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# Determination of Multi-Pesticide Residues in Dried Tea Samples using an Optimized Extraction / Clean-up Regime and the Agilent 7000 Series Tandem Quadrupole GC/MS/MS System


**Prof. Jana Hajslova, Dr. Tomas Cajka, Dr. Jana Pulkrabova, Veronika Bachanova, Lucie Drabova, Kamila Kalachova**

**Department of Food Analysis and Nutrition**
**Institute of Chemical Technology**
**Prague, Czech Republic**

***in collaboration with***

**Chris Sandy**
**EMEA GC-MS Food Applications Chemist**
**Agilent Technologies UK**


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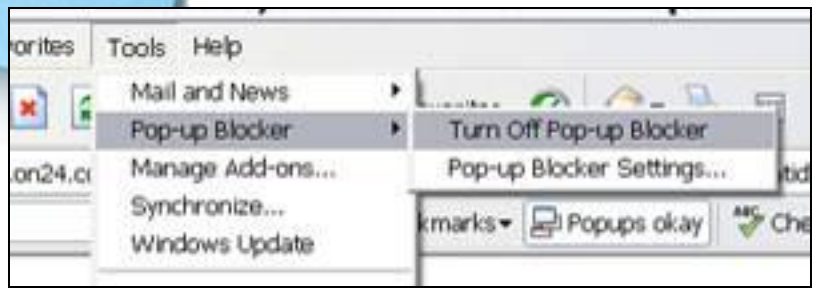


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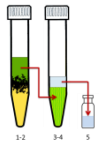
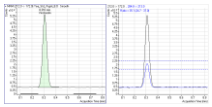
# Presentation Overview

1.  **Introduction**
2.  **Optimization of sample prep / method validation**
3.  **GC/MS/MS Analysis**
4.  **Results**
5.  **Summary**

# 1. Introduction

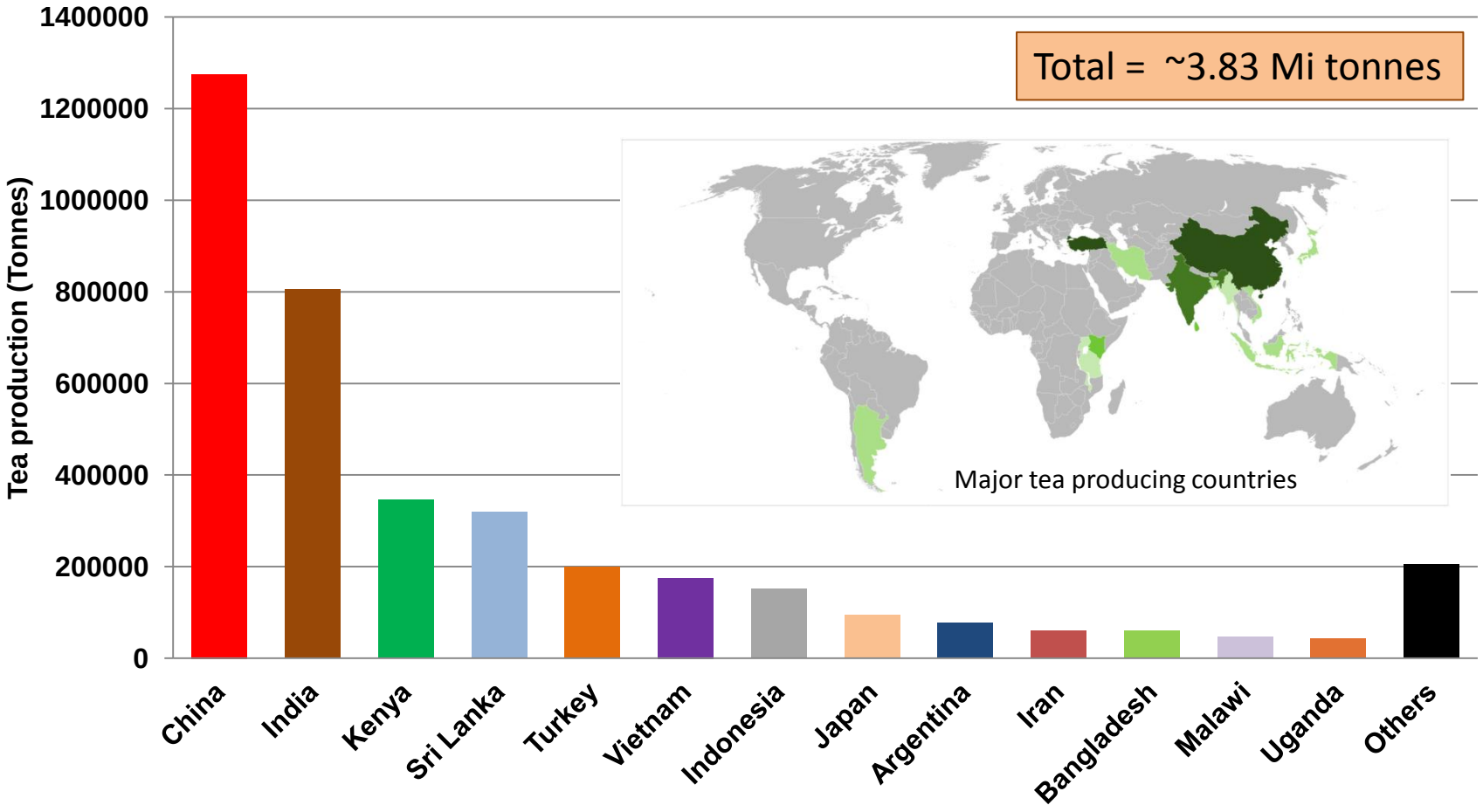
- Collaboration at ICT started March 2011
- Key objectives
  - Optimization of sample prep
  - Development of robust / rapid GC method > 100 target analytes

- All analytical development performed at ICT
- Analysis performed at ICT and in UK

**Tea – *Camelia sinensis***

# WW Tea Production by Country - 2008

## Tea – *Camelia sinensis*



# Production processes for Tea

Fresh tea leaves

Sunned wilting

Indoor wilting

Tossing in basket

Partial oxidation

Light crushing

Full oxidation

CTC with full oxidation

Steaming

Panning

Baking

Panning

Baking

Sweltering

Baking

Light Rolling

Rolling

Ball rolling

Rolling

Rolling

Forming

Rolling

Drying

Drying

Drying

Drying

Drying

Natural aging

Wet piling

Firing

Post-fermented tea

Green tea

Yellow tea

White tea

Oolong tea

Black tea

WILTING

BRUISING

OXIDATION

FIXATION

YELLOWING

SHAPING

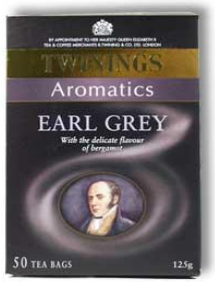
DRYING

CURING

# The many varieties of Tea…

- White Tea: Wilted and un-oxidized
- Yellow Tea: Unwilted and un-oxidized, but allowed to yellow
- Green Tea: Unwilted and un-oxidized
- Oolong Tea: Wilted, bruised and partially oxidized
- Black Tea: Wilted, sometimes crushed and fully oxidized
- Post-fermented Tea: Green Tea that has been allowed to ferment / compost

- Flavoured Tea: Fruit, herbal etc

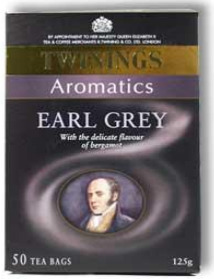



# The many varieties of Tea…













Other teas are available

# Common Pests in Tea Plantations

Major tea producing countries

| Pest | Common Name |
|---|---|
| Insects | Thrips |
| | Slug caterpillars |
| | Bunch caterpillars |
| | Leaf roller |
| | Looper |
| | Tea mosquito |
| | Aphids |
| | Jassids |
| | Helopeltis |
| | Flushworm |
| Mites | Purple, pink and red |
| Fungi | Blister blight |
| | Black rot |
| | Red rust |

# Agro-chemicals used on Tea Plantations

| Chemical Class | Example Chemicals | Pest / Disease |
|---|---|---|
| Insecticides | OCPs, OPs, Pyrethroids | Leafhoppers, plant bugs, aphids, thrips, leaf eating / leaf folding caterpillars, beetles etc |
| Acaricides | Amitraz, Azinphos ethyl, Diazinon, Naled | Mites, Ticks etc |
| Fungicides | Carbendazim, Hexaconazole, Propiconazole, Tridemorph | Fungal diseases – Blister blight, Grey blight etc |

# EU Pesticide MRL Legislation

REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 23 February 2005

on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

(Text with EEA relevance)

## Recital 5

(5) One of the most common methods of protecting plants and plant products from the effects of harmful organisms is the use of active substances in plant protection products. However, a possible consequence of their use may be the presence of residues in the treated products, in animals feeding on those products and in honey produced by bees exposed to those substances. According to Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market (1), public health should be given priority over the interests of crop protection, thus it is necessary to ensure that such residues should not be present at levels presenting an unacceptable risk to humans and, where relevant, to animals. MRLs should be set at the lowest achievable level consistent with good agricultural practice for each pesticide with a view to protecting vulnerable groups such as children and the unborn.

- Requirement for strict residue controls to safeguard public health

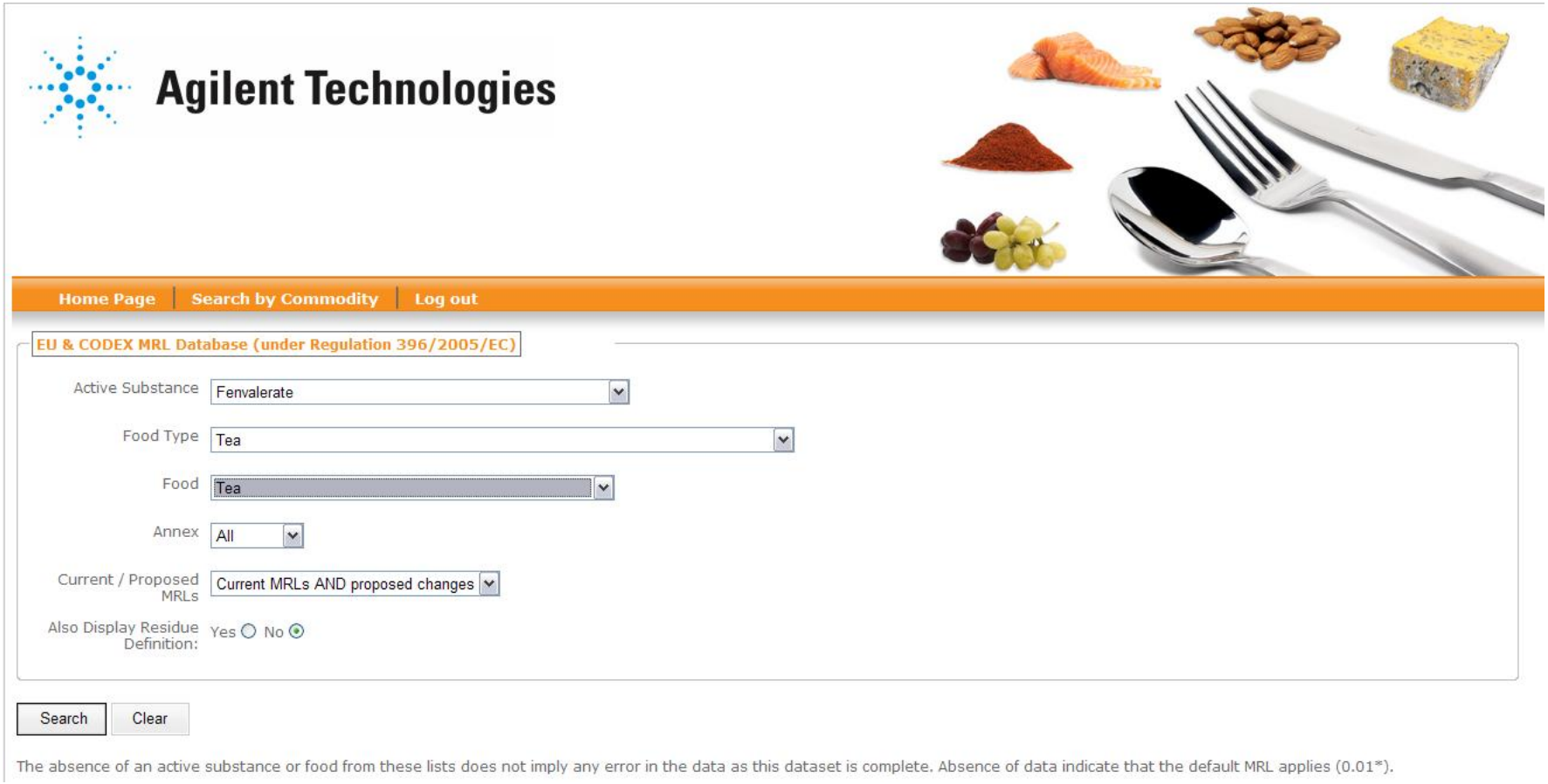
# FC24 Food Commodities and Contaminants Database

**FC24 Food Commodities and Contaminants Database.** Agilent offers our customers ***free access*** to the FERA FC24 database, which pulls together relevant food contaminants and residues information from across the EU. In a matter of seconds, you can search the database and have the information you need at your fingertips.

https://secure.fera.defra.gov.uk/foodcontaminants/agilent/index.cfm

fera
The Food and Environment Research Agency

# FC24 Food Commodities and Contaminants Database



# FC24 Food Commodities and Contaminants Database

Agilent Technologies

Home Page | Search by Commodity | Log out

**LIAISON** EU & CODEX MRL Database (under Regulation 396/2005/EC) Search Results for Fenvalerate and Esfenvalerate (Sum of RR & SS isomers)

There is 1 request matching your criteria.

| Active Substance | MRL mg/kg | Previous MRL (Date of Change) | Food type | Food | Comments | Codex MRL (derivation) | Annex |
|---|---|---|---|---|---|---|---|
| Fenvalerate and Esfenvalerate (Sum of RR & SS isomers) | 0.05* | (1 September 2008) | Tea (dried leaves and stalks, fermented or otherwise of Camellia sinensis) | Tea | | No Codex MRL has been set | II |

*** Level at or about the limit of determination (LOD)**

**(1) The code number is introduced by this Annex and is intended to set a classification under this and other related Annexes of Regulation (EC) No 396/2005.**

**(2) The scientific name of the items listed in the column "Examples of individual products within the groups to which the MRLs apply", where possible and relevant, is mentioned. As much as possible the International System of Nomenclature is followed.**

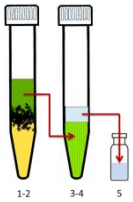
**(9) Part of the products to which the MRL apply.**

**MRLs with a green background indicate that the active has approval (in at least one product, either on-label or off-label) on the listed crop within the UK. Such approved uses at the LOD (i.e. those marked *) indicate that no detectable residue should occur under GAP**

**MRLs with no background colour indicate that no UK uses are currently approved. However, additional presence of a CODEX MRL would indicate approval somewhere in the world and residues above the MRL would not be expected under the appropriate GAP in those countries**

New Search

**Sources**



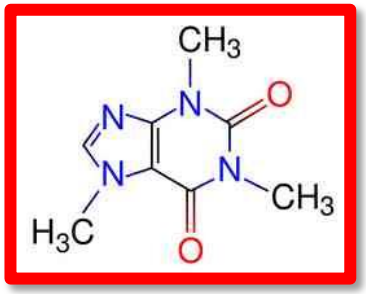

# 2. Optimization of Sample prep / Method Validation

# Tea matrix composition

## Main constituents of tea leaves

| Component | Content (% dry weight) |
|---|---|
| Polyphenols | 25–35 |
| flavanols | 80% of total polyphenols |
| Sacharides | 25 |
| polysacharides | 14–22 |
| Proteins | 15 |
| Minerals | 5 |
| Free aminoacids | 4 |
| Chlorophyll | 0.5 |
| **CAFFEINE** | **2.5–5.5** |

**CAFFEINE**




Content in cup of tea

**Black Tea**: 23–110 mg
**Oolong Tea**: 12–55 mg
**Green Tea**: 8–36 mg
**White Tea**: 6–25 mg

**40% dry matter soluble in water (fermented tea)**

# SAMPLE PREPARATION PROCEDURE

**(1)**
**Weigh 2 g of homogenized tea sample**

**(2)**
**Add 10 mL of distilled water**
**Shake for 30 s**

**(3)**
**Matrix swelling (hydration) for 30 min**

**(4)**
**Add 10 mL of MeCN**
**Shake for 1 min**

# SAMPLE PREPARATION PROCEDURE

**(5)**
**Add 1 g NaCl**

**(6)**
**Add 4 g $MgSO_4$**
**Shake for 1 min**

**(7)**
**Add internal standard (TPP)**

**(8)**
**Centrifuge 5 min at 10,000 rpm**

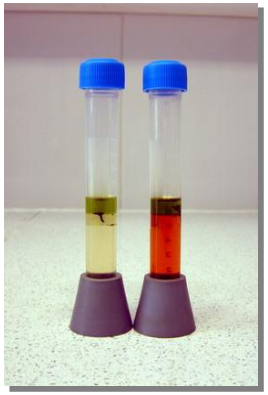
# SAMPLE PREPARATION PROCEDURE

**(9)**

**Add 1 mL of MeCN extract into a 15-mL tube containing 1 mL of hexane and 5 mL of 20% (*w*/*w*) NaCl solution, shake for 1 min**

**(10)**

**Centrifuge 1 min at 10,000 rpm**



**(11)**

**Take a part of the hexane layer into a vial**

# Bond Elut – QuEChERS Consumables

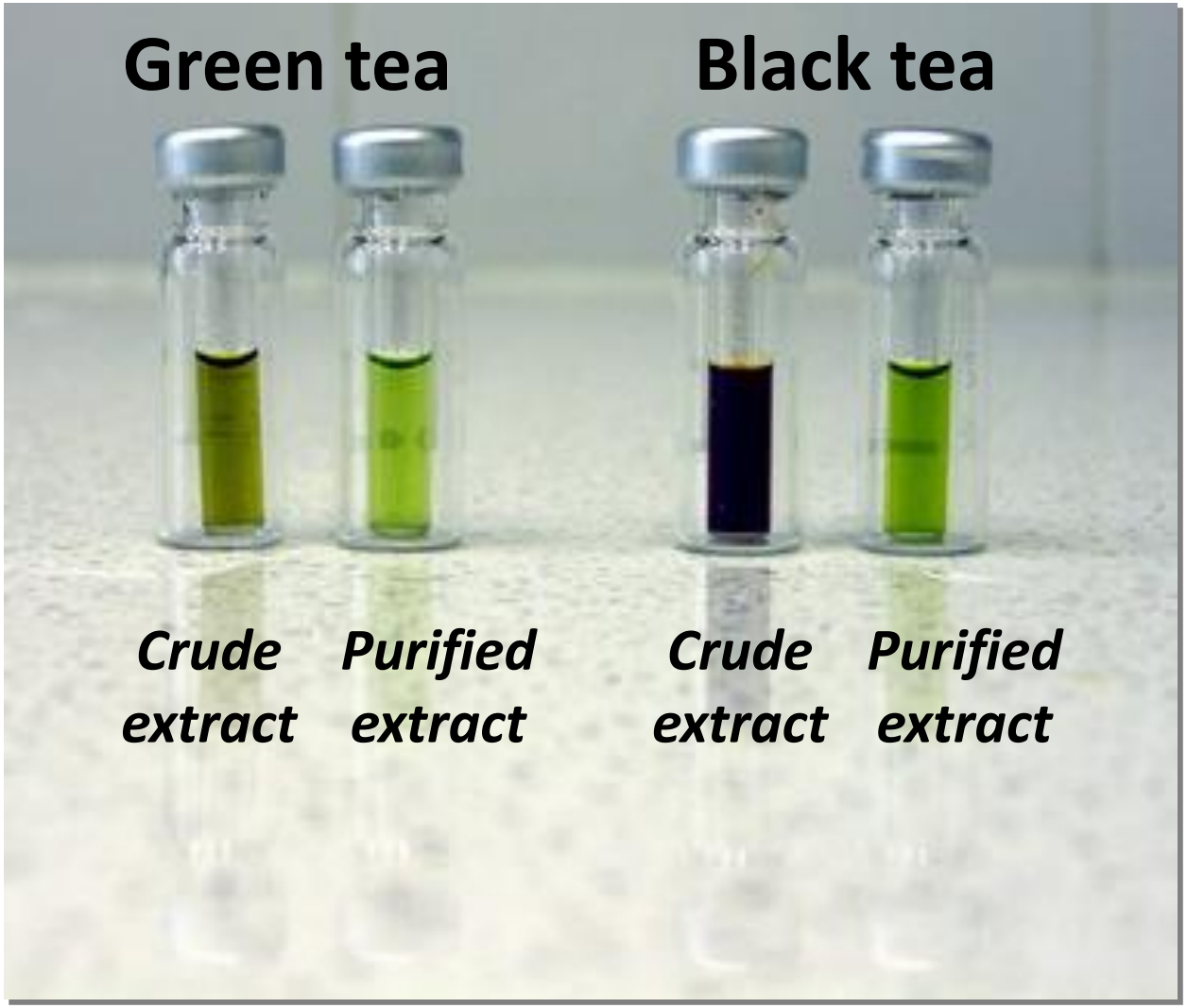
**(5)**
**Add 1 g NaCl**

**(6)**
**Add 4 g $MgSO_4$**
**Shake for 1 min**



Agilent Bond Elut : P/No. 5982-5550
50x 50mL tubes + 50x salt packets
[4g $MgSO_4$ + 1g NaCl]

# SAMPLE PREPARATION PROCEDURE



# SAMPLE PREPARATION PROCEDURE

1. 2g dried tea leaves + 10mL water in 50 mL plastic centrifuge tube, shake 30 seconds, wait 30 minutes for matrix hydration

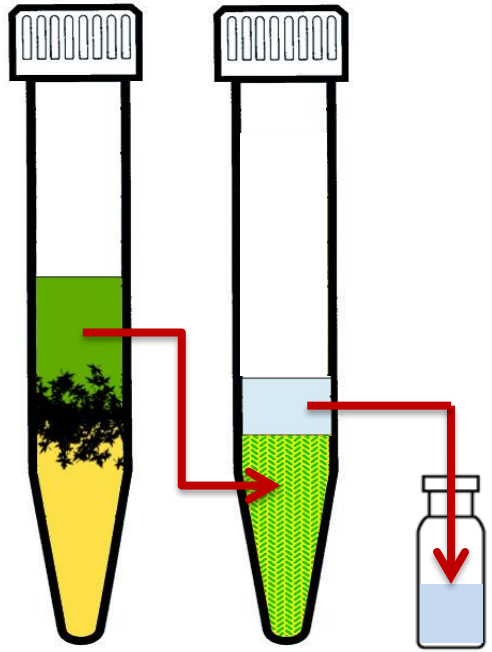
2. Add 10 mL acetonitrile; agitate 1 minute, Add 4g $MgSO_4$ + 1g NaCl; agitate 1 minute, add TPP (ISTD), centrifuge 5 minutes at 10,000 rpm

3. Take 1 mL acetonitrile layer in to a 15 mL plastic centrifuge tube, add 1 mL *n*-hexane and 5 mL 20% aqueous NaCl solution, agitate 1 minute

4. Centrifuge 1 minute at 10,000 rpm

5. Take part of *n*-hexane layer into 2 mL auto-sampler vial

**Easily prepare 6 samples / hour**




Acetonitrile Water Hexane

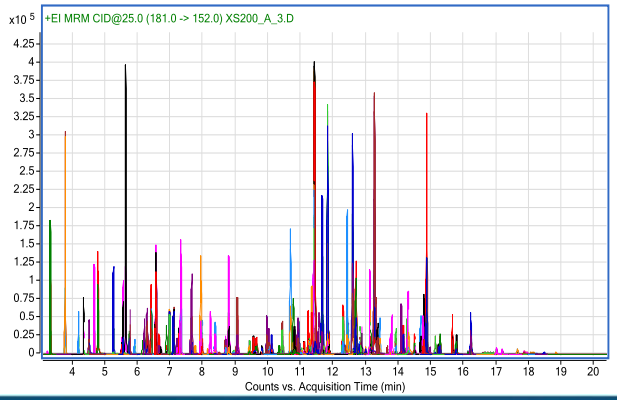
Acetonitrile / 20% Aq. NaCl

# GC/MS/MS Methodology (Summary)

## GC–MS/MS analysis

- Injection: MMI, Cold splitless (2 µL)
- Column: HP-5MSUI
  (15 m × 0.25 mm ID × 0.25 µm)
- Retention time locking
- Post-column, post-run back flush
- 2–4 MRM transitions per analyte

| | |
|---|---|
| GC run: | 20 min |
| Backflush: | 2 min |
| MMI/GC cooling: | 6 min |
| Pre-injection steps : | 1 min |
| TOTAL : | 29 min per injection |



# OPTIMIZATION OF SAMPLE PREPARATION

- (1) Purification of crude MeCN extract
  - dSPE, LLE
- (2) Checking of sample preparation efficiency
  - Gravimetric determination of co-extracts and determination of caffeine (dominating co-extract) using DART–MS
- (3) LLE with hexane and NaCl solution
  - Recovery of target analytes

# OPTIMIZATION OF SAMPLE PREPARATION

- (4) Comparison of various QuEChERS methods
  - Original, buffered, citrate
- (5) Matrix swelling prior to extraction
  - QuEChERS (with water addition) *vs*. MeCN extraction (without water addition)
- (6) Long term stability of analytes signal
  - Repeated injections
- (7) Method validation
  - Recovery, repeatability, LOQ

# (1) Purification of crude MeCN extract
# (2) Checking of sample preparation efficiency

- Tested purification approaches
  - (A) dSPE followed by LLE
  - (B) dSPE without LLE
  - (C) only LLE (dSPE omitted)

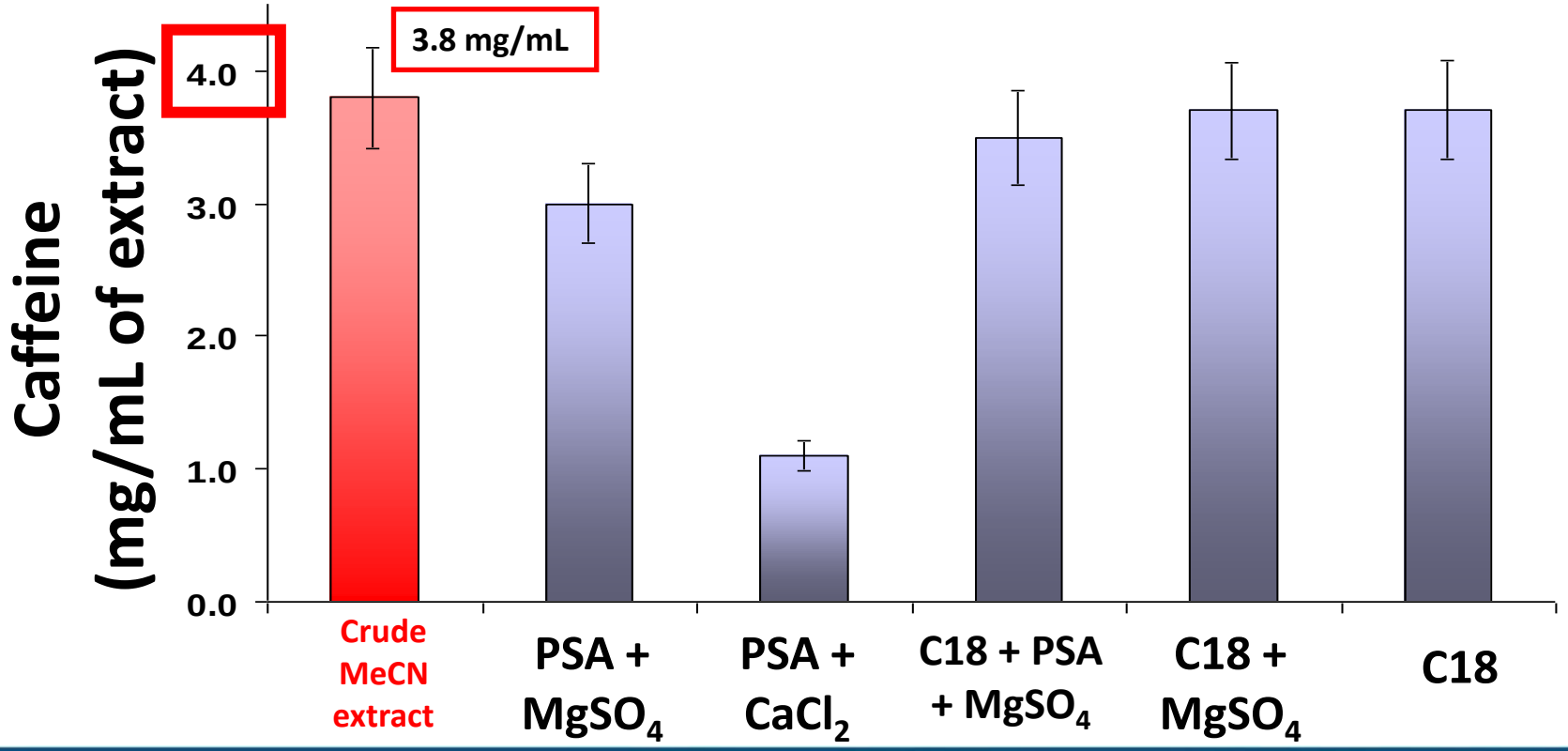
# (1) Purification of crude MeCN extract
# (2) Checking of sample preparation efficiency

- Sorbents tested
  - 50 mg PSA + 150 mg $MgSO_4$ per 1 mL
  - 50 mg PSA + 50 mg $CaCl_2$ per 1 mL
  - 10 mg C18 + 50 mg PSA + 150 $MgSO_4$ per 1 mL
  - 10 mg C18 + 150 mg $MgSO_4$ per 1 mL
  - 10 mg C18 per 1 mL
- *Matrix content: 0.2 g/mL*

# (1) Purification of crude MeCN extract

# (2) Checking of sample preparation efficiency

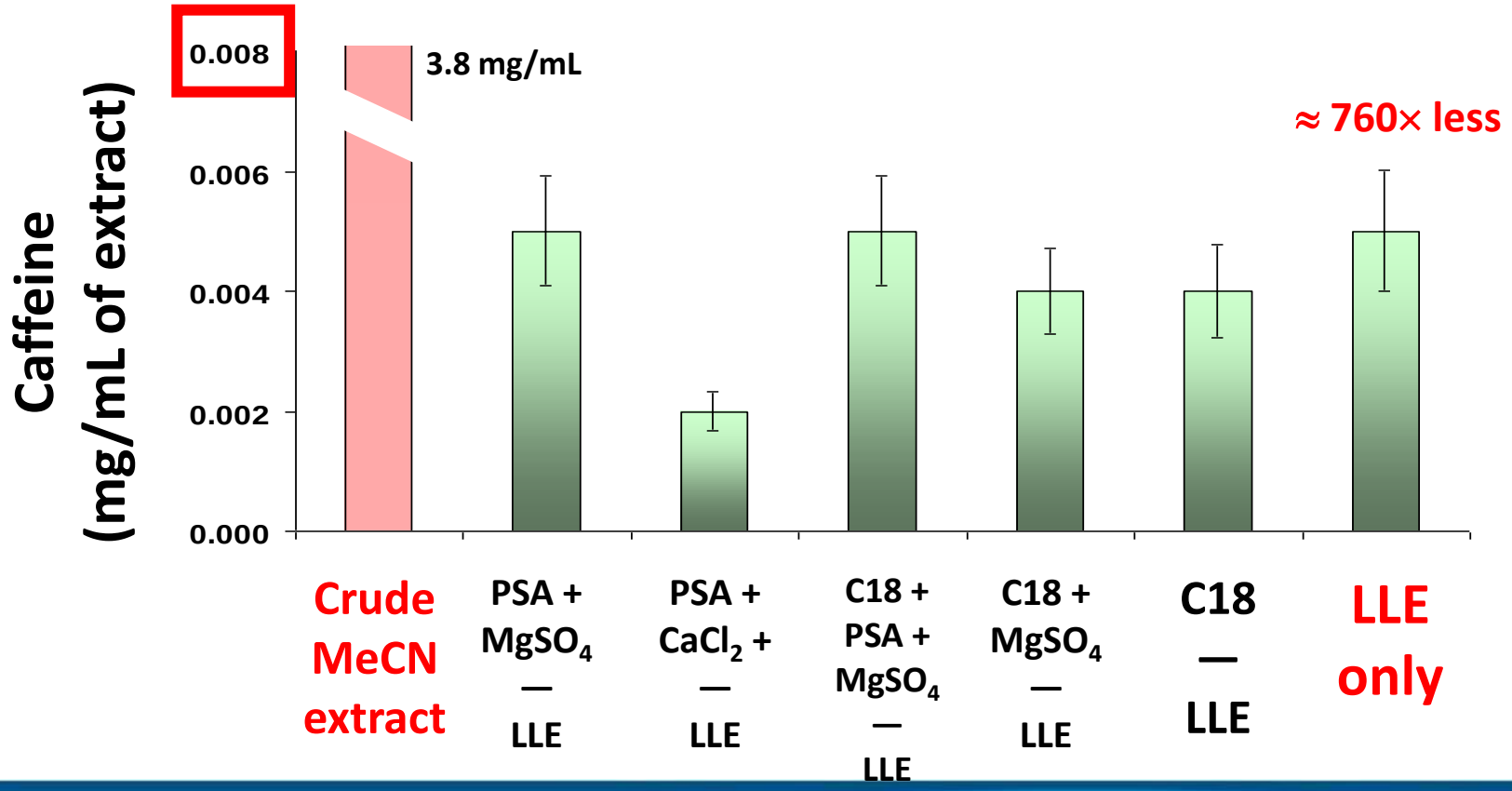
- Content of caffeine in the examined extracts



# (1) Purification of crude MeCN extract

# (2) Checking of sample preparation efficiency

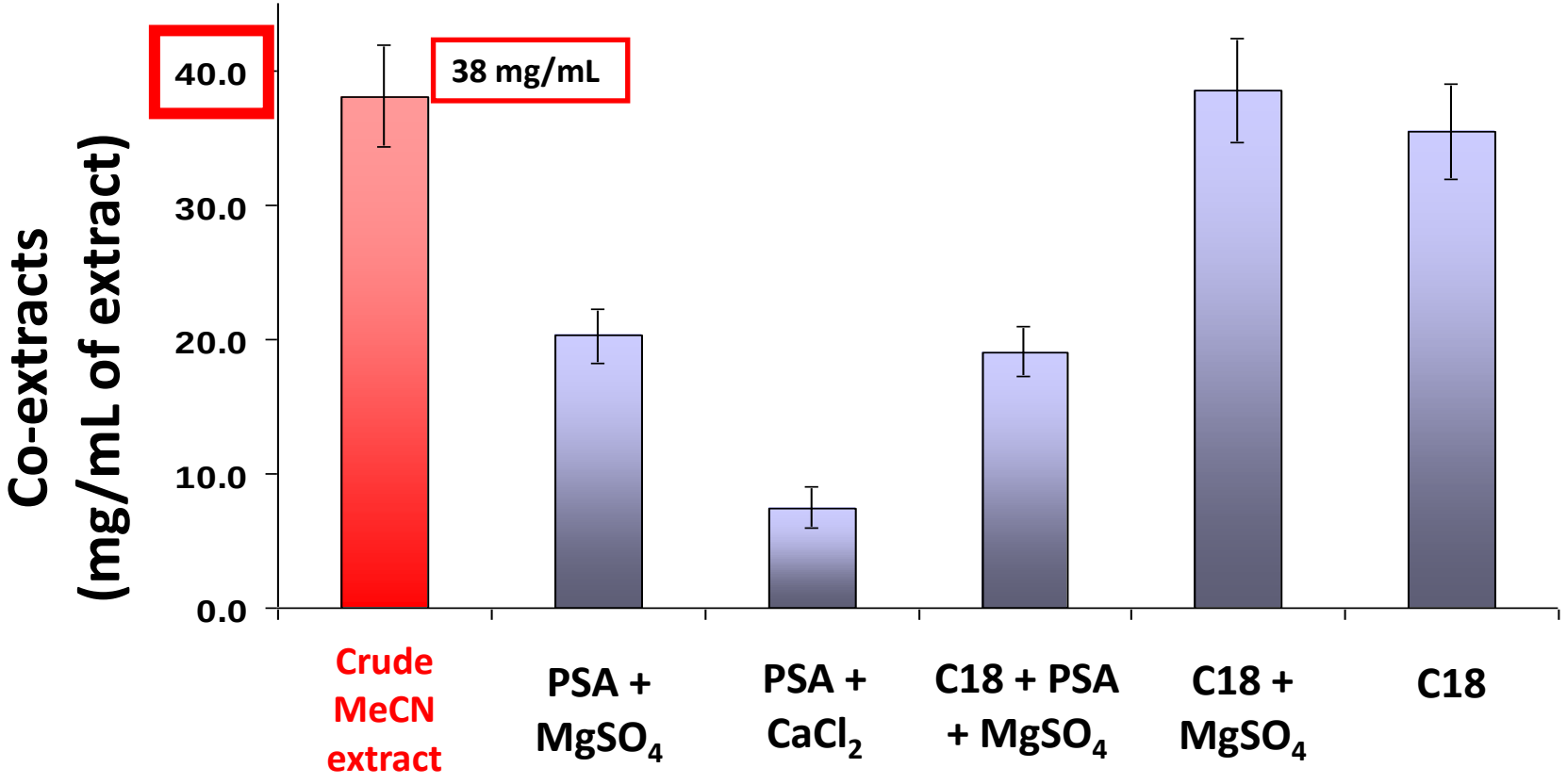
- Content of caffeine in the examined extracts



# (1) Purification of crude MeCN extract

# (2) Checking of sample preparation efficiency

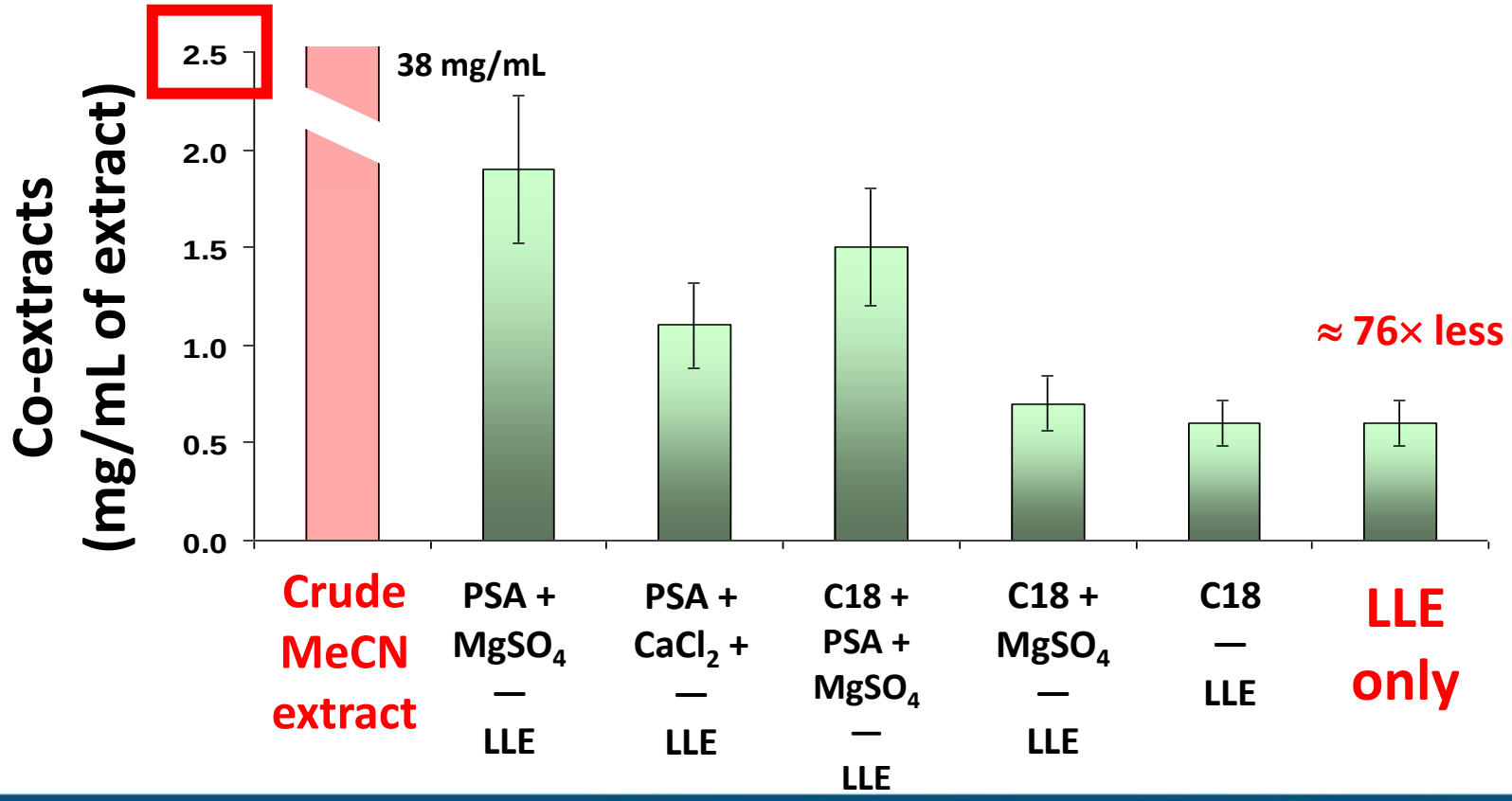
- Gravimetrically determined co-extracts



# (1) Purification of crude MeCN extract

# (2) Checking of sample preparation efficiency

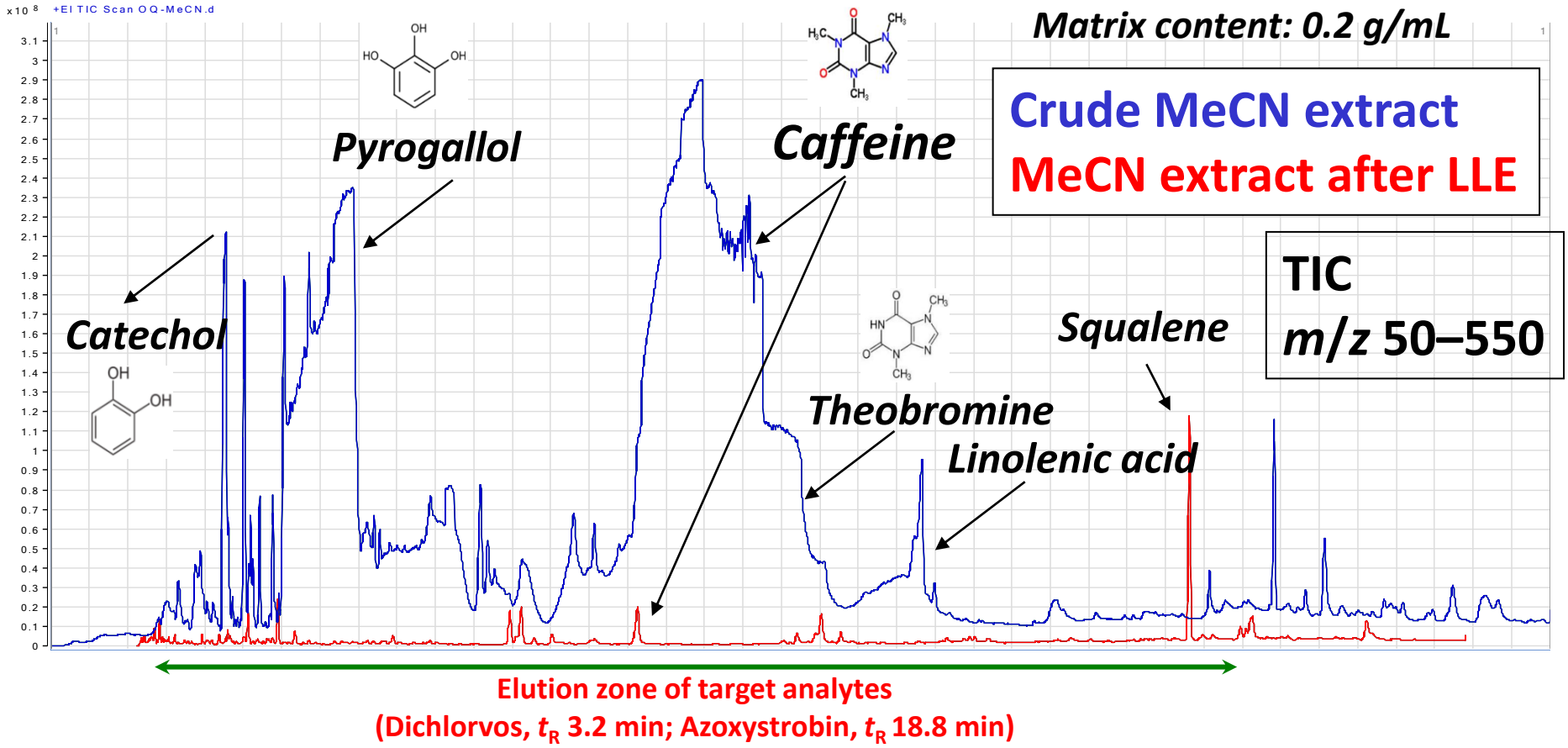
- Gravimetrically determined co-extracts



# (1) Purification of crude MeCN extract

# (2) Checking of sample preparation efficiency

- GC–MS analysis (MS$^1$ full scan) of the extracts

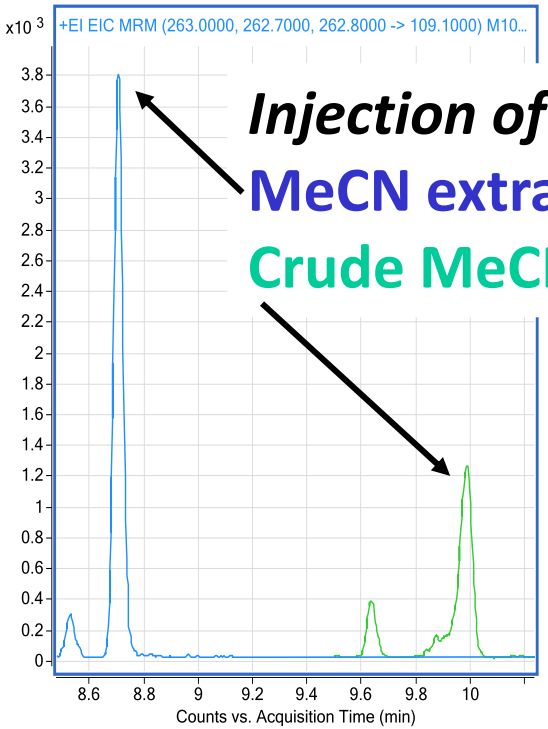


# (1) Purification of crude MeCN extract
# (2) Checking of sample preparation efficiency

- **Influence of matrix co-extracts on RT, peak shape and signal intensity**

**Chlorpyrifos-Me**

*m/z* 263.0 → 109.1




***Injection of analyte in:***

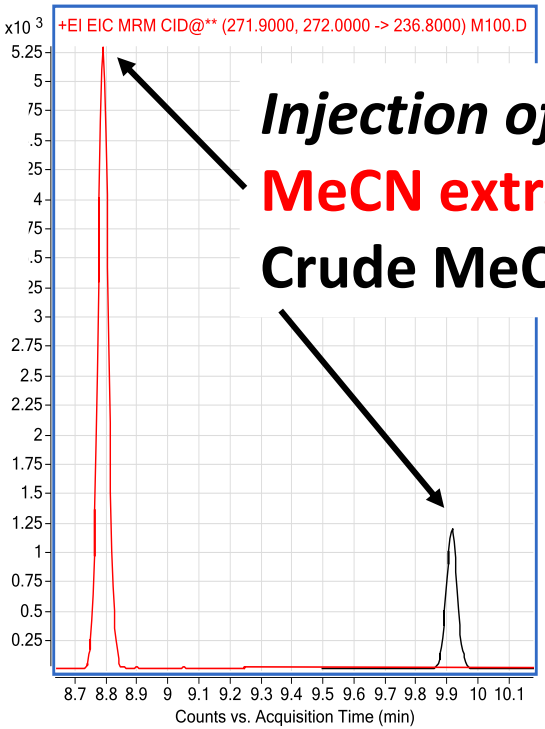
**MeCN extract after LLE**

**Crude MeCN extract**

100 ng/g
(20 ng/mL)

**Heptachlor**

*m/z* 271.9 →236.8




***Injection of analyte in:***

**MeCN extract after LLE**

**Crude MeCN extract**

# (1) Purification of crude MeCN extract
# (2) Checking of sample preparation efficiency

- **CONCLUSIONS**

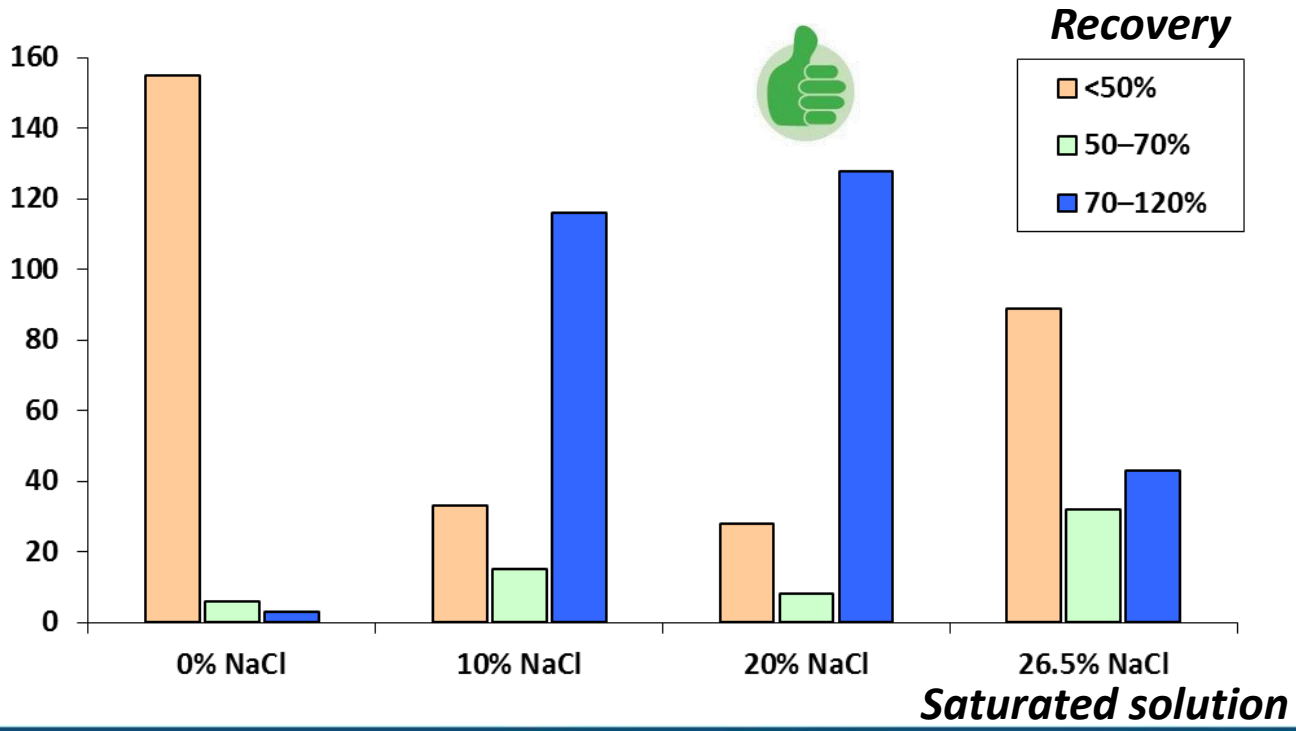
  - **dSPE (with $MgSO_4$/$CaCl_2$) is not sufficient clean-up technique for purification of tea MeCN extracts**

  - **LLE (with hexane/20% NaCl (*w*/*w*) mixture) leads to significant reduction of matrix co-extracts**

  - ***dSPE step with C18 sorbent (as proposed previously) can be omitted, thus, further increasing speed of sample preparation***

# (3) LLE with hexane and NaCl solution

- 164 pesticides involved (MeCN extracts spiked before LLE, 1 mg/kg)
- MeCN extract : hexane : NaCl solution (1:1:5, *v*/*v*/*v*)
- In the case of 0% NaCl solution an emulsion formed; results obtained by LLE with MeCN extract : hexane (1:1, *v*/*v*)



# (4) Comparison of various QuEChERS methods

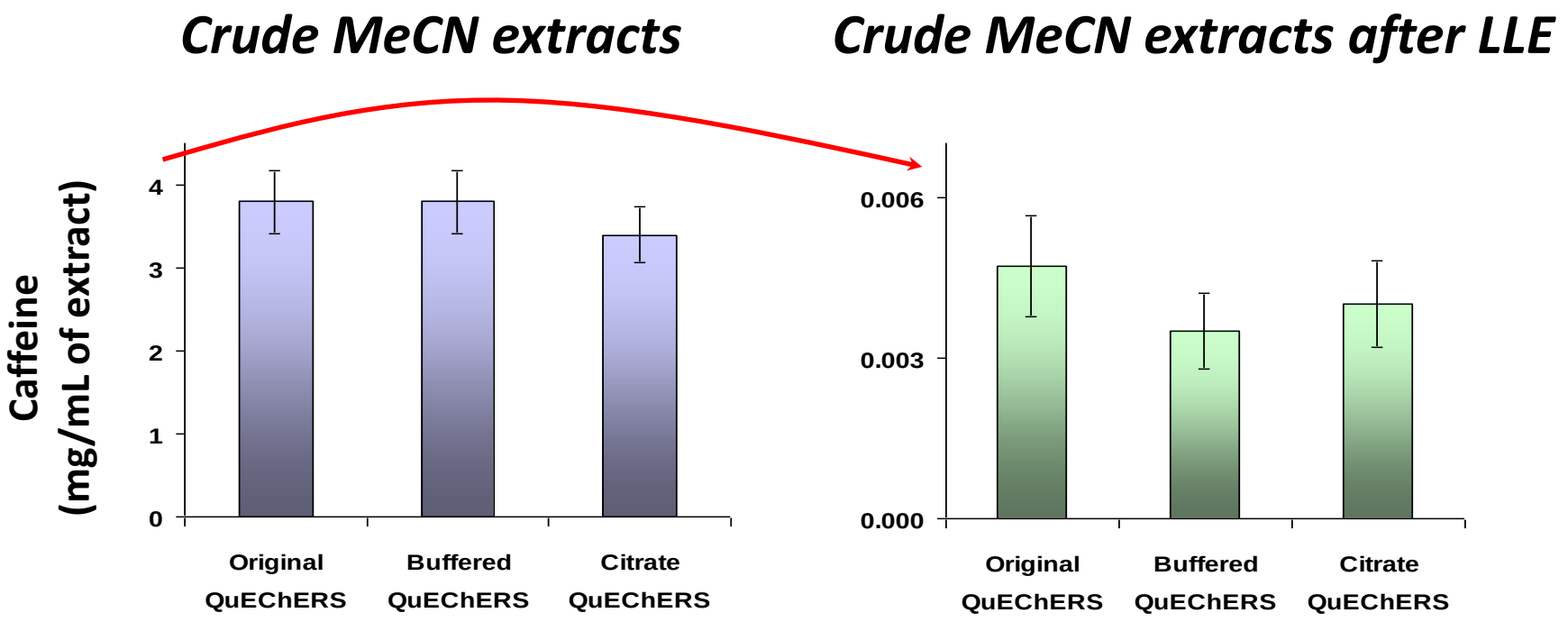
| Original QuEChERS | Buffered QuEChERS | Citrate QuEChERS |
|---|---|---|
| 2 g sample + 10 mL distilled water | | |
| matrix swelling for 30 min | | |
| 10 mL MeCN | 10 mL 1% AcA MeCN | 10 mL MeCN |
| Shake for 1 min | | |
| 4 g $MgSO_4$, 1 g NaCl | 4 g $MgSO_4$, 1.7 g $NaOAc \cdot 3 H_2O$ | 4 g $MgSO_4$, 1 g NaCl, 1 g $Na_3Citr \cdot 2 H_2O$, 0.5 g $Na_2HCitr \cdot 1.5$ |
| shake for 1 min, centrifuge 5 min at 10,000 rpm | | |
| 1 mL of MeCN per 1 mL hexane and 5 mL 20% NaCl solution | | |
| shake for 1 min, centrifuge 1 min at 10,000 rpm | | |
| hexane layer into a vial for GC–MS/MS analysis | | |

# (4) Comparison of various QuEChERS methods

- Content of caffeine in the examined extracts
- Gravimetrically determined co-extracts
- Recovery of target analytes

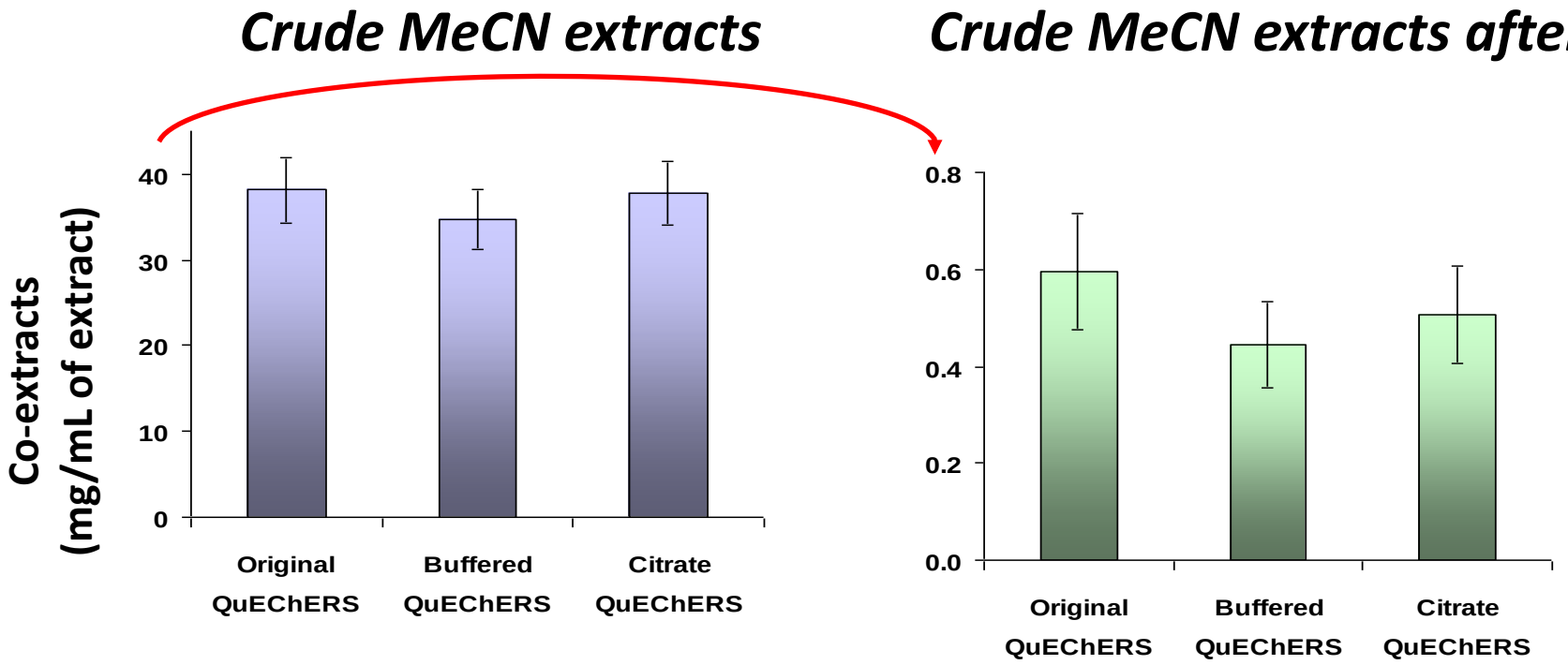
# (4) Comparison of various QuEChERS methods

- Content of caffeine in the examined extracts



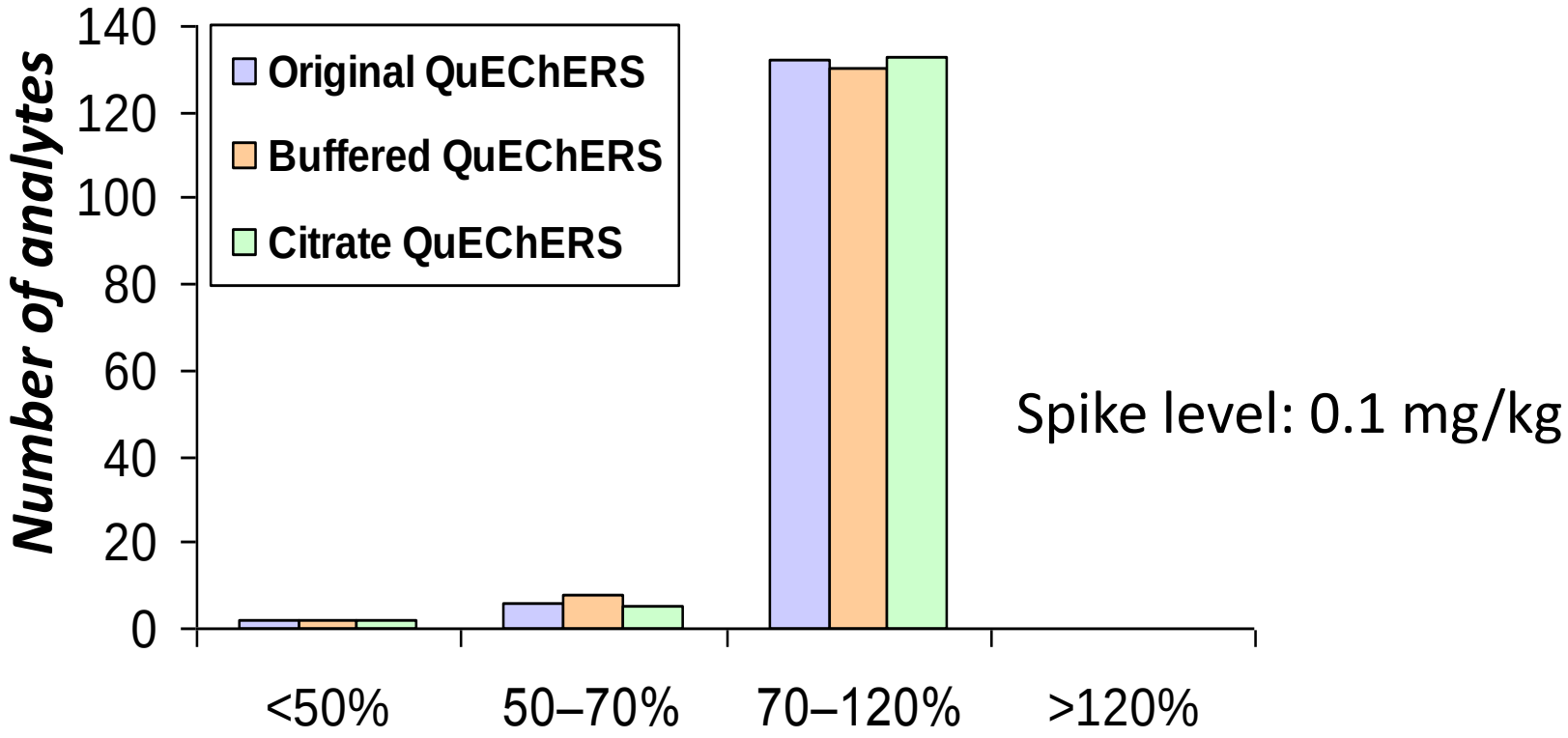
# (4) Comparison of various QuEChERS methods

- Gravimetrically determined co-extracts



# (4) Comparison of various QuEChERS methods

- **Recovery of target analytes**




***pH of tea matrix: 5.1–5.2 (2 g + 10 mL distilled water) → no buffering required***

# (4) Comparison of various QuEChERS methods

- CONCLUSIONS
  - All three QuEChERS extraction techniques provide comparable results in terms of:
    - Recovery of target analytes
    - Amount of isolated co-extracts
  - Original QuEChERS method used for validation experiments

# (5) Matrix swelling prior the extraction

- **Recently, studies dealing with the extraction of pesticide residues from tea using pure MeCN without previous addition of water for matrix hydration reported**

  G.F. Pang, *et al.*. , *J. AOAC Int.* **94** (2011) 1253–1296

  X.M. Xu, *et al.*, *J. Sep. Sci.* **34** (2011) 210–216

  Z. Huang *et al.*, *J. Sep. Sci.* **32** (2009) 1294–1301

- **SANCO/12495/2011 strictly advises to use water for swelling the matrix before the extraction**

***'To improve the extraction efficiency of low moisture containing commodities (cereals, dried fruits), it is recommended to add water to the samples before extraction is carried out.'***

# (5) Matrix swelling prior the extraction

- **In general, absence of water during the extraction of low moisture matrices results in cleaner extracts, but problem with lower recoveries of INCURRED pesticide residues**

M. E. Poulsen, H.B. Christensen, S.S. Herrmann, *Accred. Qual. Assur.* **14** (2009) 477–485

# (5) Matrix swelling prior to extraction

| Method A (with water) | Method B (without water) |
|---|---|
| 2 g sample + 10 mL distilled water | 5 g sample + 15 mL MeCN |
| matrix swelling for 30 min | Turrax for 1 min |
| 10 mL MeCN | centrifuge 5 min at 10,000 rpm |
| shake for 1 min | MeCN into 25 mL vol. flask |
| 4 g $MgSO_4$, 1 g NaCl | repeat with 15 mL MeCN |
| shake for 1 min | combine MeCN extracts (25 mL) |
| centrifuge 5 min at 10,000 rpm | |

1 mL of MeCN per 1 mL hexane and 5 mL 20% NaCl solution,

shake for 1 min, centrifuge 1 min at 10,000 rpm

hexane layer into a vial for GC–MS/MS analysis

# (5) Matrix swelling prior the extraction

- Content of caffeine in the examined extracts
- Gravimetrically determined co-extracts
- Comparison of recoveries: QuEChERS/LLE *vs*. extraction with pure MeCN
- Comparison of content of incurred residues obtained using both extraction techniques

# (5) Matrix swelling prior the extraction

- **Content of caffeine in the examined extracts**

| Method A (with water) | Method B (without water) |
|---|---|
| Caffeine: 3.8 mg/mL | Crude extract: 0.13 mg/mL |
| Extract after LLE: 0.005 mg/mL | Crude extract: 0.0001 mg/mL |

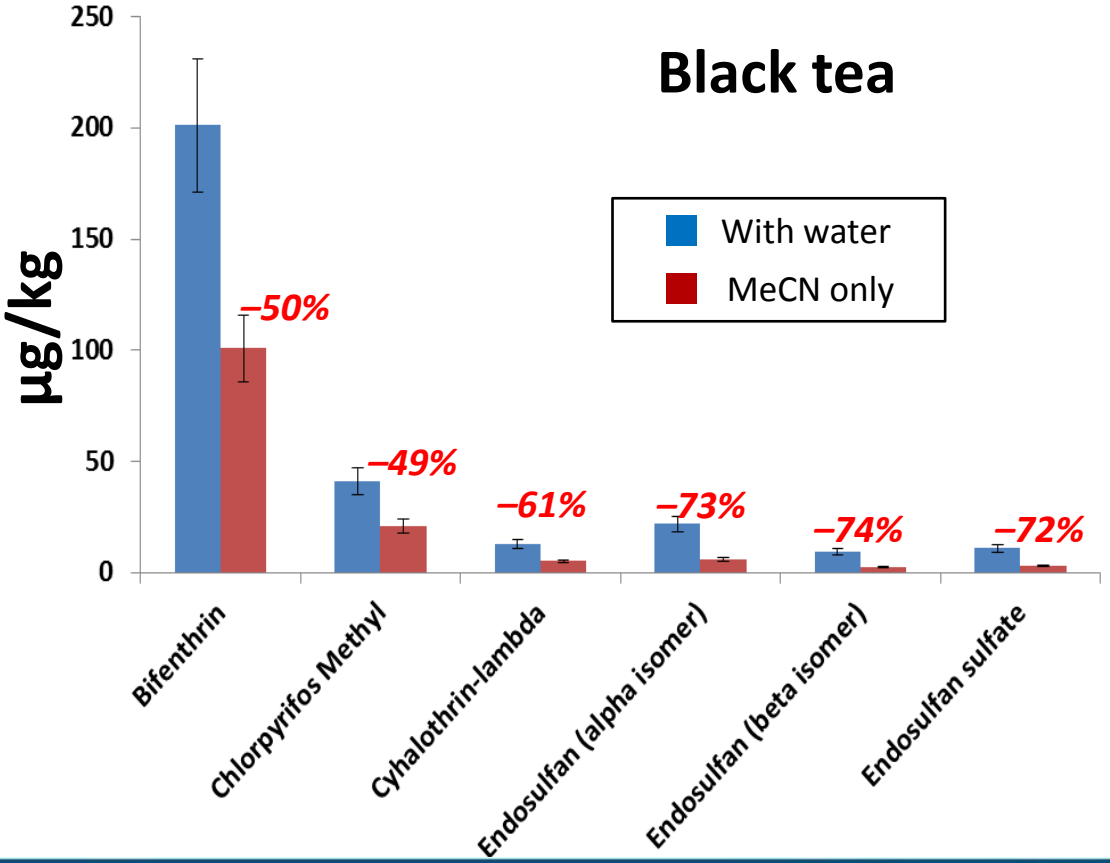
- **Gravimetrically determined co-extracts**

| Method A (with water) | Method B (without water) |
|---|---|
| Crude extract: 38 mg/mL | Crude extract: 1.3 mg/mL |
| Extract after LLE: 0.60 mg/mL | Extract after LLE: 1.3 mg/mL |

# (5) Matrix swelling prior the extraction

- **Analysis of samples with incurred residues**

*Incurred residues*



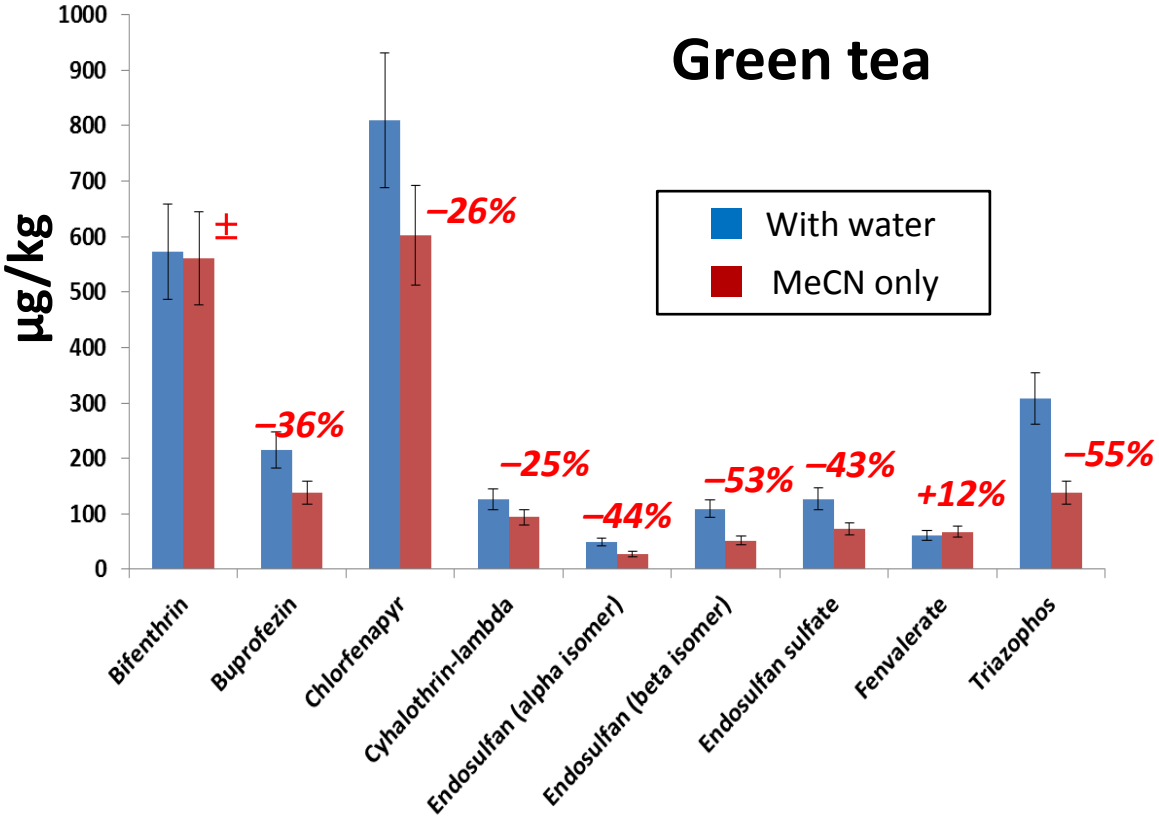

*Spikes (0.1 mg/kg)*

| Analyte | Recovery QuEChERS | Recovery Pure MeCN |
|---|---|---|
| Bifenthrin | 78% | 88% |
| Chlorpyrifos-Me | 92% | 85% |
| Cyhalothrin-λ | 101% | 86% |
| Endosulfan-α | 77% | 80% |
| Endosulfan-β | 77% | 80% |
| Endosulfan-$SO_4$ | 90% | 82% |

# (5) Matrix swelling prior the extraction

- Analysis of samples with incurred residues

*Incurred residues*




*Spikes (0.1 mg/kg)*

| Analyte | Recovery QuEChERS | Recovery Pure MeCN |
|---|---|---|
| Bifenthrin | 78% | 88% |
| Buprofezin | 87% | 78% |
| Chlorfenapyr | 121% | 115% |
| Cyhalothrin-λ | 101% | 86% |
| Endosulfan-α | 77% | 80% |
| Endosulfan-β | 77% | 80% |
| Endosulfan-$SO_4$ | 90% | 82% |
| Fenvalerate | 94% | 89% |
| Triazophos | 106% | 84% |

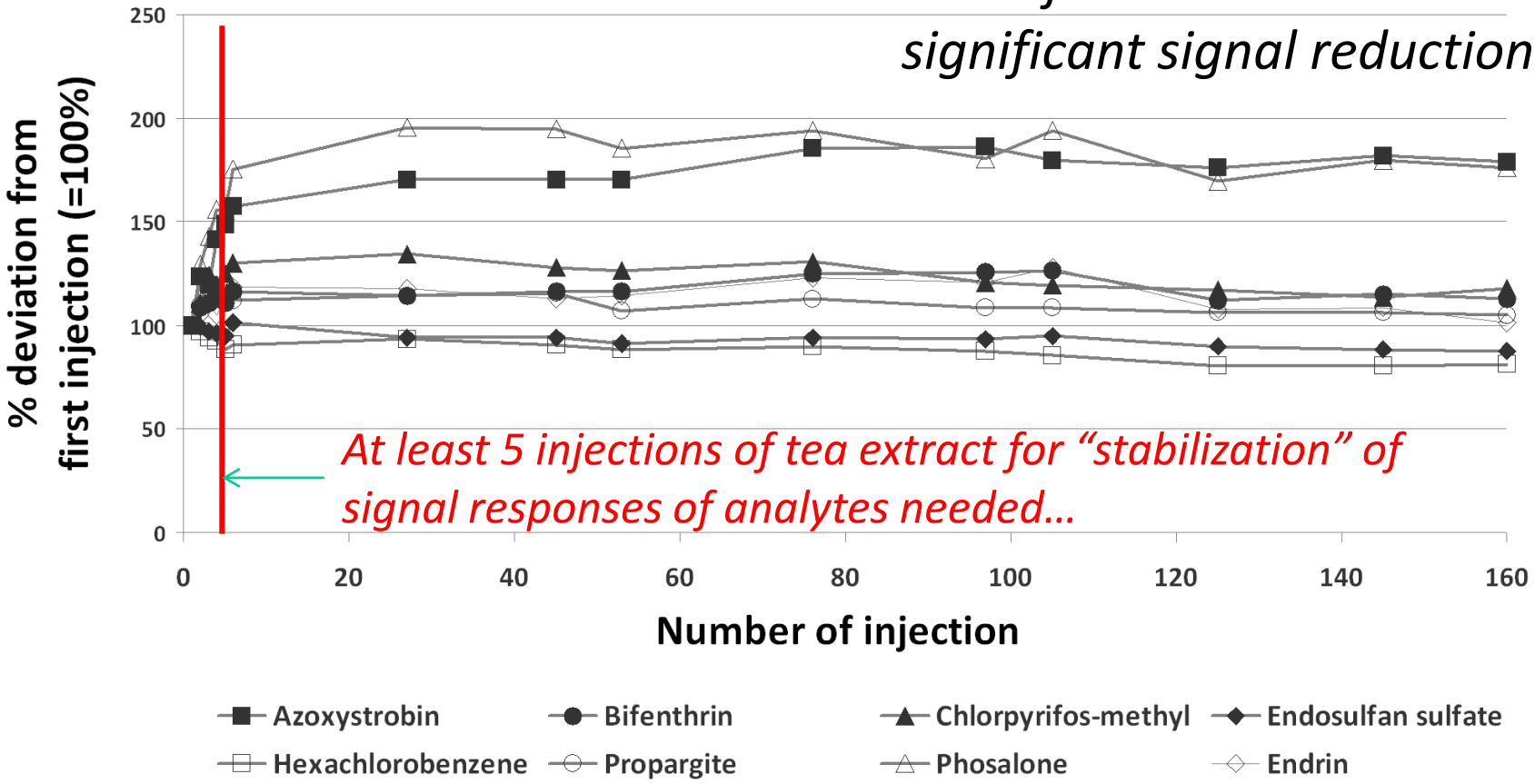
# (5) Matrix swelling prior the extraction

- **CONCLUSIONS**
  - **Matrix swelling (hydration)** represents an **important step** during the analysis of pesticide residues in tea
  - **Lower extraction efficiency** of **incurred residues** from tea samples if **pure MeCN** without matrix hydration is used although acceptable recoveries can be obtained by the analysis of spikes

# (6) Long-term stability of analytes signal

- Repeated injections of 0.1 mg/kg spiked tea extracts

*160 injections without significant signal reduction*



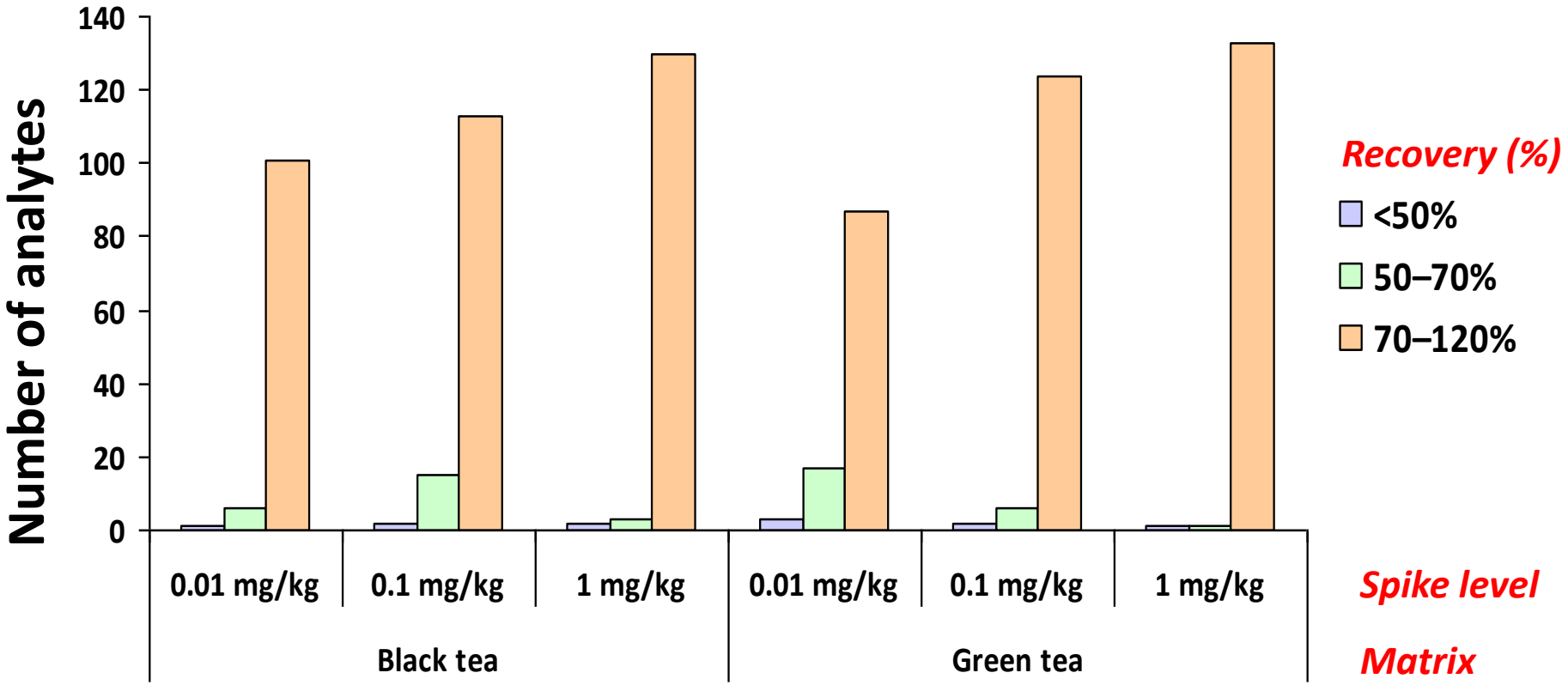
# (7) Method validation

- Recovery (%) & Repeatability (RSD, %)
  - Matrix: Black tea (levels: 0.01 , 0.1 , 1 mg/kg) *n*=6

    Green tea (levels: 0.01 , 0.1 , 1 mg/kg) *n*=6
- LOQ
  - Expressed as the lowest calibration level (LCL)

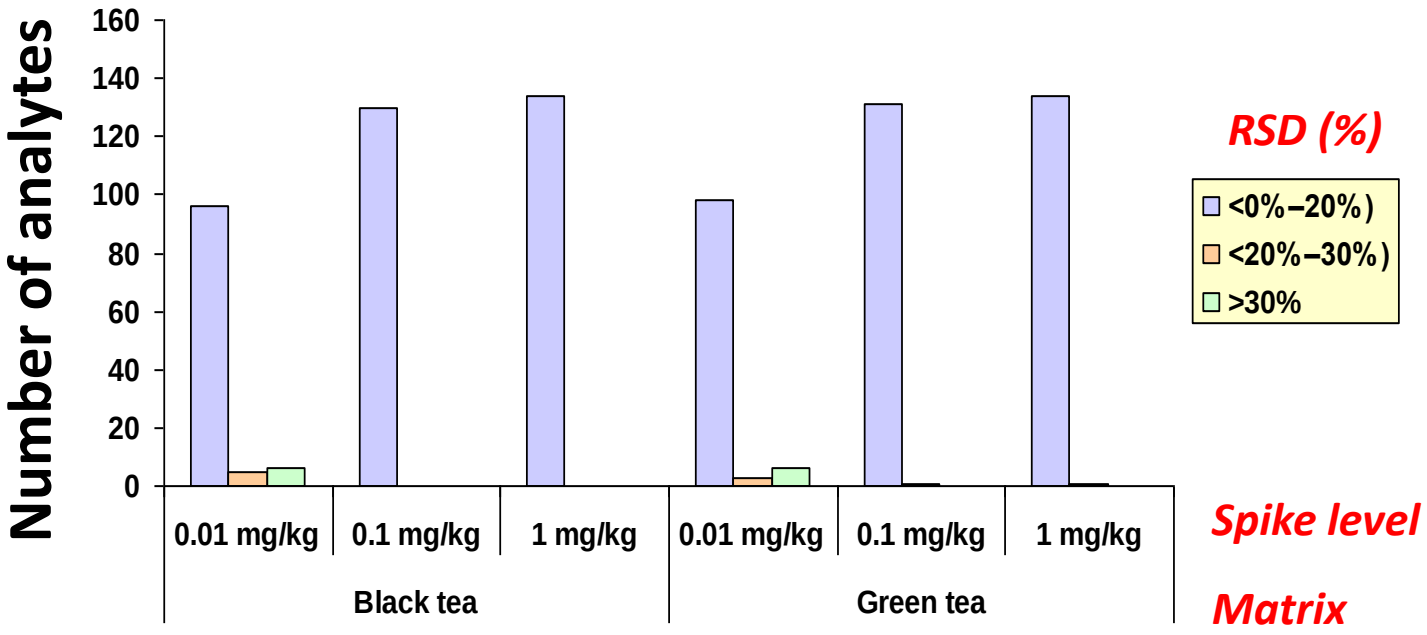
# (7) Method validation

- Recovery (%)



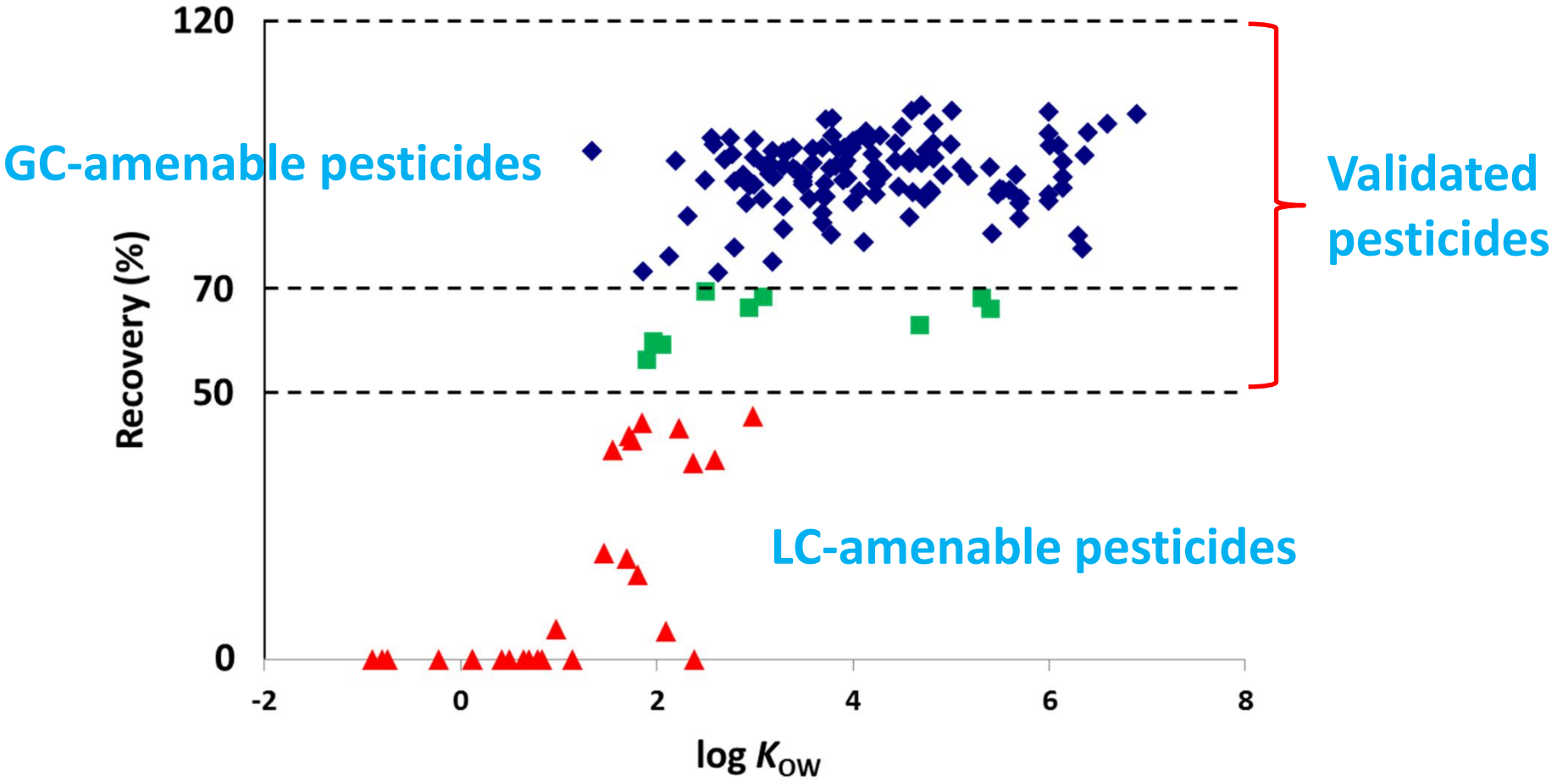
# (7) Method validation

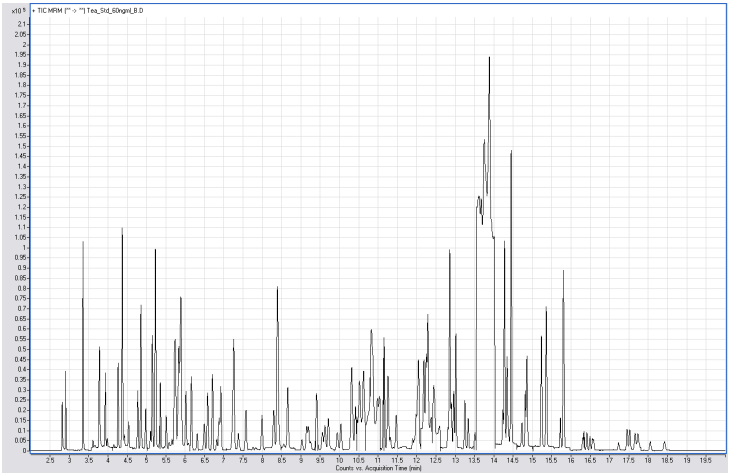
- Repeatability (RSD, %)




- LCLs For most of analytes ≤ 0.005 mg/kg (Corresponding to ≤ 0.25 ng/mL)

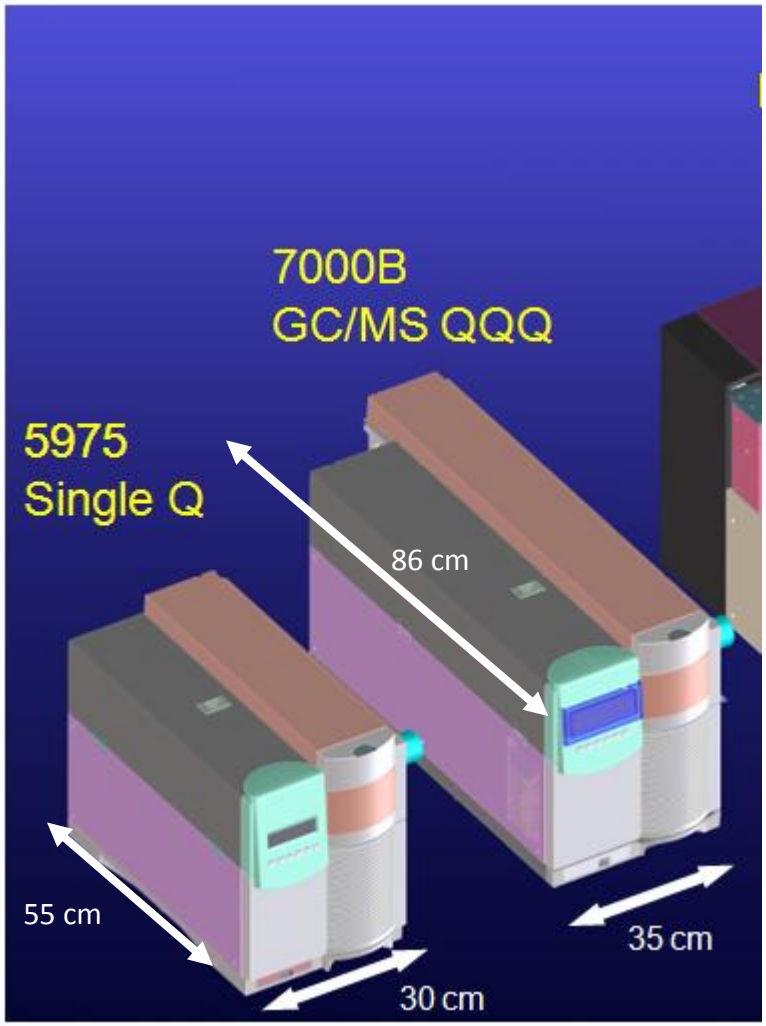
# (7) Method validation





# 3. GC/MS/MS Analysis

# 7000 GC/MS/MS – Compact Design

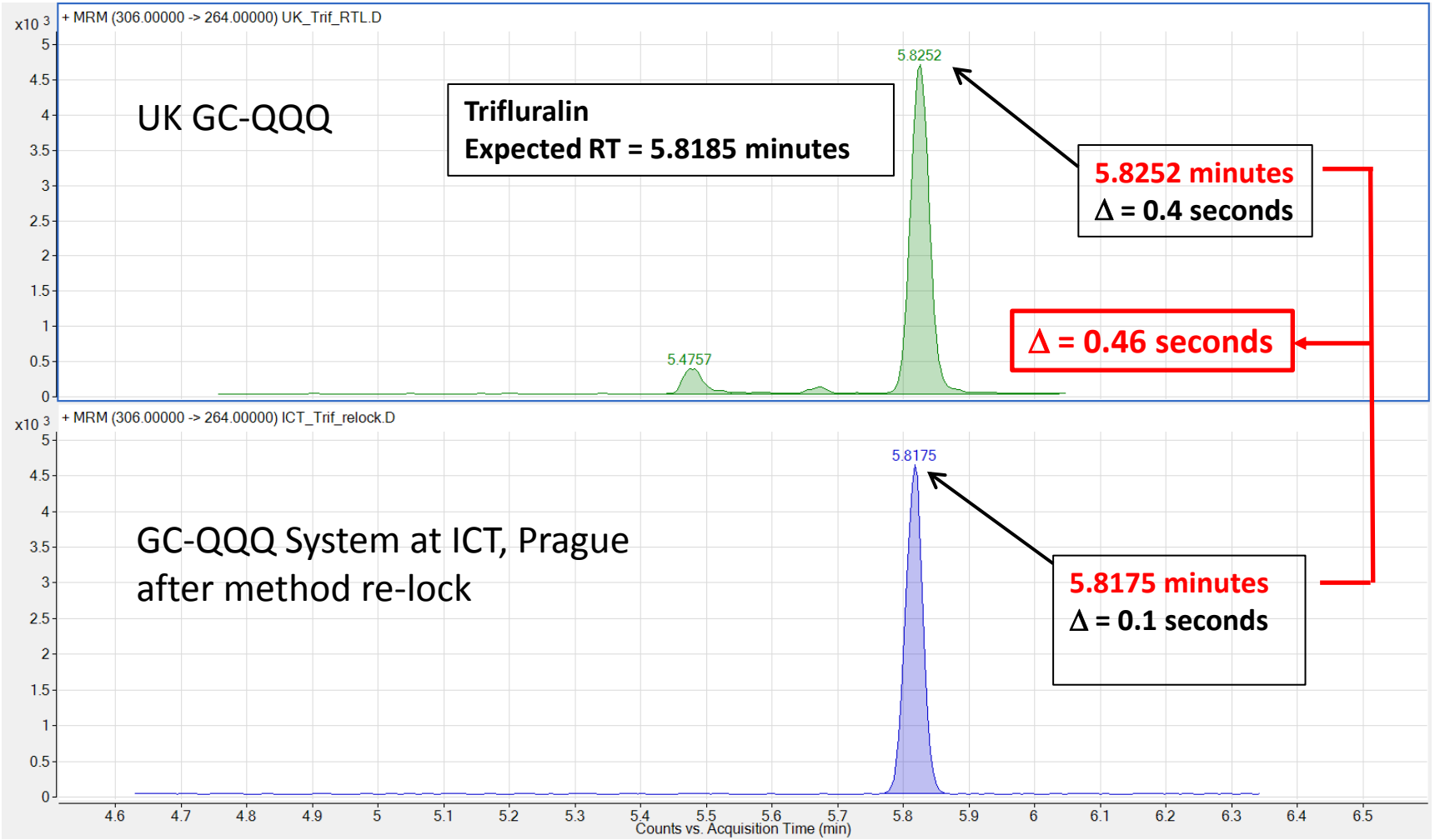


# 7890 / 7000B GC/MS/MS System

# Target analytes for Tea analysis by GC/MS/MS

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Aldrin | 26 | Chlorpyrifos-methyl | 51 | Endosulfan-sulfate | 76 | Isofenphos | 101 | Phenothrin (sum) | 126 | Tetradifon |
| 2 | Ametryn | 27 | Chlozolinate | 52 | Endrin | 77 | Isofenphos-methyl | 102 | Phenthoate | 127 | Thiometon |
| 3 | Azinphos-ethyl | 28 | Cyfluthrin (sum) | 53 | Ethion | 78 | Kresoxim-methyl | 103 | Phenylphenol, *o* - | 128 | Tolclofos-methyl |
| 4 | Azinphos-methyl | 29 | Cyhalothrin-lambda | 54 | Ethoprophos | 79 | Lindane | 104 | Phosalone | 129 | Tolylfluanid |
| 5 | Azoxystrobin | 30 | Cypermethrin (sum) | 55 | Etrimfos | 80 | Malaoxon | 105 | Phosmet | 130 | Triadimefon |
| 6 | BHC-alpha | 31 | Cyprodinil | 56 | Fenamidone | 81 | Malathion | 106 | Pirimiphos-ethyl | 131 | Triadimenol |
| 7 | BHC-beta | 32 | DDD, *o* ,*p* '- | 57 | Fenamiphos | 82 | Mecarbam | 107 | Pirimiphos-methyl | 132 | Triazophos |
| 8 | BHC-delta | 33 | DDD, *p* ,*p* '- + DDT, *o* ,*p* '- | 58 | Fenarimol | 83 | Metazachlor | 108 | Procymidone | 133 | Trifloxystrobin |
| 9 | Bifenthrin | 34 | DDE, *o* ,*p* '- | 59 | Fenchlorphos | 84 | Methacrifos | 109 | Profenofos | 134 | Trifluralin |
| 10 | Bromophos-methyl | 35 | DDE, *p* ,*p* '- | 60 | Fenitrothion | 85 | Methidathion | 110 | Propargite | 135 | Vinclozolin |
| 11 | Bromophos-ethyl | 36 | DDT, *p* ,*p* '- | 61 | Fenoxycarb | 86 | Methiocarb | 111 | Propham | | |
| 12 | Bromopropylate | 37 | Deltamethrin | 62 | Fenthion | 87 | Methoxychlor | 112 | Prothiofos | | |
| 13 | Bupirimate | 38 | Diazinon | 63 | Fenvalerate (sum) | 88 | Myclobutanil | 113 | Pyrazophos | | |
| 14 | Buprofezin | 39 | Dichlobenil | 64 | Flucythrinate (sum) | 89 | Naled | 114 | Pyridaben | | |
| 15 | Cadusafos | 40 | Dichlofluanid | 65 | Fludioxonil | 90 | Nitrofen | 115 | Pyridaphenthion | | |
| 16 | Carbophenothion | 41 | Dichlorvos | 66 | Fluvalinate (sum) | 91 | Nuarimol | 116 | Quinalphos | | |
| 17 | Chinomethionat | 42 | Diclofop-methyl | 67 | Fonofos | 92 | Oxychlordane | 117 | Quintozene | | |
| 18 | Chlordane, *cis* - | 43 | Dicloran | 68 | Haloxyfop-ethoxyethyl | 93 | Oxyfluorfen | 118 | Resmethrine (sum) | | |
| 19 | Chlordane, *trans* - | 44 | Dieldrin | 69 | Haloxyfop-methyl | 94 | Paraoxon | 119 | Sulfotep | | |
| 20 | Chlorfenapyr | 45 | Difenoconazol (sum) | 70 | Heptachlor | 95 | Parathion-ethyl | 120 | Tebuconazole | | |
| 21 | Chlorfenvinphos | 46 | Diphenylamine | 71 | Heptachlor-epoxide (endo) | 96 | Parathion-methyl | 121 | Tecnazene | | |
| 22 | Chlorobenzilate | 47 | Disulfoton | 72 | Heptachlor-epoxide (exo) | 97 | Penconazole | 122 | Tefluthrin, *cis* - | | |
| 23 | Chlorothalonil | 48 | Disulfoton-sulfone | 73 | Heptenophos | 98 | Pencycuron | 123 | Terbufos | | |
| 24 | Chlorpropham | 49 | Endosulfan-alpha | 74 | Hexachlorobenzene | 99 | Pendimethalin | 124 | Terbufos-sulfone | | |
| 25 | Chlorpyrifos | 50 | Endosulfan-beta | 75 | Iprodione | 100 | Permethrin (sum) | 125 | Tetraconazole | | |

# RTL GC/MS/MS Method – Set up

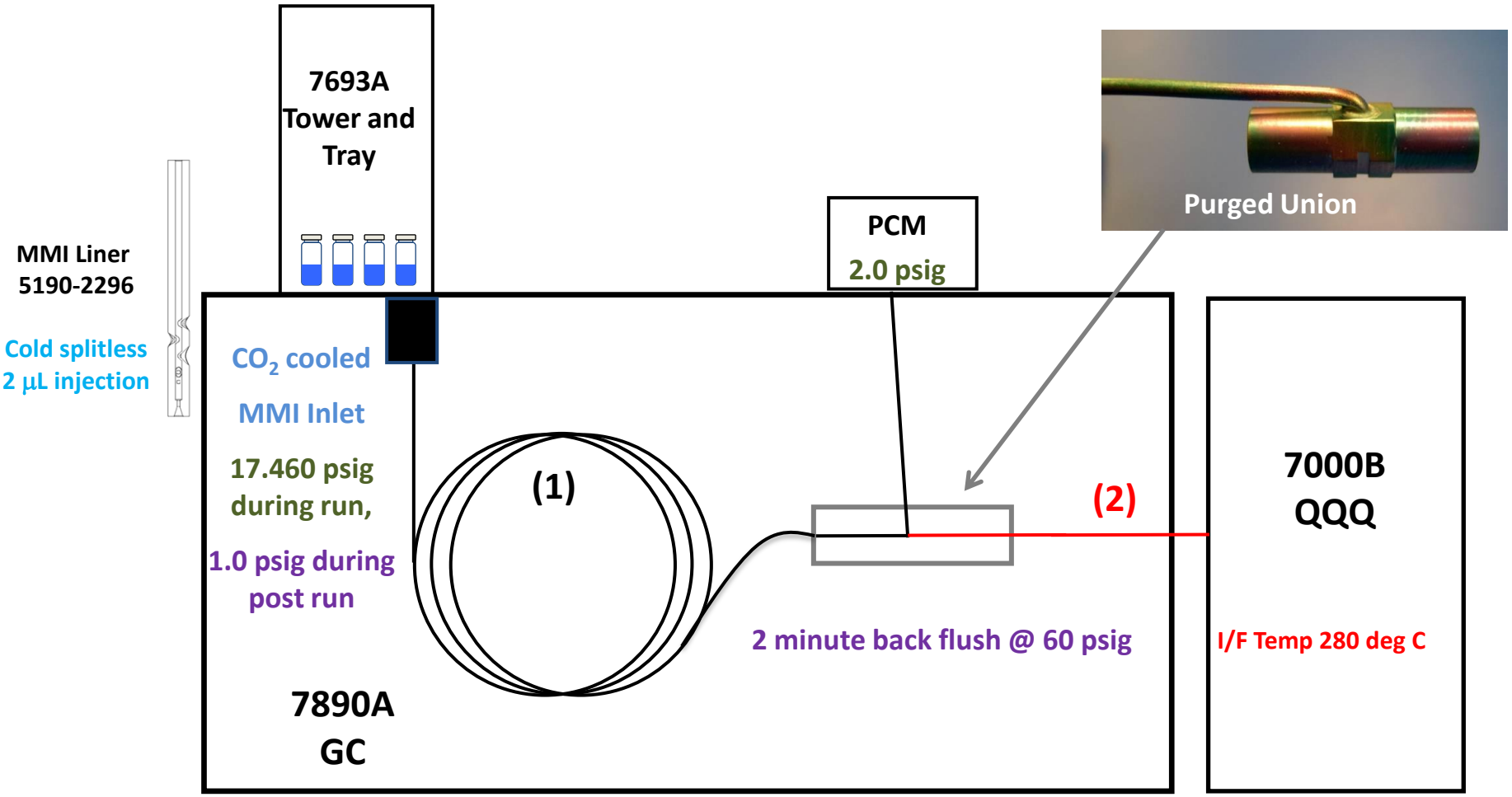



Load method, re-lock method on customer site and acquisition method is set-up

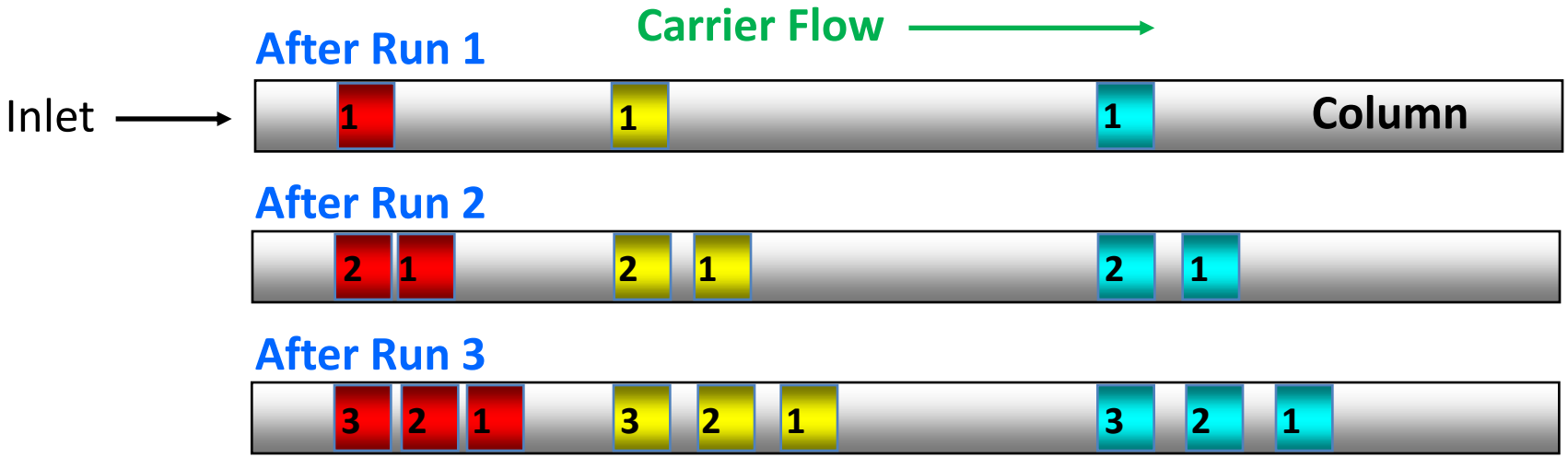
# RTL 2x Pesticide Method + Post-Column Backflush

(1) Constant Pressure, Column 15 m X 0.25 mm id X 0.25 μm HP-5MSUI

(2) Constant Pressure, Restrictor 0.50 m X 0.15 mm id x 0.15 μm DB-5MSUI

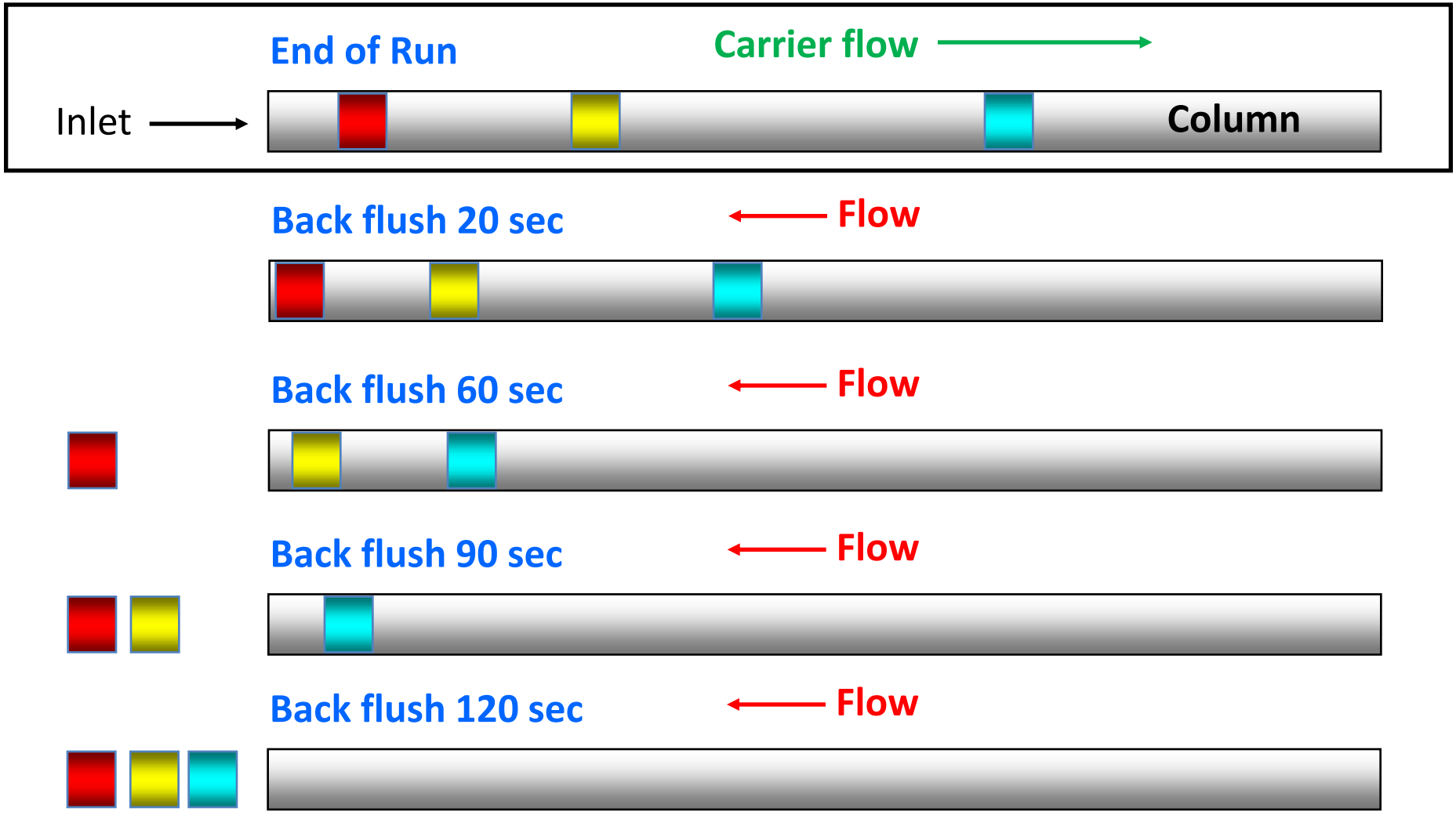


# Matrix Compounds may be left in the Column after each Injection



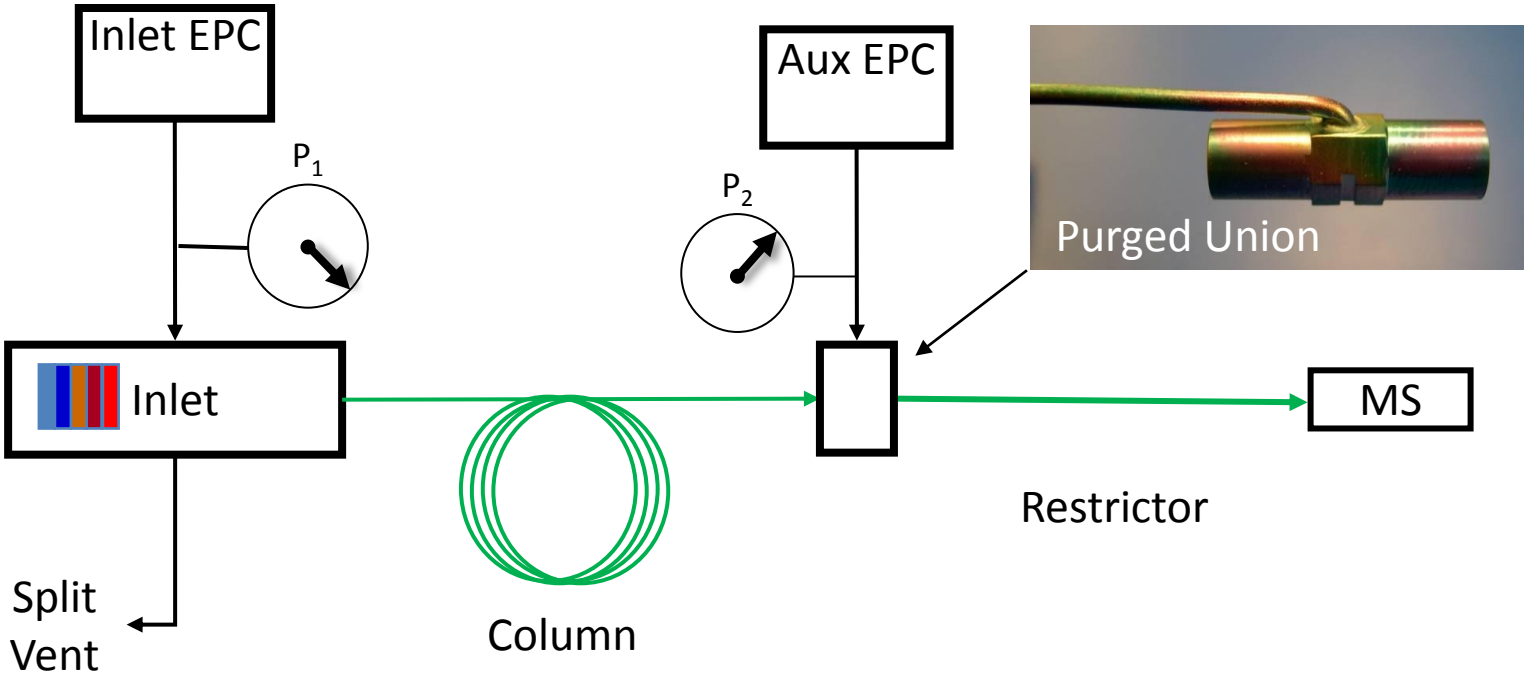

- These heavy matrix materials build up and travel further into the column with each injection.
- This build-up of heavy materials can cause retention time shifts, peak distortion, higher bleed, and loss of sensitivity

# Back flushing After Each Injection



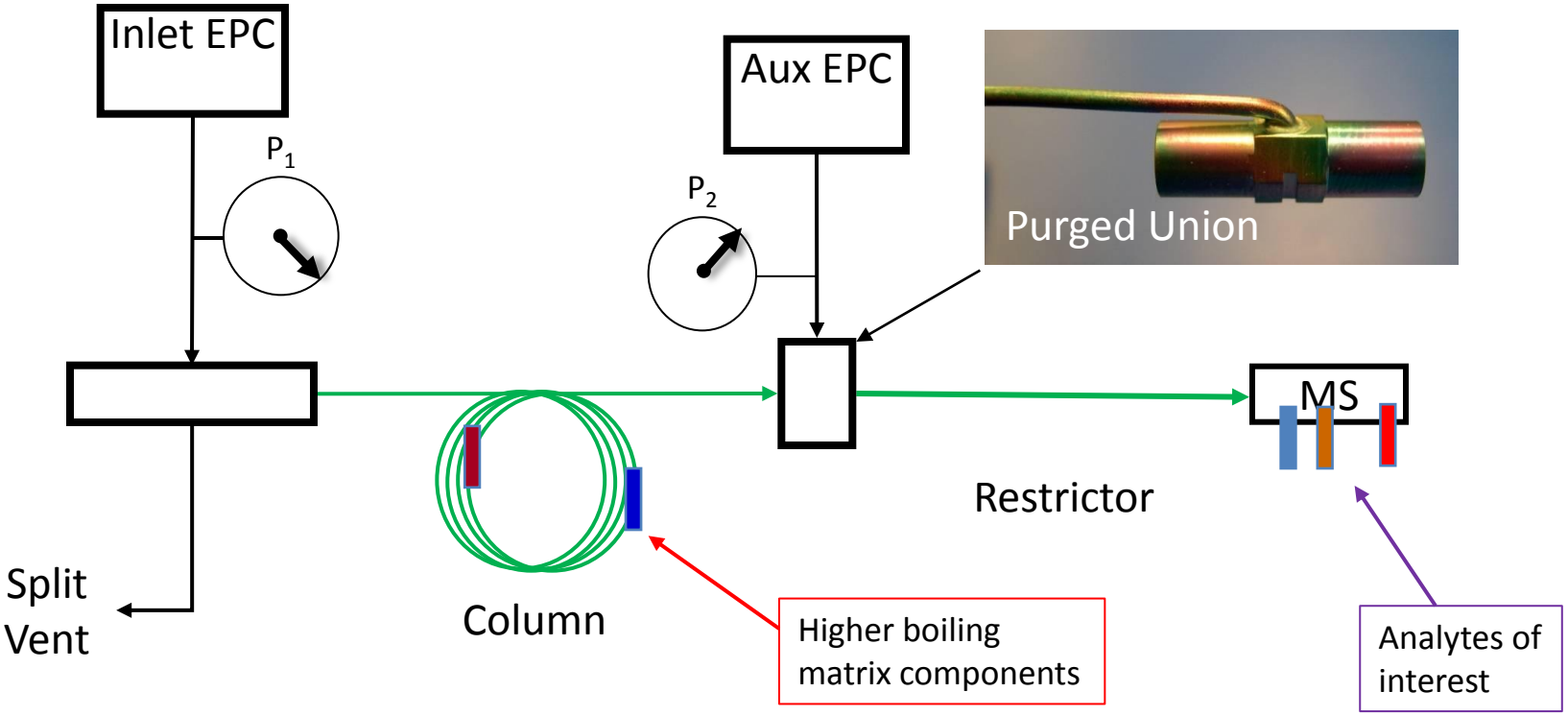

- Back flushing removes heavy materials after each injection, heaviest first

# Post column, post run back flush



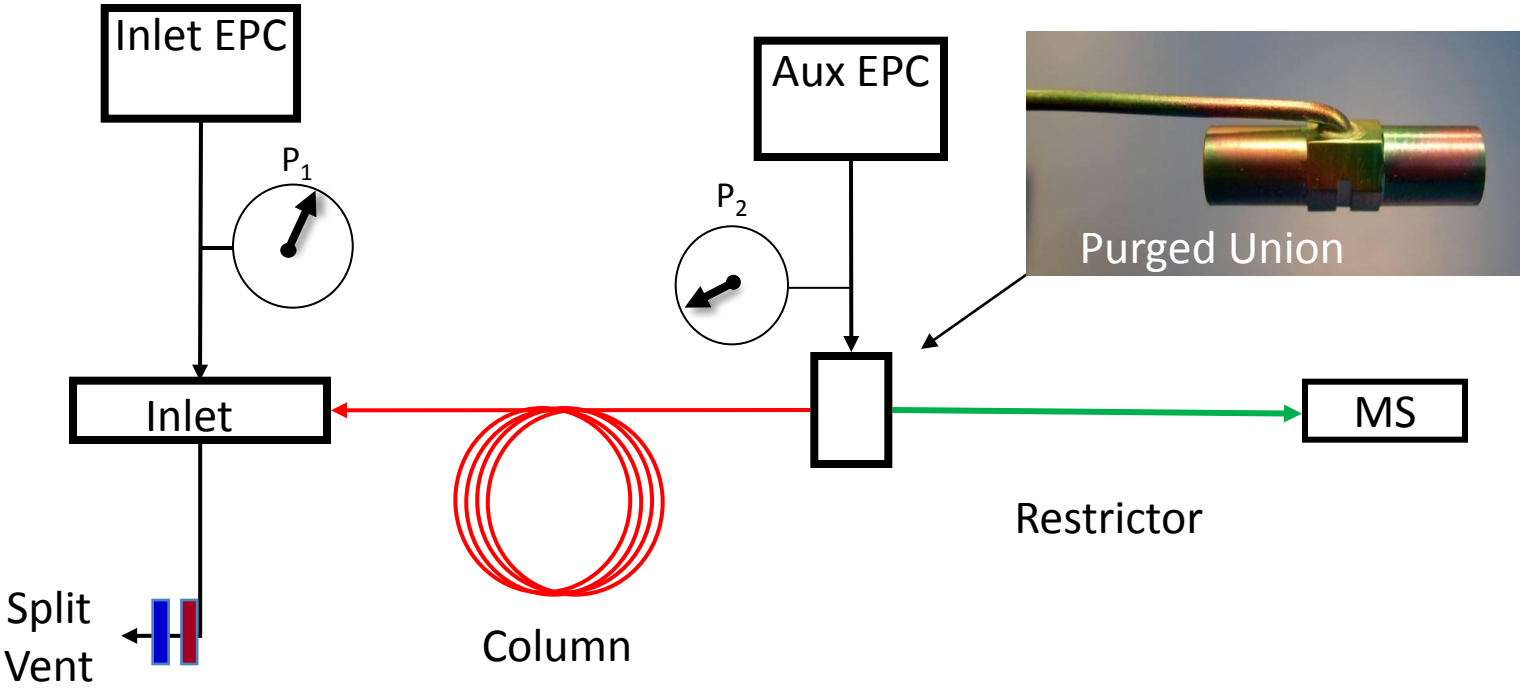

During the run, $P_1 > P_2$, Carrier gas flows towards MS

# Post column, post run back flush



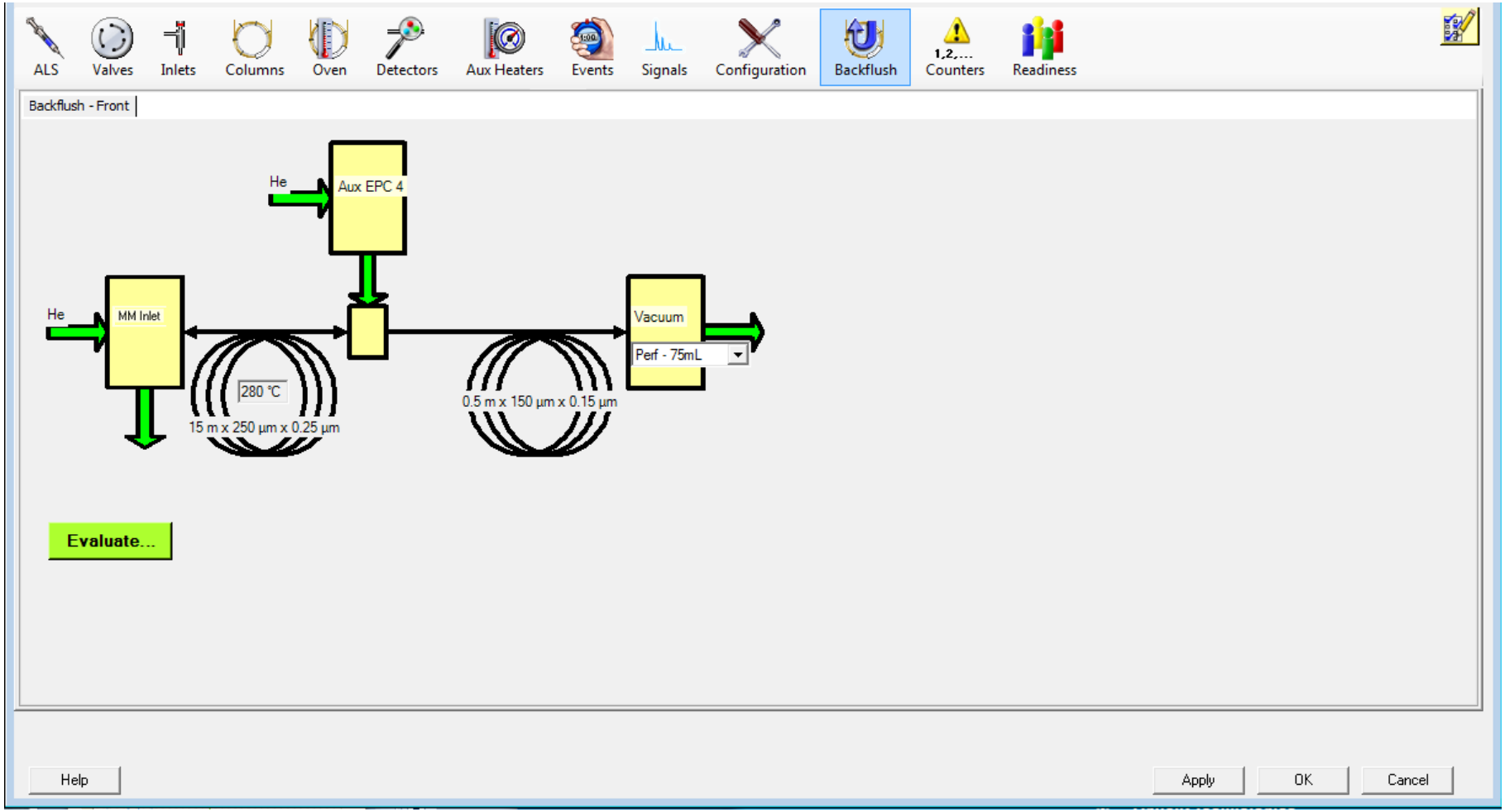

During the run, $P_1 > P_2$, Carrier gas flows towards MS

# Post column, post run back flush

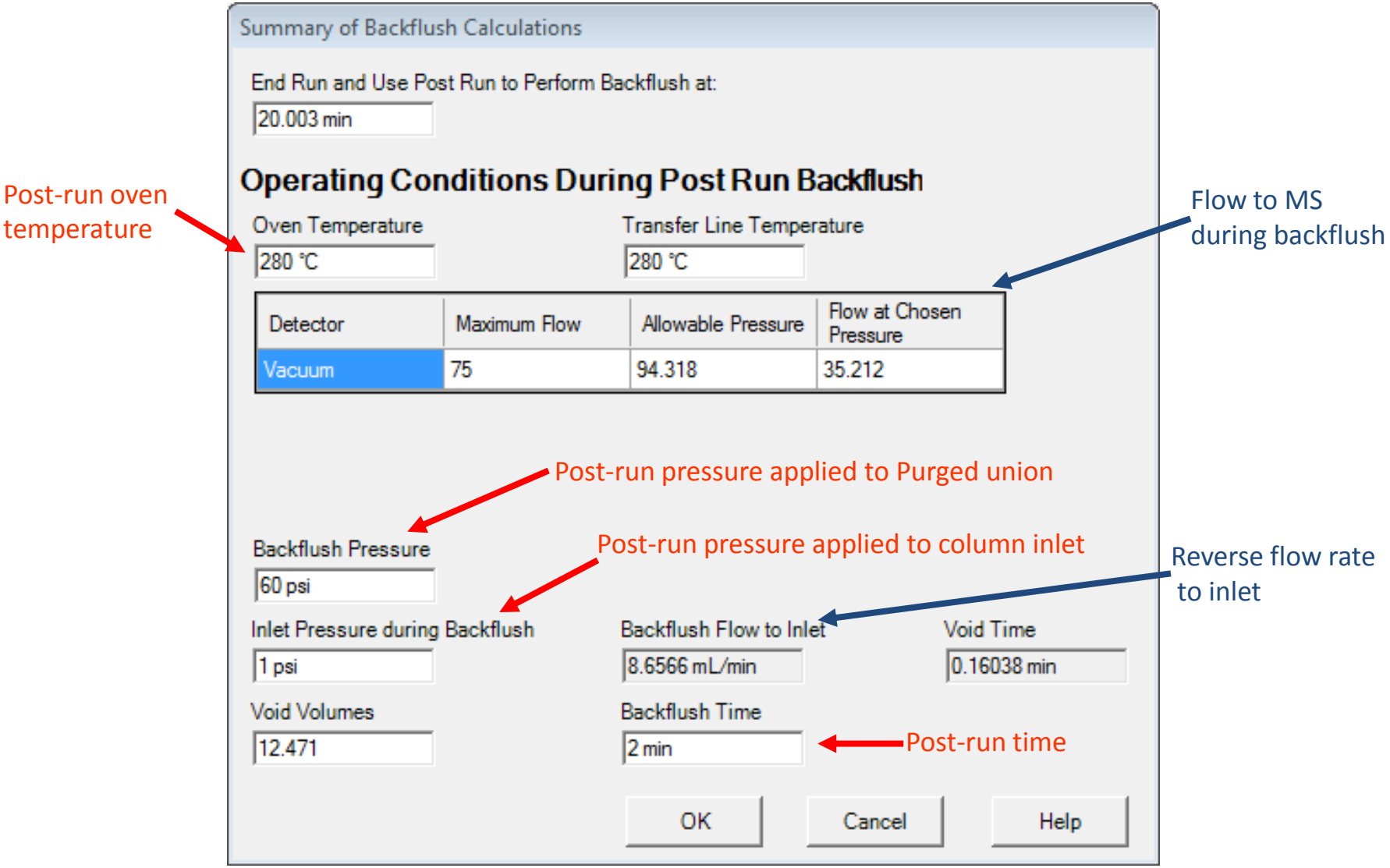



During post run back flush $P_1 < P_2$, Carrier gas flow reversed in column

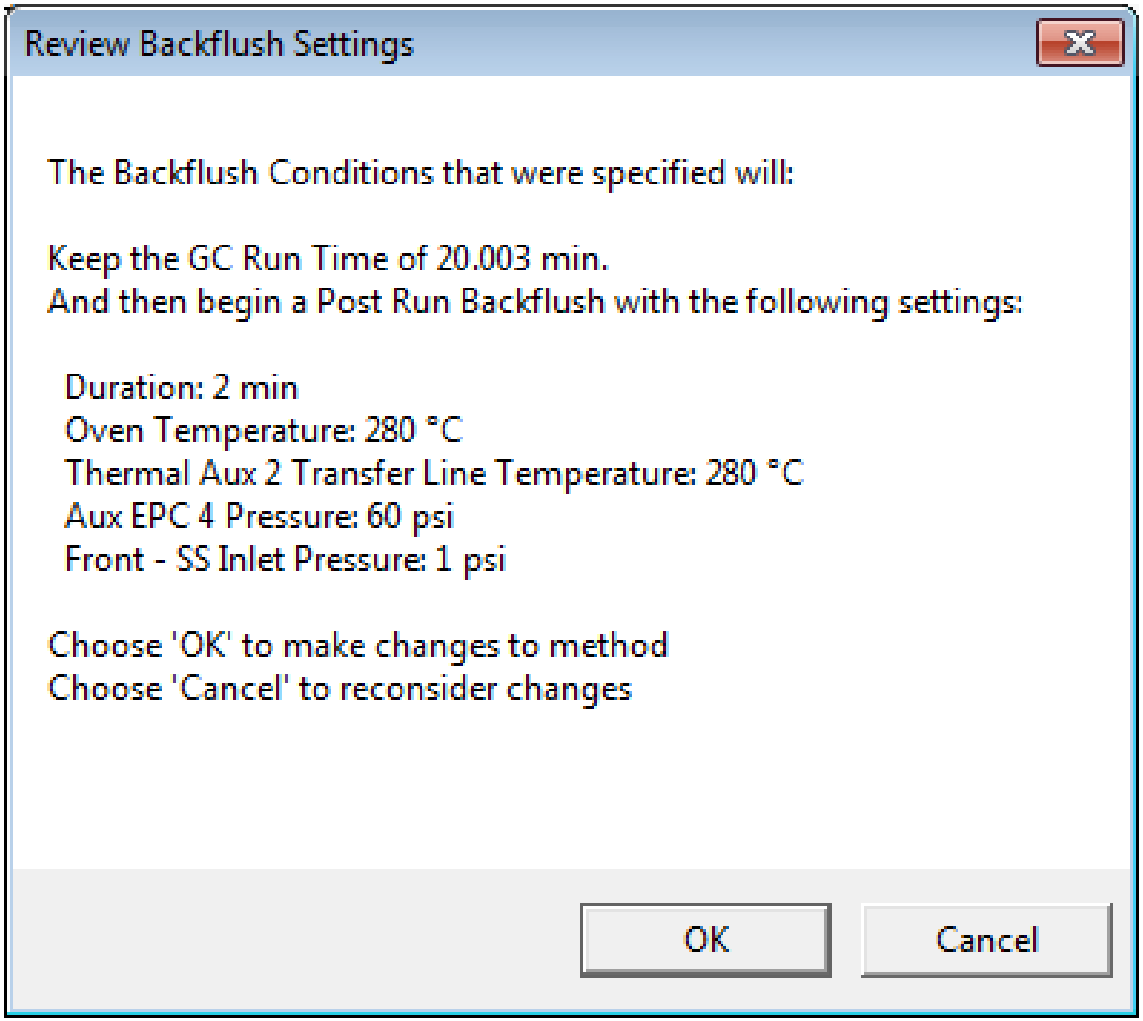
# Post-Column Back flush set-up – Step 1



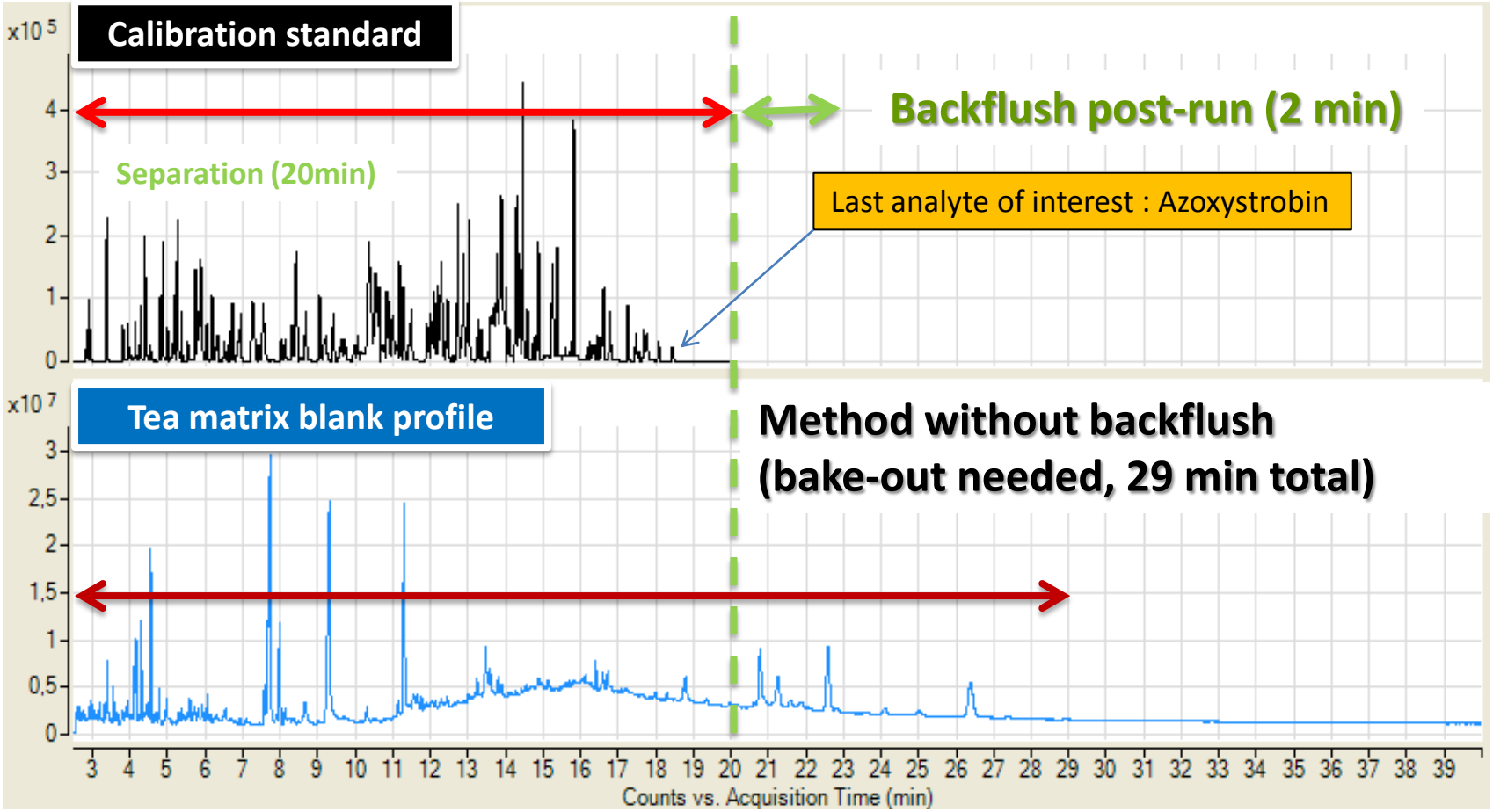
# Post-Column Back flush set-up – Step 2



# Post-Column Back flush set-up – Step 3



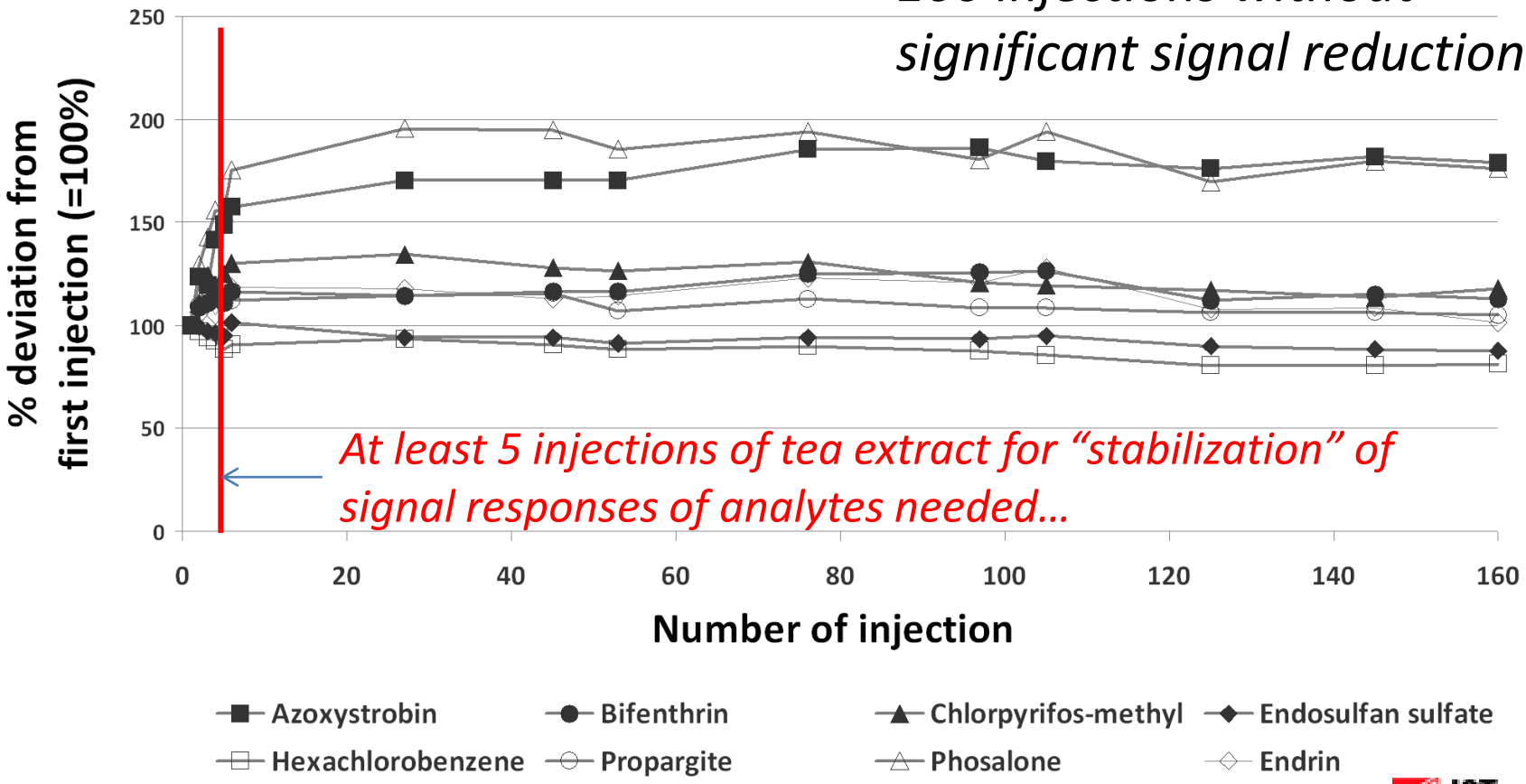
# Back flush saves cycle time



# Long-term stability of analytes signal

- **Repeated injections of 0.1 mg/kg spiked tea extracts**

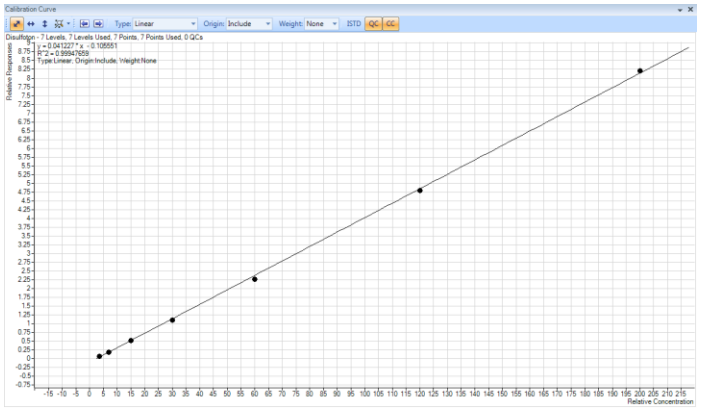
*160 injections without significant signal reduction*

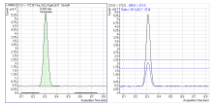



ICT PRAGUE

# Benefits of Backflush

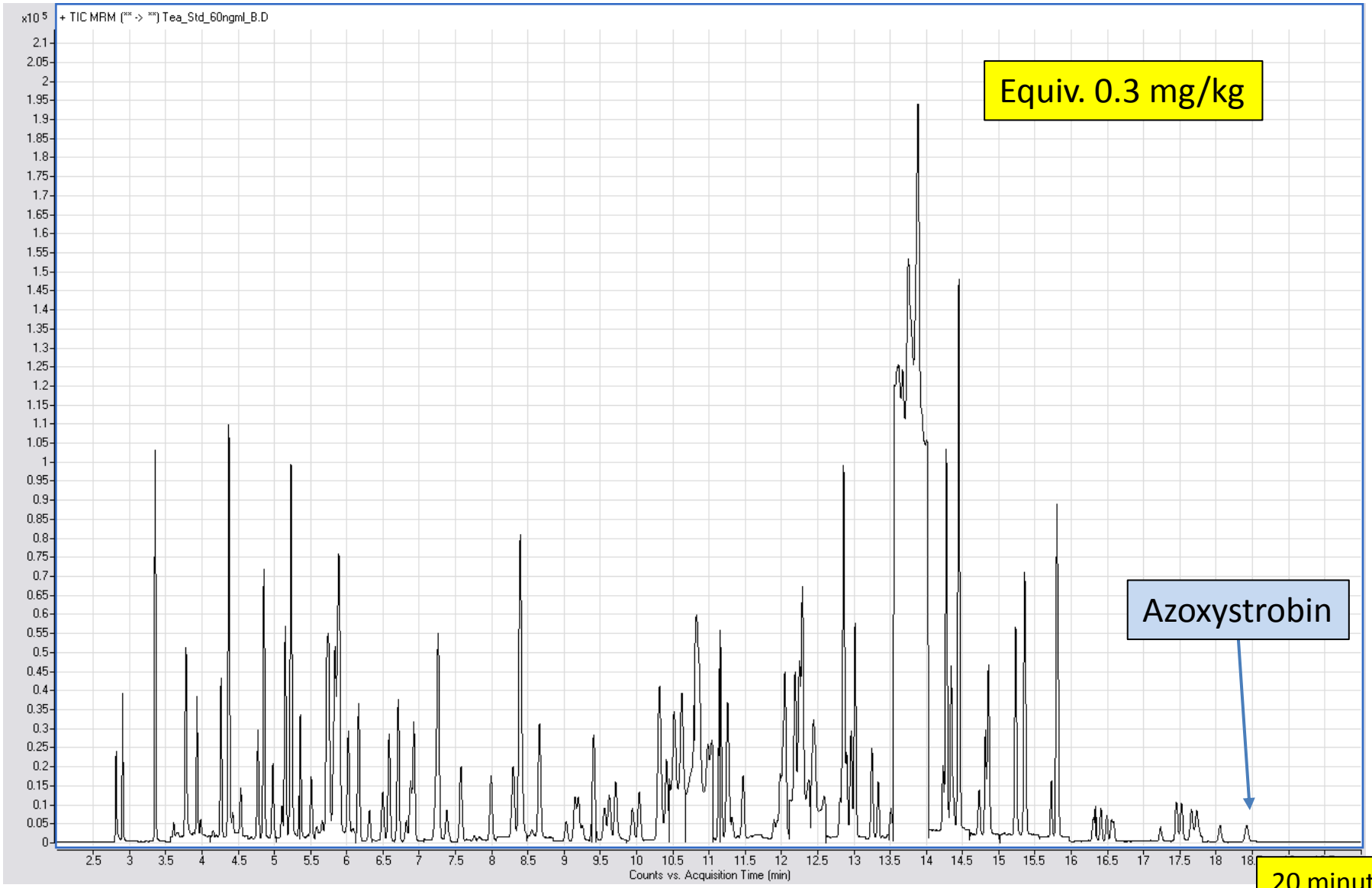
- Consistent analyte retention times and responses
- Robust chromatography and consistent analyte chromatographic peak shapes
- Prevention of high boiling matrix from contaminating the MS ion source
- Extended column life-time and reduced cycle times by removing the need for high-temperature bake-out between runs





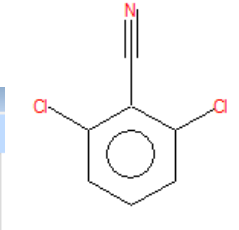
# 4. Results

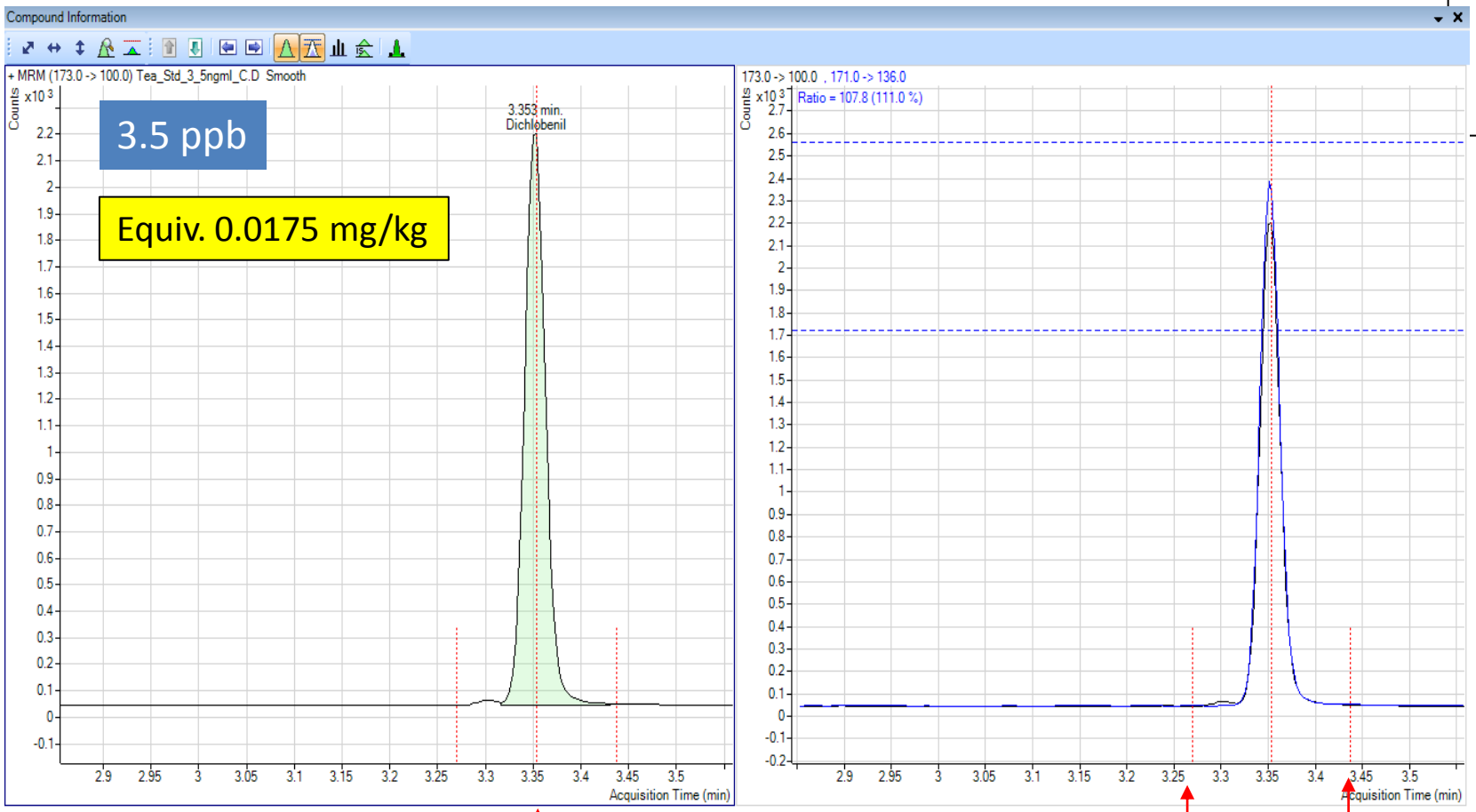
# TIC MRM :Tea Matrix Spiked at 60 ng/mL




20 minutes

# Matrix matched Tea Standard - Dichlobenil



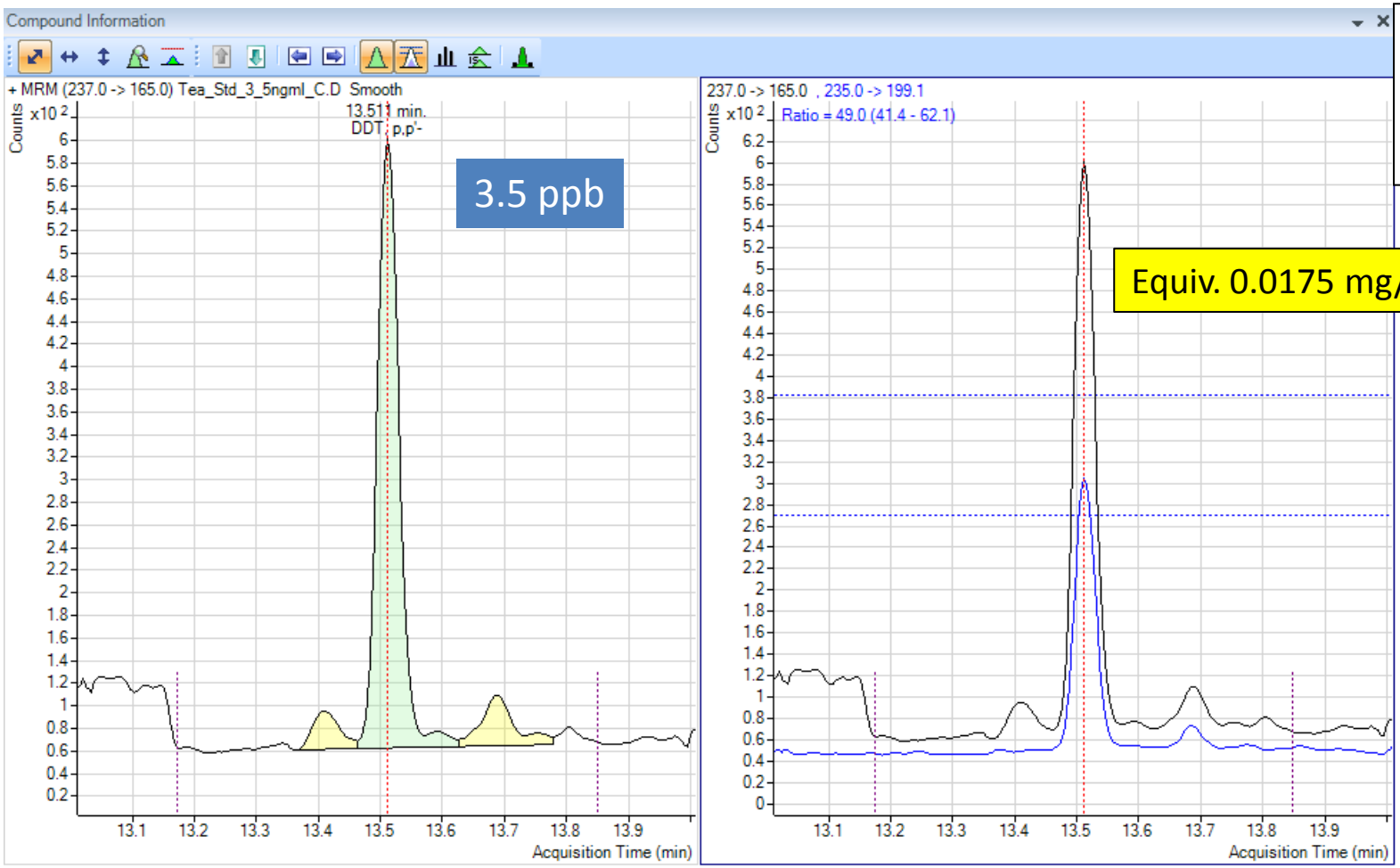

Ion ratio
+/- 20%

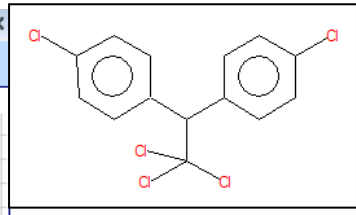
Expected . RT

ID Window

Early eluting Benzo-nitrile herbicide

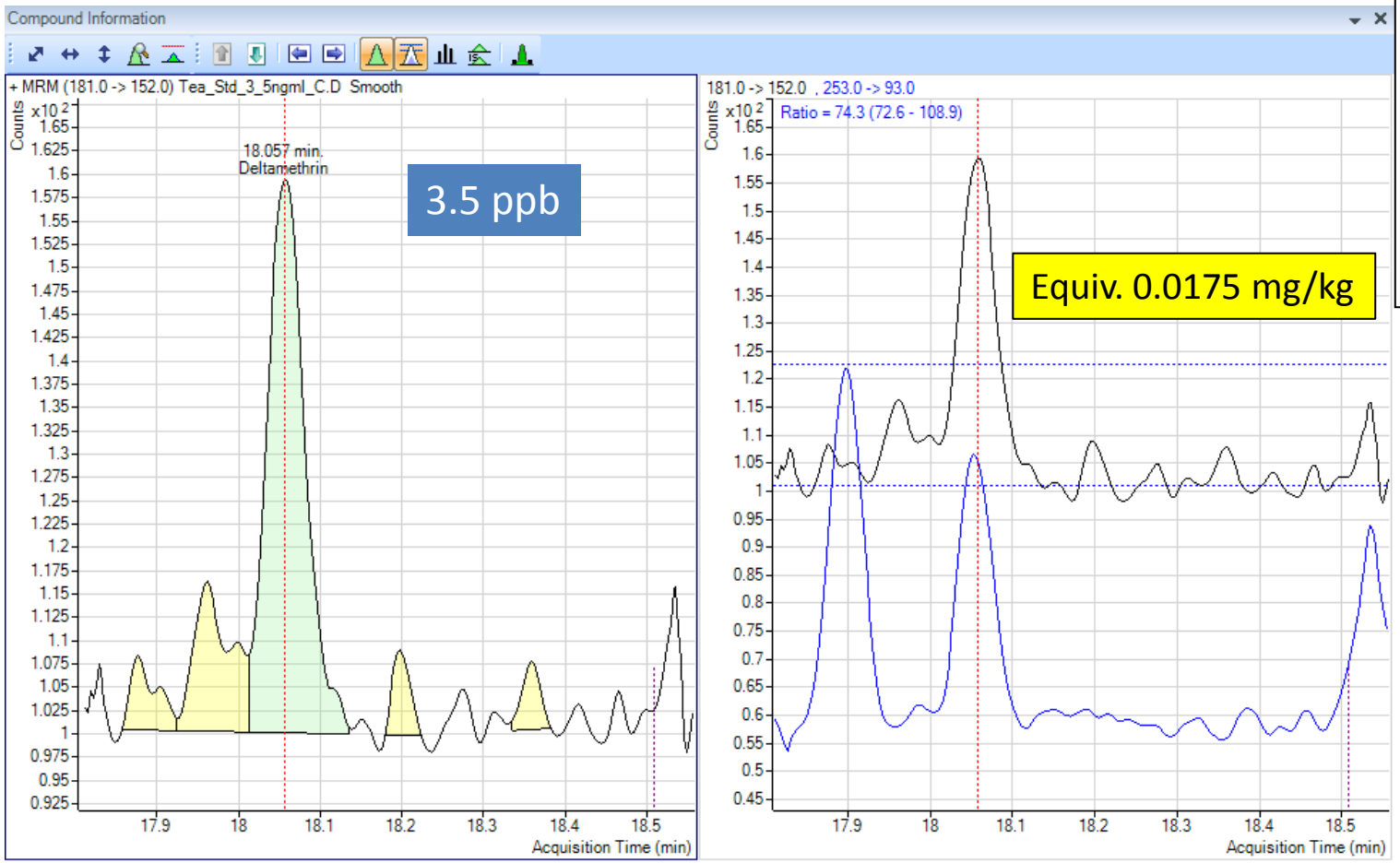
# Matrix matched Tea Standard – p,p'-DDT

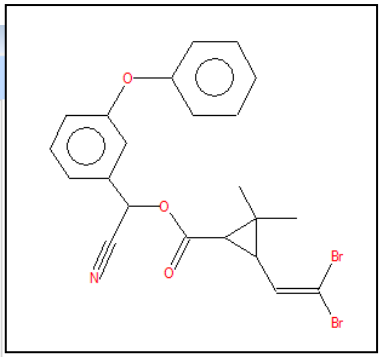



Mid- eluting Organo-chlorine insecticide

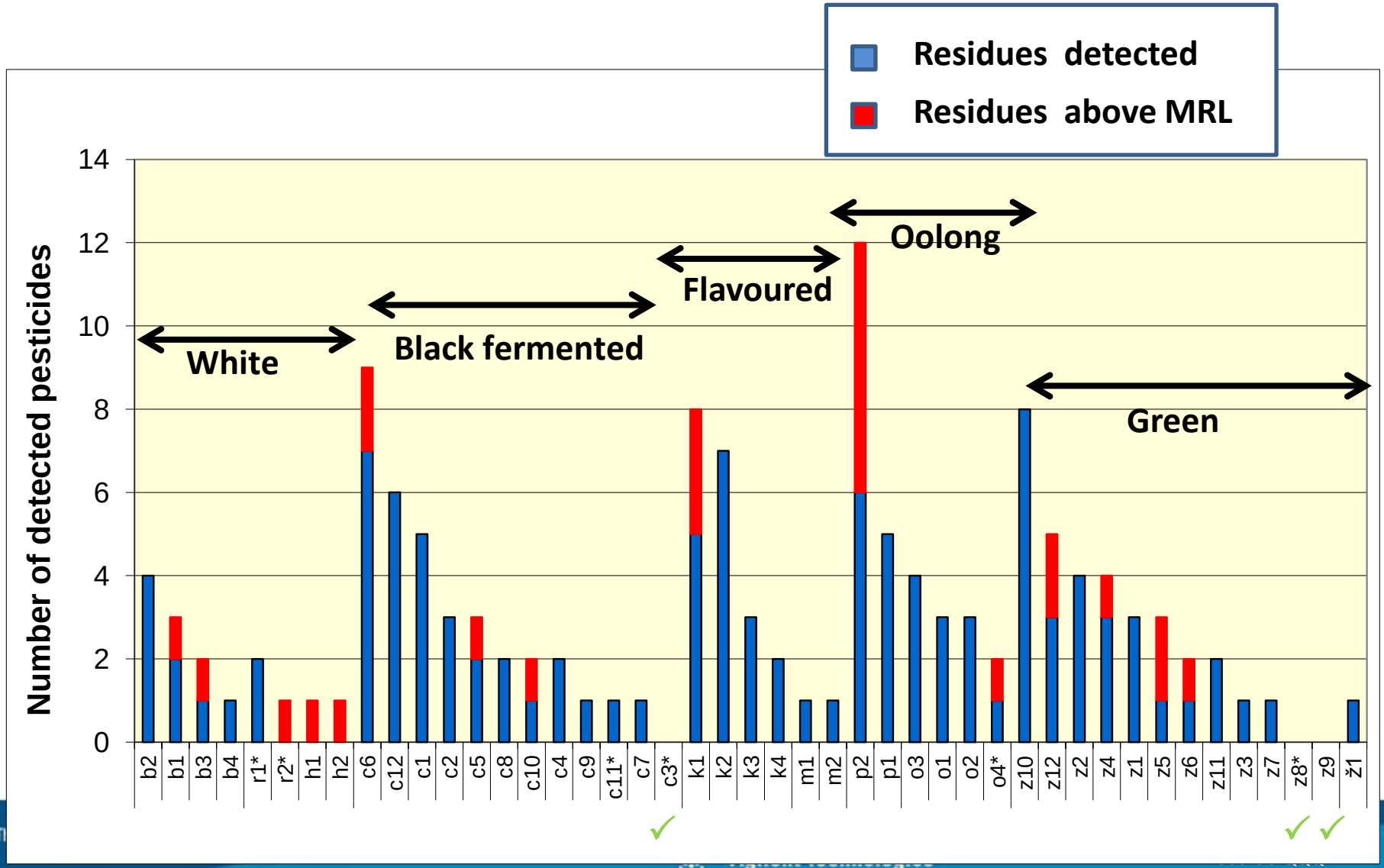
# Matrix matched Tea Standard - Deltamethrin




Late eluting Pyrethroid insecticide

# Pesticide residues in Tea from Czech market: Results of pilot study (*n*=45)



# Pesticide residues in tea samples (*n*=45)

## Data from GC–MS/MS and LC–MS/MS analyses

| Pesticide | Number of positive samples (45 Tea samples) | Maximum concentration (mg/kg) | EU MRL* (mg/kg) | Number of MRL exceedances |
|---|---|---|---|---|
| Chlorpyrifos-methyl | 22 | 1.763 | 0.1 | 8 |
| Imidacloprid | 12 | 0.294 | 0.05 | 7 |
| Acetamiprid | 7 | 0.623 | 0.1 | 2 |
| Myclobutanil | 1 | 0.459 | 0.05 | 1 |
| Pyraclostrobin | 1 | 0.052 | 0.05 | 1 |
| Thiophanate-Me | 1 | 1.329 | 0.1 | 1 |
| Acephate | 1 | 0.091 | 0.05 | 1 |
| Metolcarb | 1 | 0.05 | 0.01 | 1 |
| Monocrotophos | 1 | 0.855 | 0.1 | 1 |
| Triazophos | 1 | 0.461 | 0.02 | 1 |
| Paclobutrazol | 1 | 0.056 | 0.02 | 1 |
| Carbendazim | 2 | 0.284 | 0.1 | 1 |
| Buprofezin | 2 | 0.09 | 0.05 | 1 |
| Methomyl | 2 | 0.303 | 0.1 | 1 |
| Thiametoxam | 2 | 0.169 | 0.1 | 1 |

* Regulation (EC) No 396/2005, Tea (dried leaves and stalks, fermented or otherwise, of *Camelia sinensis*)

# Pesticide residues in tea samples (*n*=45)

**Confirmed, quantified but compliant with EU MRL**

| Pesticide | Number of positive samples | Maximum concentration (mg/kg) | EU MRL* (mg/kg) |
|---|---|---|---|
| Endosulfan | 16 | 0.107 | 30 |
| Bifenthrin | 13 | 0.862 | 5 |
| Cyhalothrin-lambda | 10 | 0.123 | 1 |
| Propargite | 6 | 0.119 | 5 |
| Cypermethrin | 5 | 0.312 | 0.5 |
| Chlorpyrifos | 3 | 0.081 | 0.1 |
| DDT | 3 | 0.03 | 0.2 |
| Ethion | 2 | 0.838 | 3 |
| Metalaxyl | 2 | 0.021 | 0.1 |
| Pyridaben | 2 | 0.048 | 0.05 |

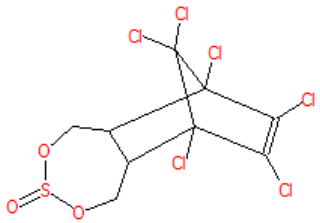
* Regulation (EC) No 396/2005, Tea (dried leaves and stalks, fermented or otherwise, of Camelia sinensis )

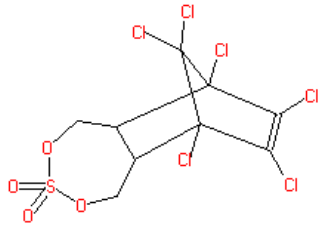
# Finding a Tea Matrix Blank - Analysis of Organic Tea Samples

# Pesticide Residues in 4 Organic Tea Extracts by GC/MS/MS

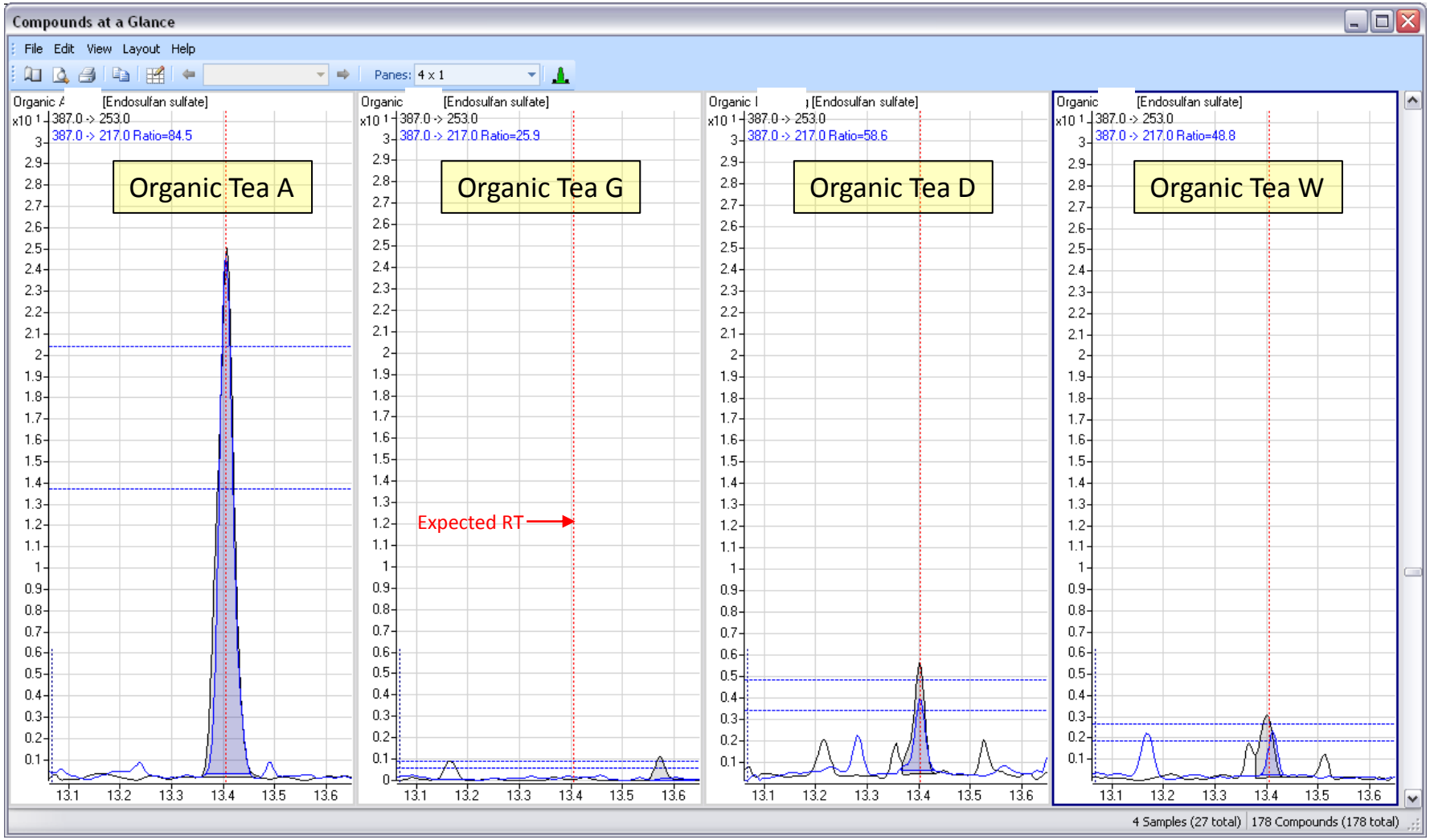
| Analyte | Ret Time (min) | Organic A | Organic G | Organic D | Organic W |
|---|---|---|---|---|---|
| | | ppb | ppb | ppb | ppb |
| Biphenyl | 3.55 | 0.33 | 0.67 | 0.24 | 0.41 |
| o-Phenyl phenol | 4.42 | 0.25 | 0.45 | 0.24 | 0.24 |
| a-Endosulfan | 11.32 | 0.89 | | | |
| p,p'-DDE | 12.02 | 0.18 | | | |
| b-Endosulfan | 12.59 | 1.15 | | | |
| Endosulfan sulfate | 13.40 | 3.31 | | 0.60 | |
| pp'-DDT | 13.51 | 0.45 | | 0.27 | |

Σ Endosulfans = 5.35 ppb, Equivalent to 0.027 mg/kg
EU MRL (Tea) = 30 mg/kg





# Endosulfan Sulfate Transitions in 4 Organic Tea Extracts

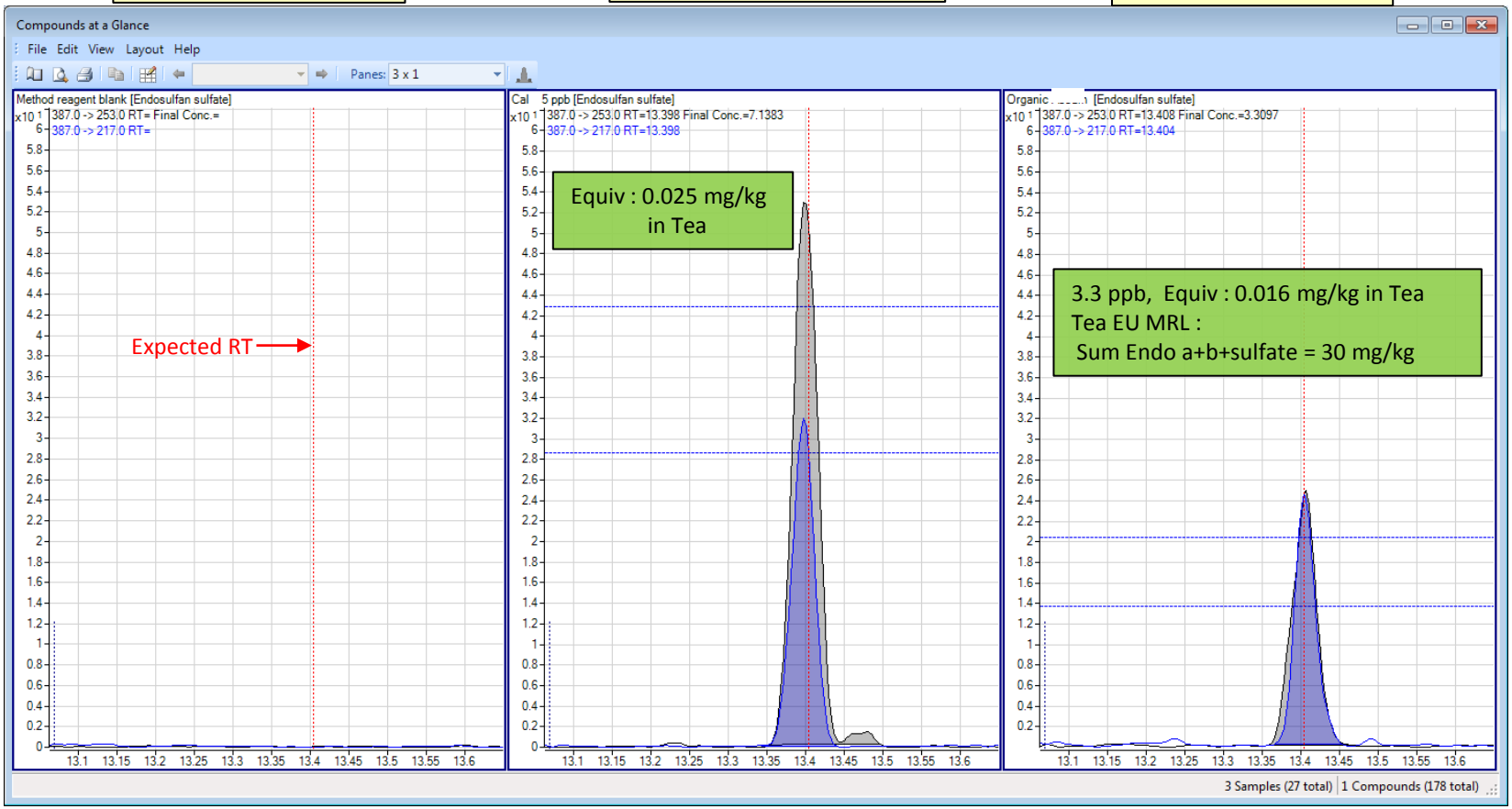


# Endosulfan Sulfate (?) in Organic Tea Extract – Using 2 MRM Transitions

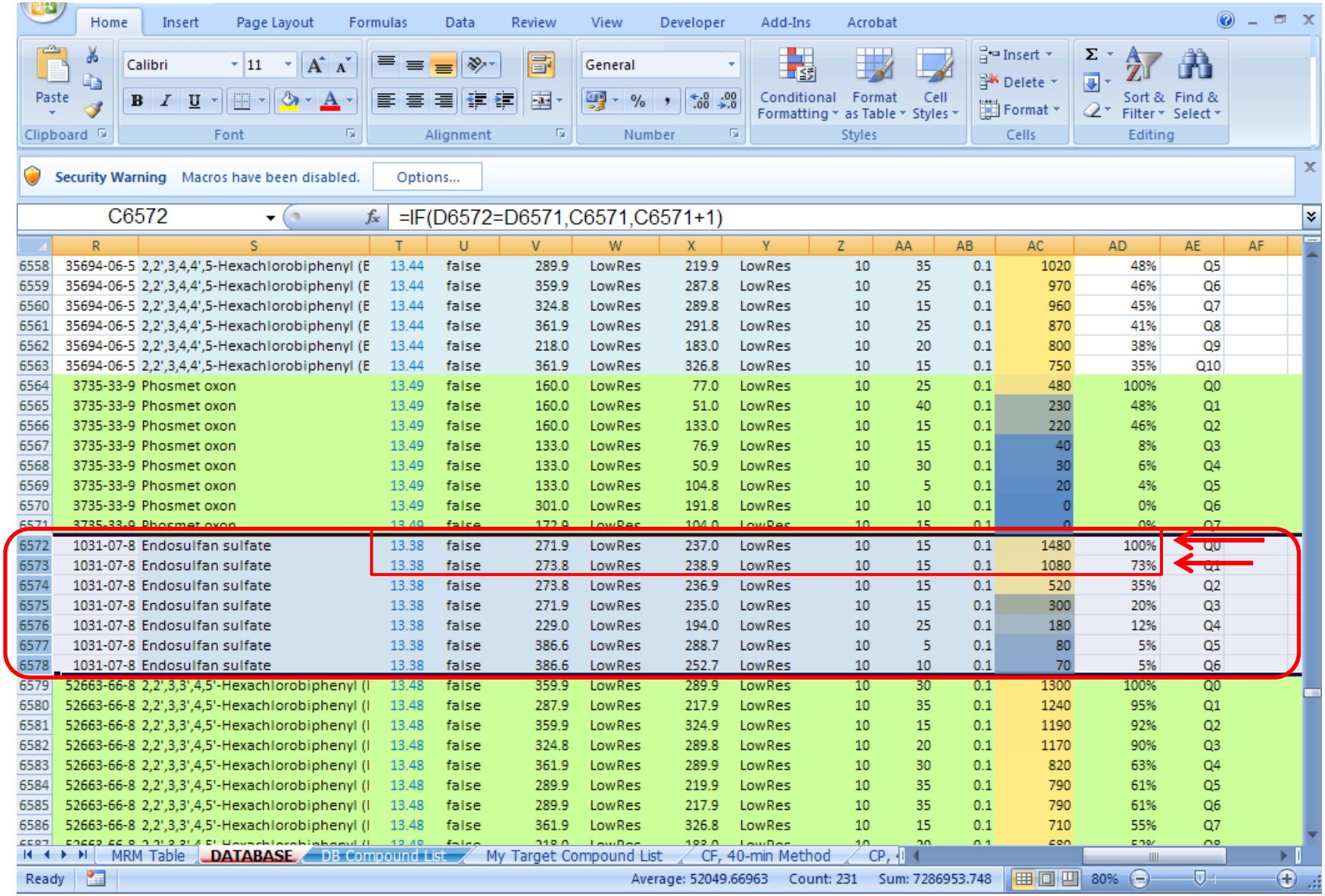
Reagent blank

Matrix Matched Std 5 ppb

Organic Tea A



# Optimized Endosulfan Sulfate MRM Transitions in the G9250AA Database



Security Warning Macros have been disabled. Options...

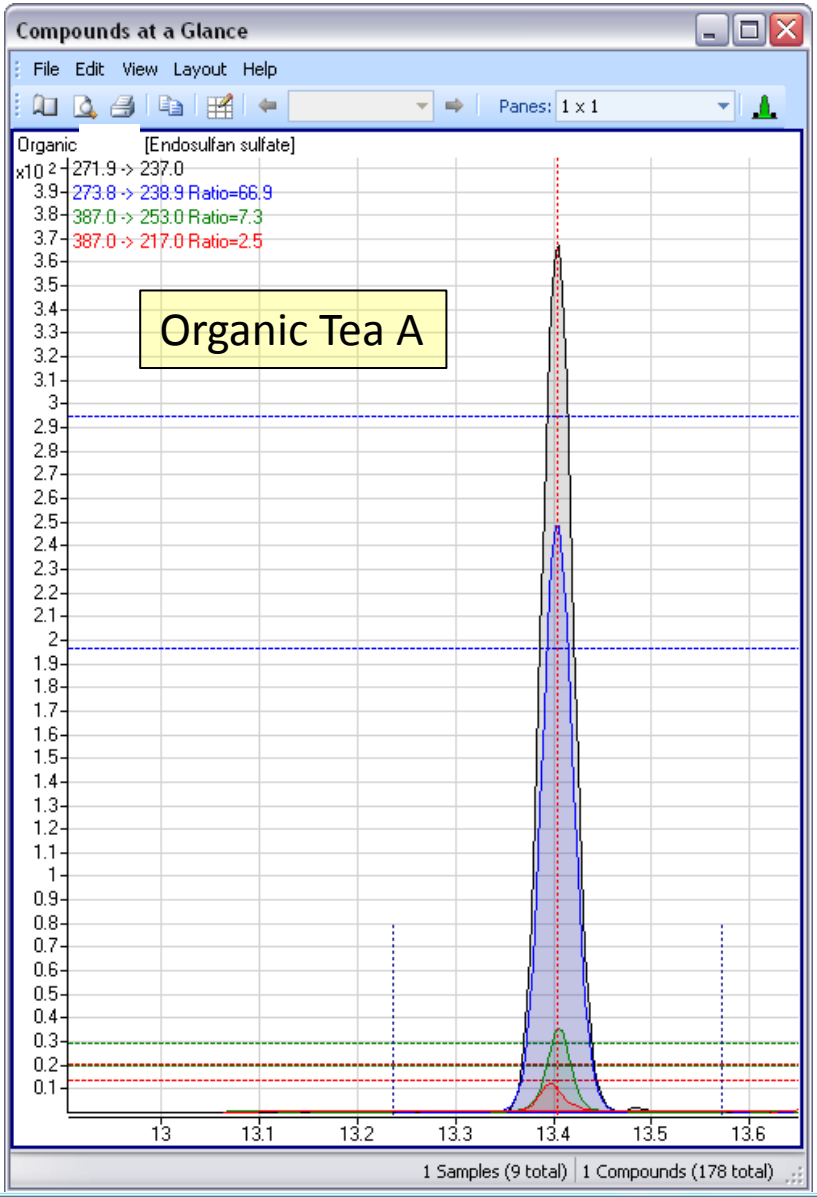
C6572 =IF(D6572=D6571,C6571,C6571+1)

| | R | S | T | U | V | W | X | Y | Z | AA | AB | AC | AD | AE | AF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6558 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 289.9 | LowRes | 219.9 | LowRes | 10 | 35 | 0.1 | 1020 | 48% | Q5 | |
| 6559 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 359.9 | LowRes | 287.8 | LowRes | 10 | 25 | 0.1 | 970 | 46% | Q6 | |
| 6560 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 324.8 | LowRes | 289.8 | LowRes | 10 | 15 | 0.1 | 960 | 45% | Q7 | |
| 6561 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 361.9 | LowRes | 291.8 | LowRes | 10 | 25 | 0.1 | 870 | 41% | Q8 | |
| 6562 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 218.0 | LowRes | 183.0 | LowRes | 10 | 20 | 0.1 | 800 | 38% | Q9 | |
| 6563 | 35694-06-5 | 2,2',3,4,4',5-Hexachlorobiphenyl (E | 13.44 | false | 361.9 | LowRes | 326.8 | LowRes | 10 | 15 | 0.1 | 750 | 35% | Q10 | |
| 6564 | 3735-33-9 | Phosmet oxon | 13.49 | false | 160.0 | LowRes | 77.0 | LowRes | 10 | 25 | 0.1 | 480 | 100% | Q0 | |
| 6565 | 3735-33-9 | Phosmet oxon | 13.49 | false | 160.0 | LowRes | 51.0 | LowRes | 10 | 40 | 0.1 | 230 | 48% | Q1 | |
| 6566 | 3735-33-9 | Phosmet oxon | 13.49 | false | 160.0 | LowRes | 133.0 | LowRes | 10 | 15 | 0.1 | 220 | 46% | Q2 | |
| 6567 | 3735-33-9 | Phosmet oxon | 13.49 | false | 133.0 | LowRes | 76.9 | LowRes | 10 | 15 | 0.1 | 40 | 8% | Q3 | |
| 6568 | 3735-33-9 | Phosmet oxon | 13.49 | false | 133.0 | LowRes | 50.9 | LowRes | 10 | 30 | 0.1 | 30 | 6% | Q4 | |
| 6569 | 3735-33-9 | Phosmet oxon | 13.49 | false | 133.0 | LowRes | 104.8 | LowRes | 10 | 5 | 0.1 | 20 | 4% | Q5 | |
| 6570 | 3735-33-9 | Phosmet oxon | 13.49 | false | 301.0 | LowRes | 191.8 | LowRes | 10 | 10 | 0.1 | 0 | 0% | Q6 | |
| 6571 | 3735-33-9 | Phosmet oxon | 13.49 | false | 172.9 | LowRes | 104.0 | LowRes | 10 | 15 | 0.1 | 0 | 0% | Q7 | |
| 6572 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 271.9 | LowRes | 237.0 | LowRes | 10 | 15 | 0.1 | 1480 | 100% | Q0 | |
| 6573 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 273.8 | LowRes | 238.9 | LowRes | 10 | 15 | 0.1 | 1080 | 73% | Q1 | |
| 6574 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 273.8 | LowRes | 236.9 | LowRes | 10 | 15 | 0.1 | 520 | 35% | Q2 | |
| 6575 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 271.9 | LowRes | 235.0 | LowRes | 10 | 15 | 0.1 | 300 | 20% | Q3 | |
| 6576 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 229.0 | LowRes | 194.0 | LowRes | 10 | 25 | 0.1 | 180 | 12% | Q4 | |
| 6577 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 386.6 | LowRes | 288.7 | LowRes | 10 | 5 | 0.1 | 80 | 5% | Q5 | |
| 6578 | 1031-07-8 | Endosulfan sulfate | 13.38 | false | 386.6 | LowRes | 252.7 | LowRes | 10 | 10 | 0.1 | 70 | 5% | Q6 | |
| 6579 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 359.9 | LowRes | 289.9 | LowRes | 10 | 30 | 0.1 | 1300 | 100% | Q0 | |
| 6580 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 287.9 | LowRes | 217.9 | LowRes | 10 | 35 | 0.1 | 1240 | 95% | Q1 | |
| 6581 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 359.9 | LowRes | 324.9 | LowRes | 10 | 15 | 0.1 | 1190 | 92% | Q2 | |
| 6582 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 324.8 | LowRes | 289.8 | LowRes | 10 | 20 | 0.1 | 1170 | 90% | Q3 | |
| 6583 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 361.9 | LowRes | 289.9 | LowRes | 10 | 30 | 0.1 | 820 | 63% | Q4 | |
| 6584 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 289.9 | LowRes | 219.9 | LowRes | 10 | 35 | 0.1 | 790 | 61% | Q5 | |
| 6585 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 289.9 | LowRes | 217.9 | LowRes | 10 | 35 | 0.1 | 790 | 61% | Q6 | |
| 6586 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 361.9 | LowRes | 326.8 | LowRes | 10 | 15 | 0.1 | 710 | 55% | Q7 | |
| 6587 | 52663-66-8 | 2,2',3,3',4,5'-Hexachlorobiphenyl (I | 13.48 | false | 218.0 | LowRes | 183.0 | LowRes | 10 | 20 | 0.1 | 680 | 52% | Q8 | |

MRM Table | DATABASE | DB Compound List | My Target Compound List | CF, 40-min Method | CP,

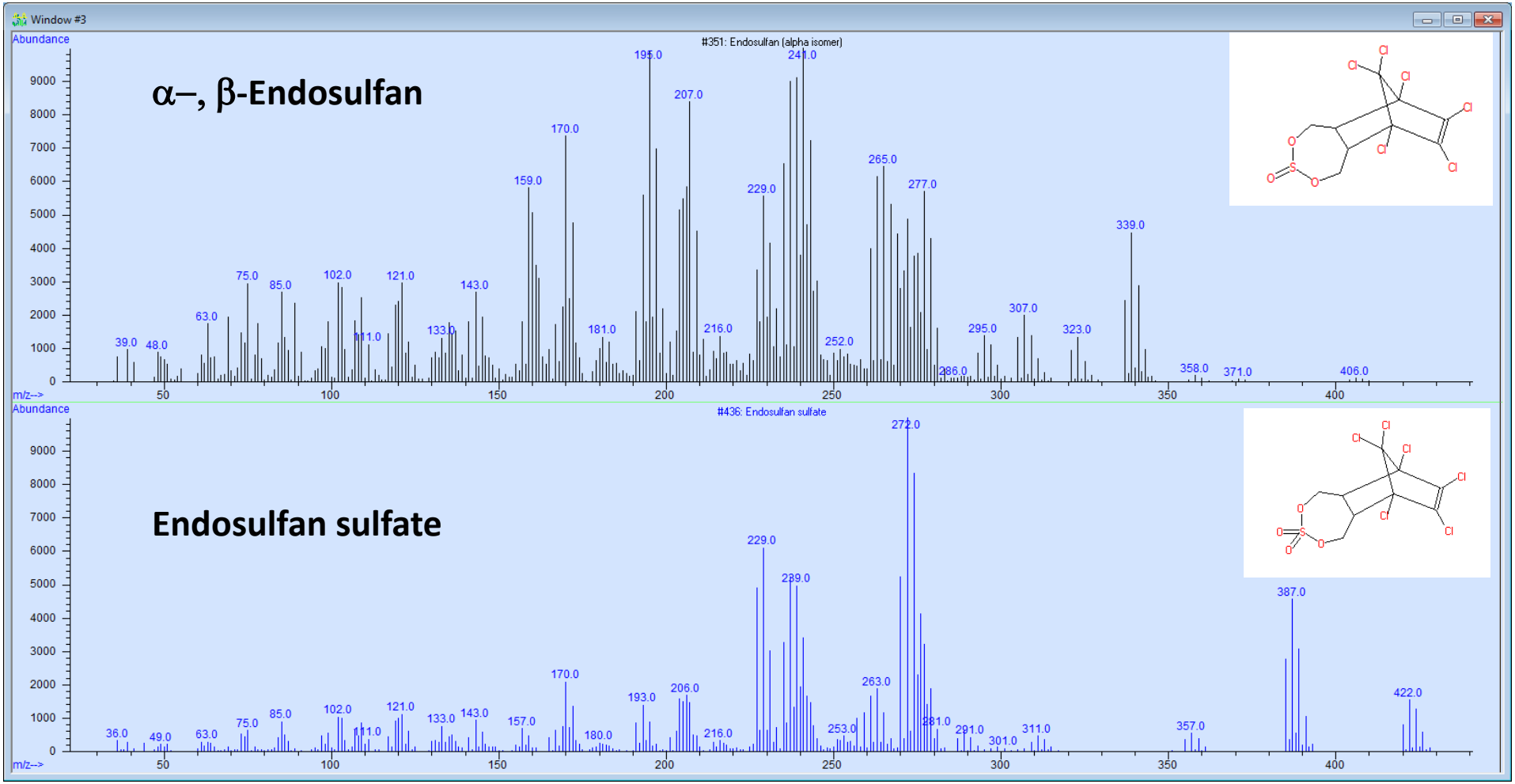
Ready Average: 52049.66963 Count: 231 Sum: 7286953.748 80%

# Endosulfan Sulfate (✓) in Organic Tea Extract – Using 4 MRM Transitions

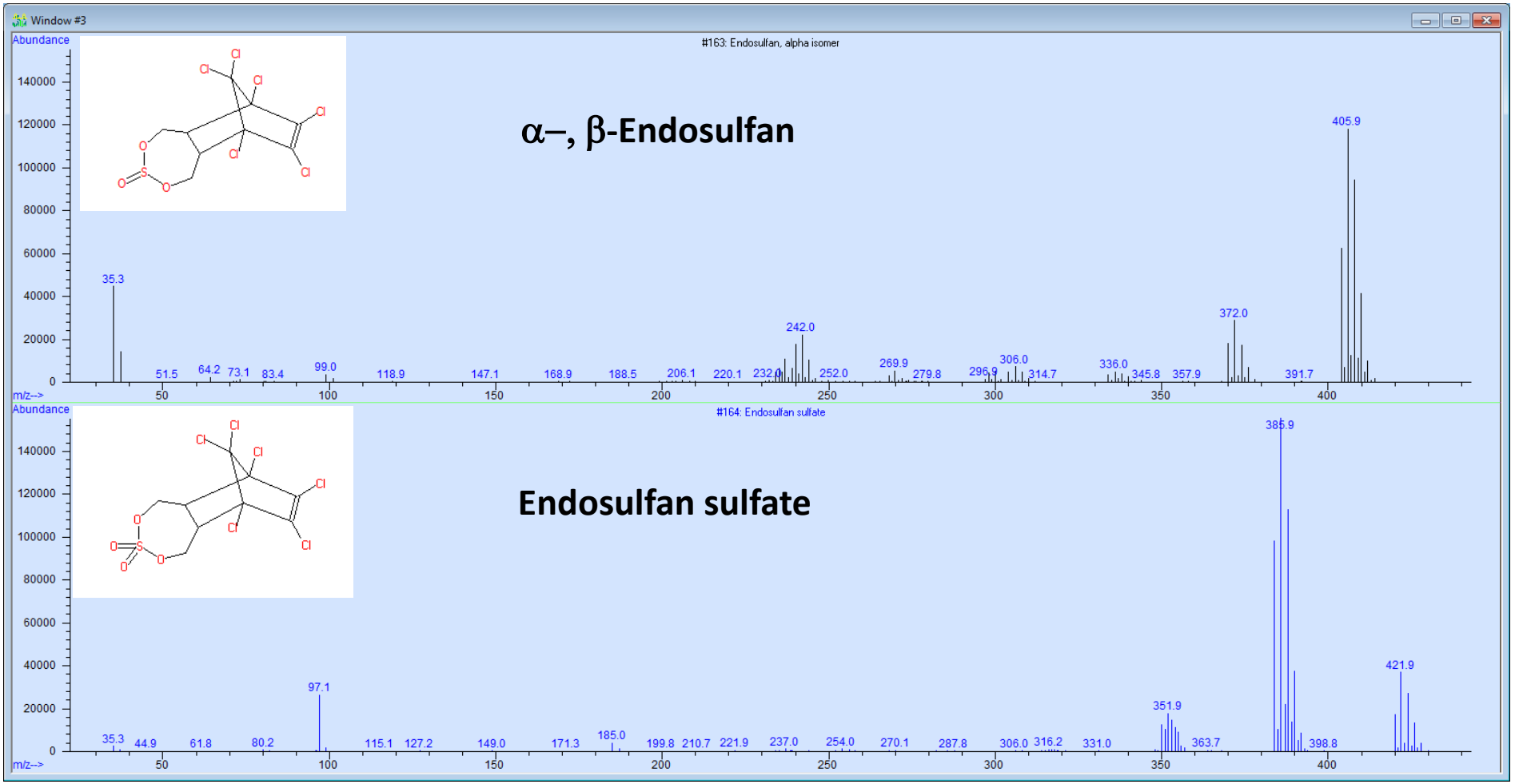



… demonstrates the value of access to multiple optimized transitions in the G9250AA MRM Database
-Not just to avoid matrix interferences but also for additional confirmation

# Endosulfan α, β and Sulfate -
# 5975 EI Mode

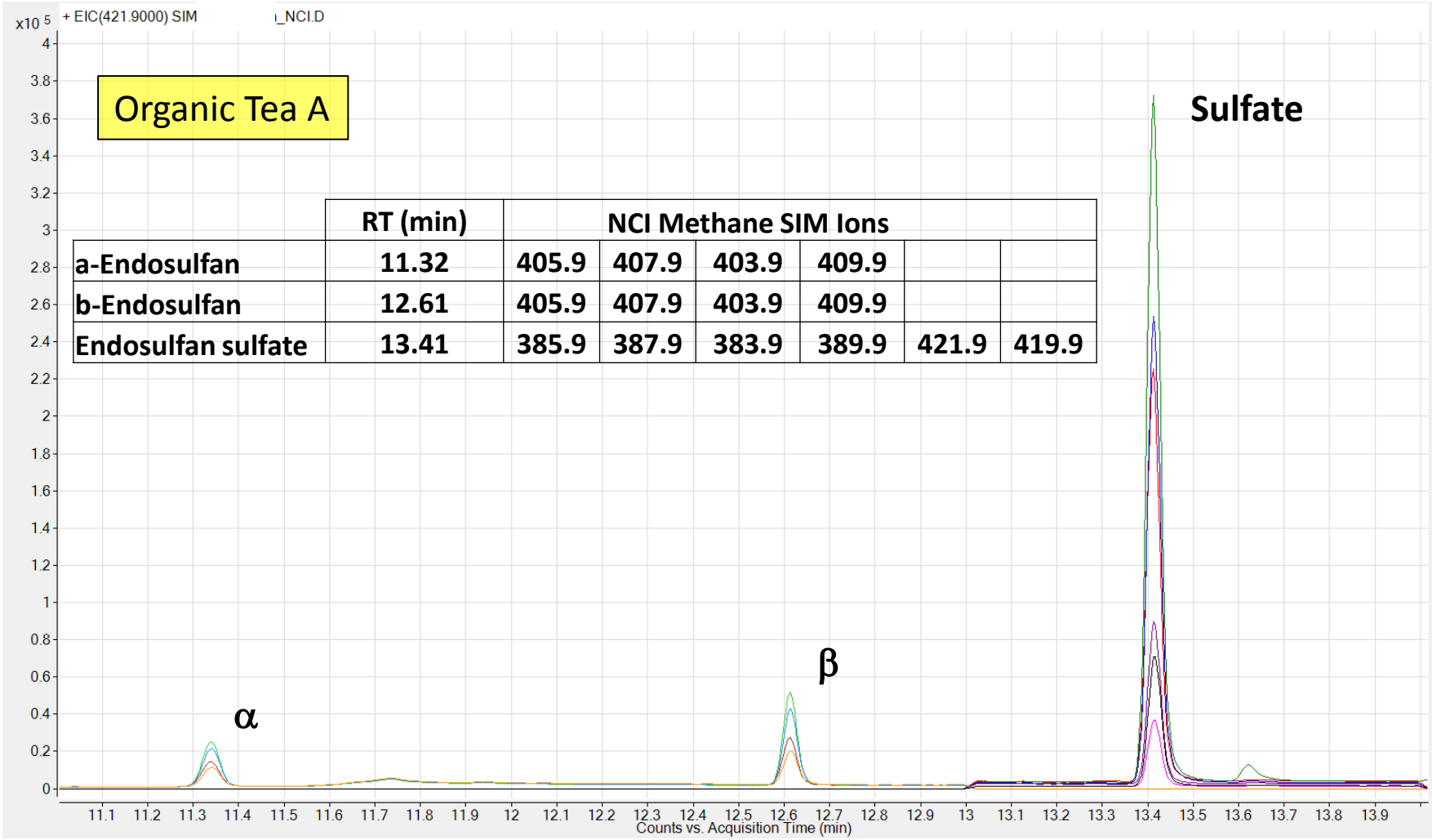


# Endosulfan α, β and Sulfate -
# 5975 NCI Methane Mode



# Endosulfan α, β and Sulfate (✓) in Organic Tea Extract A

## 5975 NCI Methane SIM




| | RT (min) | NCI Methane SIM Ions | | | | | |
|---|---|---|---|---|---|---|---|
| a-Endosulfan | 11.32 | 405.9 | 407.9 | 403.9 | 409.9 | | |
| b-Endosulfan | 12.61 | 405.9 | 407.9 | 403.9 | 409.9 | | |
| Endosulfan sulfate | 13.41 | 385.9 | 387.9 | 383.9 | 389.9 | 421.9 | 419.9 |

# Summary

- Simplified, rapid and optimized sample preparation for tea based on a modified QuEChERS regime with Liq/Liq extraction
- Sensitive detection – LCLs for most analytes ≤0.005 mg/kg
- Excellent recoveries (70–120%) down to 0.01 mg/kg level
- Excellent repeatability ≤ 20%
- RTL for ease of method set-up / maintenance
- Capillary Flow Technology: Back flush – GC method robustness
- MS/MS Selectivity – low matrix interferences
- Method suitable for detection and confirmation of pesticide residues well below EU MRLs (Regulation (EC) No.396/2005)

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

Agilent Technologies

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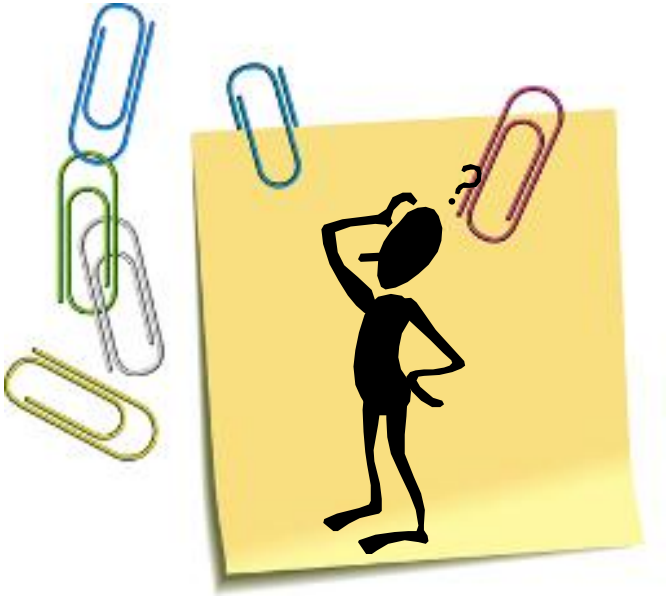
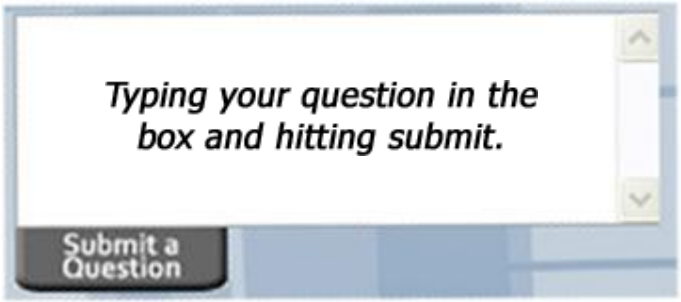
**Faculty of Food and Biochemical Technology**
**Institute of Chemical Technology**
**Prague**
**Czech Republic**

**jana.hajslova@vscht.cz**

# QUESTIONS?



*Please Submit your questions by:*




E:mail: chris_sandy@agilent.com

# THANK YOU!

*We apologize for this brief interruption but we are experiencing technical difficulties and will resume shortly.*