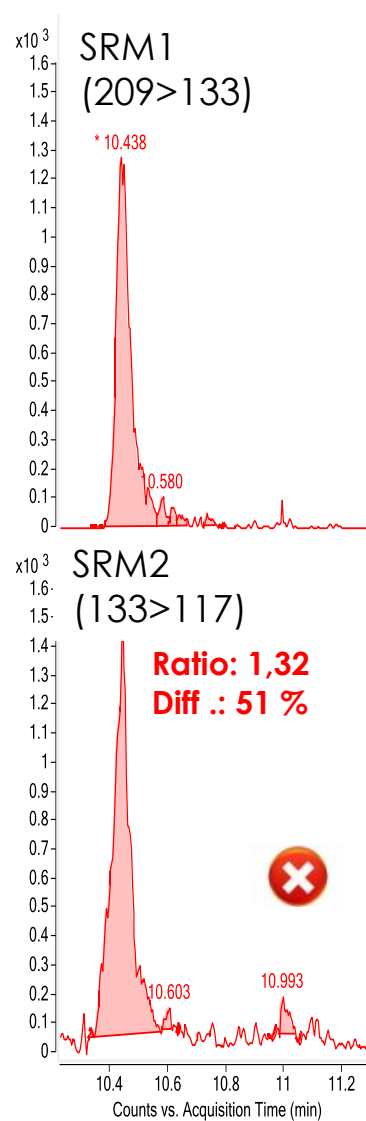


# Metazachlor at 10 µg/kg in Celery. GC-MS/MS

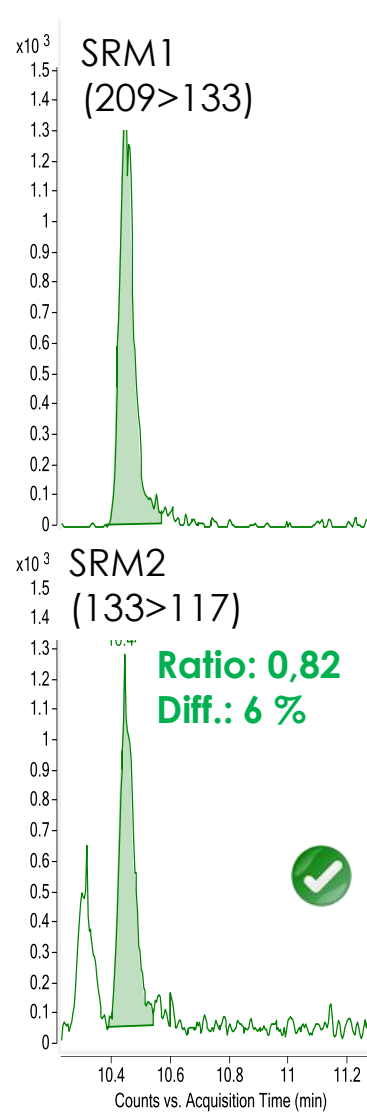
Std in solvent  
at 10 µg/L



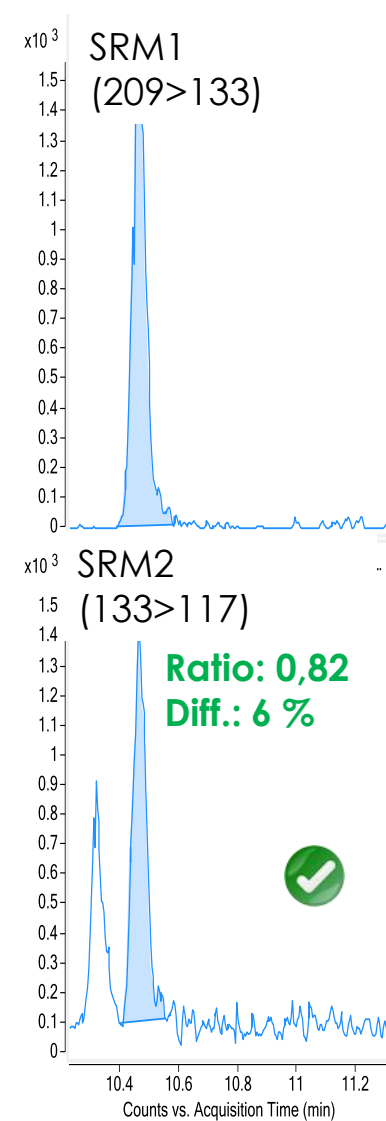
Citrate QuEChERS  
method



Ethyl Acetate  
method



NL method

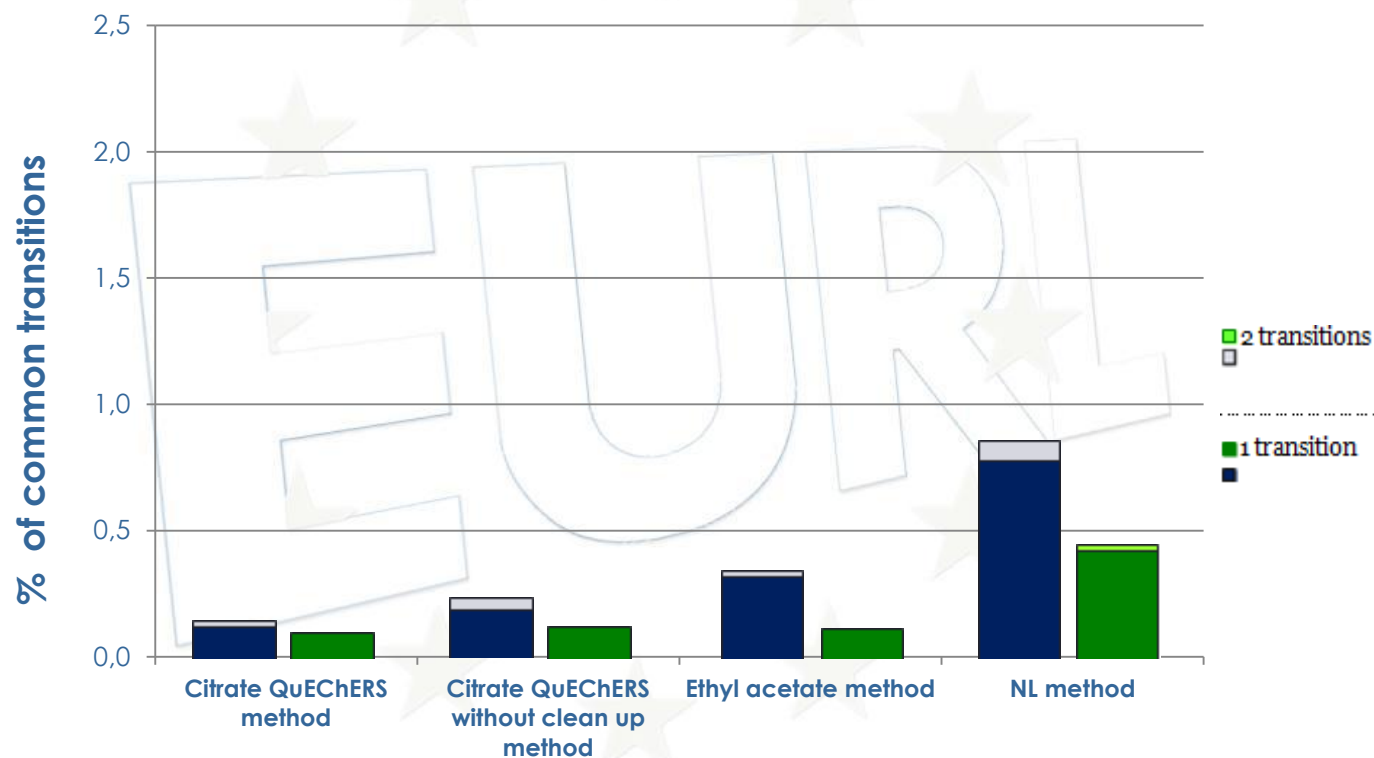






# LC-QqQ-MS/MS

Percentage of Common Transitions by method  
(same transtion(s) at different retention time windows)



Blue 0,2 min. Green 0,1 min.

0.2 g sample /mL extract

# Haloxyfop at 10 µg/kg in Aubergine. LC-MS/MS

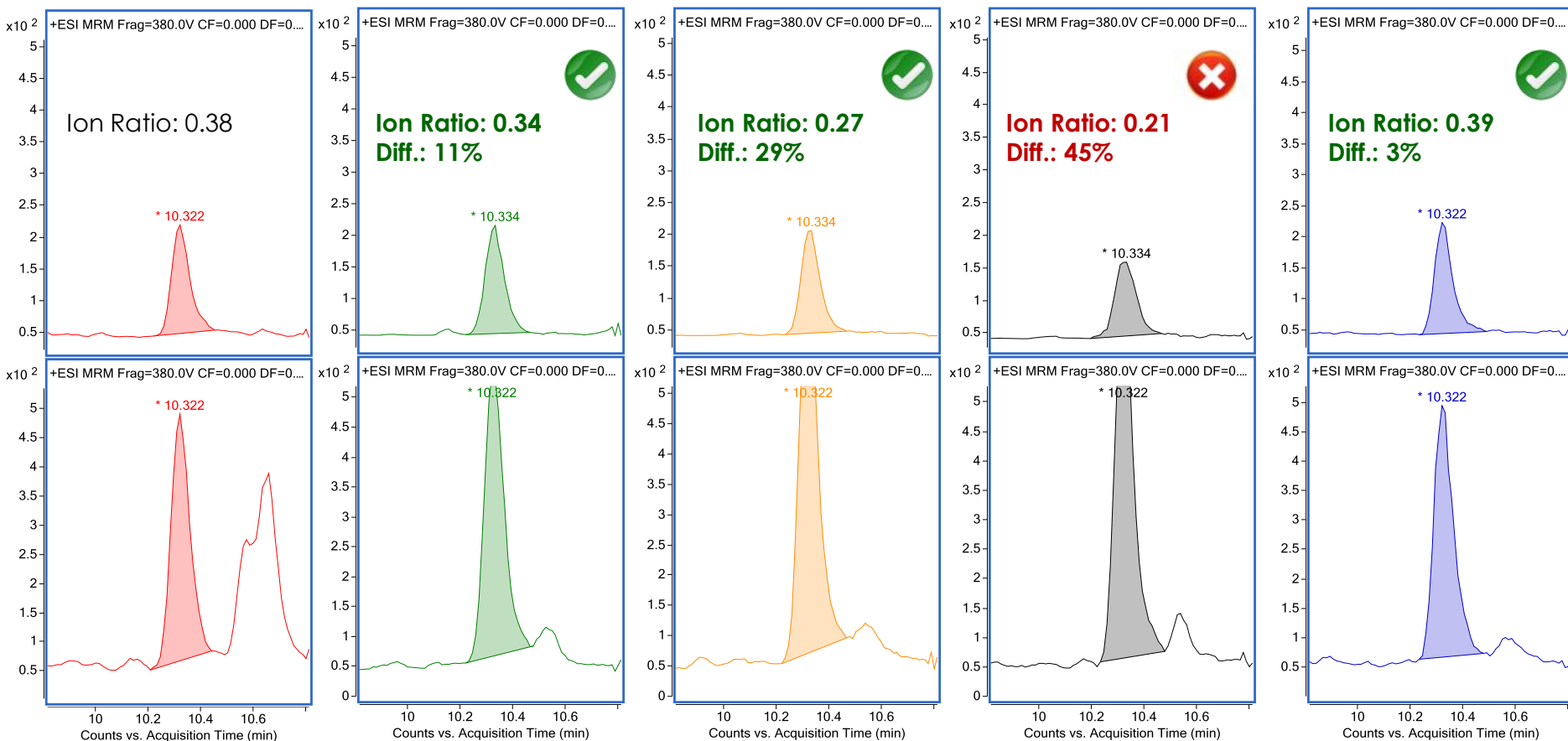
Std in solvent  
at 10 µg/L

Ethyl Acetate  
method

NL method

Citrate QuEChERS  
without clean-up  
method

Citrate QuEChERS  
method



# Azinphos methyl at 10 µg/kg in Onion. LC-MS/MS

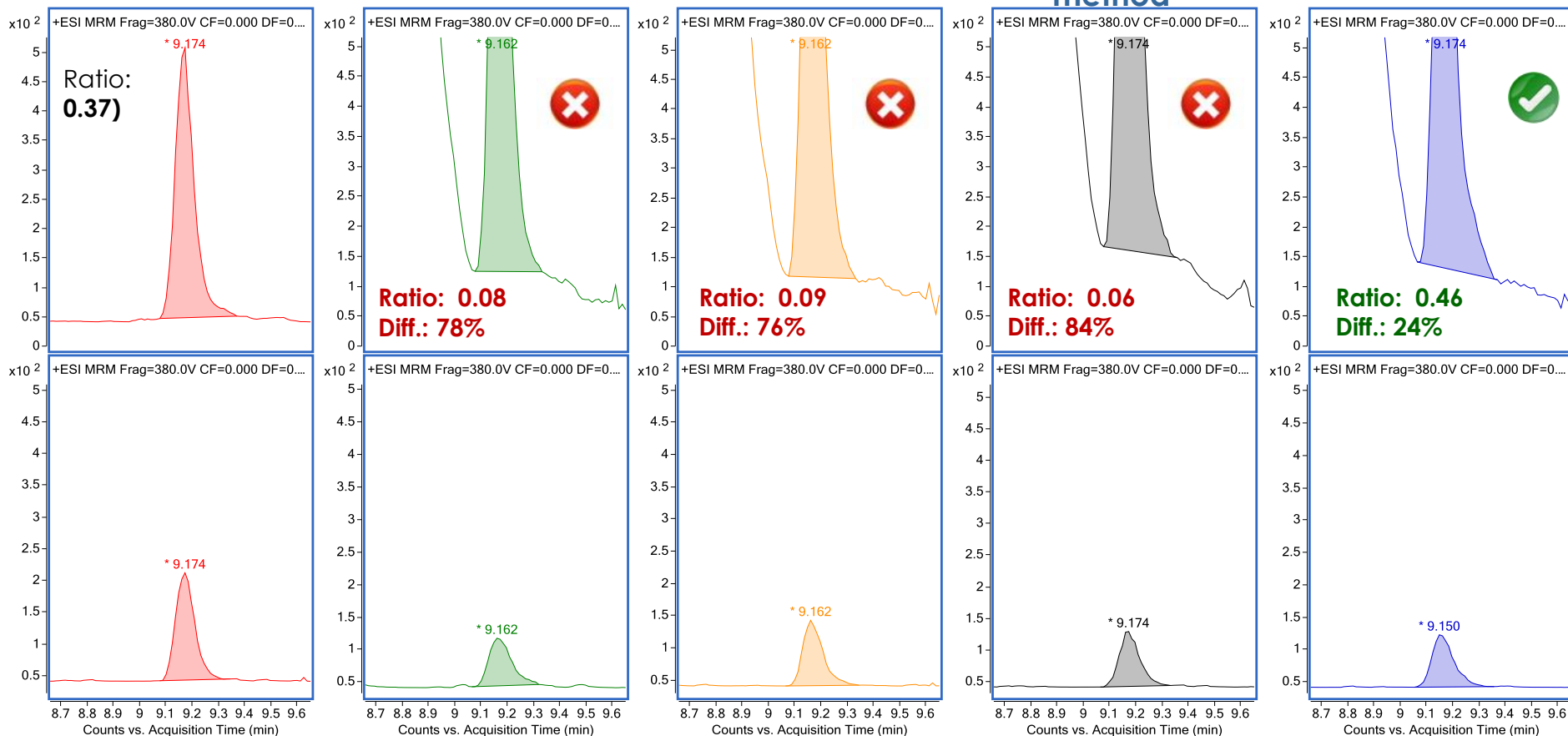
Std in solvent  
at 10 µg/L

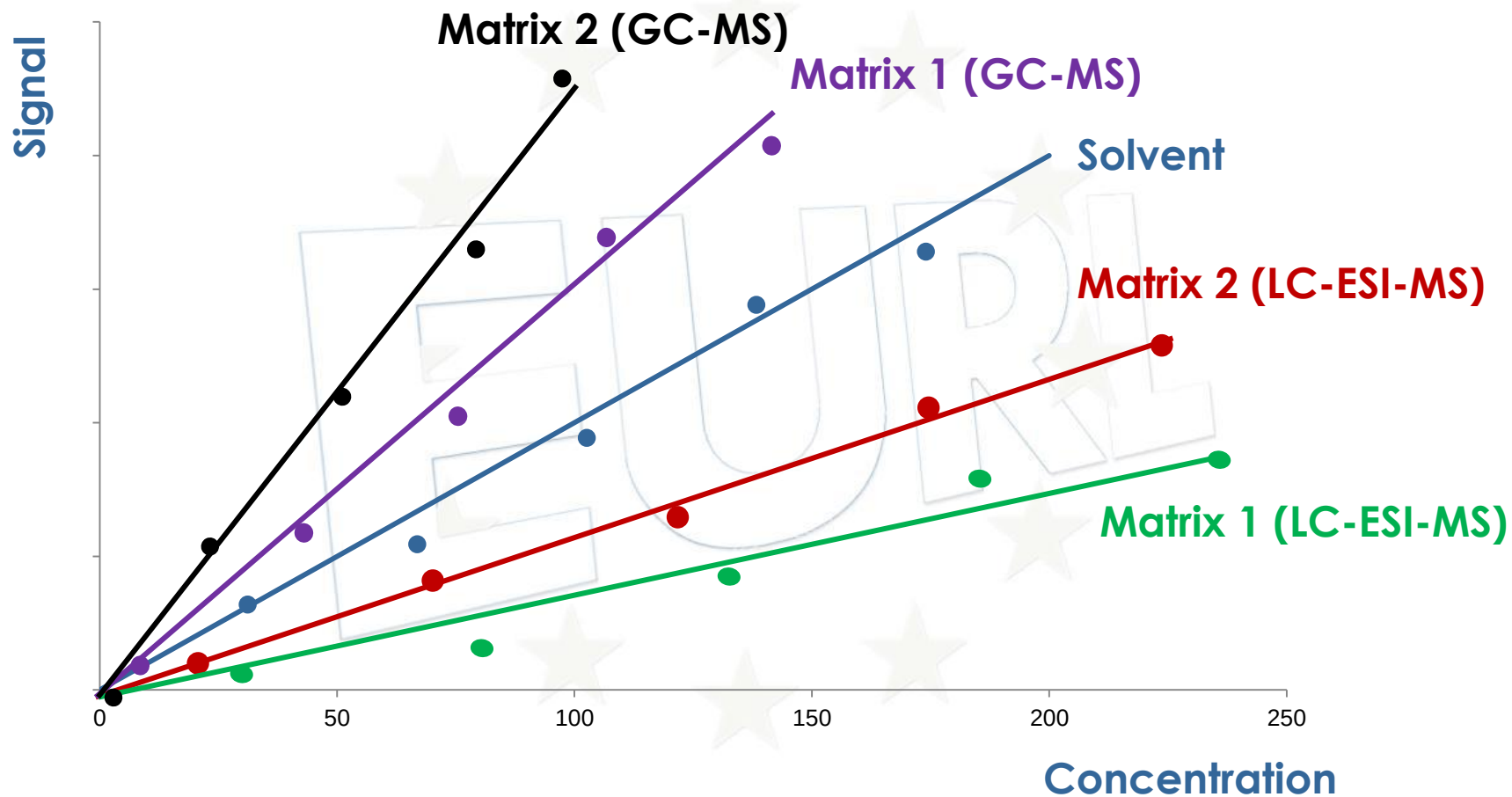
Ethyl Acetate  
method

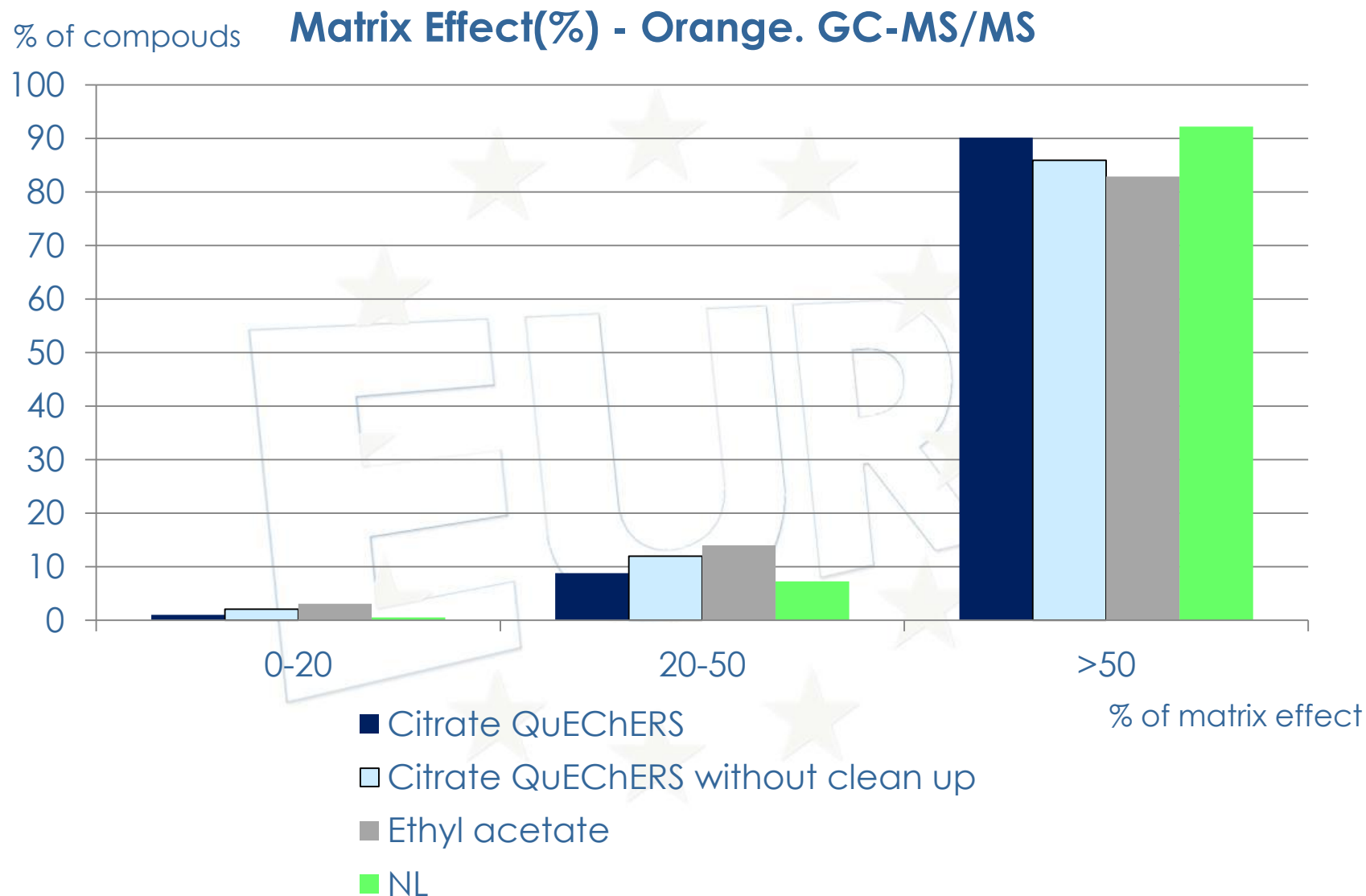
NL mehod

Citrate QuEChERS  
without clean-up  
method

Citrate QuEChERS  
method

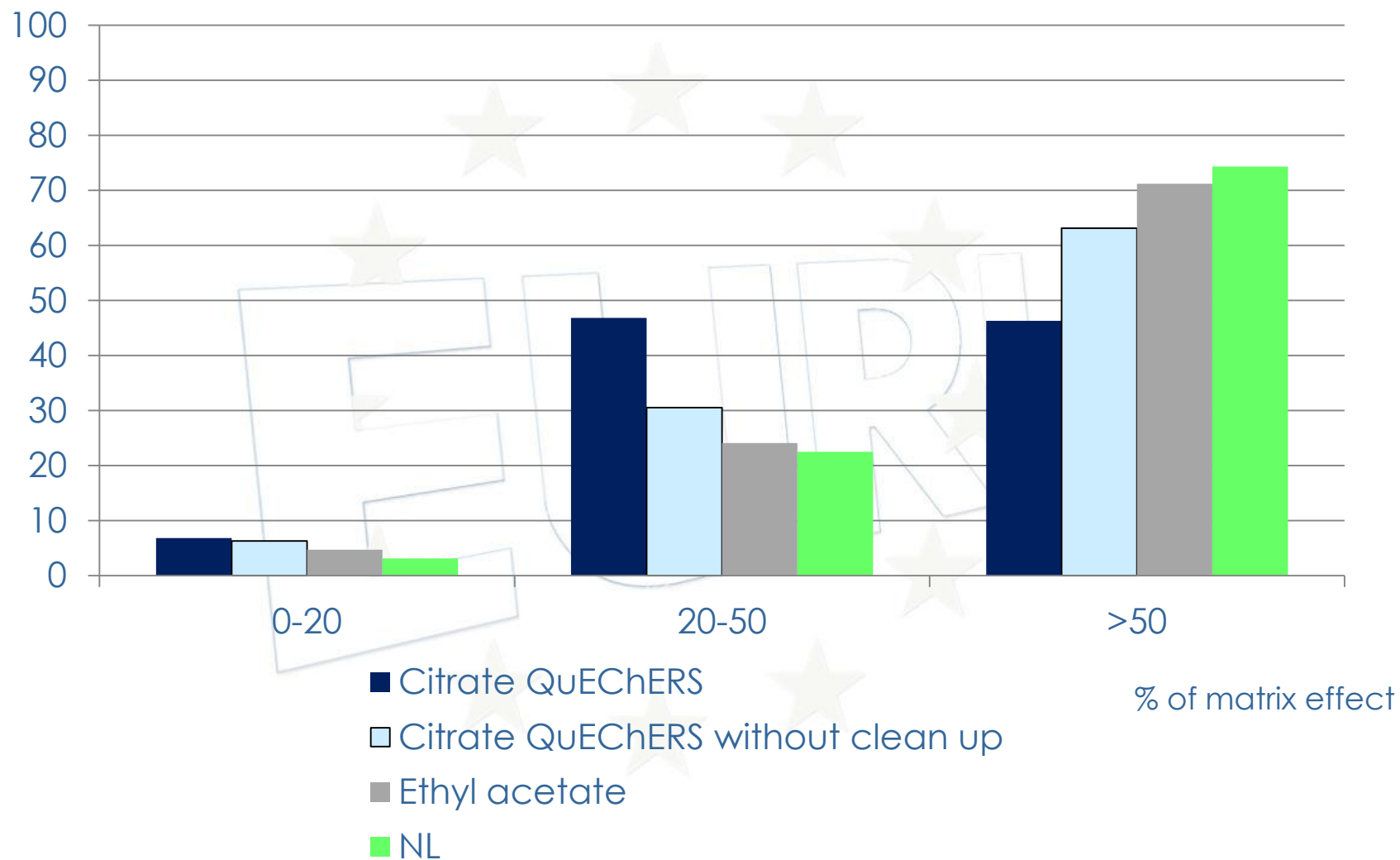






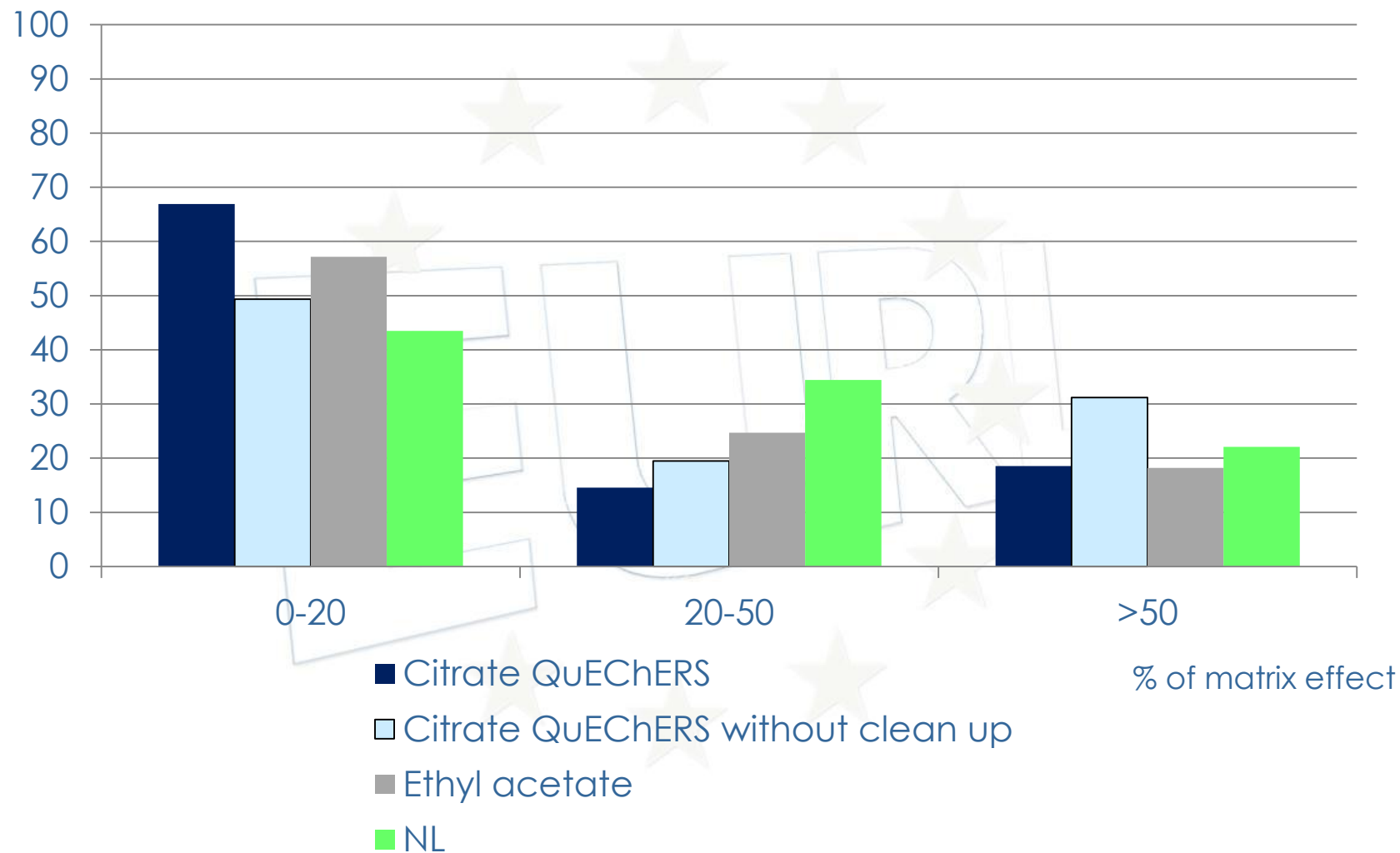
% of compounds

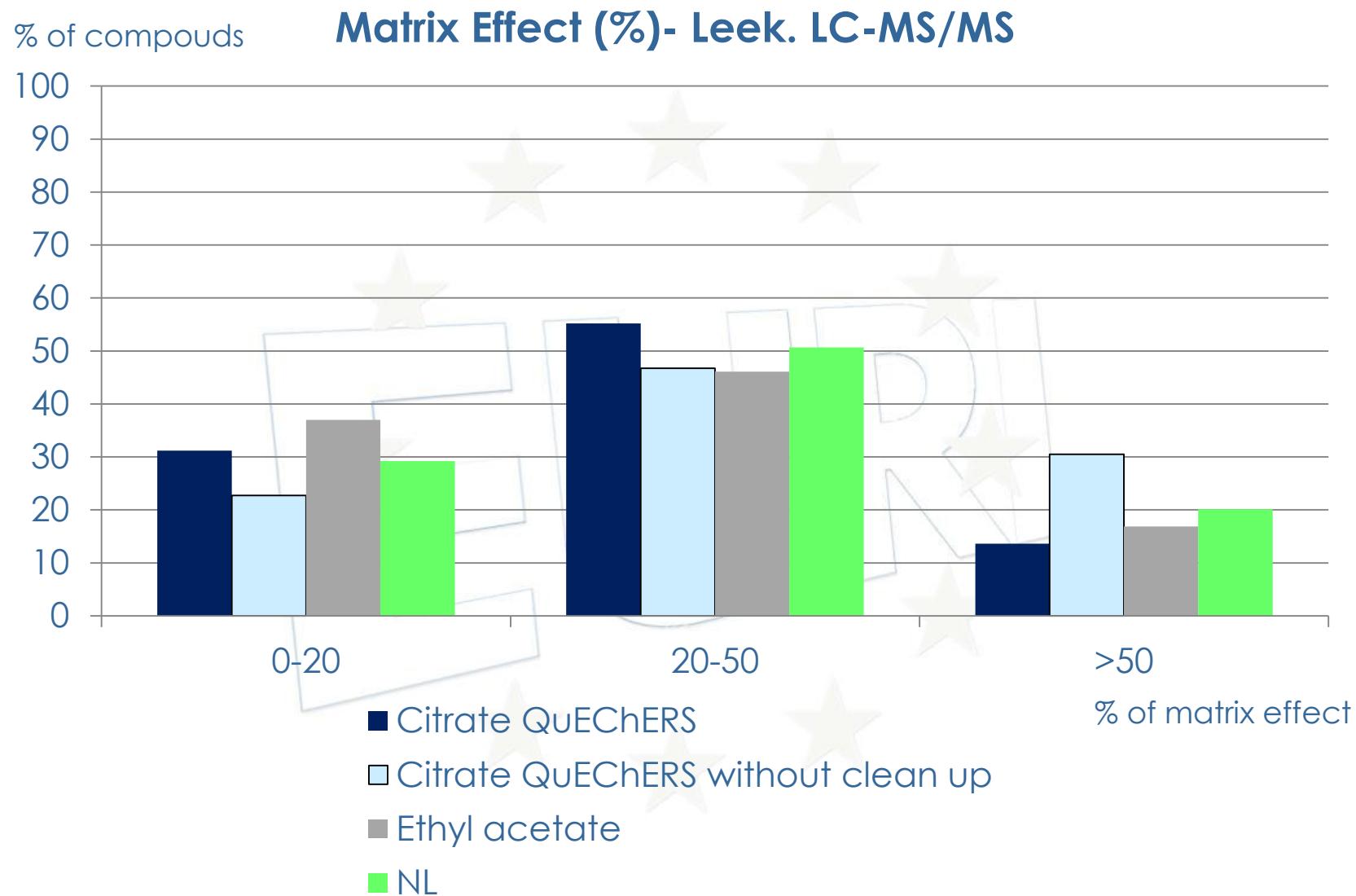
## Matrix Effect(%) - Leek. GC-MS/MS



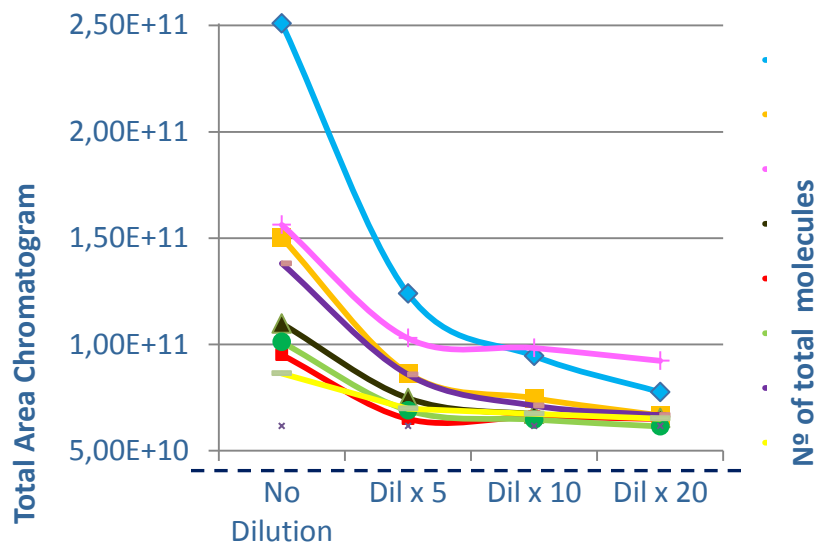


## % of compounds      **Matrix Effect (%) - Broccoli. LC-MS/MS**

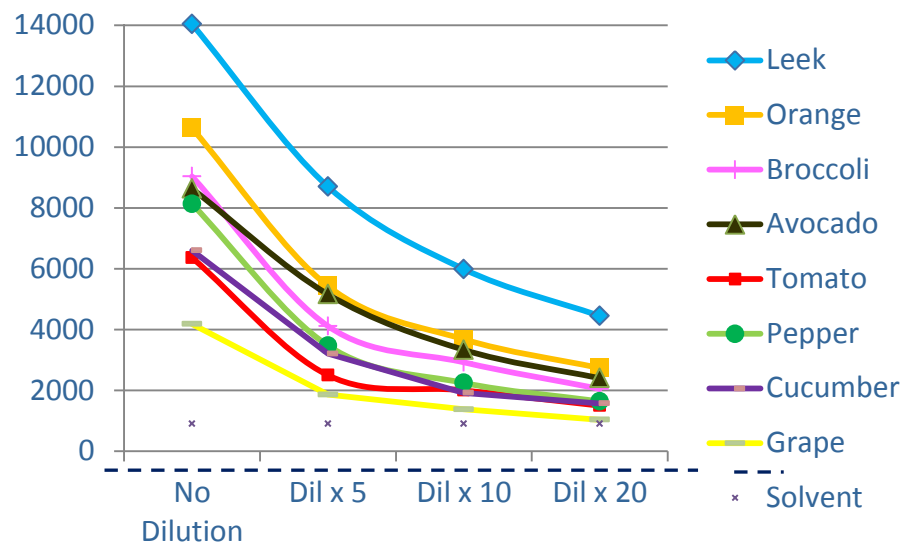




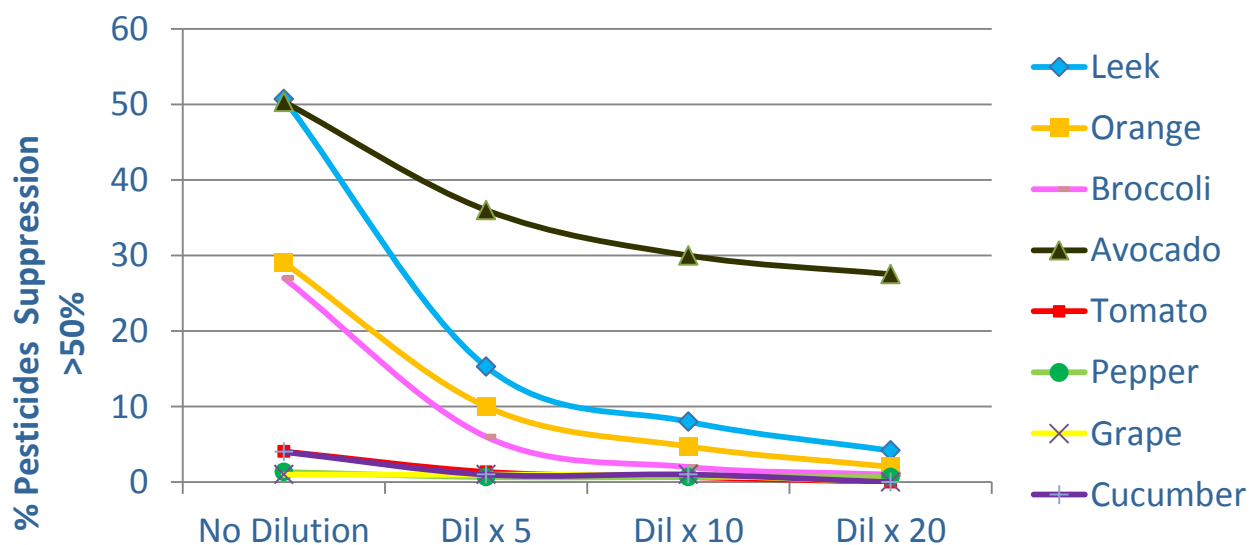
### Total Area Chromatogram



### Nº of total molecules

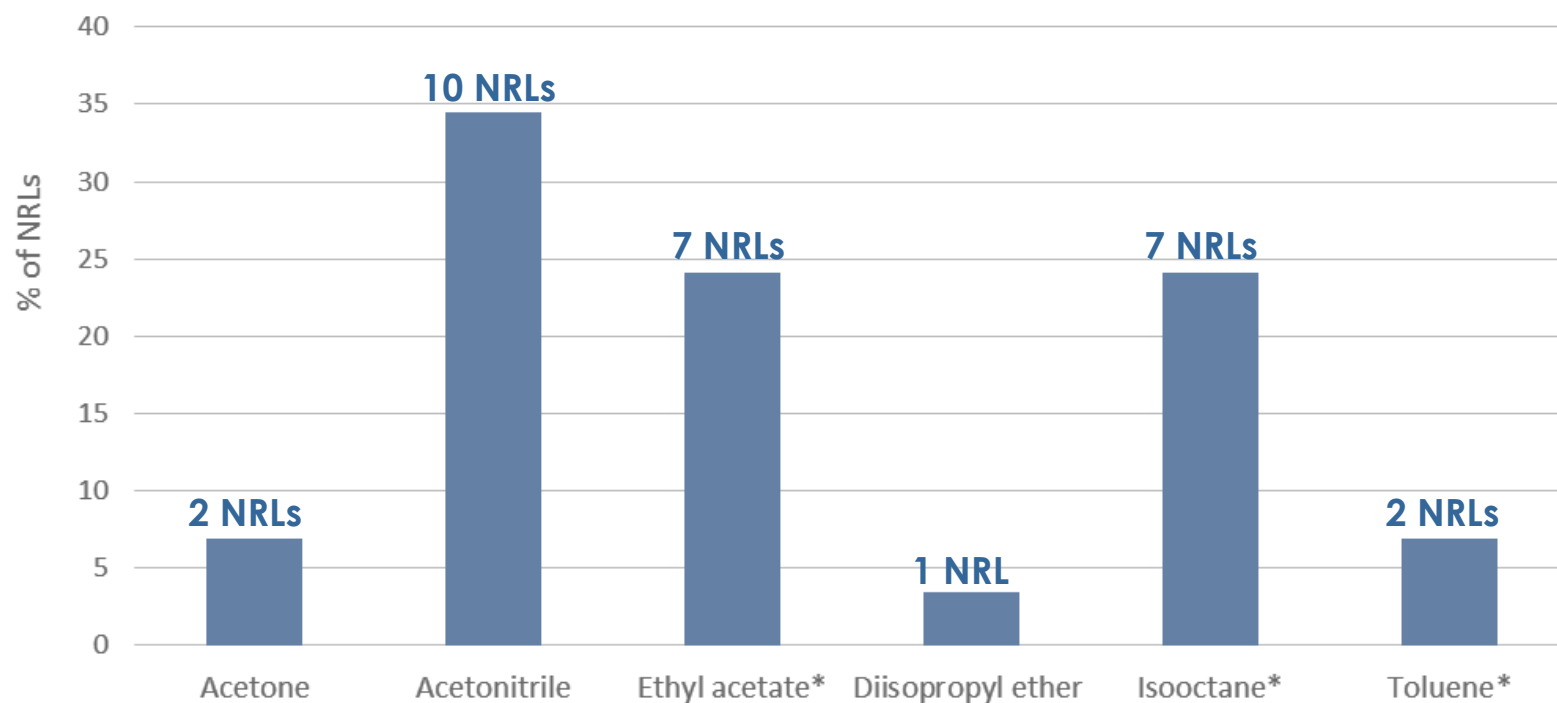


### % of compounds >50% suppression

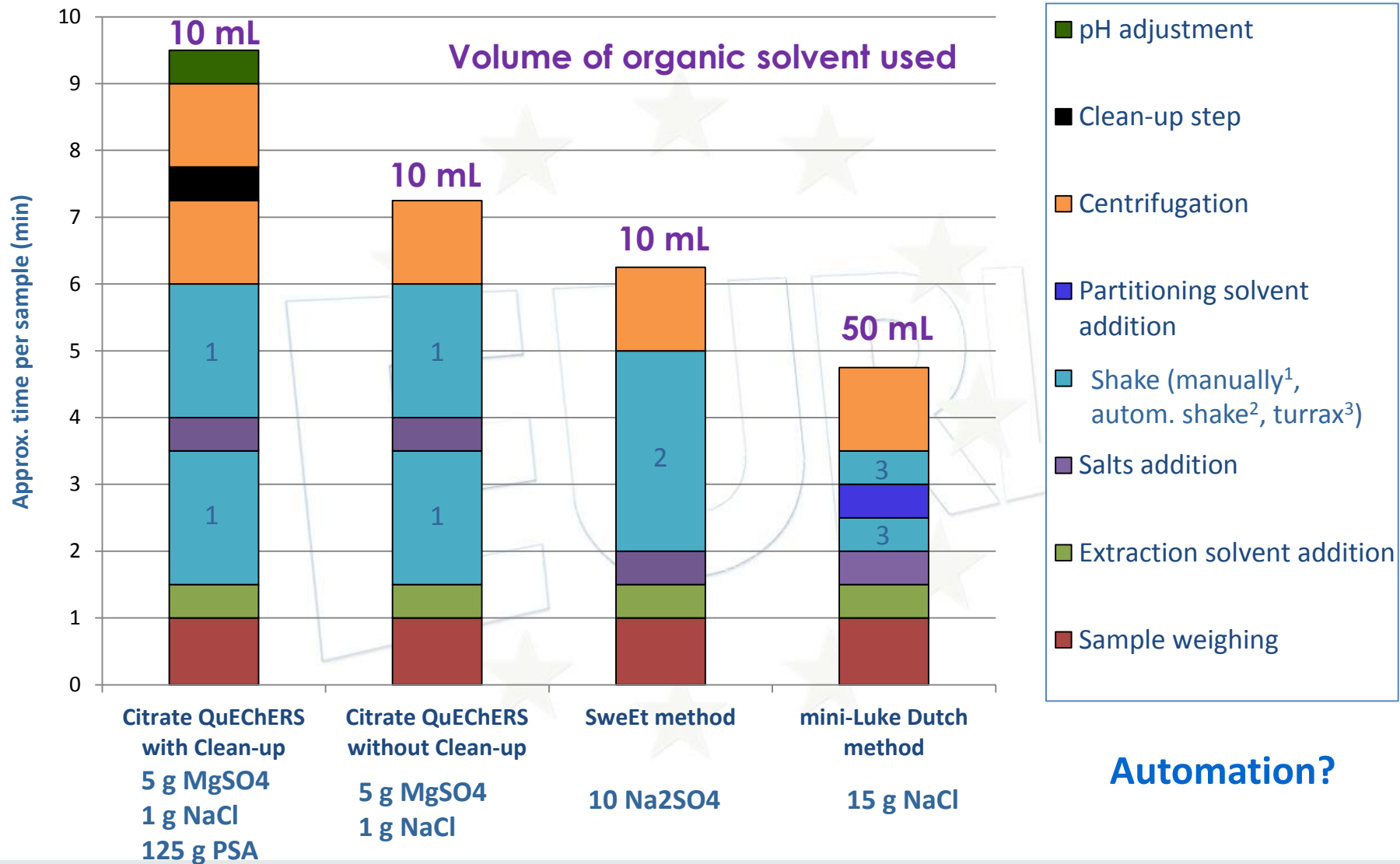




### Solvent used in GC systems



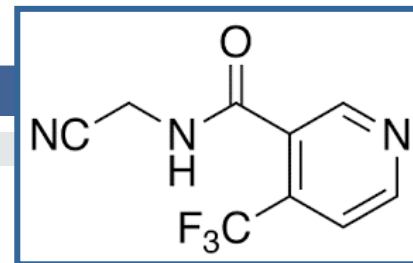
# Multiresidue extraction methods



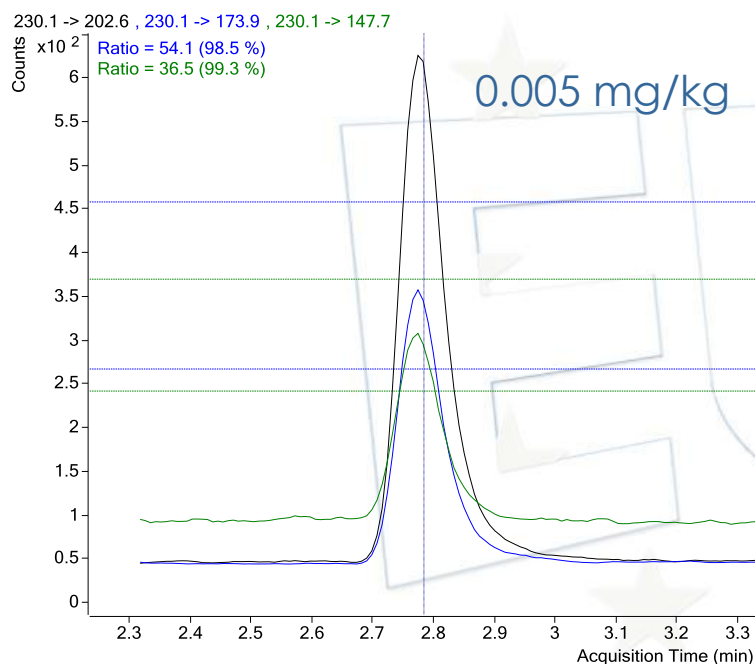
Tricky  
compounds



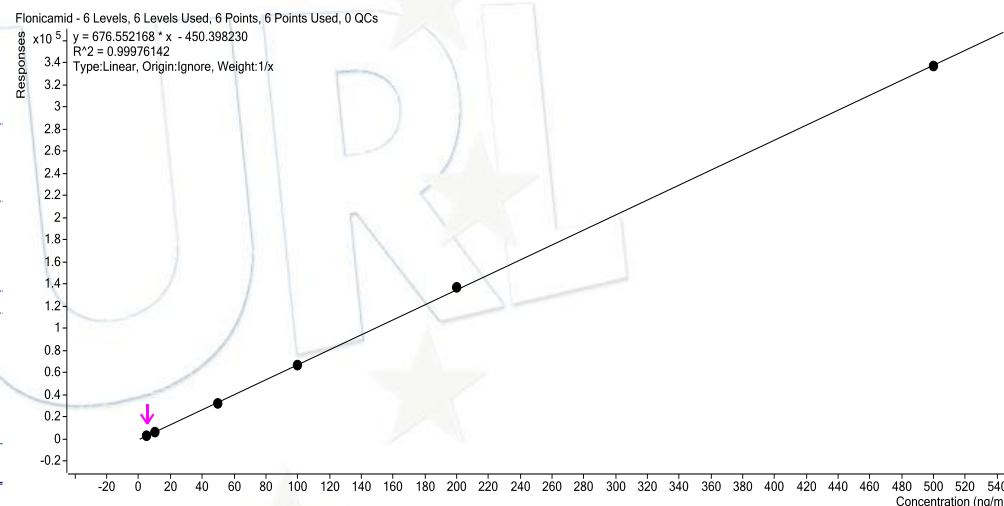
Tricky  
compounds



# Flonicamid in tomato by LC-MS/MS

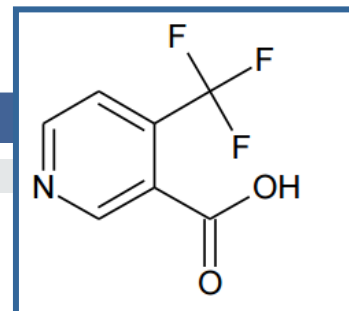


Calibration curve



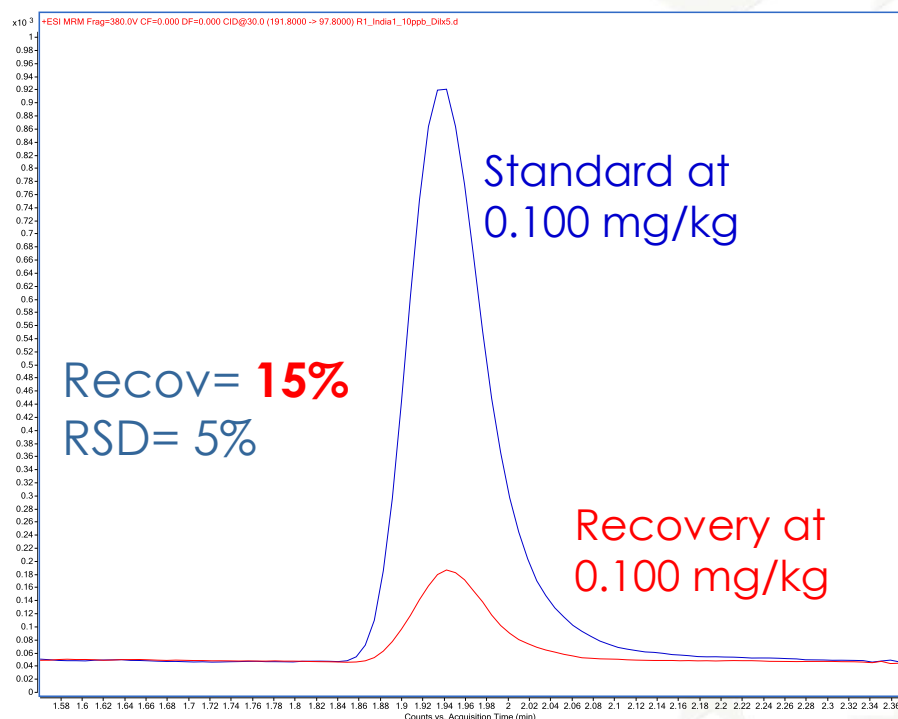
Recovery at 0.010 mg/kg (citrate QuEChERS with PSA)= **102%**  
RSD= 5%





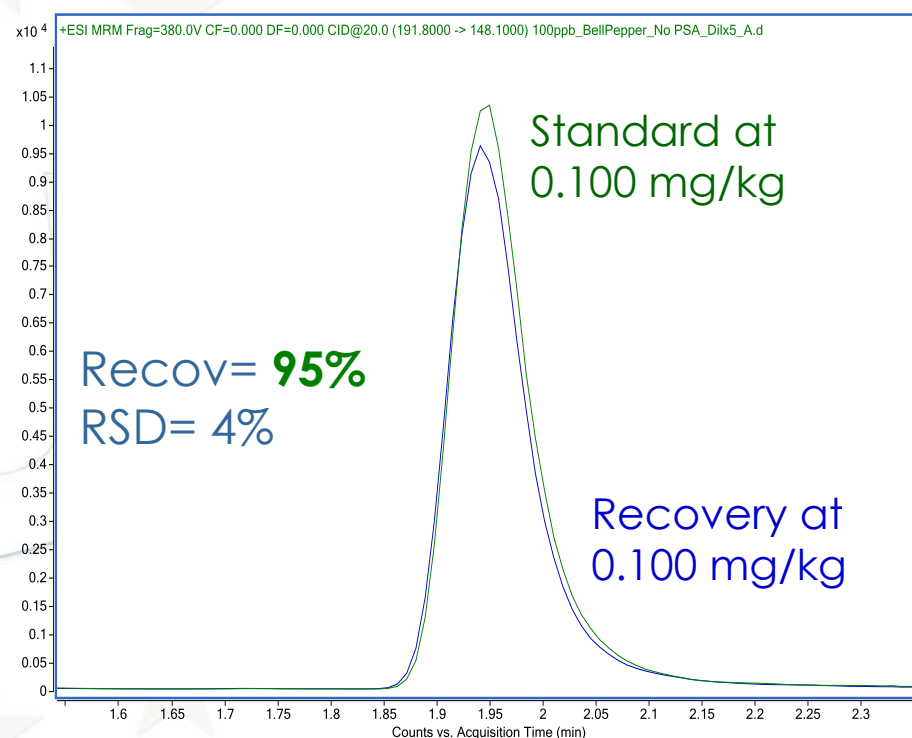
# TFNA in tomato by LC-MS/MS

Citrate QuEChERS (with PSA)



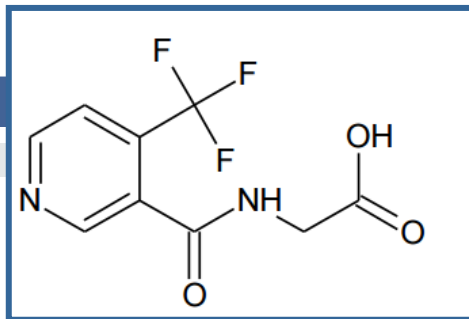
Low recovery

Citrate QuEChERS (No PSA)

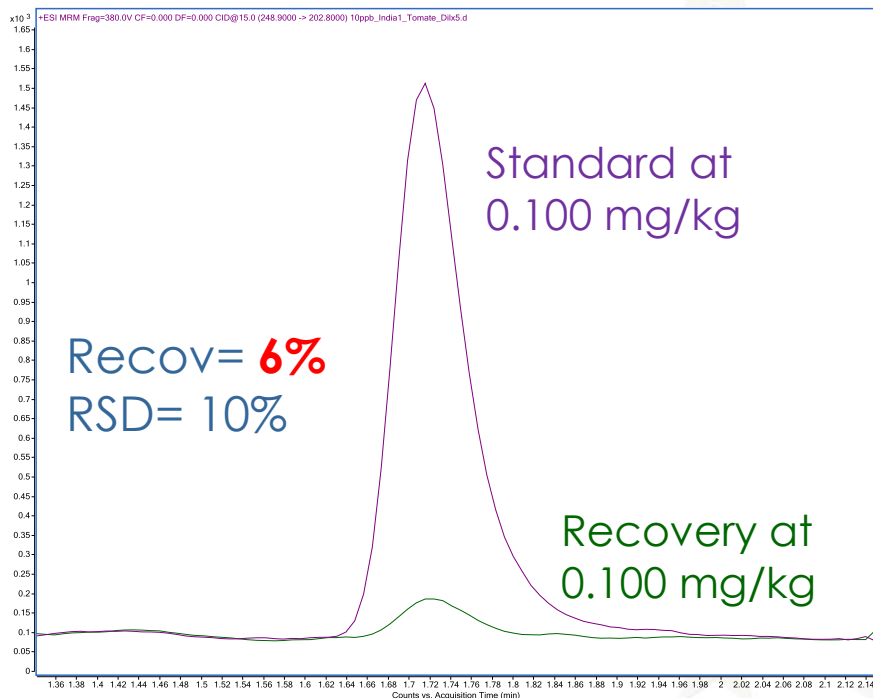


Recovery at 0.010 mg/kg with  $\text{ZrO}_2$  instead of PSA= **7%**  
RSD= 13%

# TFNG in tomato by LC-MS/MS

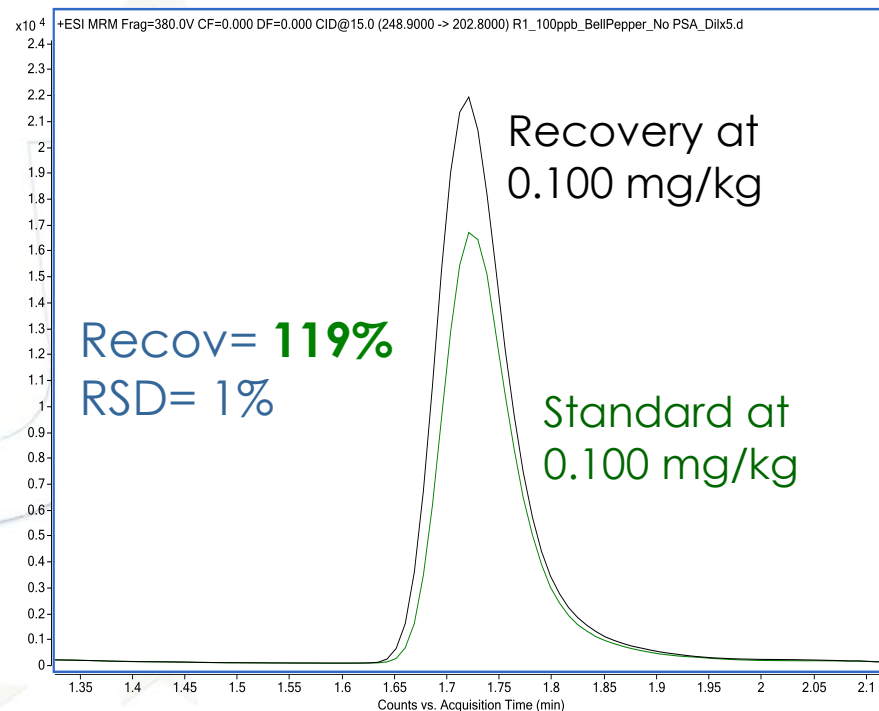


## Citrate QuEChERS (with PSA)



Low recovery

## Citrate QuEChERS (No PSA)



Recovery at 0.010 mg/kg with **ZrO<sub>2</sub>** instead of PSA= **5%**  
RSD= 14%

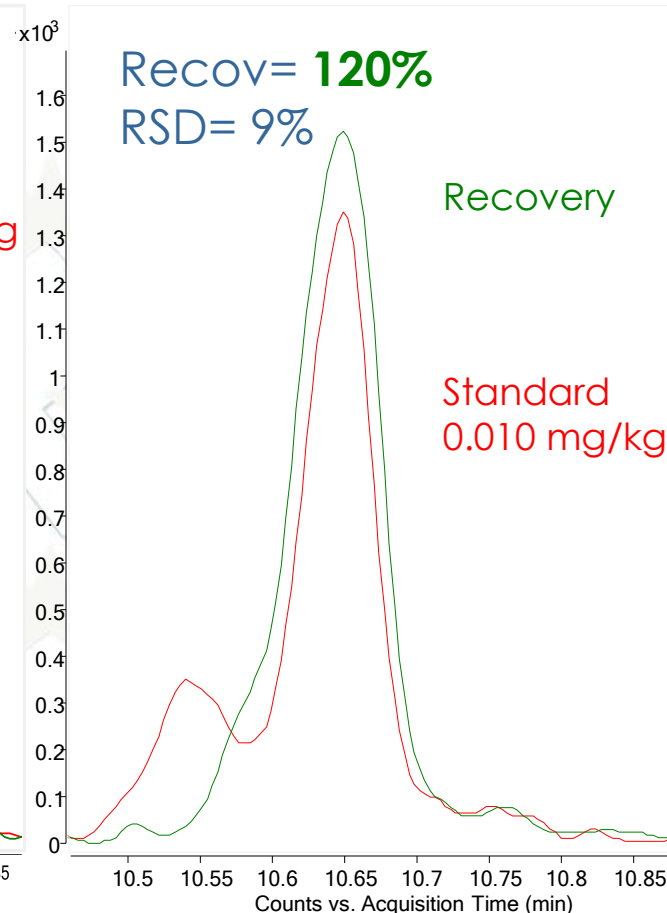
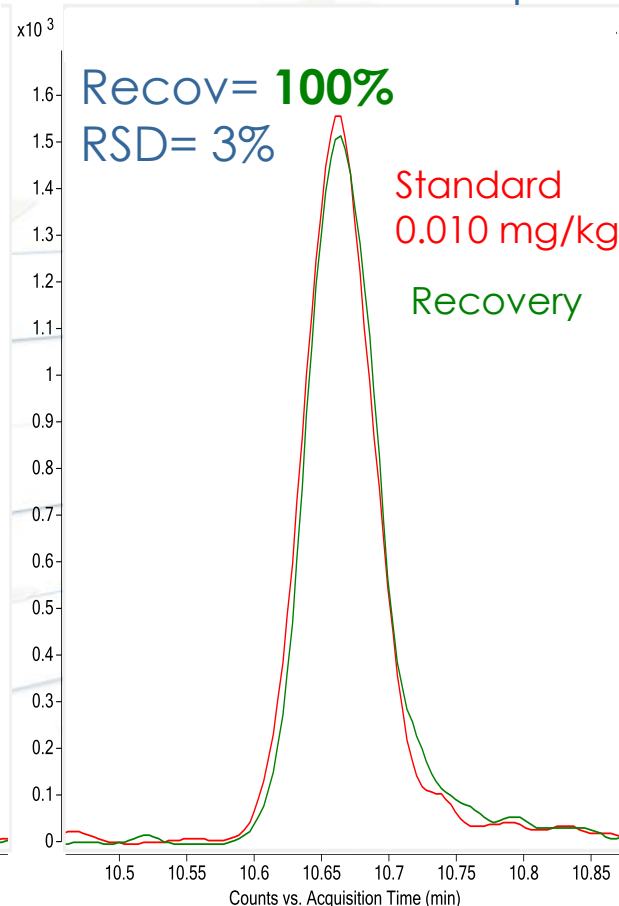
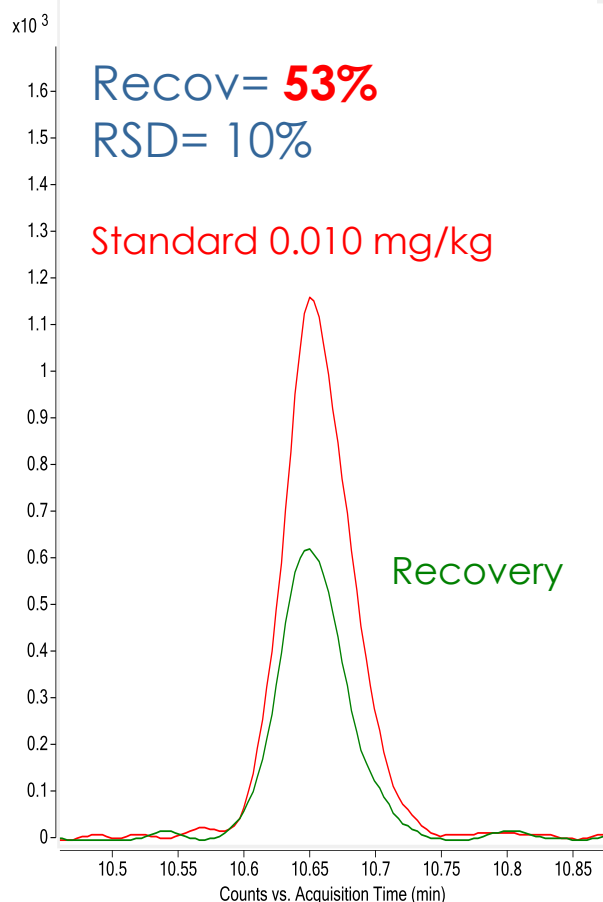


## Orange QuEChERS

without clean-up

NL-Method

Citrate QuEChERS



Citrate QuEChERS with  $\text{ZrO}_2$  instead of PSA:

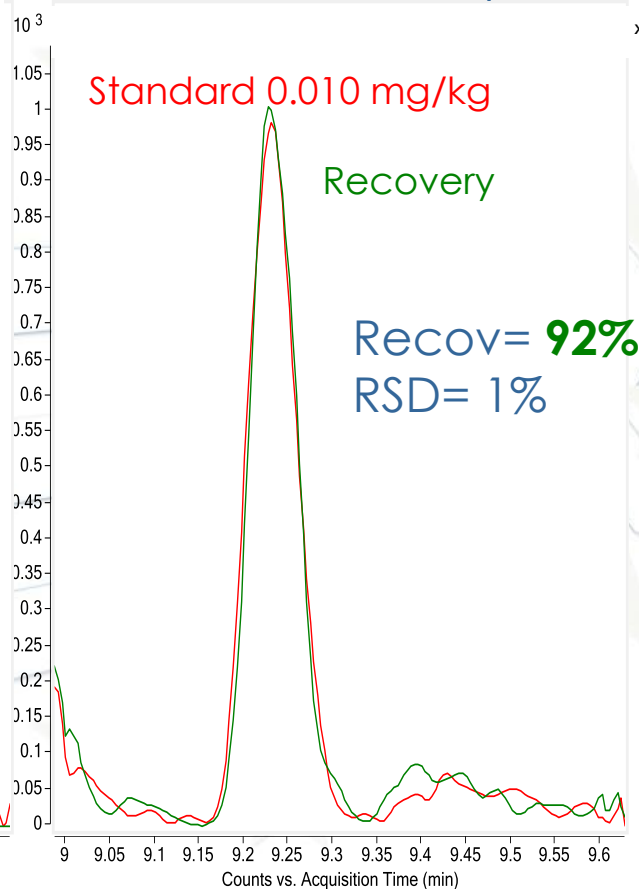
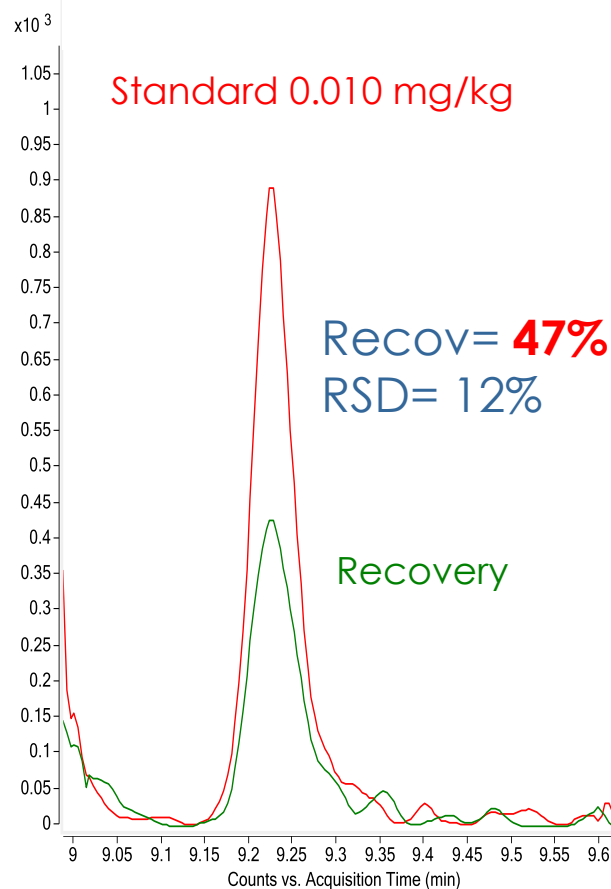
Recov = **94%**      RSD = 4%

## Orange

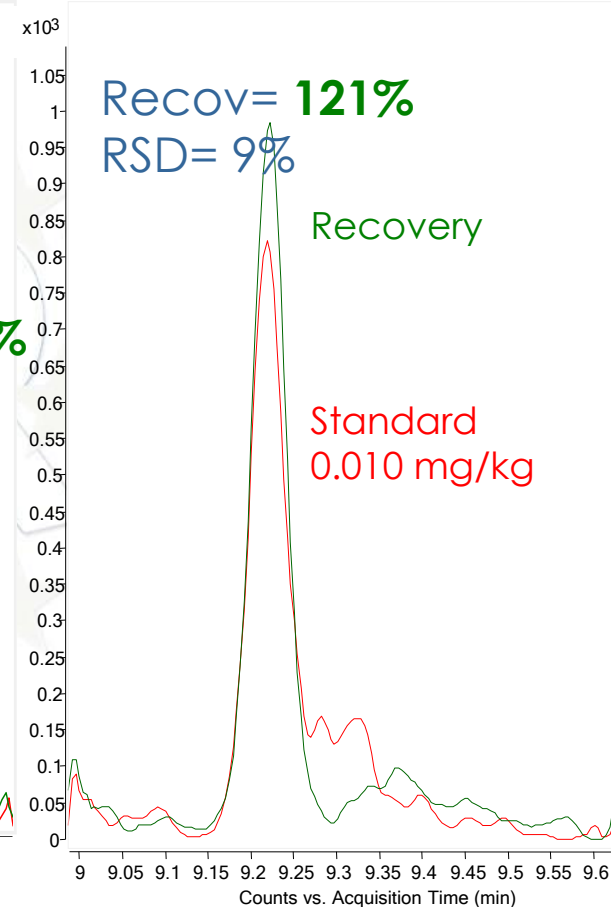
### QuEChERS

without clean-up

Citrate QuEChERS



NL-Method



Citrate QuEChERS with  $\text{ZrO}_2$  instead of PSA:

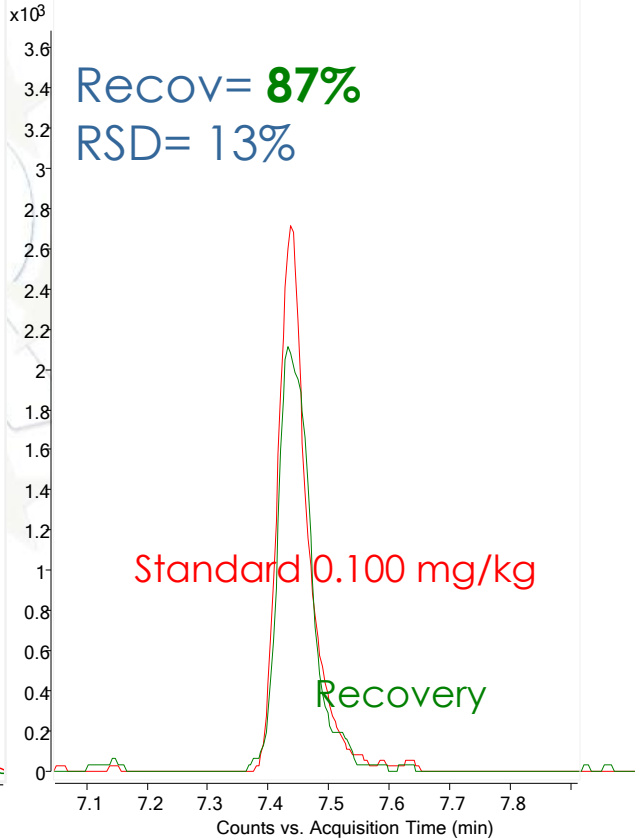
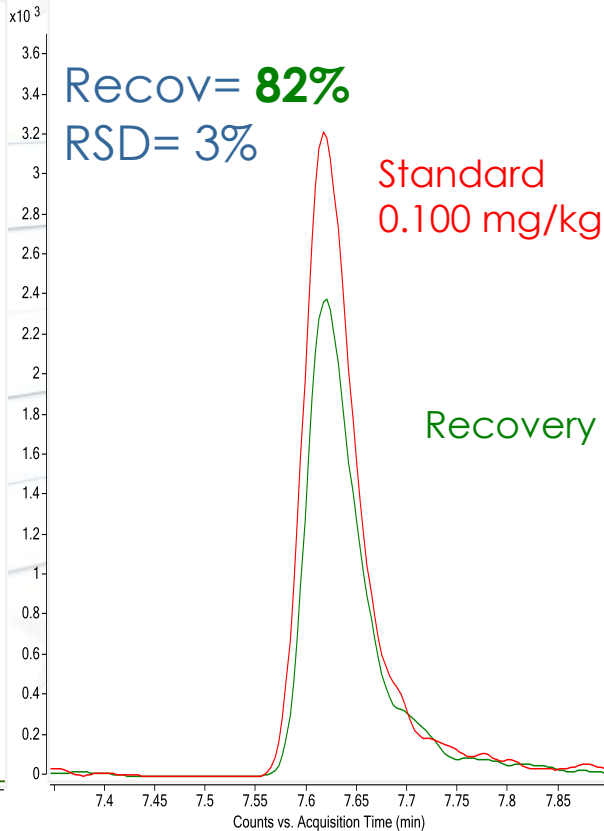
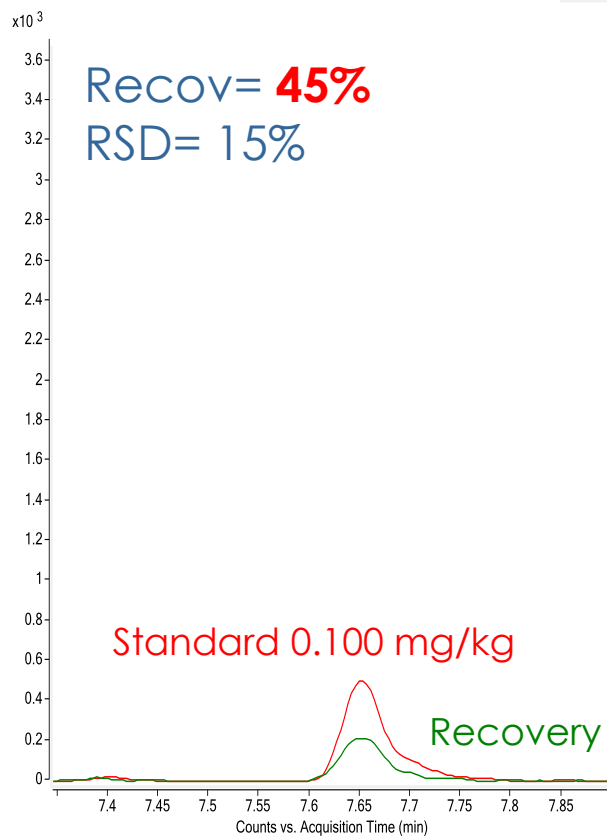
Recov = **117%**      RSD = 11%

## Broccoli

QuEChERS with conjugate  
acid hydrolysis

Citrate QuEChERS

NL-Method

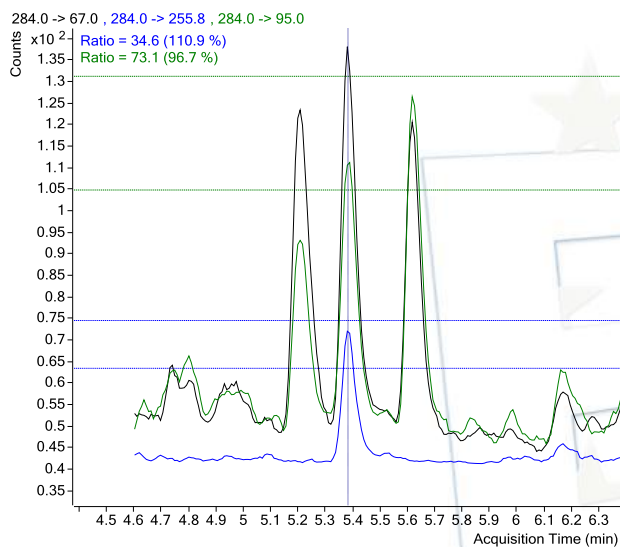


| <b>Recoveries in Avocado (0.01 mg/kg)</b> |                 |                 |
|-------------------------------------------|-----------------|-----------------|
|                                           | <b>miniLuke</b> | <b>QuEChERS</b> |
| <b>Chlorpyriphos-methyl</b>               | <b>0 %</b>      | <b>97 %</b>     |
| <b>Phenthoate</b>                         | <b>0 %</b>      | <b>92 %</b>     |

# Procymidone LC-MS/MS in tomato

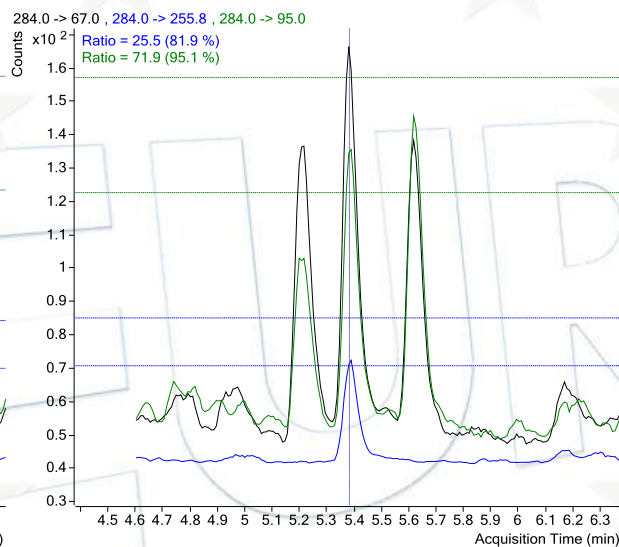
## INSTRUMENT A

0.200 mg/kg



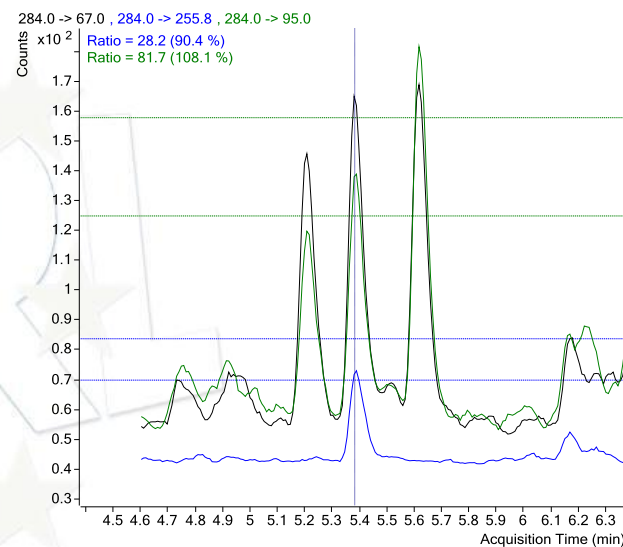
Sheath gas  
temperature:  
200°C

Area: 329 counts



Sheath gas  
temperature:  
275°C

Area: 397 counts



Sheath gas  
temperature:  
375°C

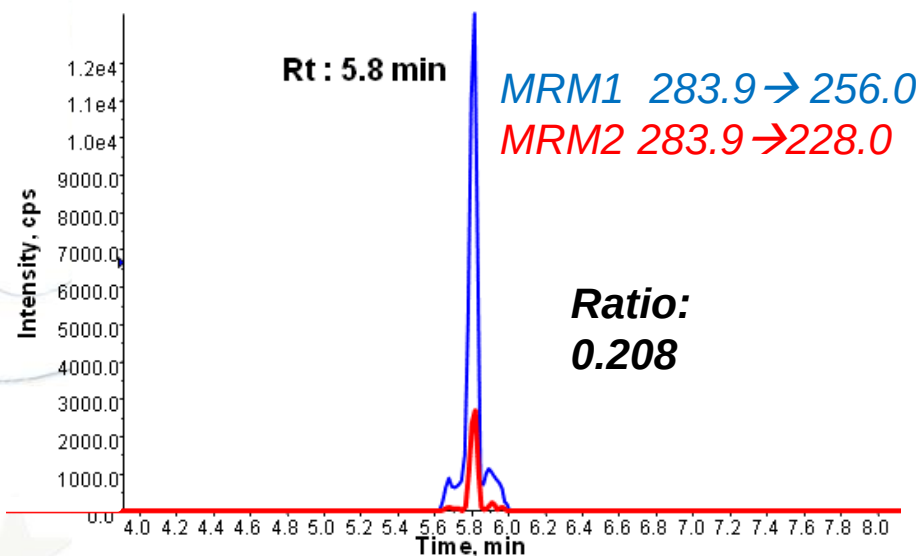
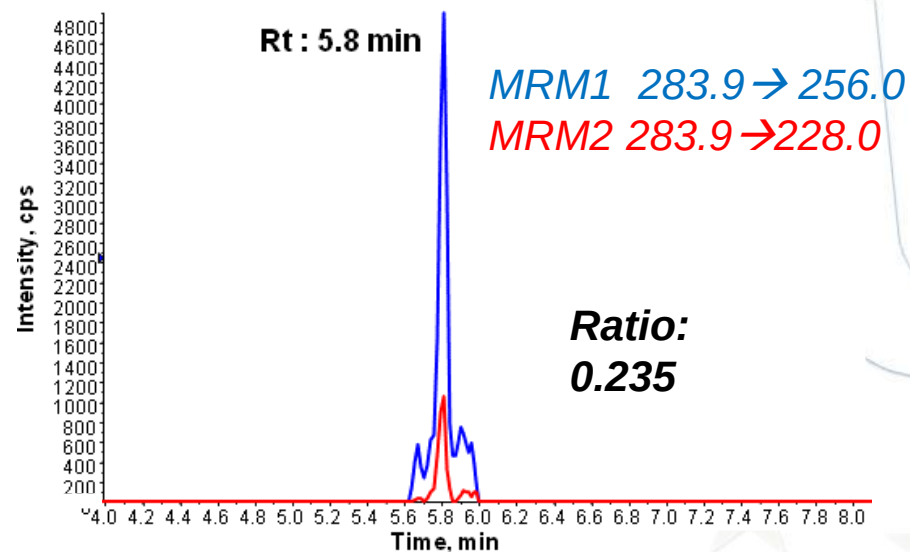
Area: 384 counts

# Procymidone LC-MS/MS in tomato

INSTRUMENT B

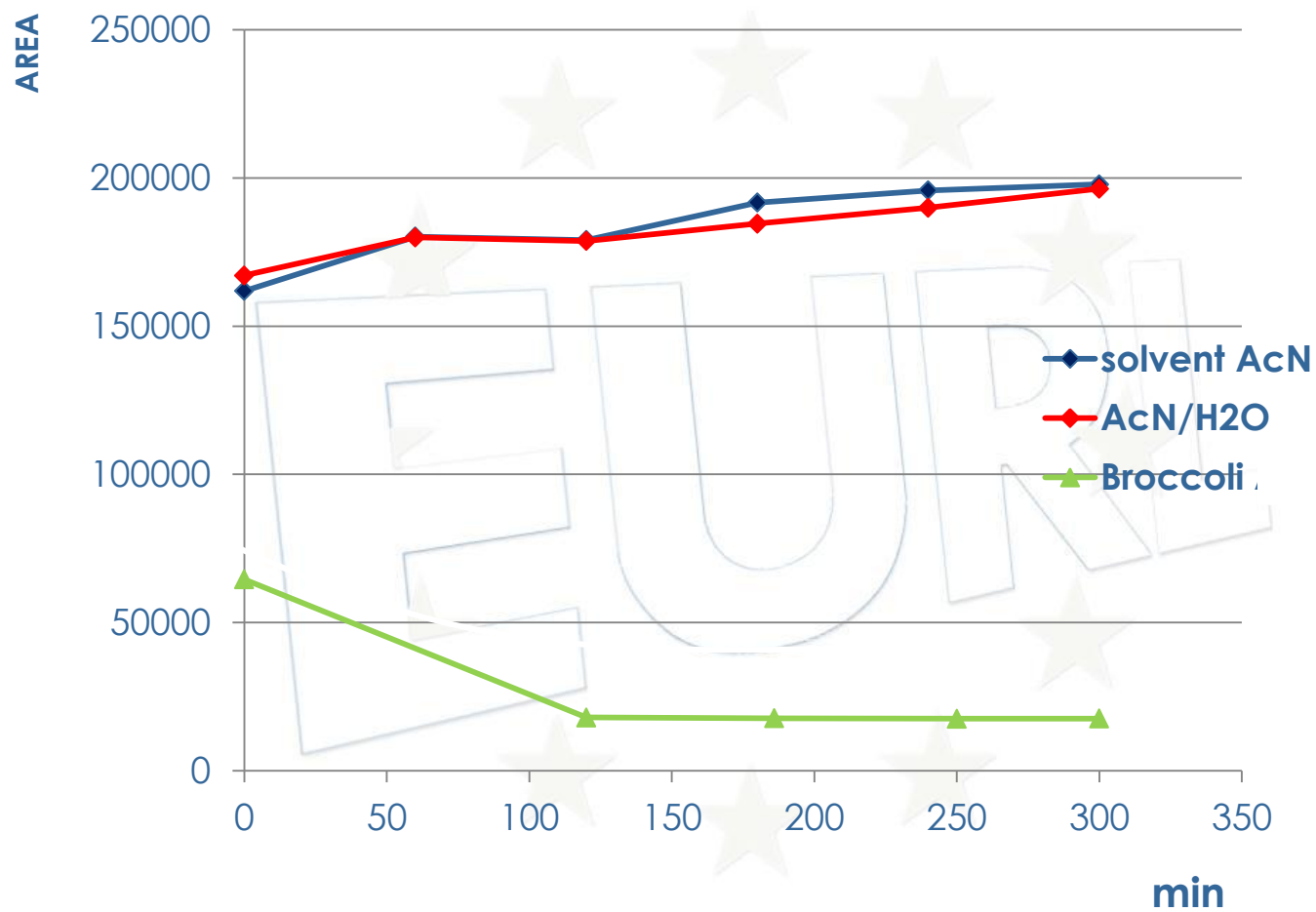
10ppb Procymidone dilx30

20ppb Procymidone dilx30



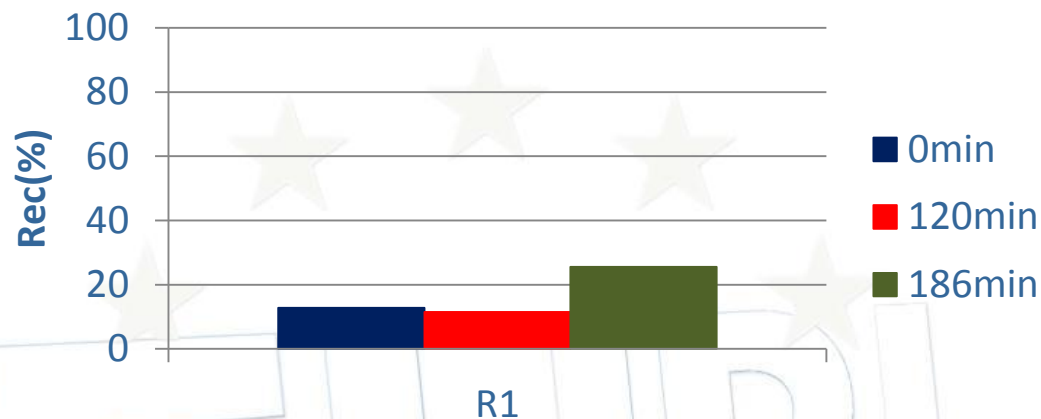


## Diafenthiuron

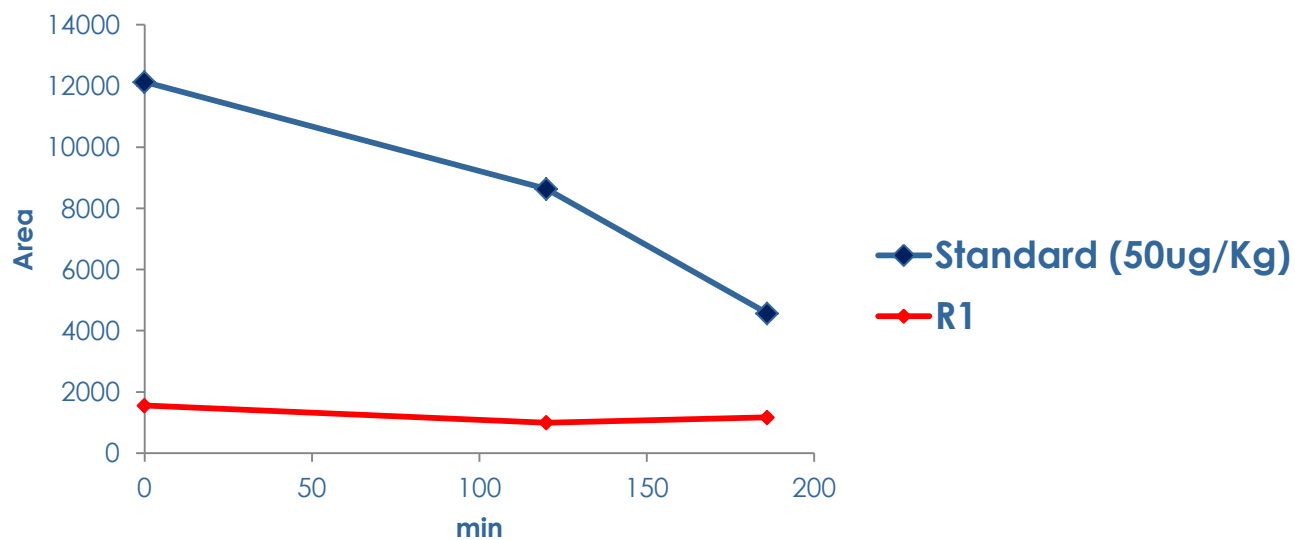


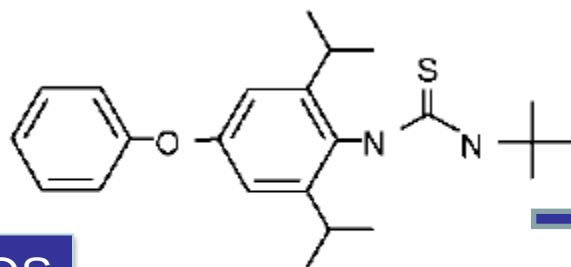
# LC-QQQ

## Recoveries at different injection time



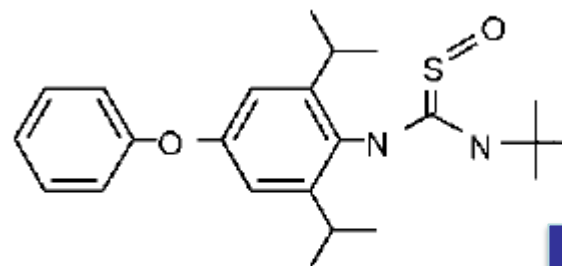
## Diafenthuron Broccoli





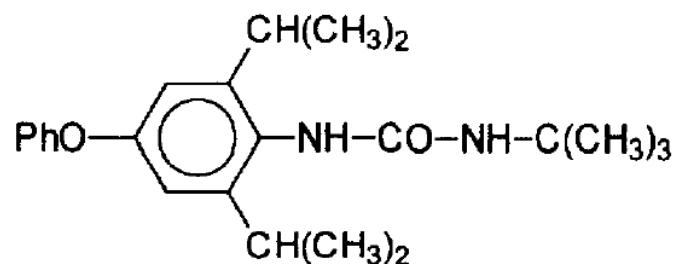
Diafenthiuron (I)

$C_{23}H_{32}N_2OS$   
385.2308



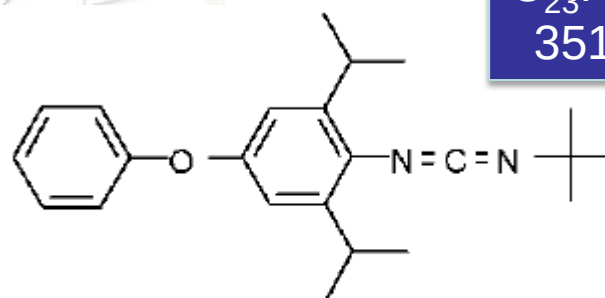
Sulfomonoxyde (IV)

$C_{23}H_{32}N_2O_2S$   
401.2257



urea

$C_{23}H_{32}N_2O_2$   
369.2536

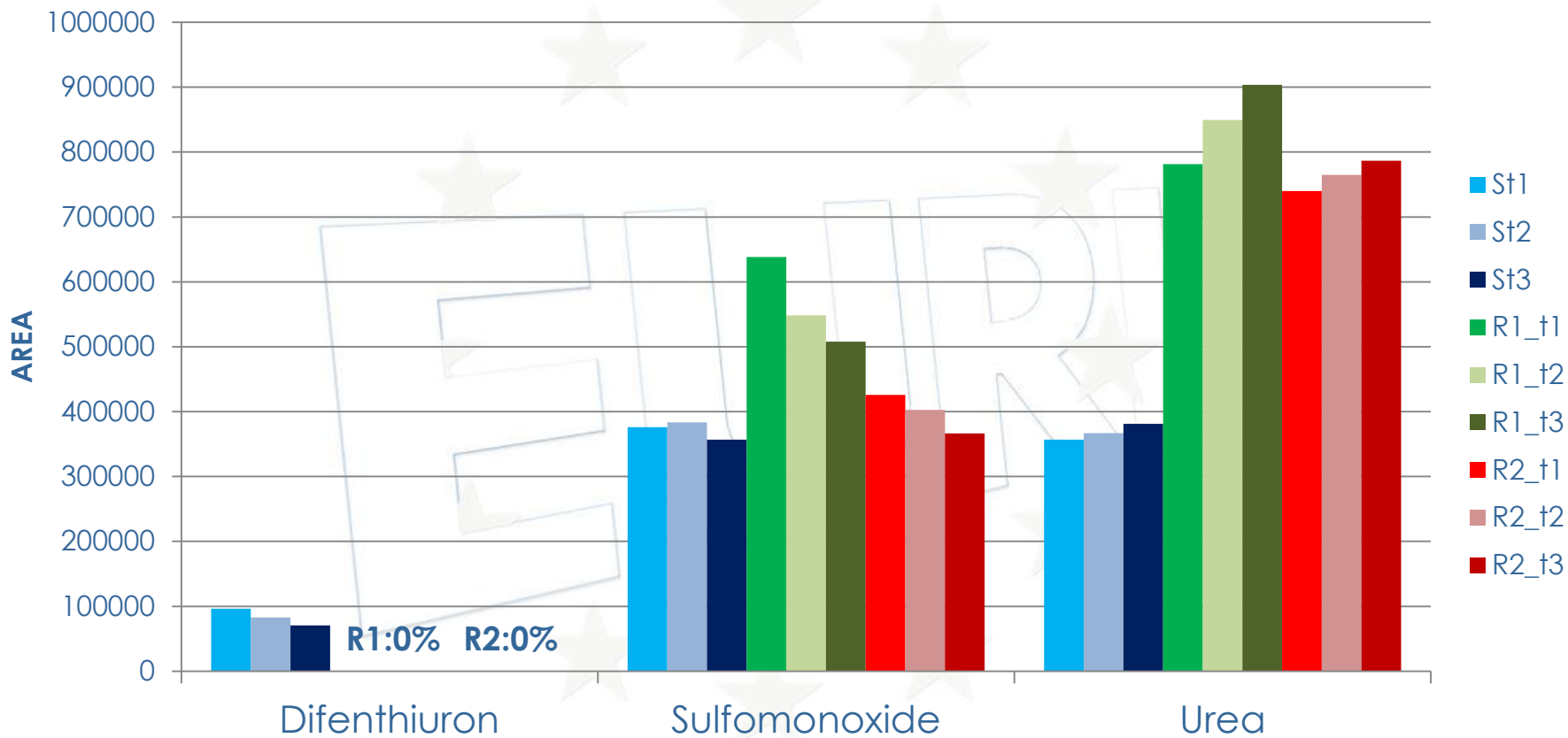


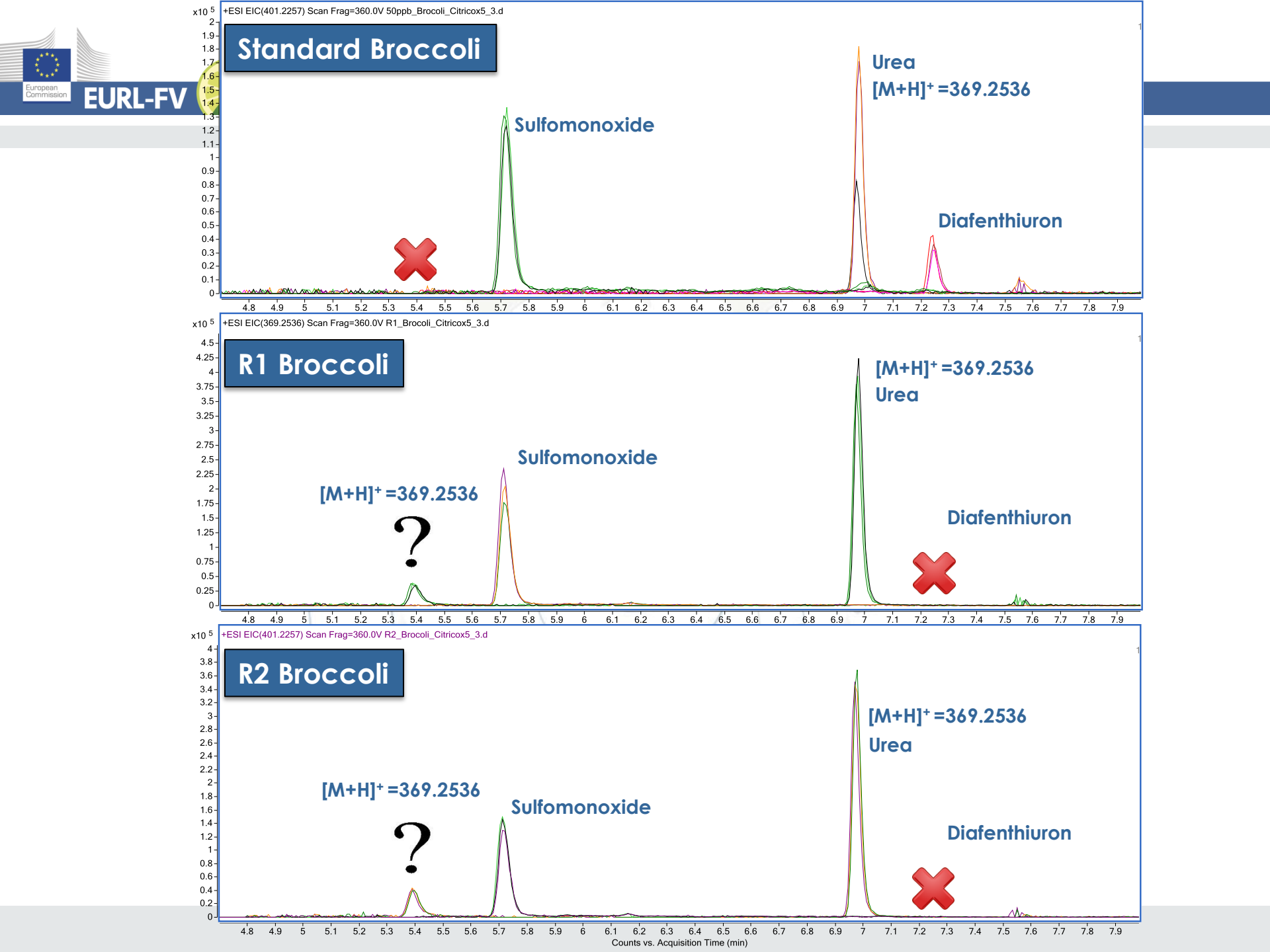
Carbodiimide (II)

$C_{23}H_{30}N_2O_2$   
351.2430

# LC-QToF

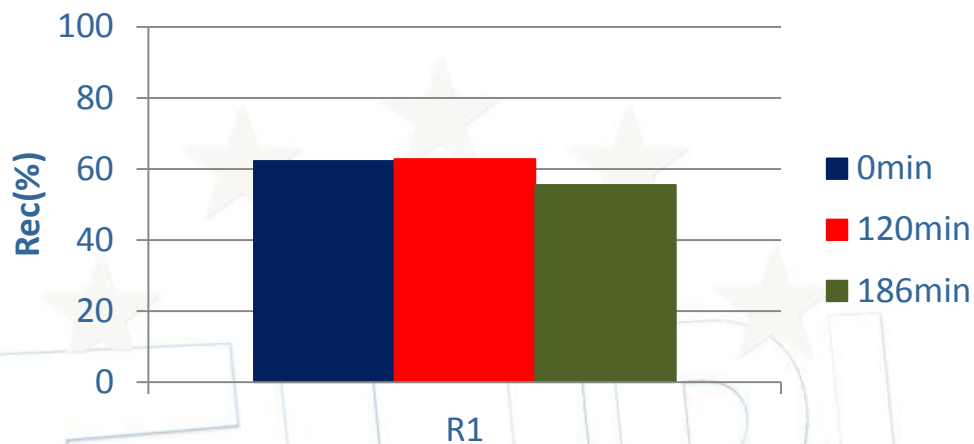
## Diafenthiuron Broccoli



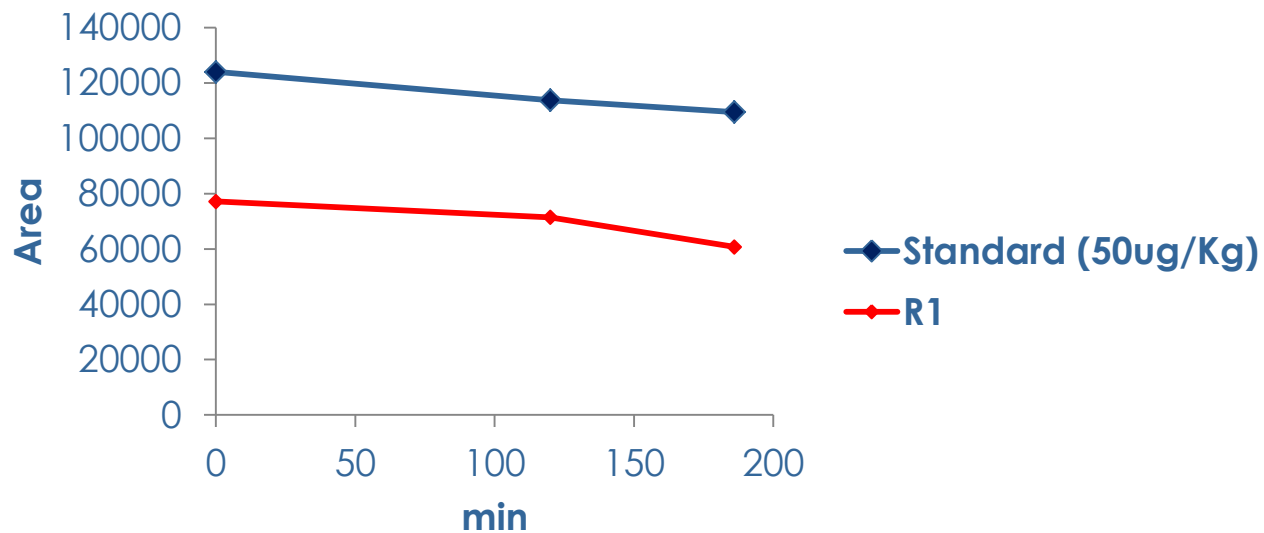


# LC-QQQ

## Recoveries at different injection time

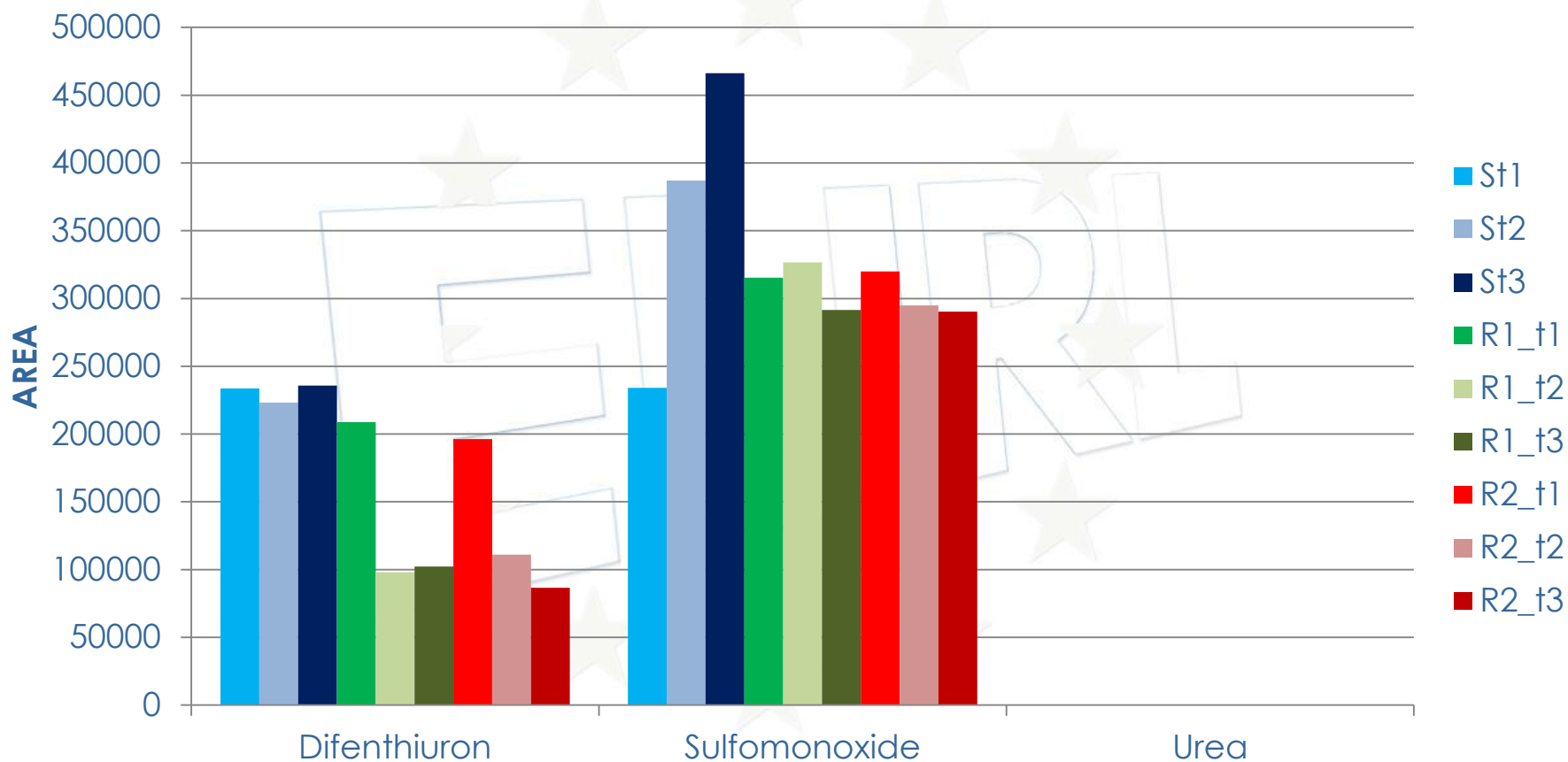


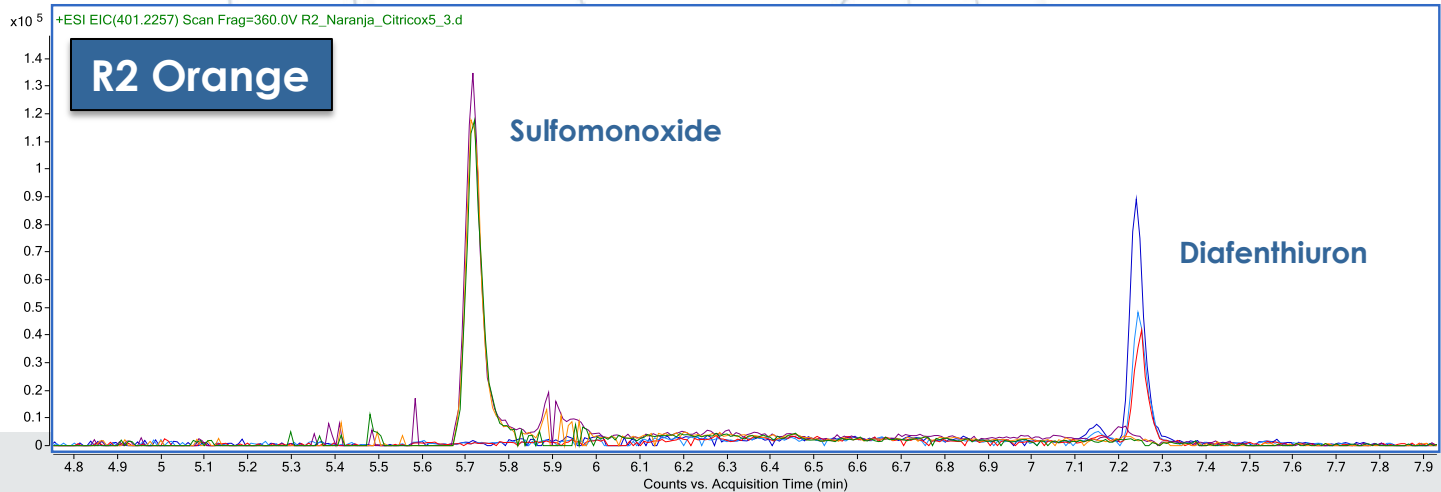
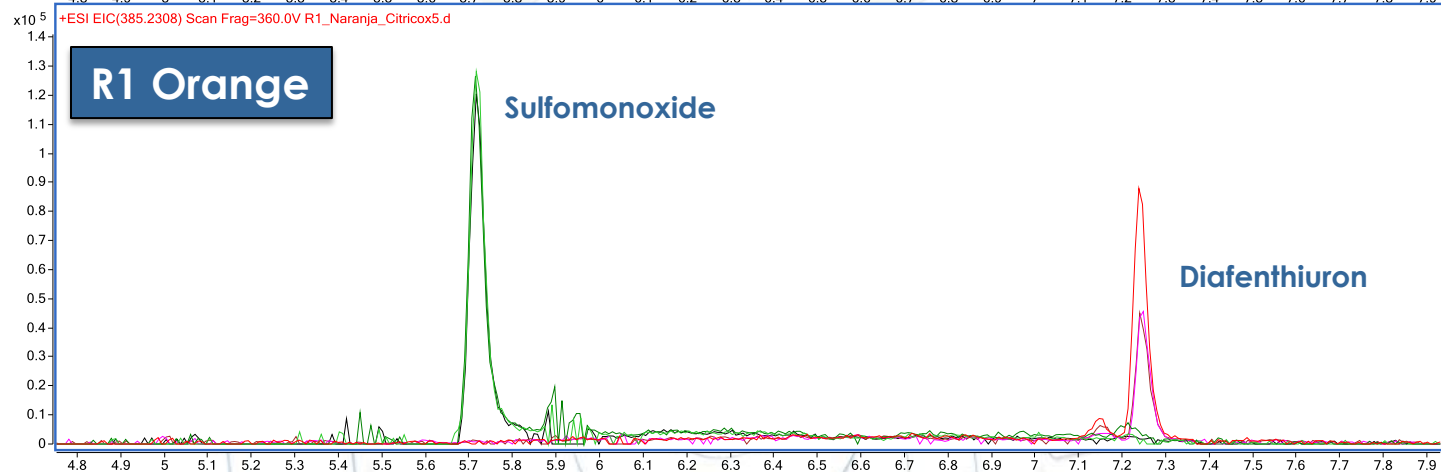
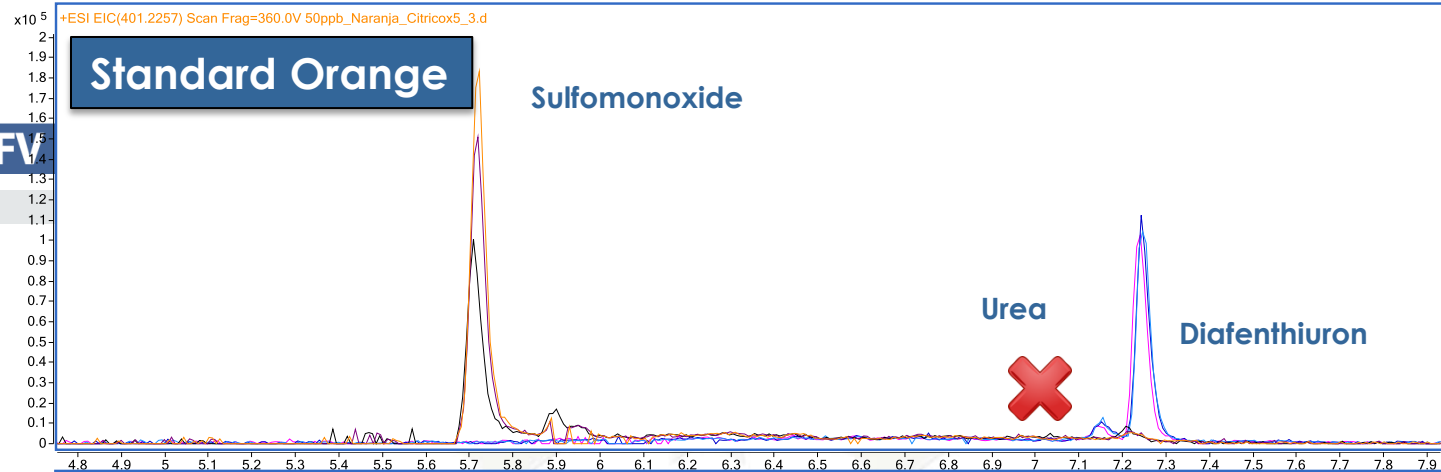
## Diafenthuron Orange



# LC-QToF

# Diafenthiuron Orange

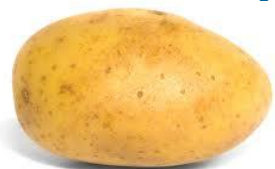








## Residue definition: Methomyl and thiodicarb (sum of methomyl and thiodicarb expressed as methomyl)

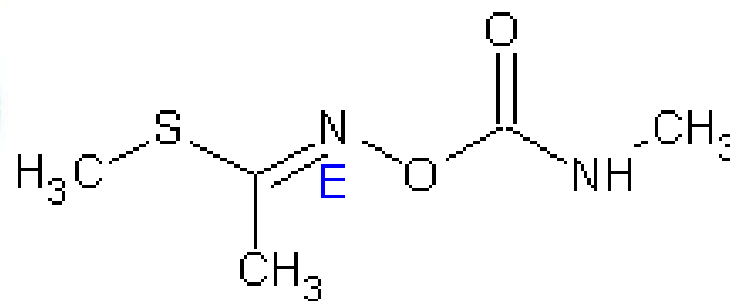
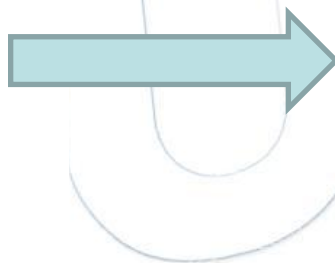
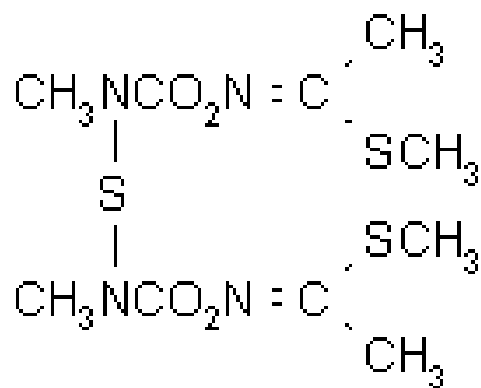


ARfD: 0,0025

**Thiodicarb** ARfD: 0,01

Present in the sample

**Methomyl**



Not Approved

Approved

0.07 mg/Kg

0.03 mg/Kg



## What are we looking for in a MRM?

- 1-Acceptable performance for X? Compounds
- 2-Compatible with LC and GC-MS/MS (no evaporation)
- 3-Selective
- 4-Low matrix effects
- 5-Low organic solvent consumption
- 6-Cheap and quick
- 7-Extensive information available



*[www.eurl-pesticides.eu](http://www.eurl-pesticides.eu)*

**Thank You  
for Your Attention**

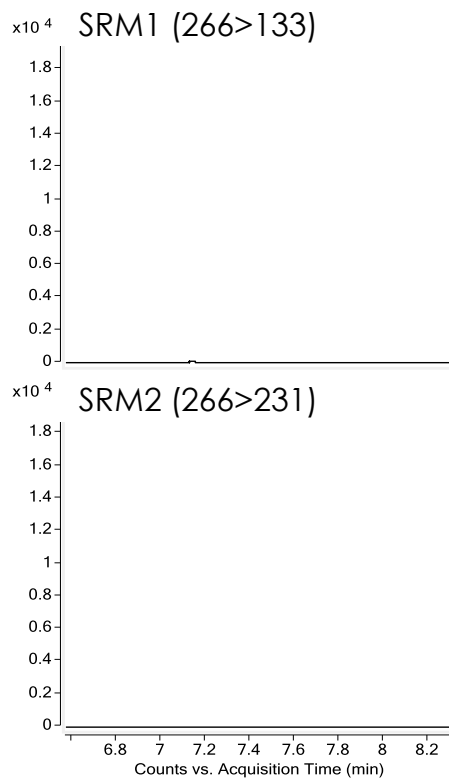


**EURL**

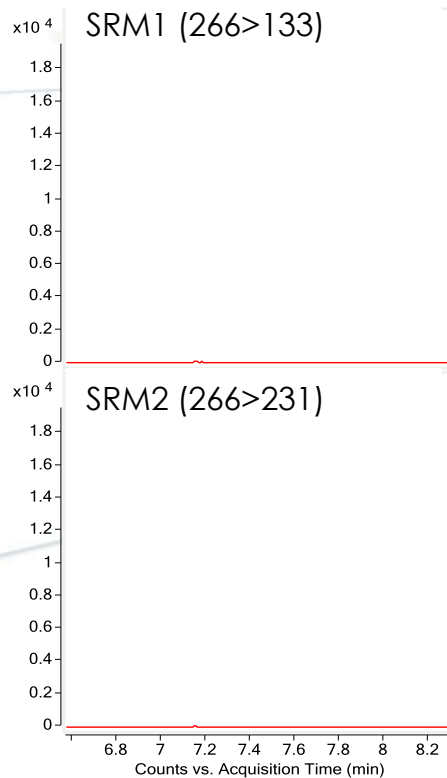
EUROPEAN  
UNION  
REFERENCE  
LABORATORY

## Citrate QuEChERS

**100 µg/kg  
Std-Chlorothalonil  
in Broccoli**

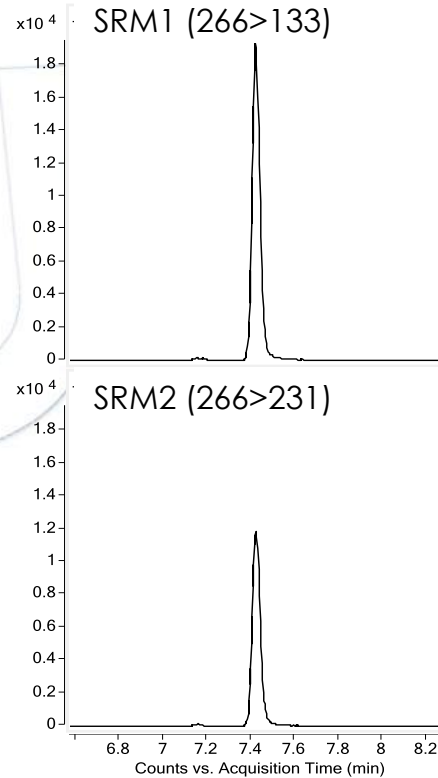


**Recovery at 100 µg/kg  
Chlorothalonil in Broccoli**

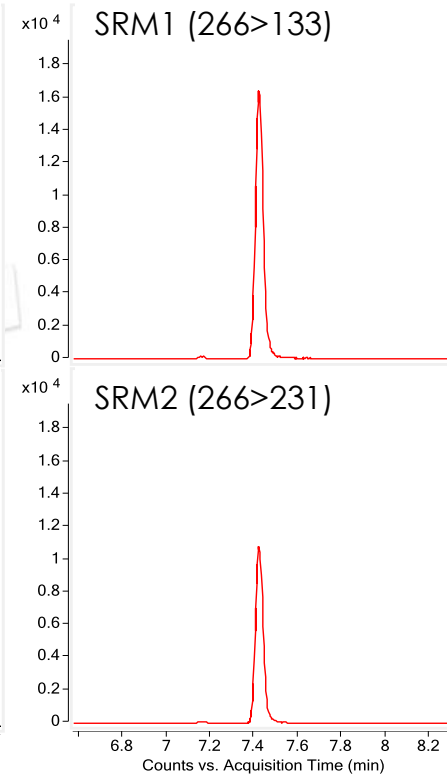


## QuEChERS with acid hydrolysis

**100 µg/kg  
Std-Chlorothalonil  
in Broccoli**

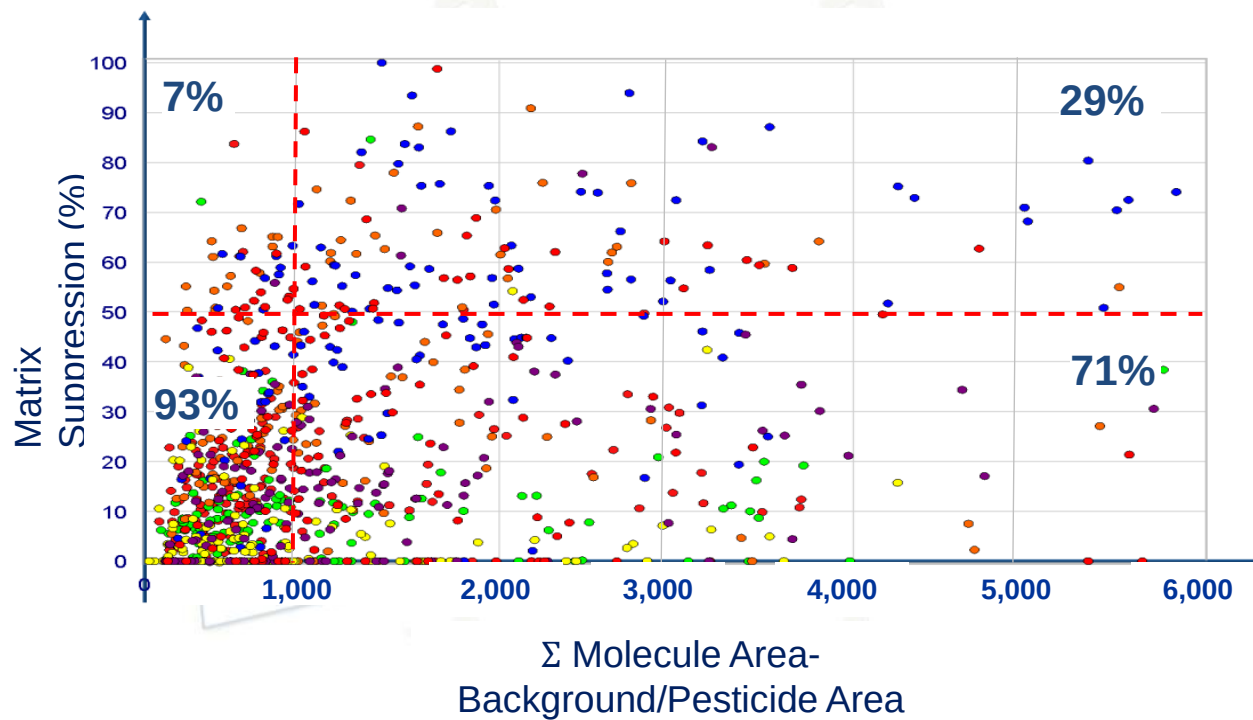


**Recovery at 100 µg/kg  
Chlorothalonil in Broccoli**





Cucumber ■ Pepper ■ Tomato ■ Grape ■ Leek ■ Orange ■ Broccoli ■



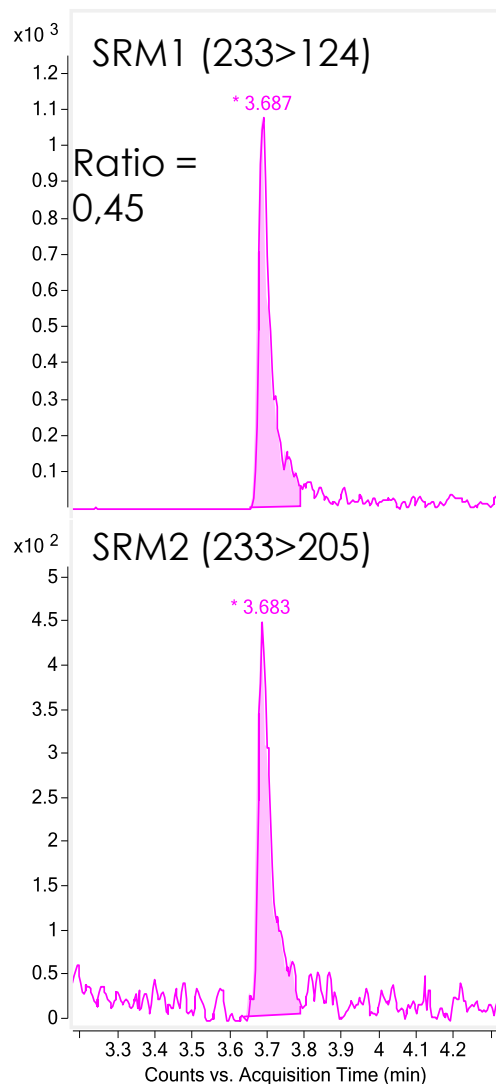


**Evaluation of Matrix effects  
vs  
Multi Residue Methods  
Evaluation of specific steps**

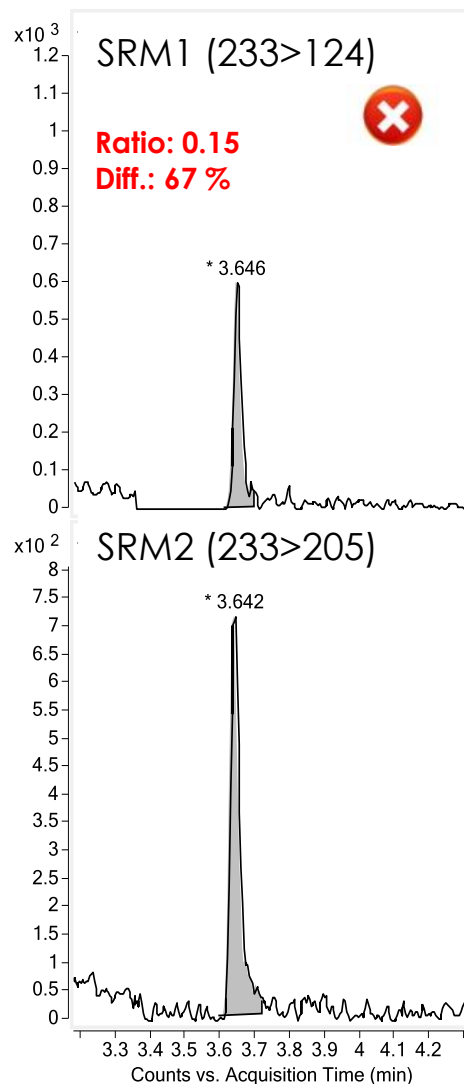


# Chlorbromuron at 10 µg/kg in Cherry. GC-MS/MS

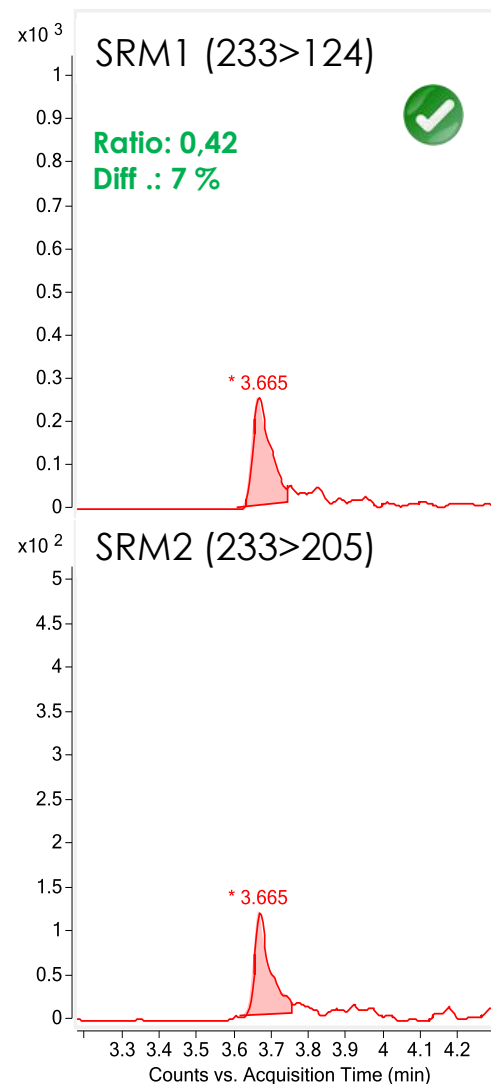
Tomato spiked at  
10 µg/L



QuEChERS without  
clean-up

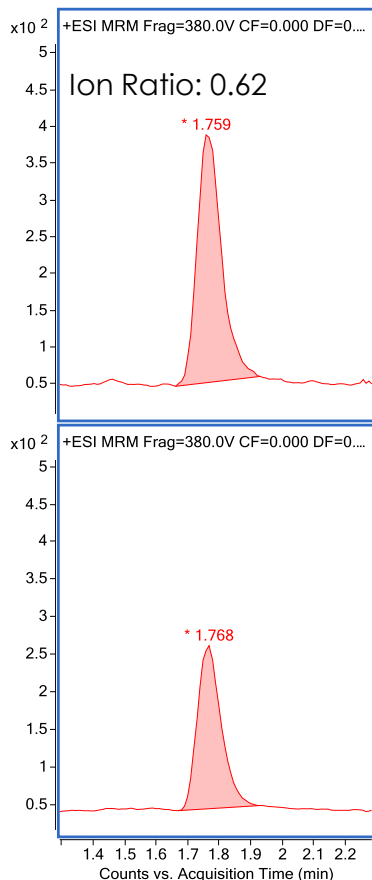


Citrate QuEChERS  
method

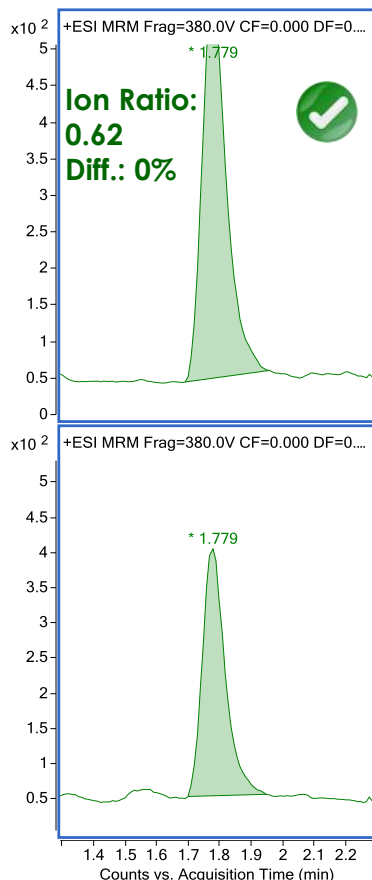


# Aldicarb sulfone at 10 µg/kg in Parsley. LC-MS/MS

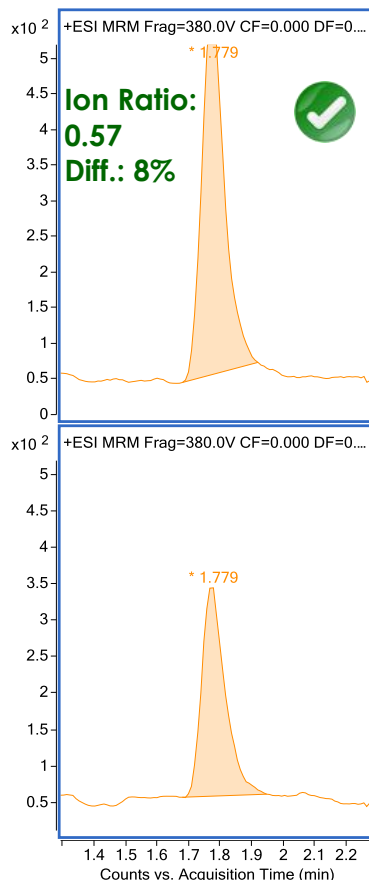
Tomato spiked  
at 10 µg/L



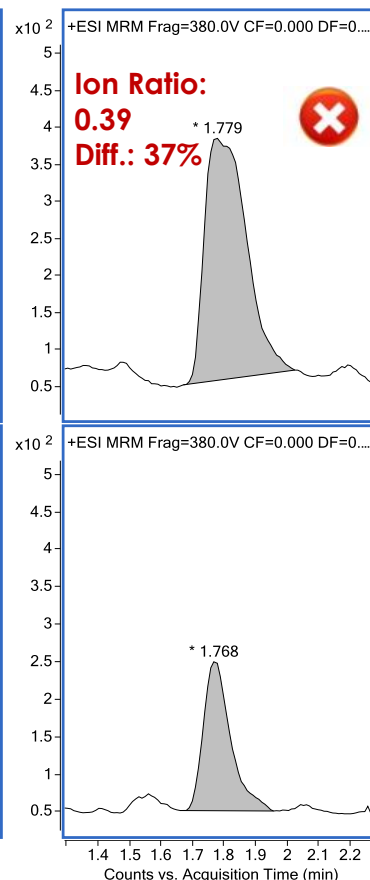
Ethyl Acetate  
method



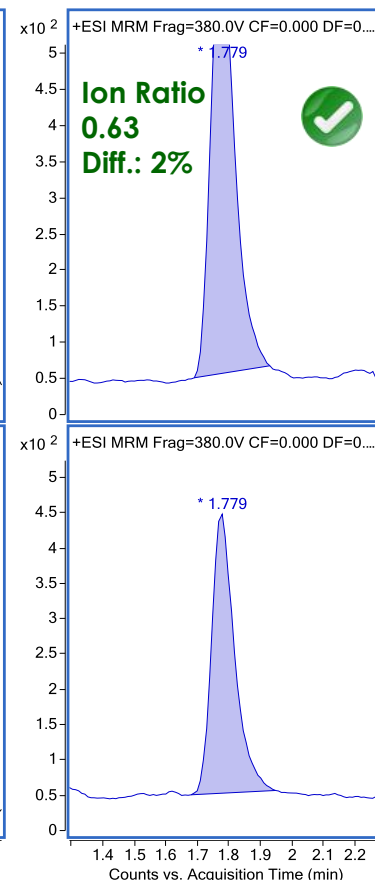
NL method



Citrate QuEChERS  
without clean-up  
method



QuEChERS  
clean-up



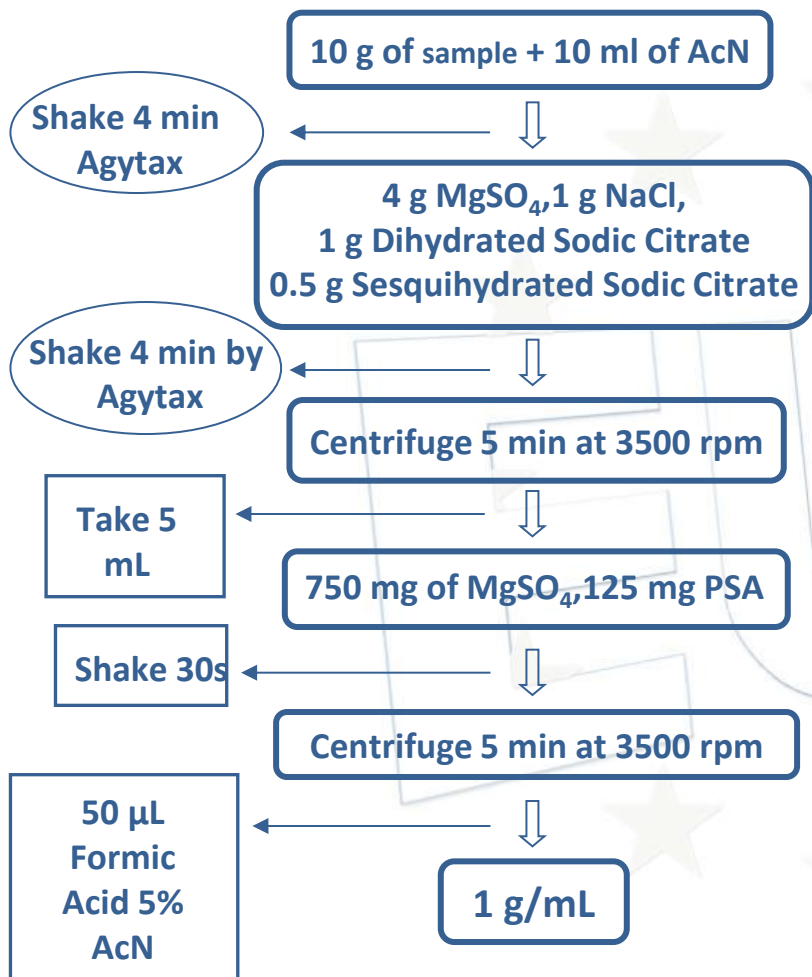


# Chlorothalonil

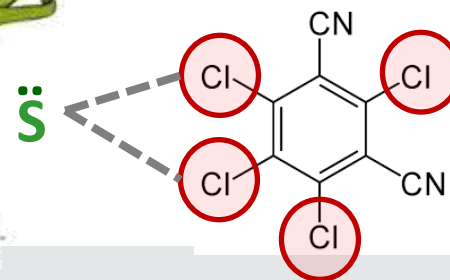
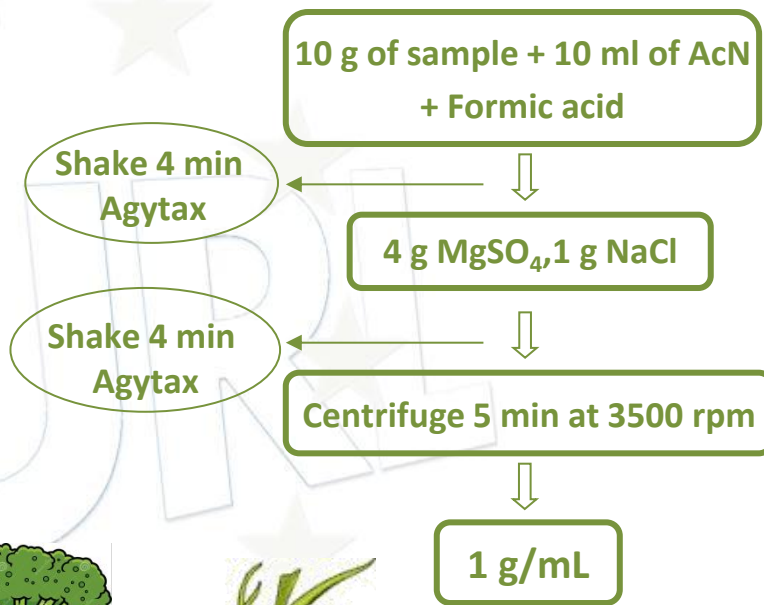
## NL-Method Results

| Matrix     | Recov. (%) | RSD (%) |
|------------|------------|---------|
| Broccoli   | 87         | 13      |
| Red Pepper | 96         | 9       |
| Orange     | 119        | 13      |
| Avocado    | 85         | 6       |

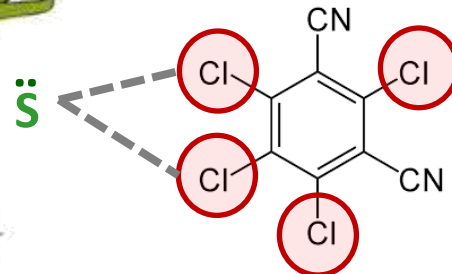
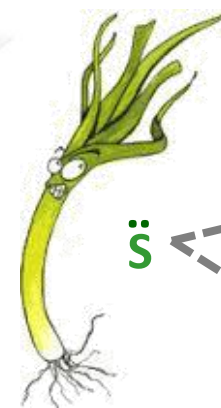
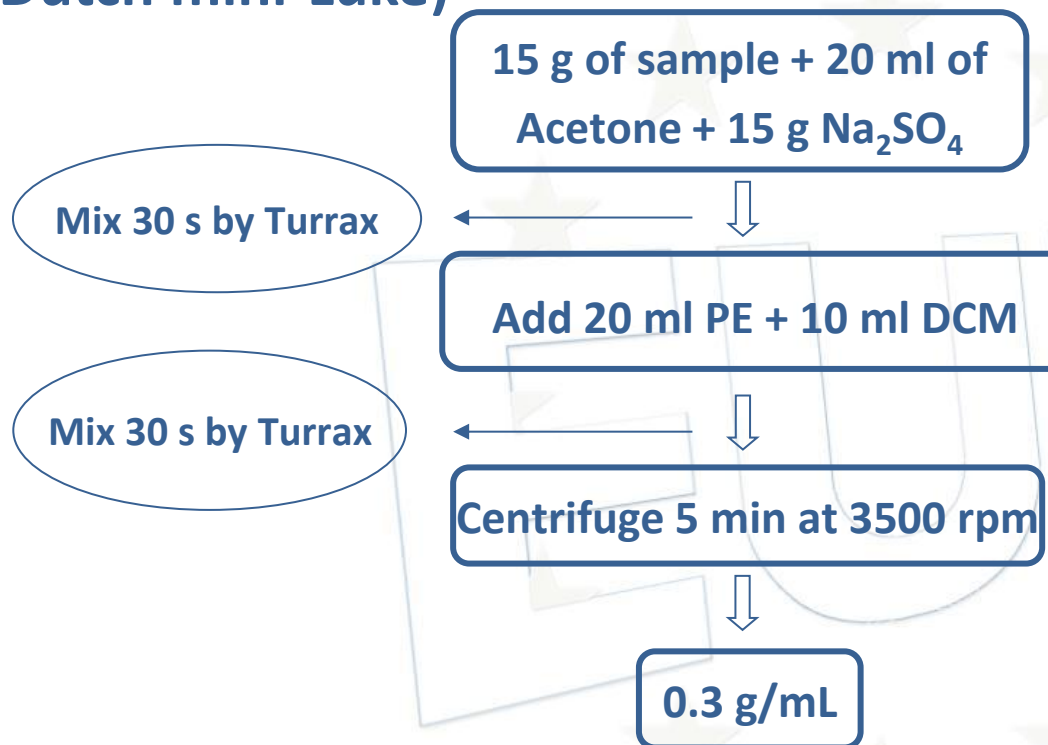
## Citrate QuEChERS



## QuEChERS with conjugate acid hydrolysis

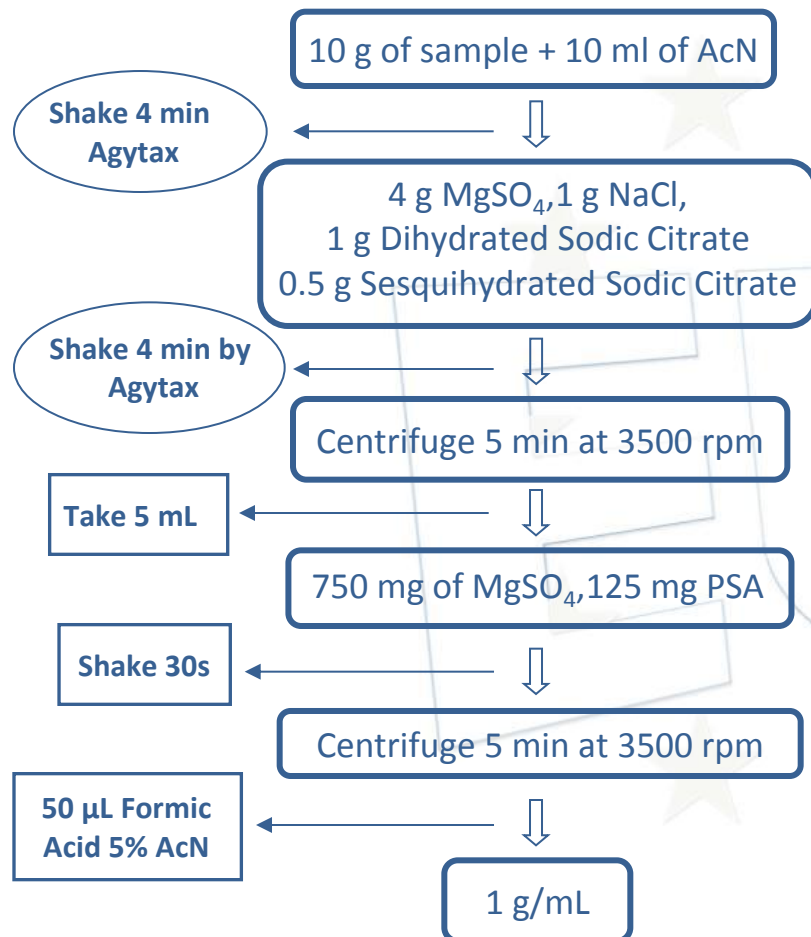


# NL-Method (Dutch mini-Luke)

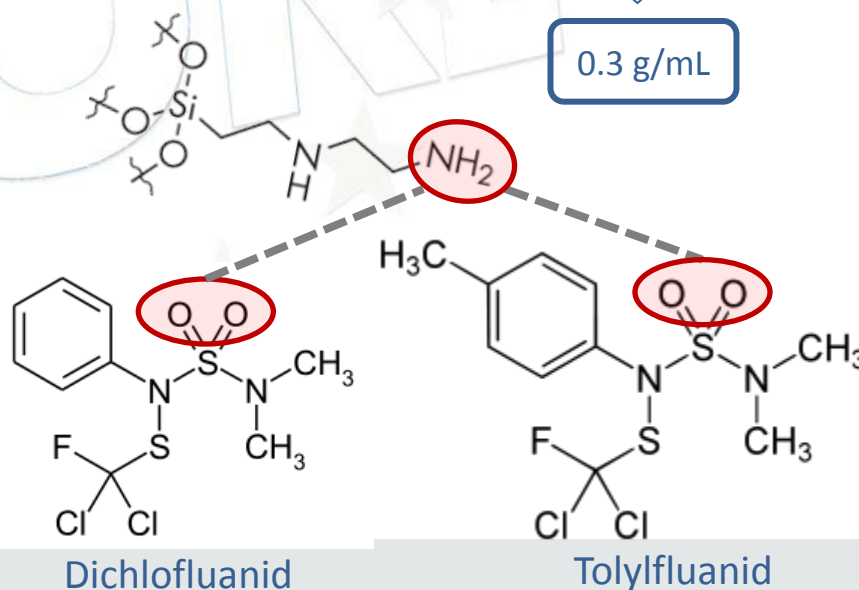
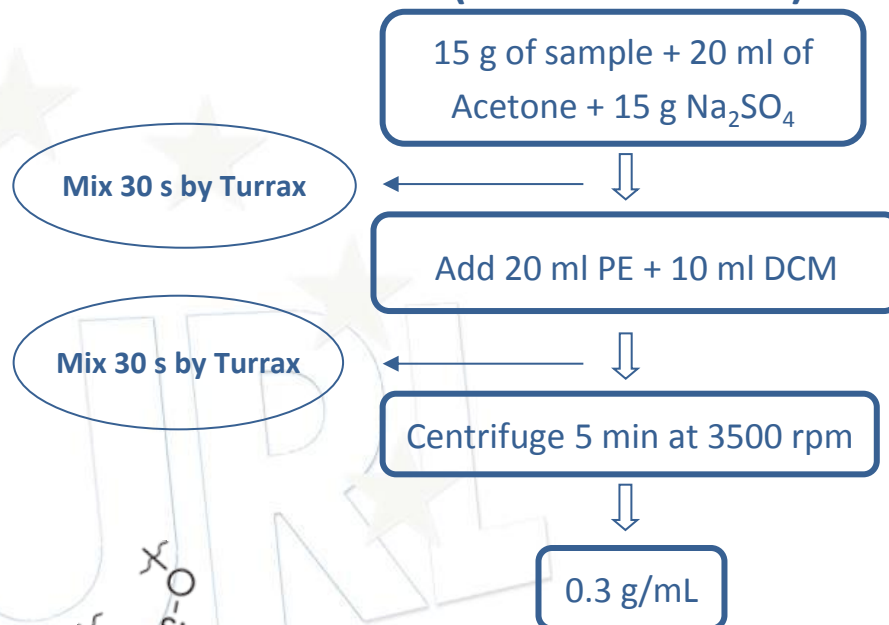




## Citrate QuEChERS without clean-up



## NL-Method (Dutch mini-Luke)

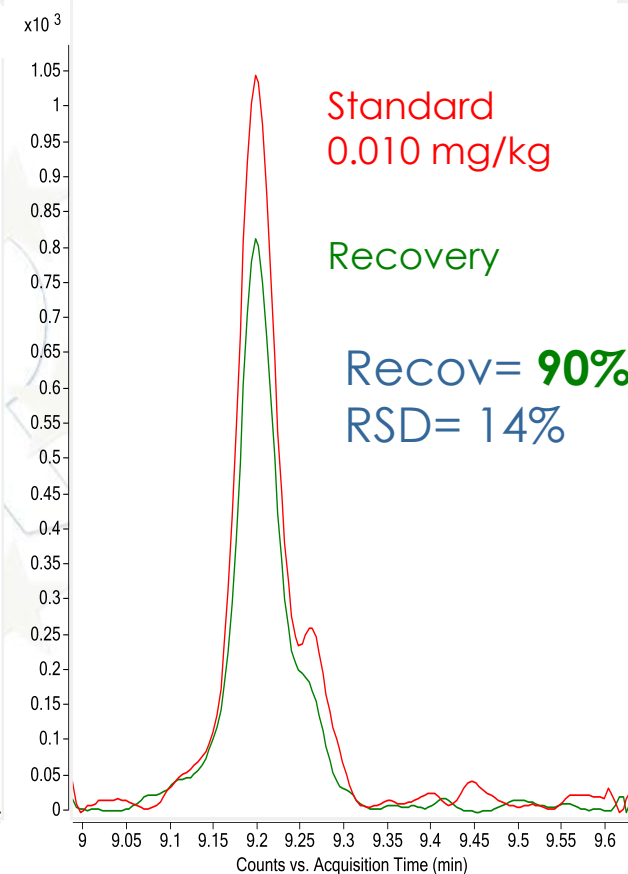
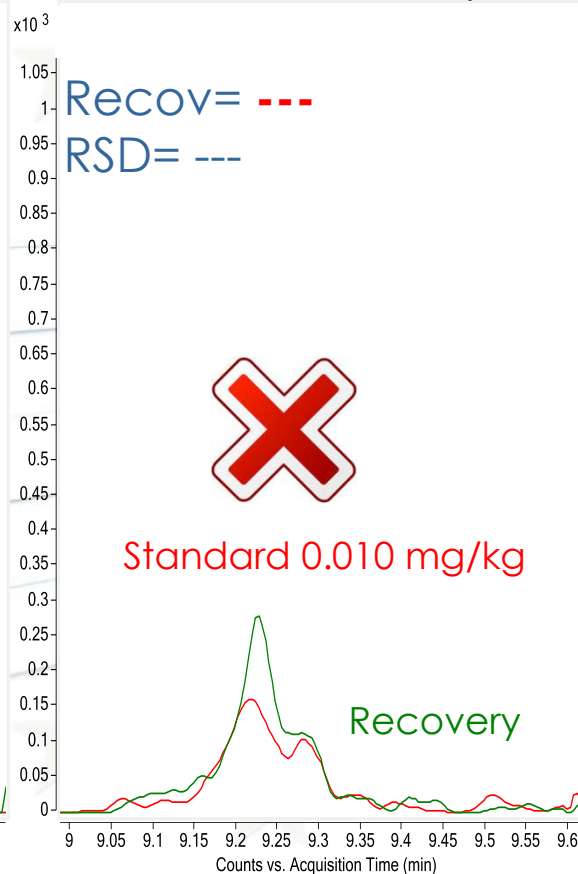
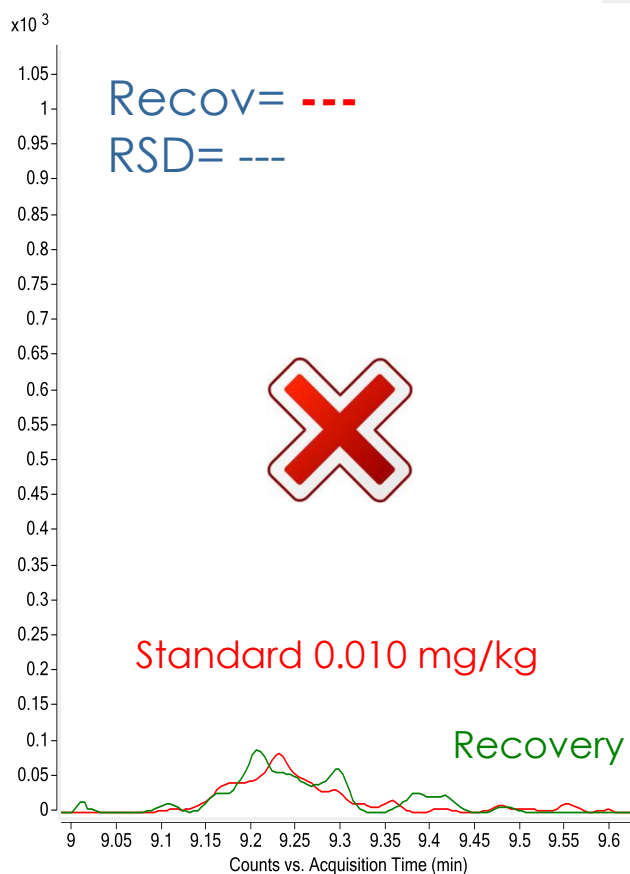


## Broccoli QuEChERS

without clean-up

NL-Method

Citrate QuEChERS



Citrate QuEChERS with  $\text{ZrO}_2$  instead of PSA:

Recov = 61%      RSD = 6%

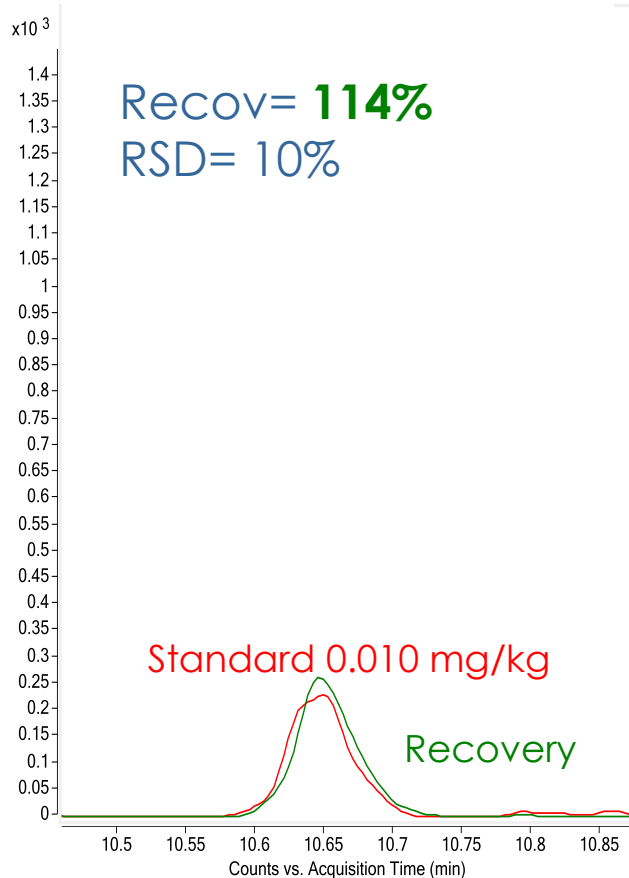
## Broccoli

### QuEChERS

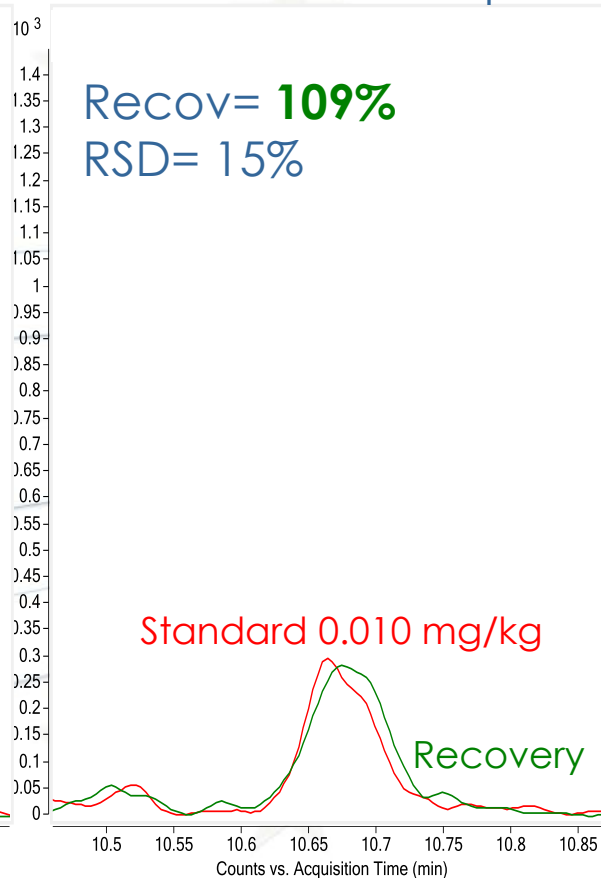
without clean-up

### Citrate QuEChERS

Recov= **114%**  
RSD= 10%



Recov= **109%**  
RSD= 15%

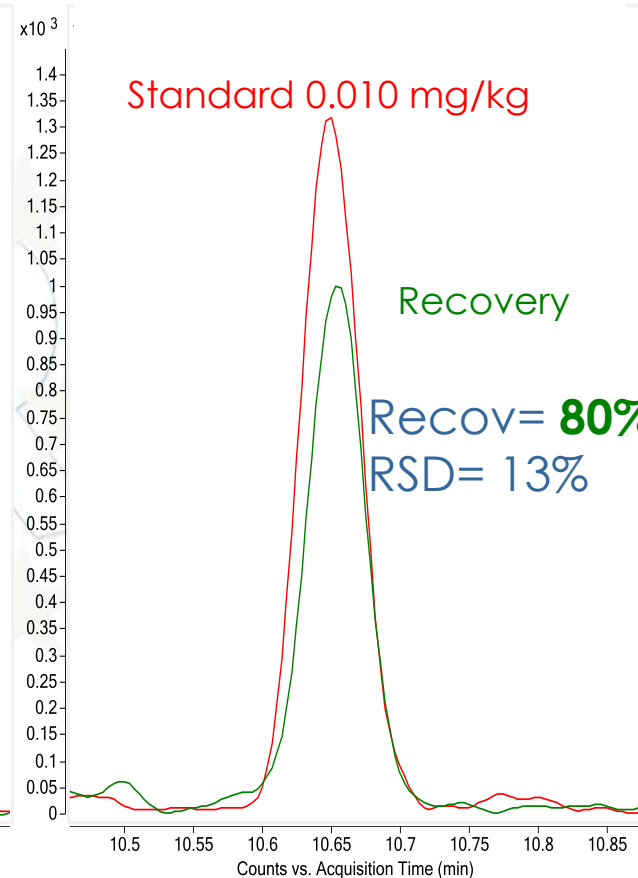


### NL-Method

Standard 0.010 mg/kg

Recovery

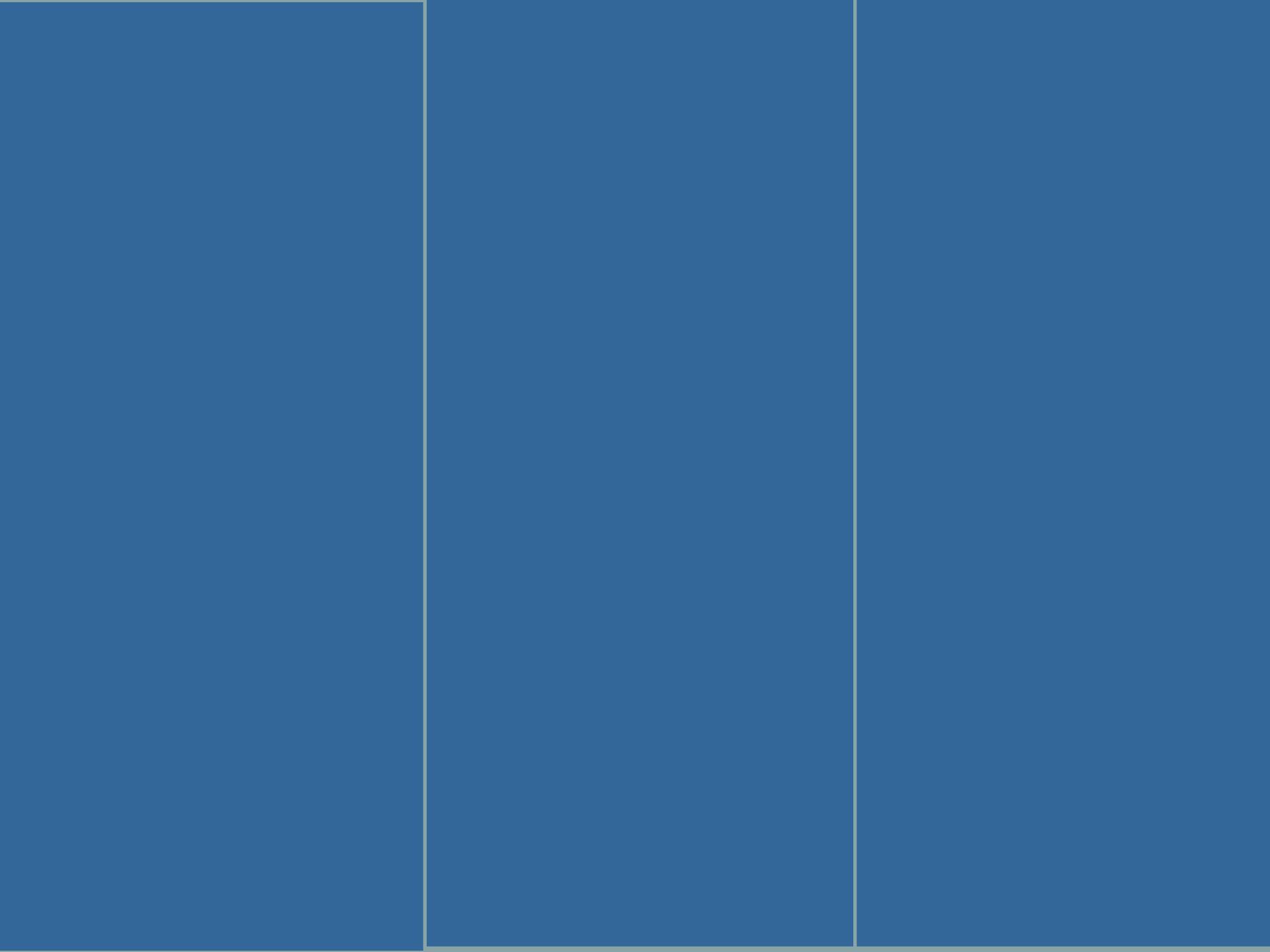
Recov= **80%**  
RSD= 13%

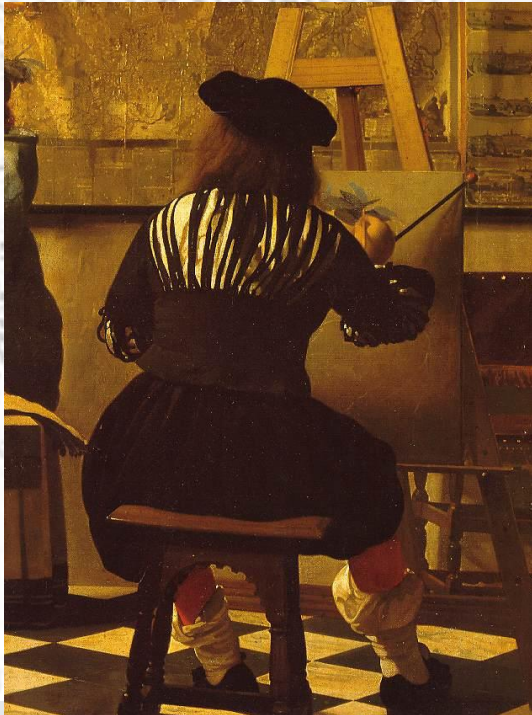


Citrate QuEChERS with **ZrO<sub>2</sub>** instead of PSA:

Recov = **80%**      RSD = 4%







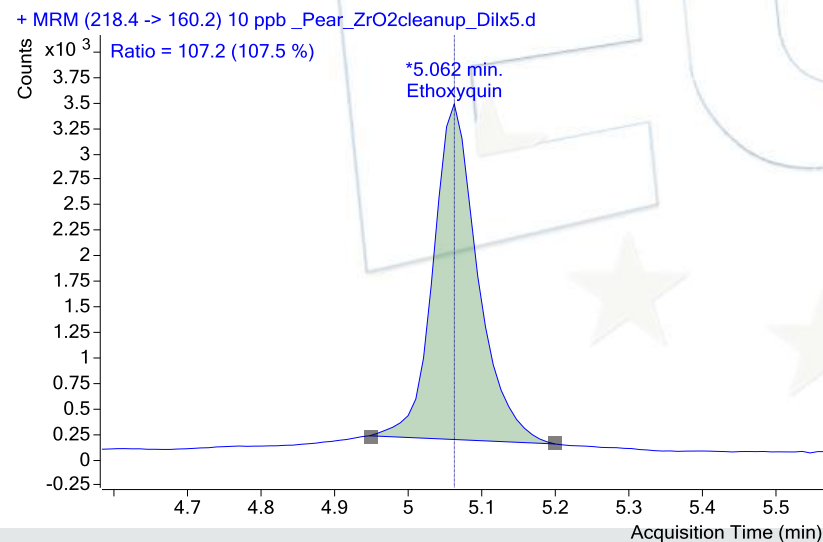
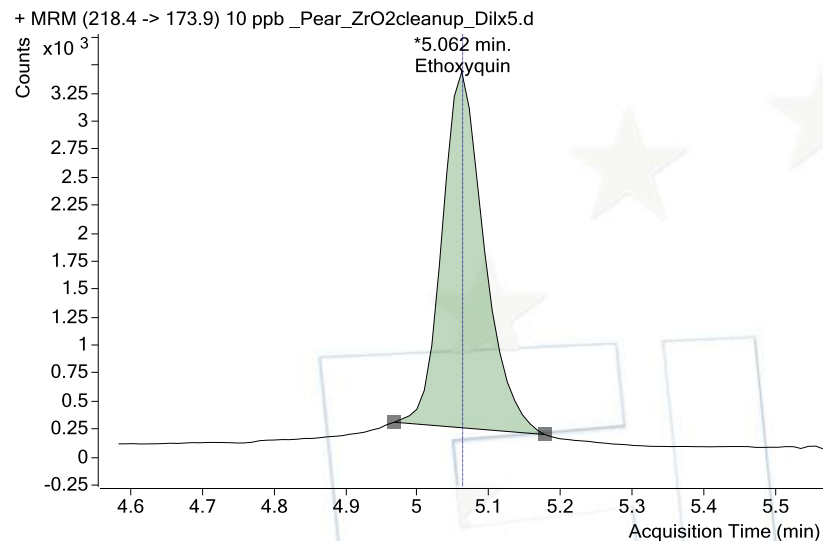


SKETSA MODEL PESAWAT UDARA LEO  
LSM GREENNUSA – SAMSUIR HUS

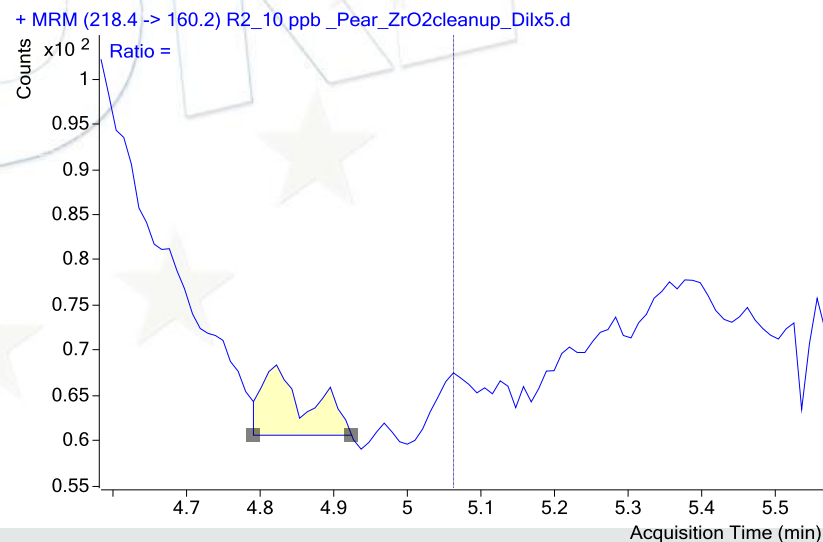
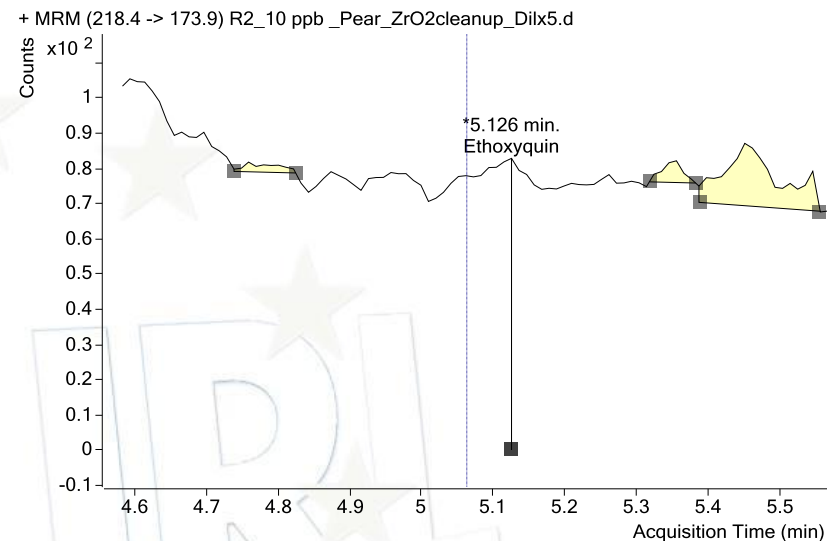




## Standard 10 ppb in Pear



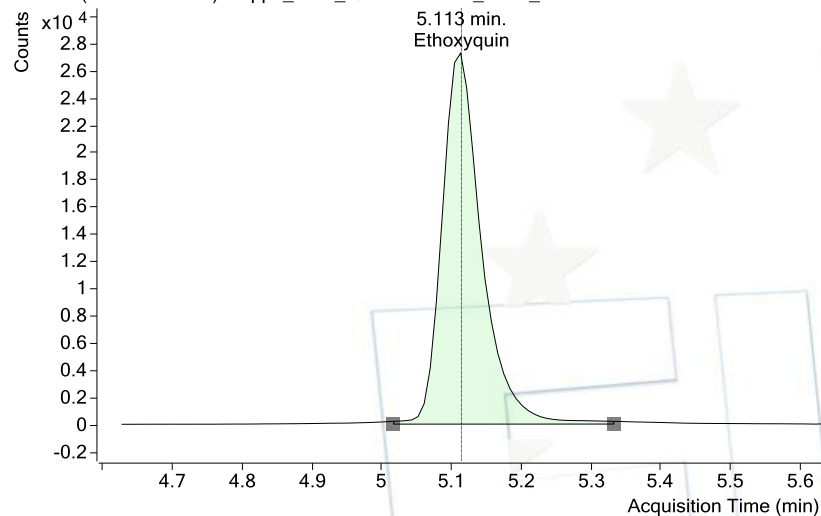
## Recovery 10 ppb in Pear





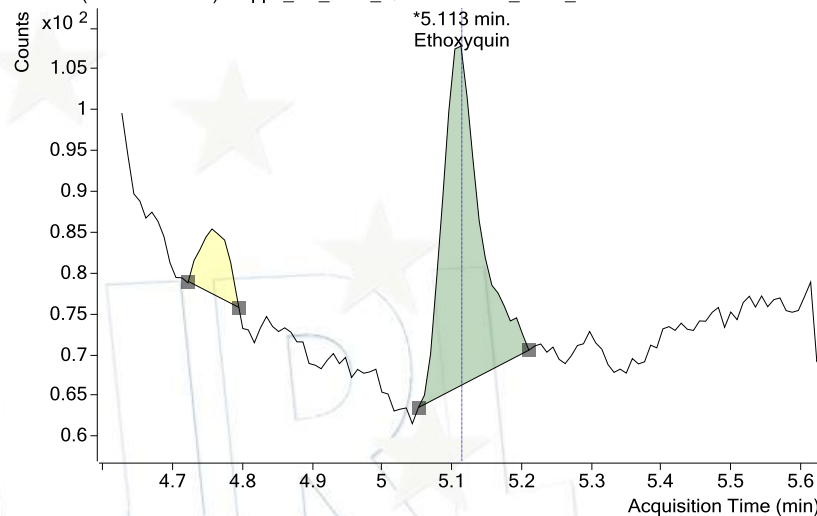
## Standard 50 ppb in Pear

+ MRM (218.4 -> 160.2) 50 ppb\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d

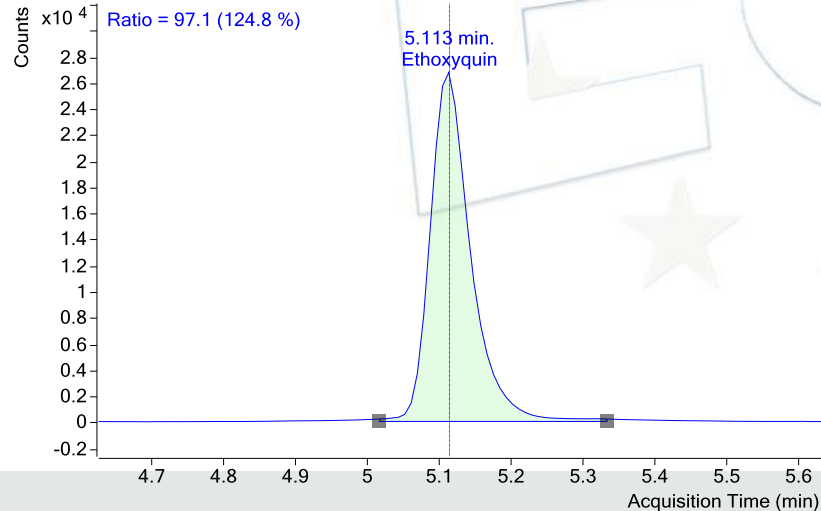


## Recovery 50 ppb in Pear

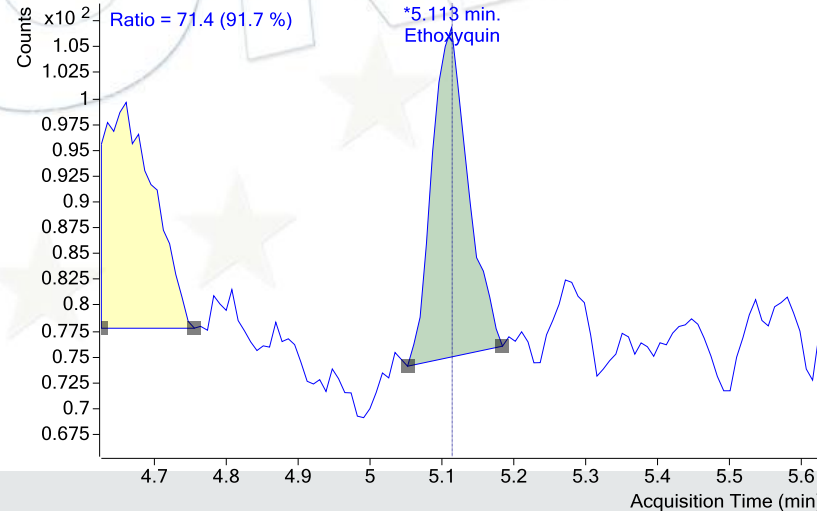
+ MRM (218.4 -> 160.2) 50 ppb\_R2\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d



+ MRM (218.4 -> 173.9) 50 ppb\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d

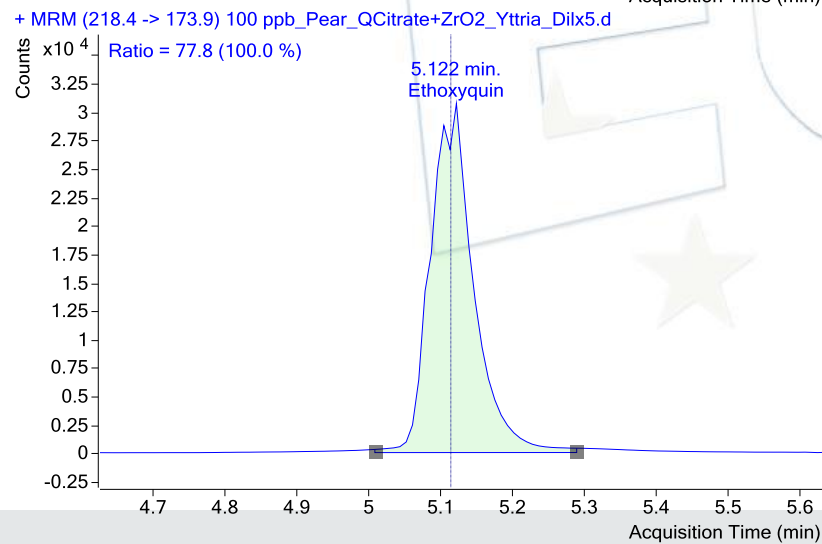
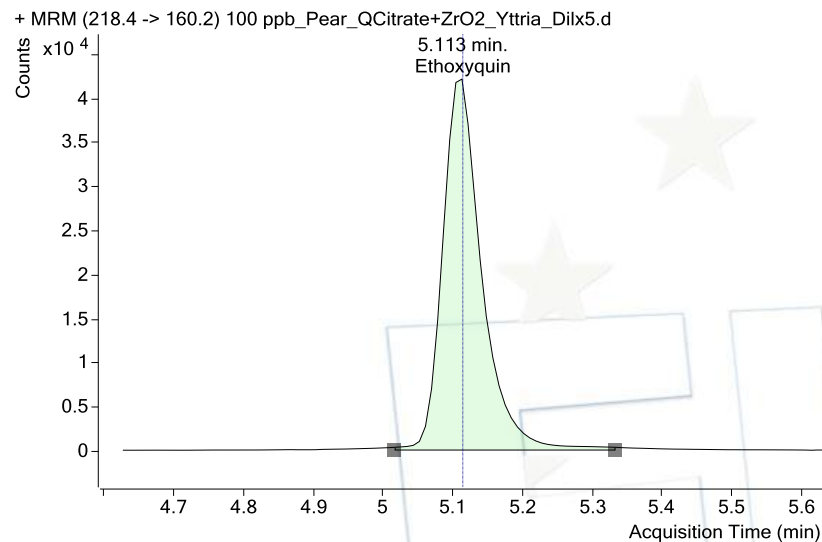


+ MRM (218.4 -> 173.9) 50 ppb\_R2\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d

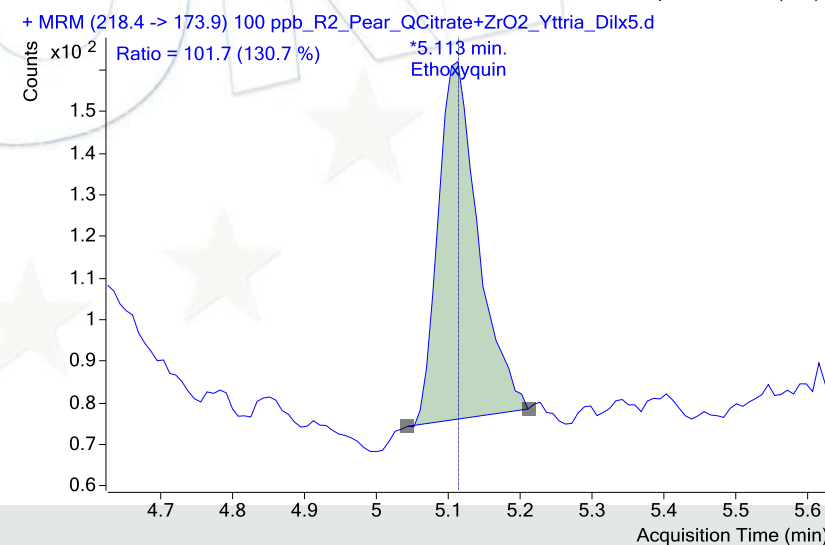
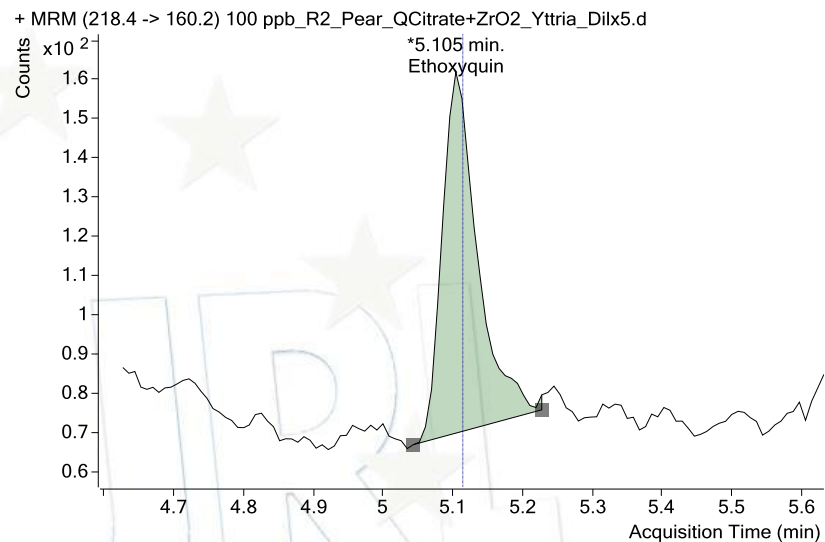




## Standard 100 ppb in Pear



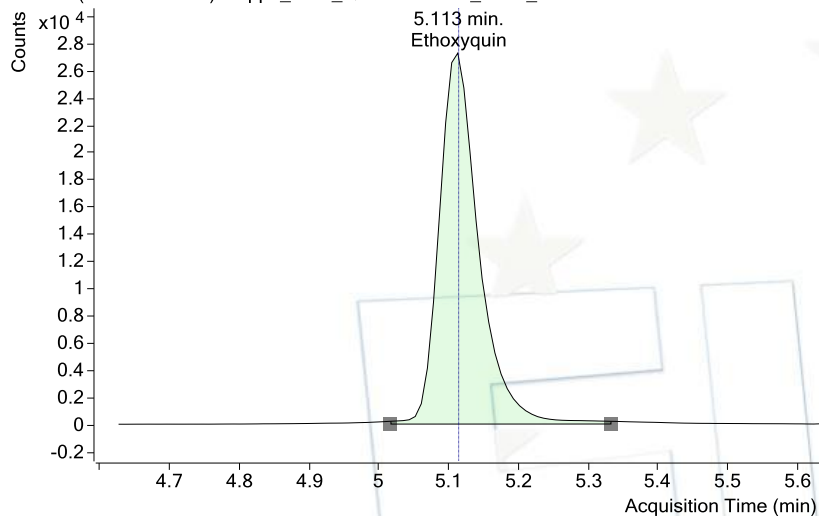
## Recovery 100 ppb in Pear



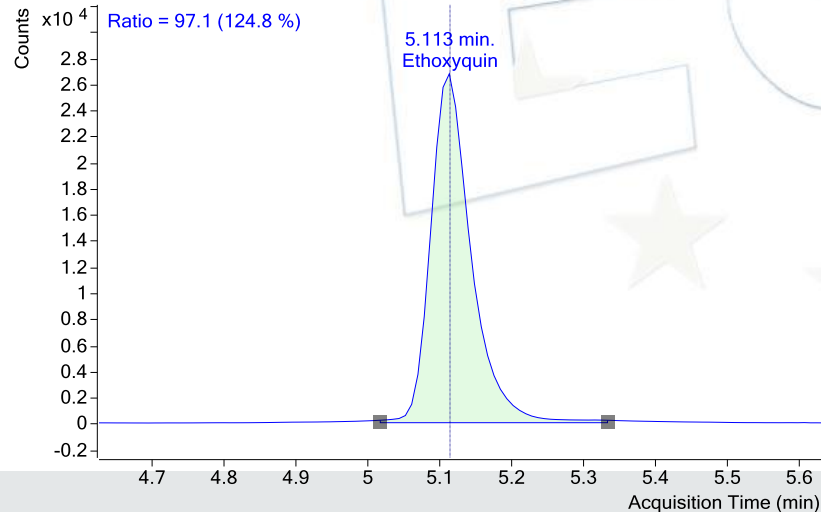


## Standard 50 ppb in Pear

+ MRM (218.4 -> 160.2) 50 ppb\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d

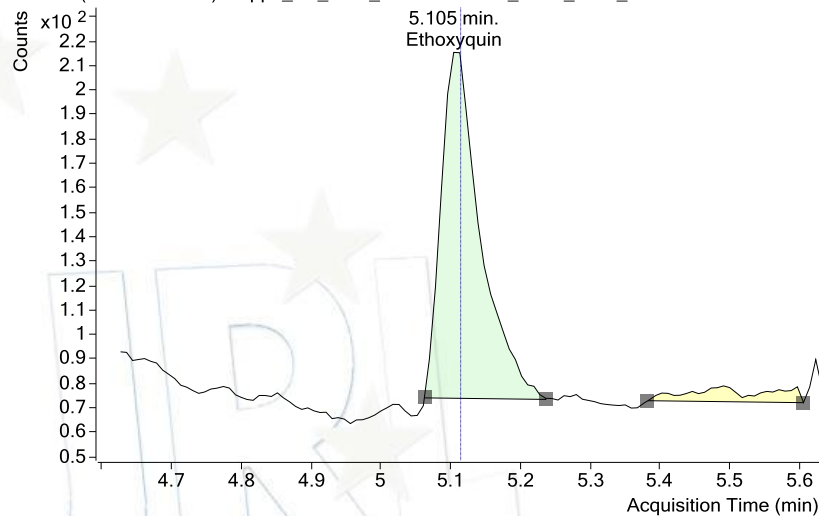


+ MRM (218.4 -> 173.9) 50 ppb\_Pear\_QCitrate+ZrO2\_Yttria\_Dilx5.d

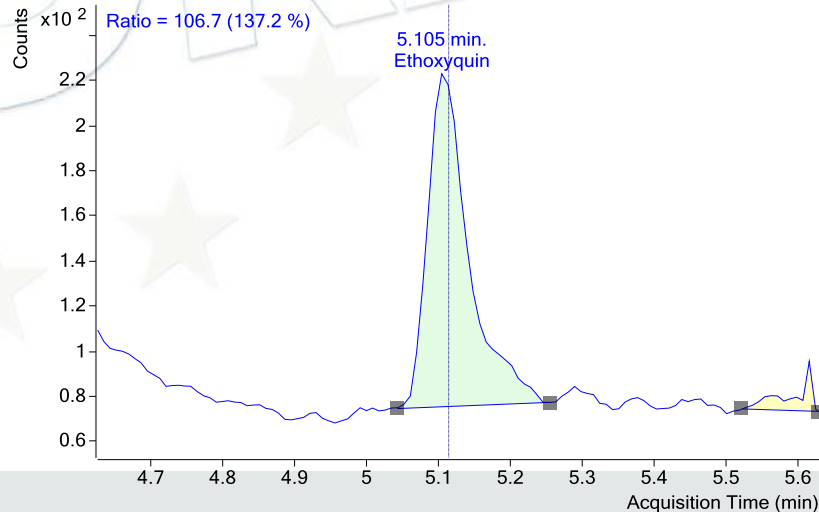


## Recovery 50 ppb in Pear

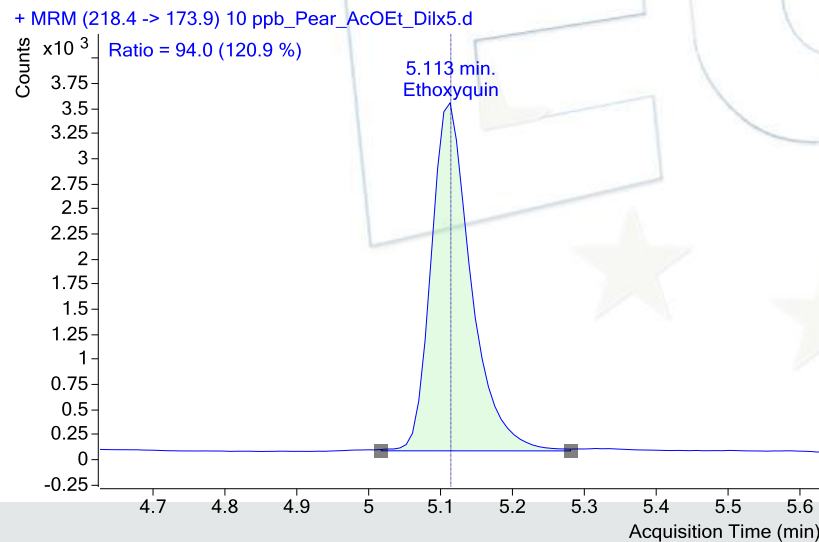
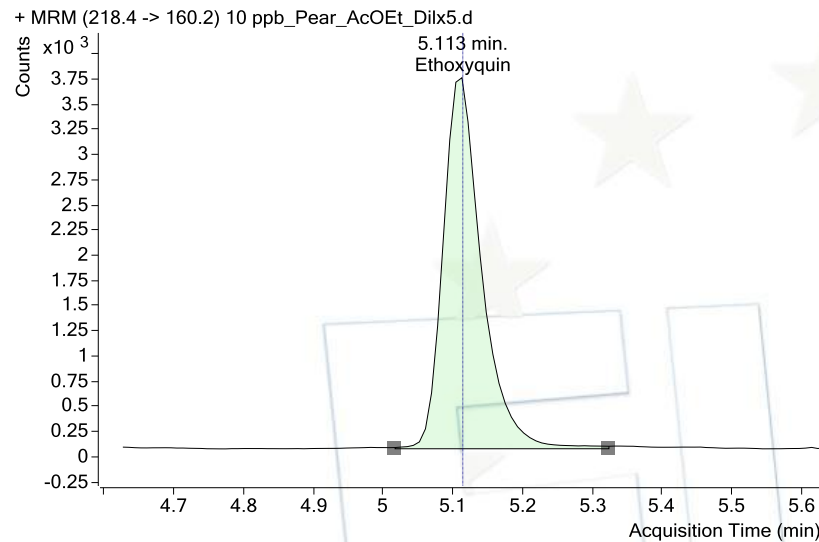
+ MRM (218.4 -> 160.2) 50 ppb\_R2\_Pear\_QCitrate+ZrO2\_Yttria\_3min\_Dilx5.d



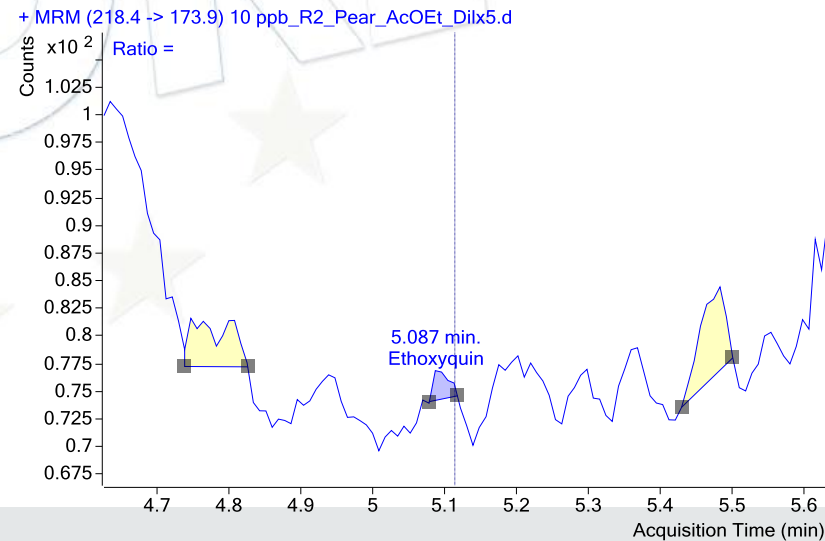
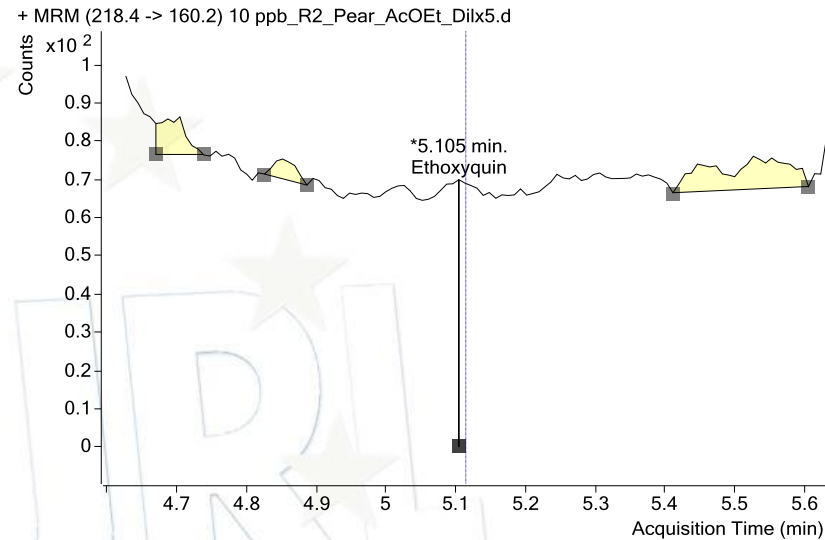
+ MRM (218.4 -> 173.9) 50 ppb\_R2\_Pear\_QCitrate+ZrO2\_Yttria\_3min\_Dilx5.d



## Standard 10 ppb in Pear

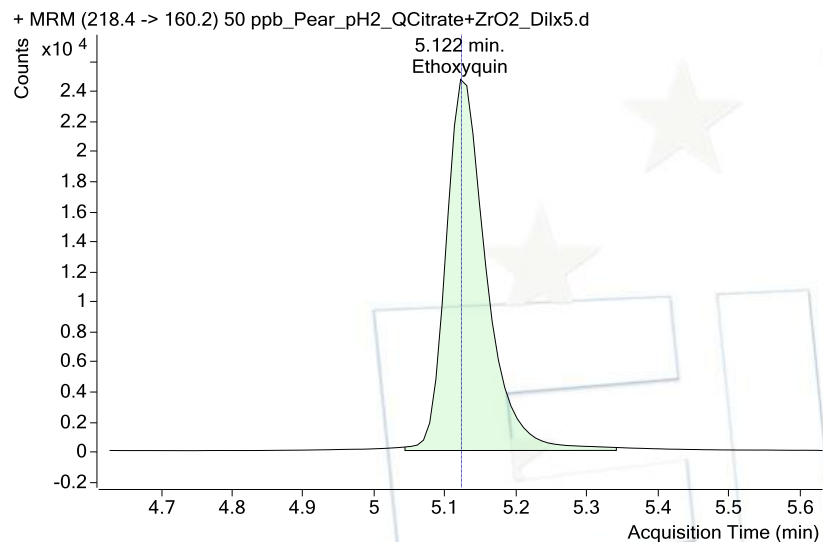


## Recovery 10 ppb in Pear

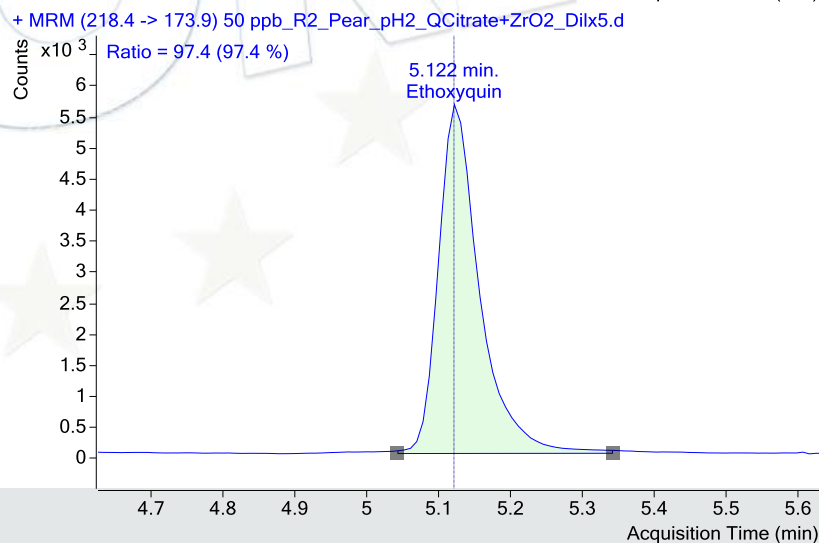
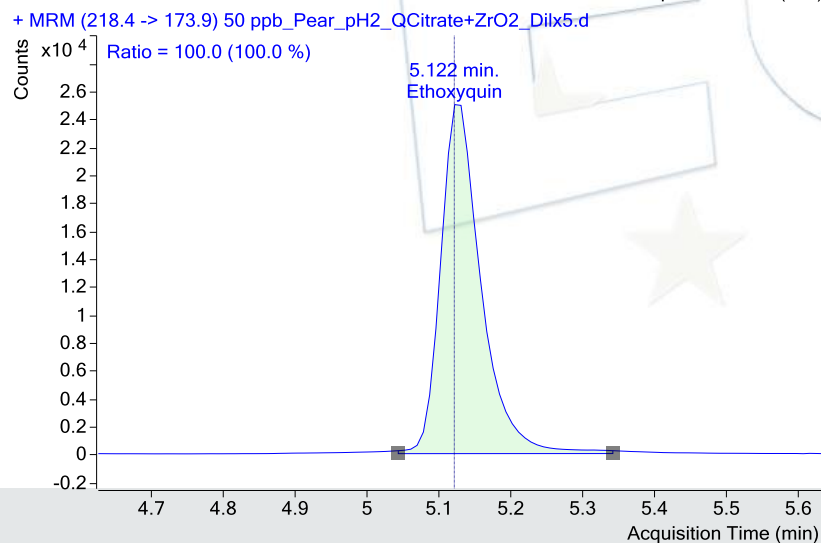
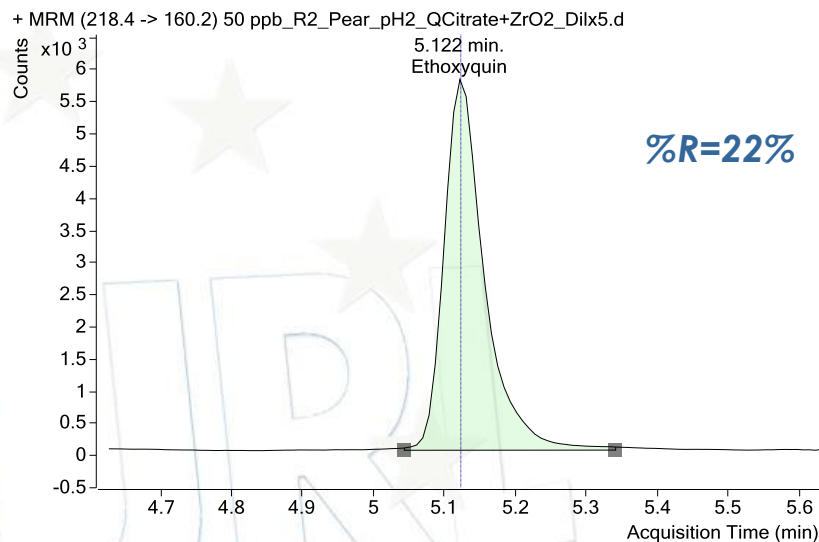




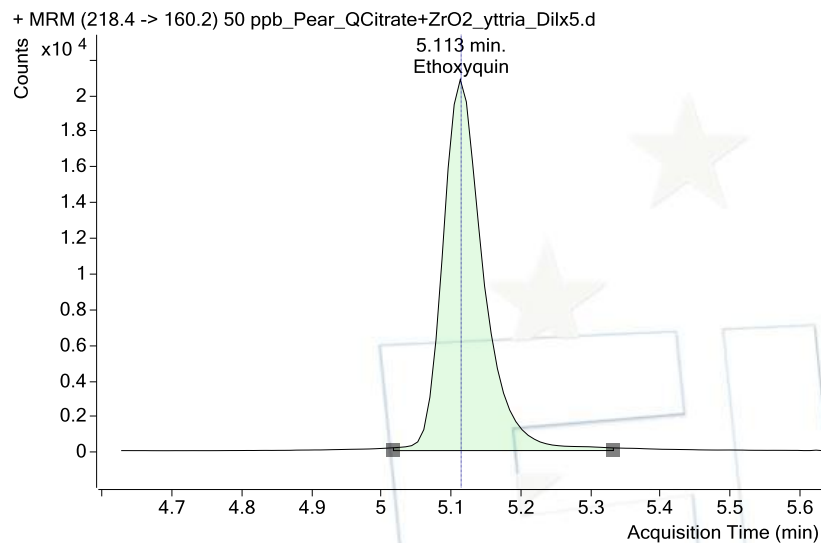
## Standard 50 ppb in Pear



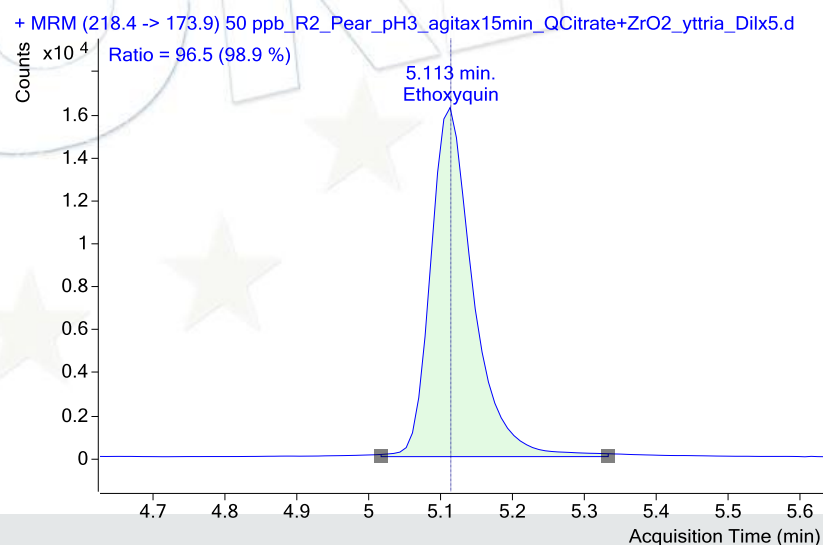
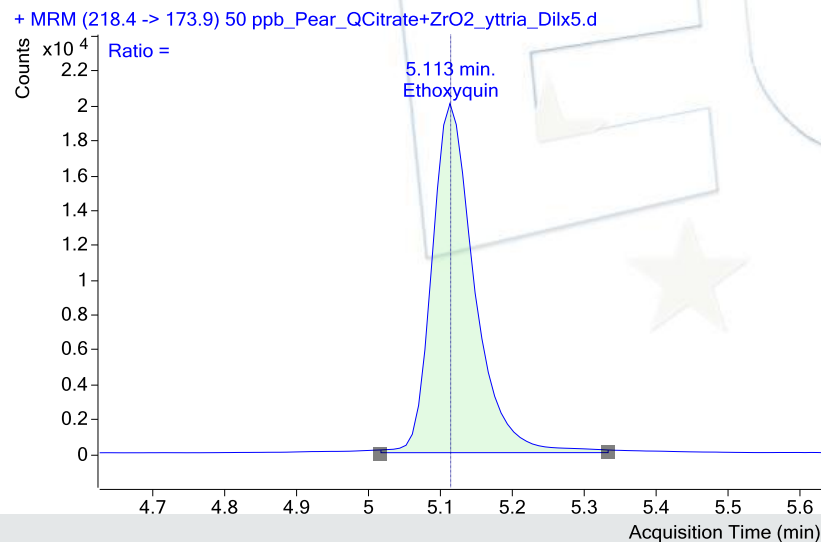
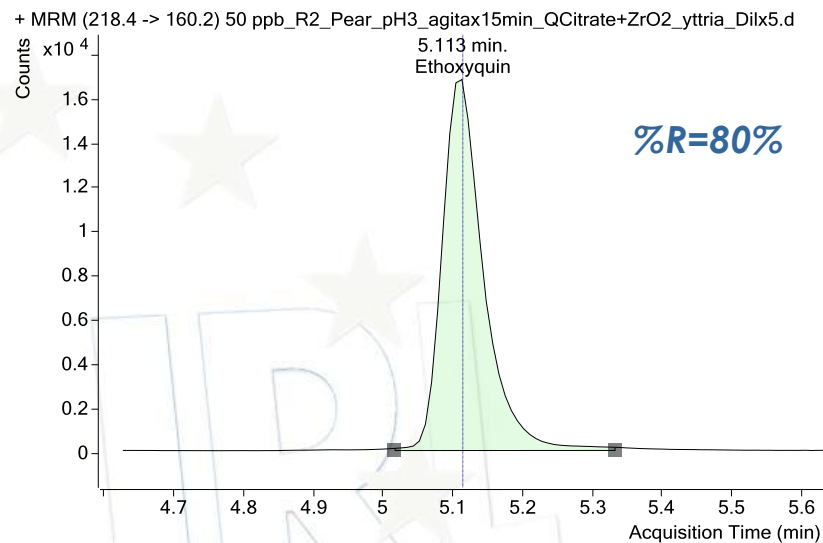
## Recovery 50 ppb in Pear

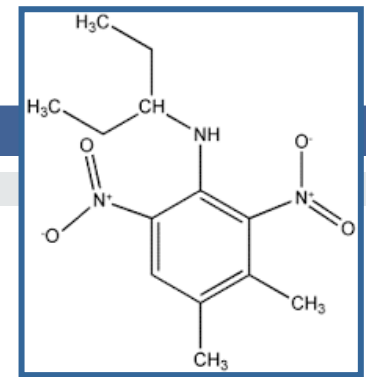


## Standard 50 ppb in Pear



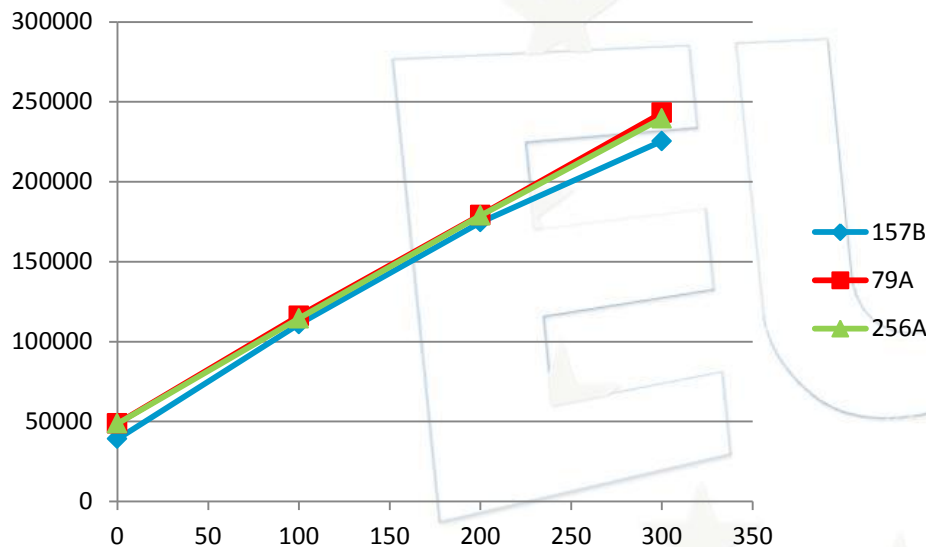
## Recovery 50 ppb in Pear





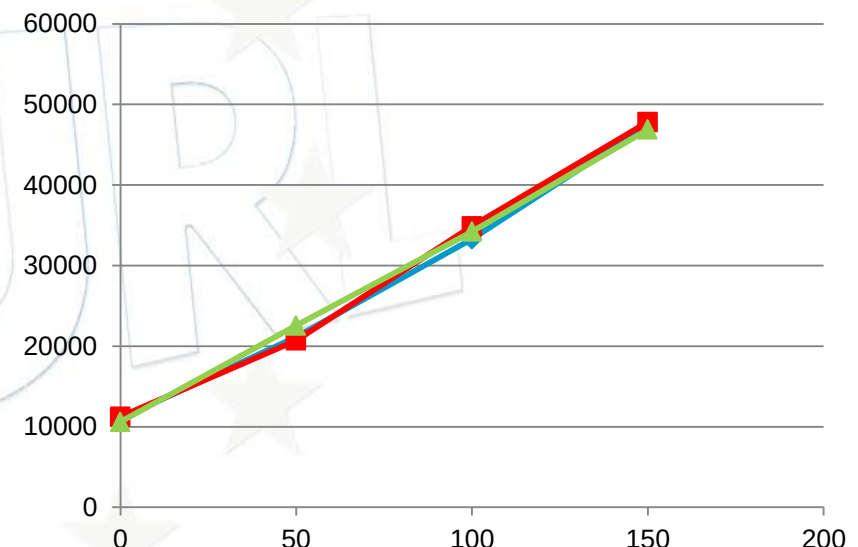
## Pendimethalin in green pepper

Standard addition  
EUP-FV 17 samples  
by **LC-MS/MS**



Average concentration: 0.076 mg/kg

Standard addition  
EUP-FV 17 samples  
by **GC-MS/MS**



Average concentration: 0.042 mg/kg

**Assigned value: 0.062 mg/kg**