FEDERAL INSTITUTE
FOR RISK ASSESSMENT

Risiken erkennen – Gesundheit schützen

Matrix effects in Electrospray Mass Spectrometry – not a hopeless case

Helen Stahnke

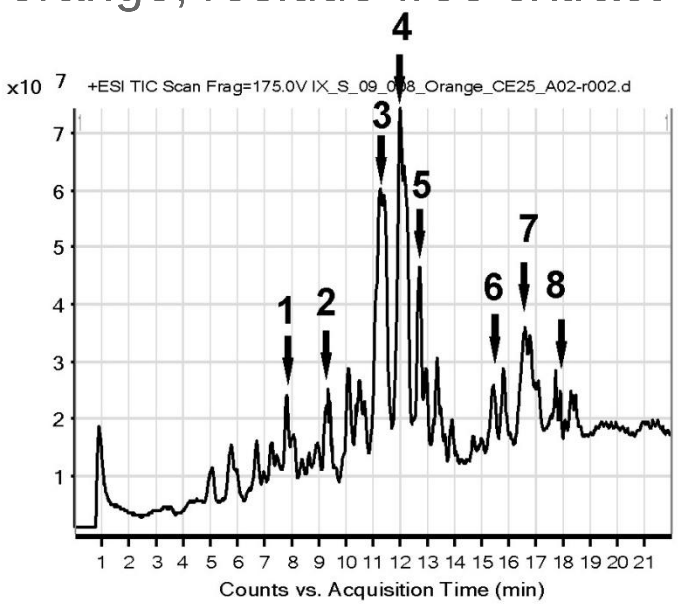
outline

- How differently are pesticides influenced by matrix?
- Can matrix effects be avoided by an improved clean-up?
- Dilute-and-shoot – How much dilution is needed?

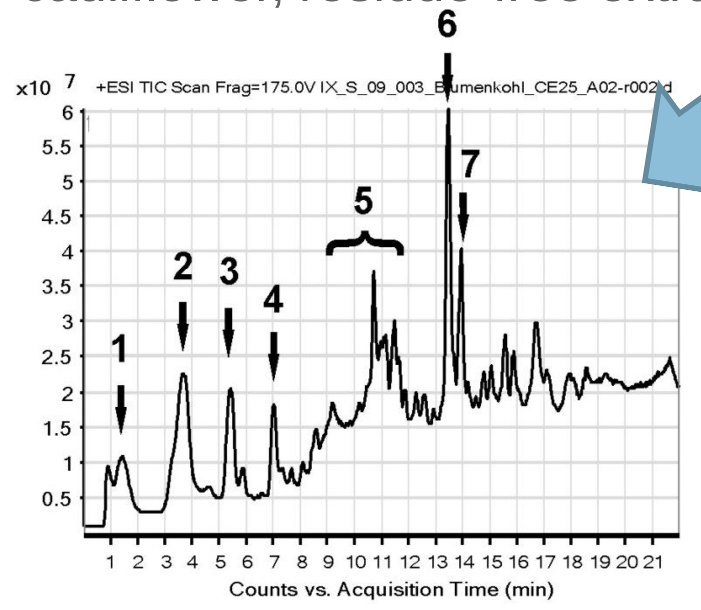
⇒ How should we handle matrix effects in pesticide residue analysis?

Matrix effects are caused by competition for charges during ESI

orange, residue-free extract



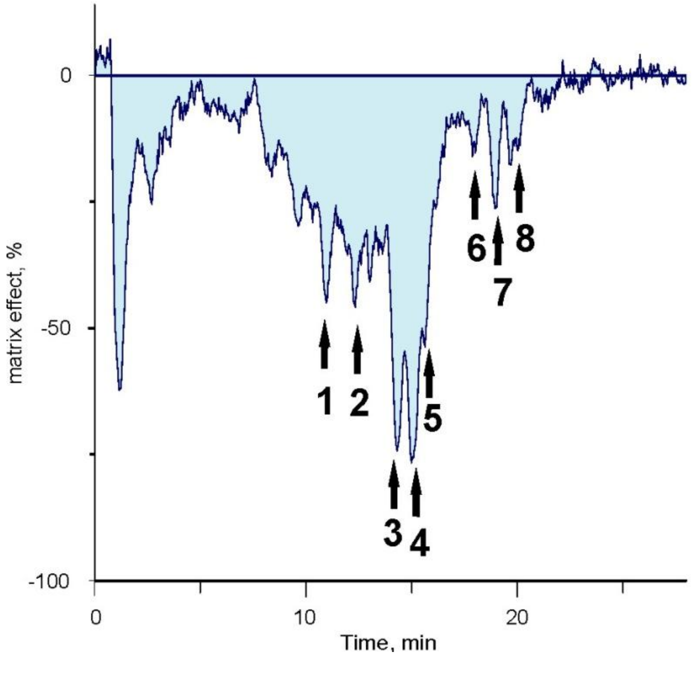
cauliflower, residue-free extract

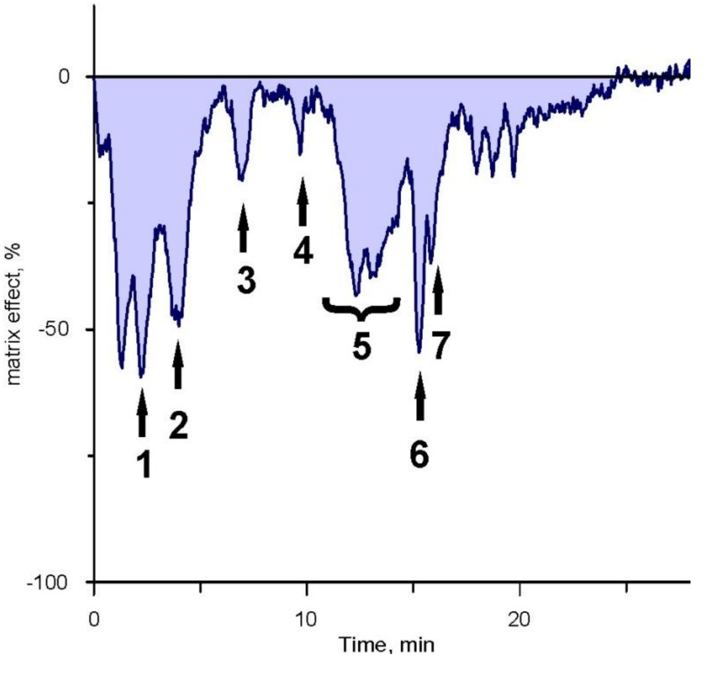


Every matrix effect is visible as a peak in the TIC of a full scan

TIC of a full scan, 100-2000 amu

(Agilent Accurate Mass Q-TOF 6530)

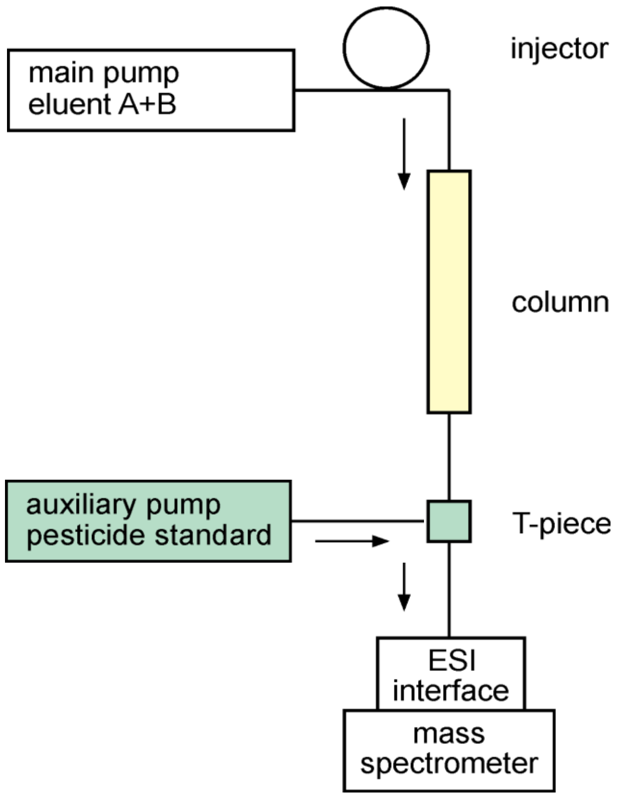




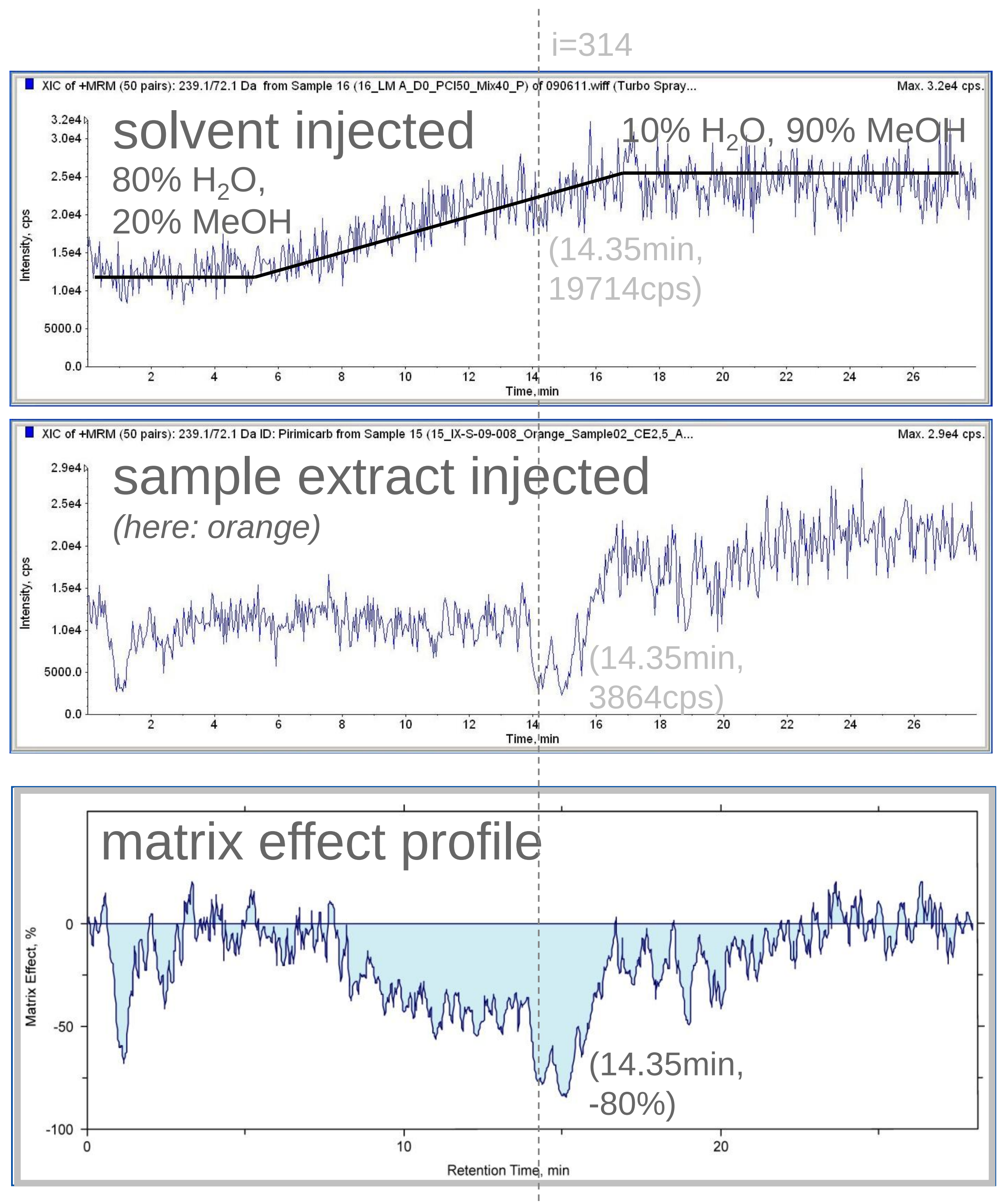
Matrix Effect Profile

(API2000)

What are “matrix effect profiles”?



Instrumental setup for permanent postcolumn infusion



<u>Calculation of matrix effect (ME):</u>

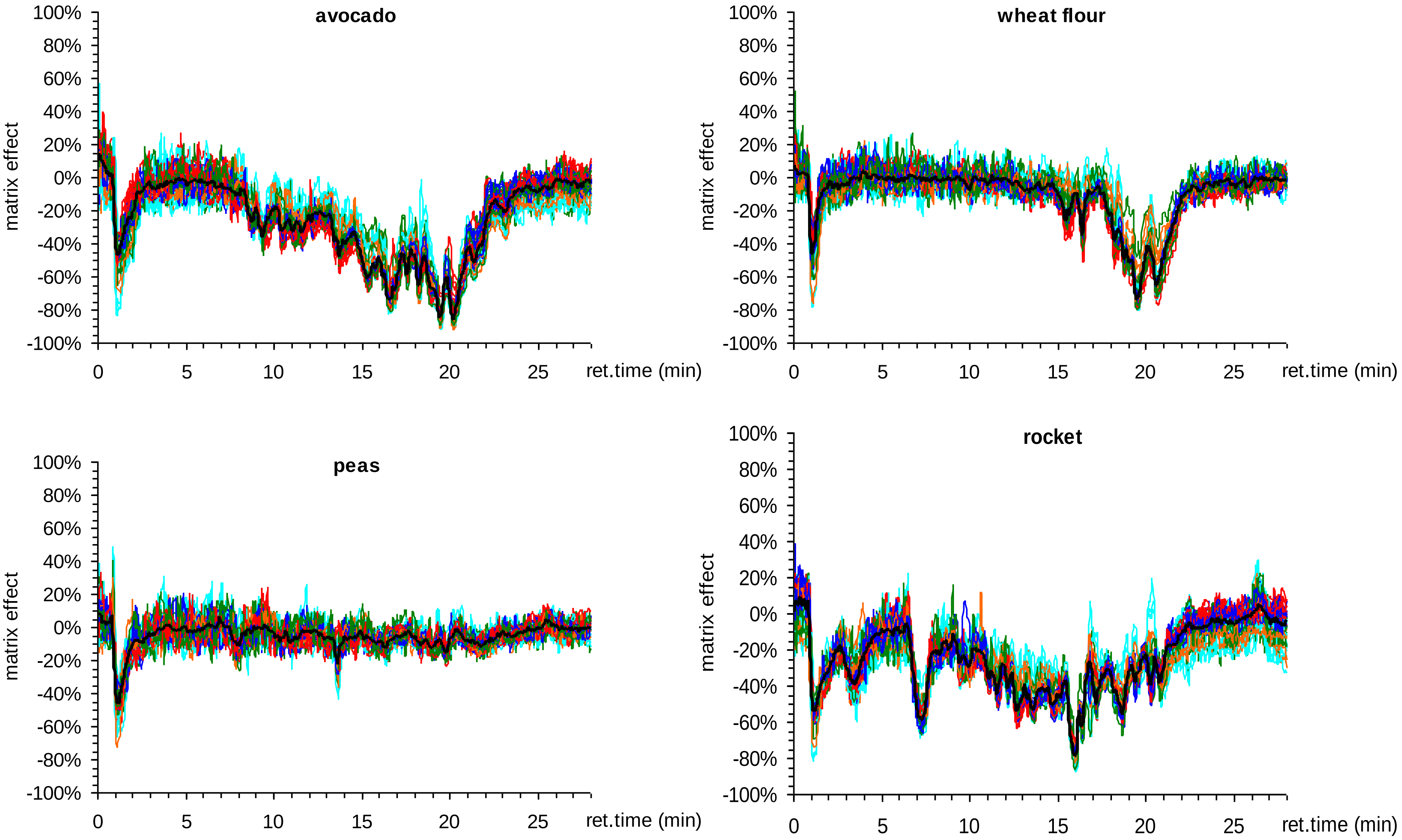
$$ME_i\,(\%) = \left(\frac{SI_i\,(\text{extract})_{\text{smooth.}}}{SI_i\,(\text{solvent})_{\text{smooth.}}} - 1 \right) \times 100\%$$

How differently are pesticides influenced by matrix?

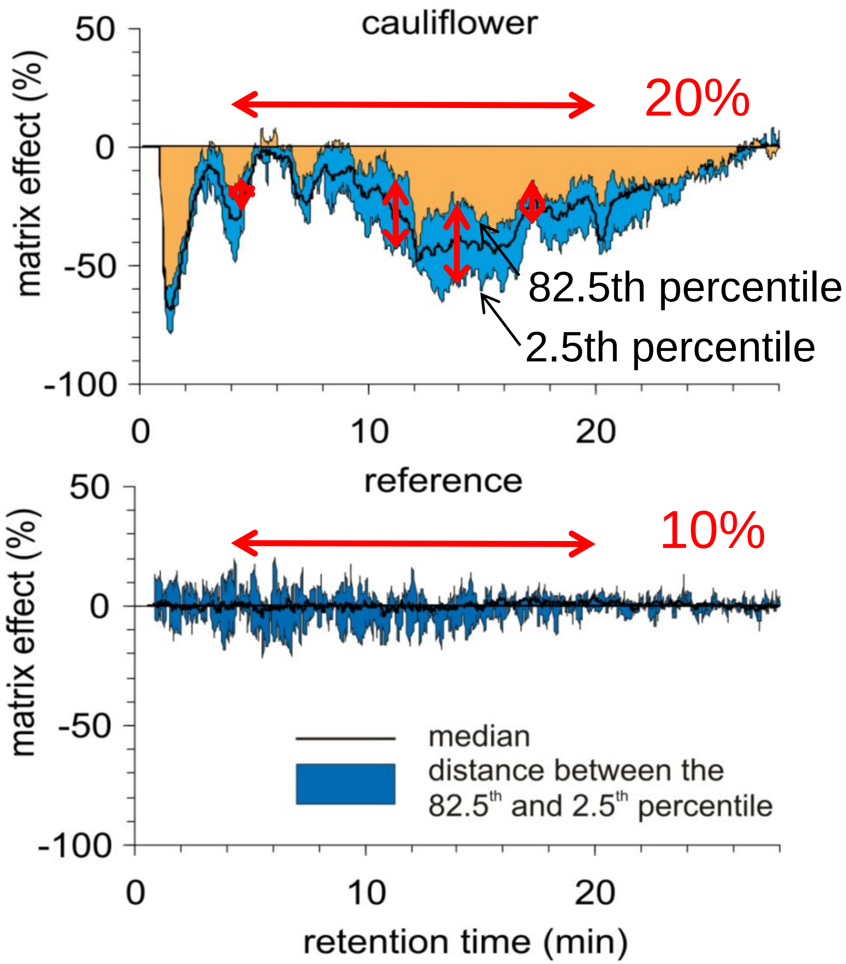
How differently are pesticides influenced by matrix?

- Determintation of matrix effects with **permanent postcolumn infusion**
- **140 pesticides in ESI(+)**
  - pK_a 3.4 to pK_b 9.3
  - log K_{ow} -1.7 to +6.9
  - surface tension 29 to 133 mN/m
  - 35 substance classes (carbamates, organophosphorus pesticides, pyrethroides, sulfonylureas, imidazoles, phenylamides …)
- **20 crops / matrixes of plant origin:**
  - high water content: apple, pear, plum, aubergine, sweet pepper, rocket, peas, onion, potato, cauliflower, carrot, leek
  - dry: wheat flour, raisins
  - high oil content: avocado, linseed
  - high acid content: orange, grapefruit, raspberries
  - difficult: black tea
- **Evaluation of 2560 analyte / matrix combinations**

Matrix effect profiles of 50 pesticides (simultaneously infused)



Similar influence of matrix on many different pesticides

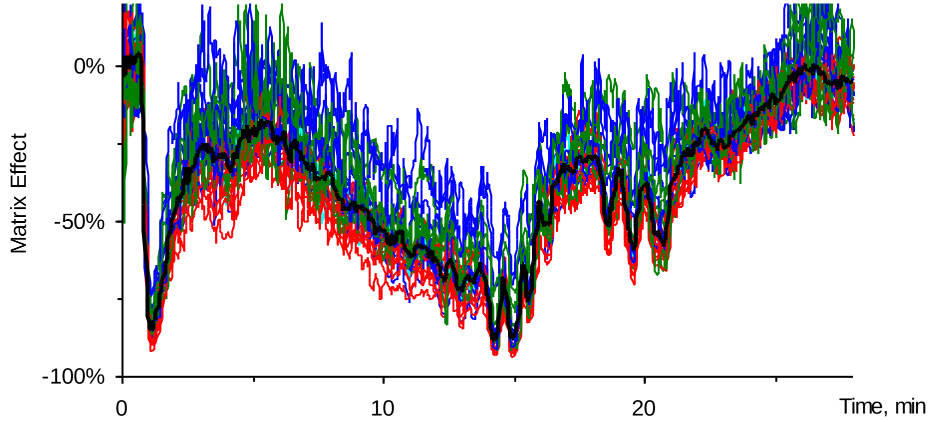


Differences between pesticides with strong effects (*2.5th percentile*) and with weak effects (*82.5th percentile*)

reference measurement without matrix	10%		
peas	10%	avocado	19%
apple	11%	leek	19%
onion	11%	rocket	19%
sweet pepper	12%	cauliflower	20%
raisins	13%	grapefruit	22%
wheat flour	14%	pear	22%
aubergine	15%	linseed	23%
plum	15%	raspberries	23%
carrot	17%	black tea	26%
potato	18%	orange	26%

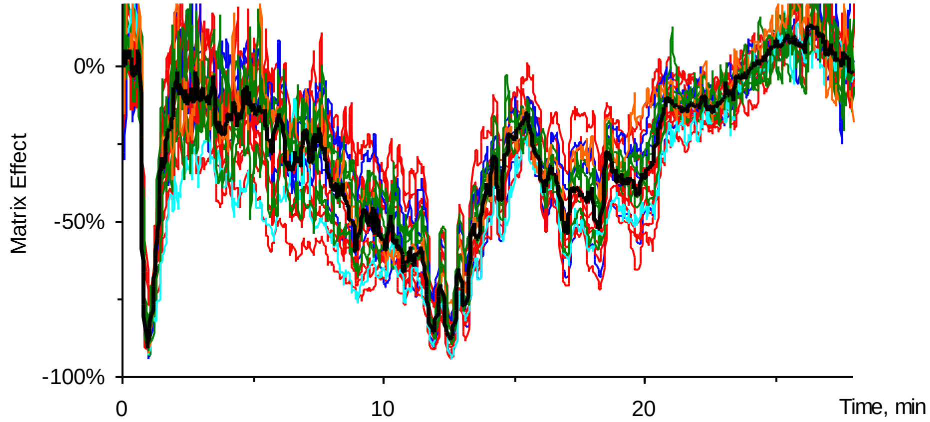
Matrix effects depend mainly on the retention time. Other analyte properties have only a minor influence.

Matrix effect profiles obtained with different LC-MS systems



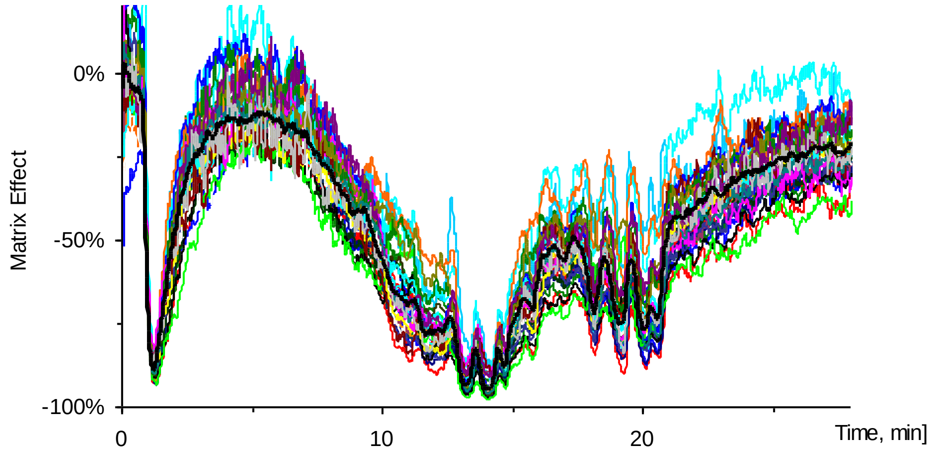
API2000

off axis spray



API3200 QTrap

orthogonal spray



Micromass Quattro LC

Z-spray

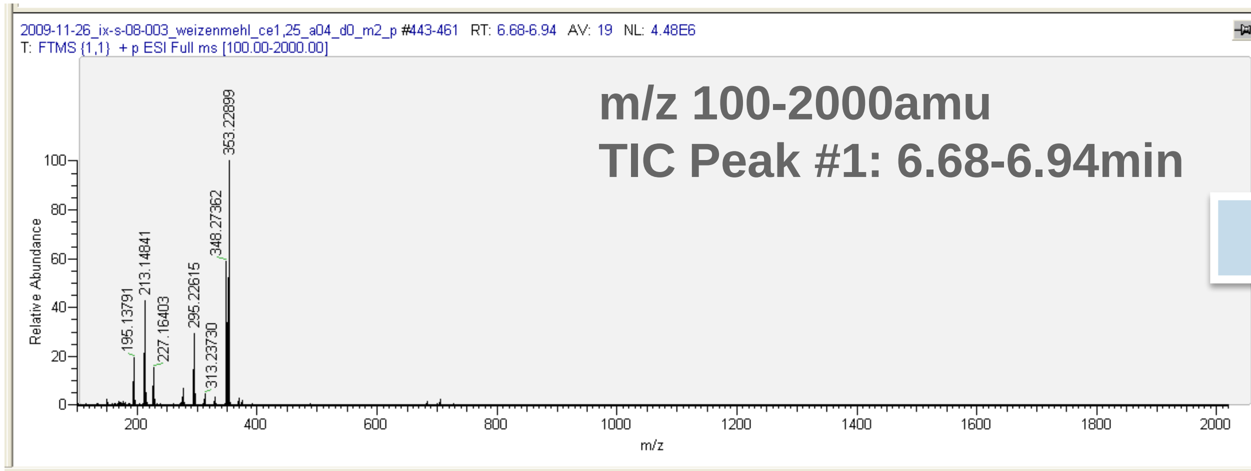
- Similar profiles obtained for 50 simultaneously infused pesticides with an identical orange extract
- All pesticides are simultaneously affected by matrix

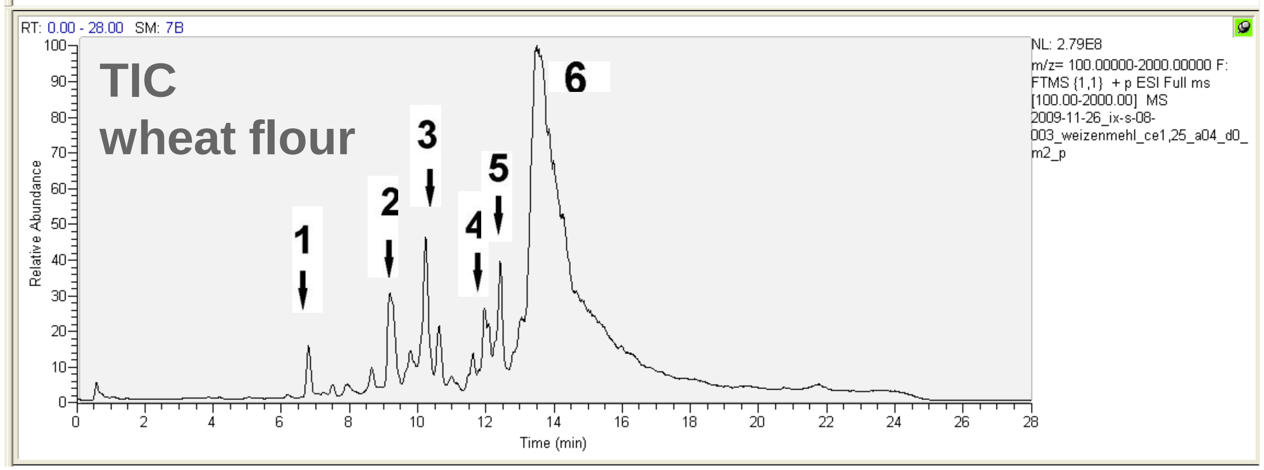
Matrix effects mainly depend on retention time.

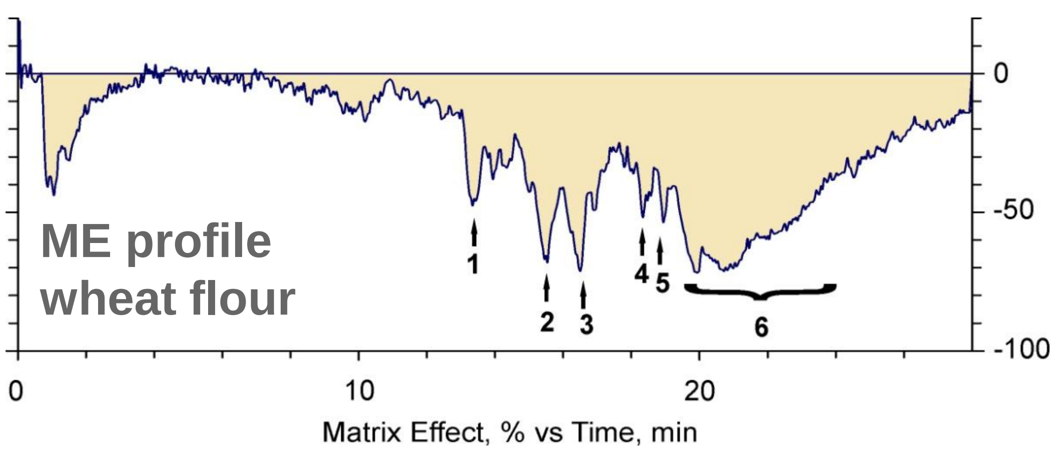
This finding is independent from the instrument platform used.

Can matrix effects be avoided by an improved clean-up?

Identification of food ingredients that cause matrix effects







List of masses for Peak #1:

#	mass, amu	Intensity, cps	
1	195.13791	2.33E6	
2	213.14841	4.97E6	
3	227.16403	1.76E6	
4	277.21612	9.14E5	
5	295.22615	3.58E6	
6	313.23730	5.96E5	$[X+H]^+$
7	330.26402	3.93E5	$[X+NH_4]^+$
8	**348.27362**	**6.66E6**	$[Y+NH_4]^+$
9	**353.22899**	**1.08E7**	$[Y+Na]^+$
10	369.20384	3.06E5	
11	683.47238	2.70E5	$[Z+H]^+$
12	705.45422	3.62E5	$[Z+Na]^+$

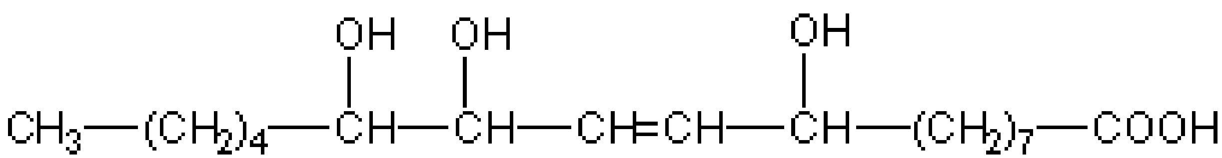
database search

Number of co-extracted food ingredients in final sample extracts[1]:

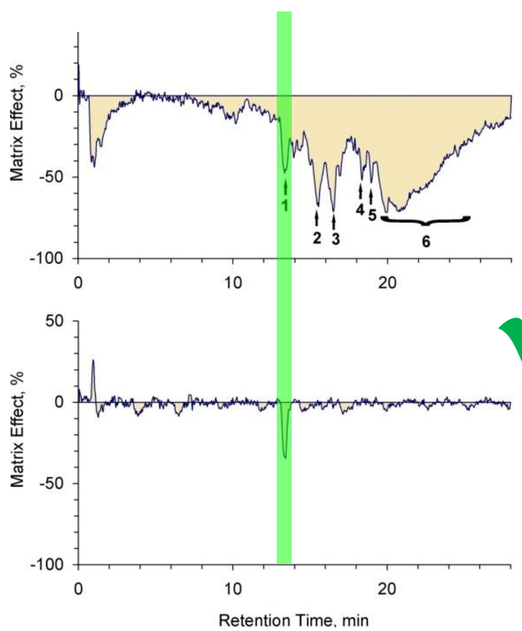
ChemElut	3246 – 6524
QuEChERS (without SPE)	3289 – 5924
QuEChERS (with SPE)	1648 – 4427

[1] derived from orange, wheat flour, cauliflower, carrot, avocado and onion

9(S),12(S),13(S)-trihydroxy-10(E)-octadecenoic acid causes signal suppressions in extracts of wheat flour *(Peak #1)*

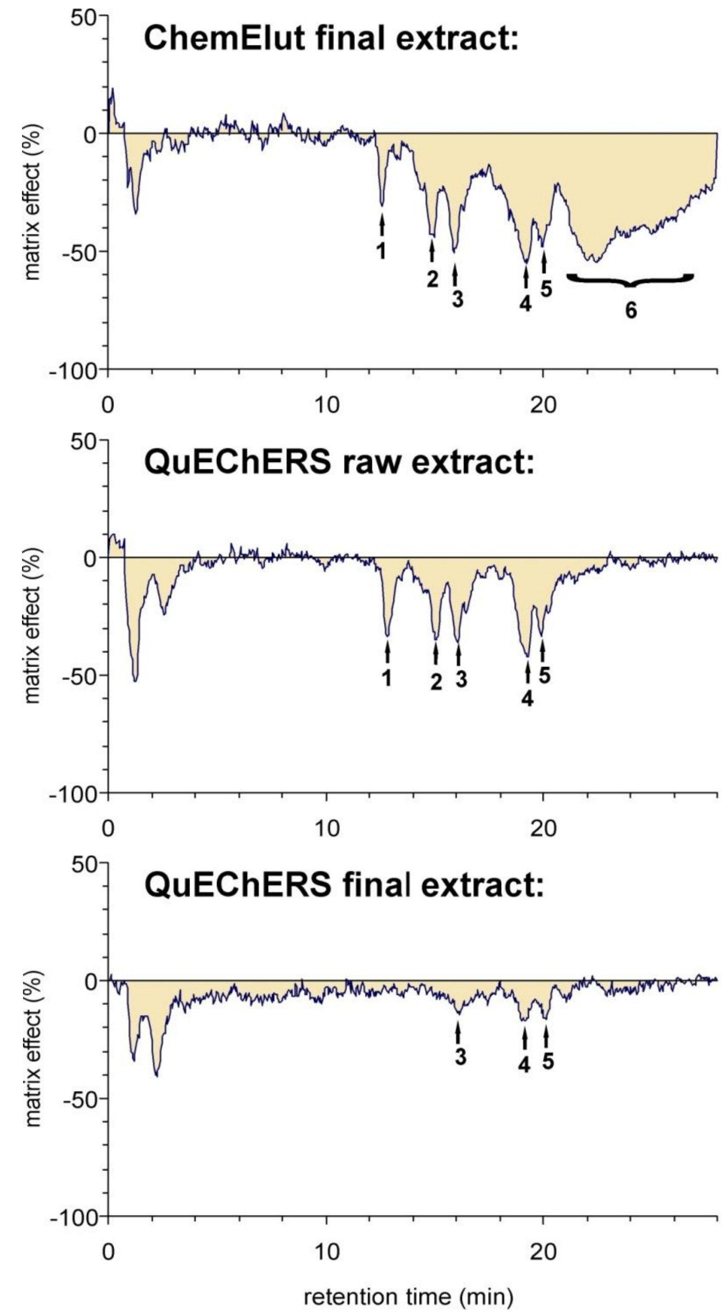


$C_{18}H_{34}O_5$, neutral mass 330.2406amu, detected as $[M+NH_4]^+$ 348.2744amu and/or $[M+Na]^+$ 353.2293amu, oxidat. product of linolenic acid, expected conc. 10ppm

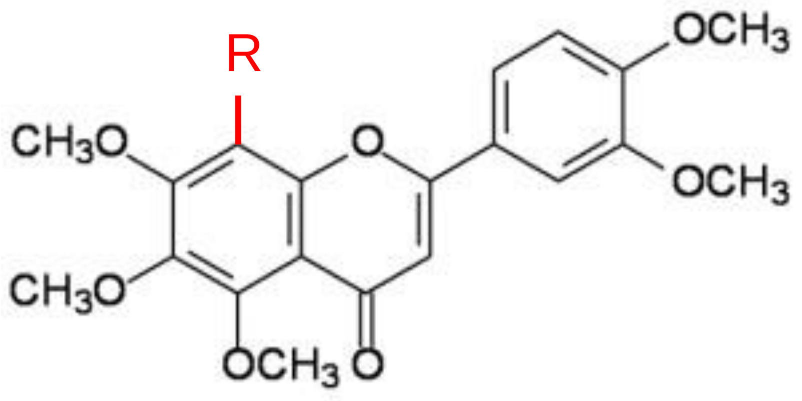


ChemElut extract of wheat flour, 10mg matrix on column

solvent standard, 100ng 9(S),12(S),13(S)-trihydroxy-10(E)-octadecenoic acid on column



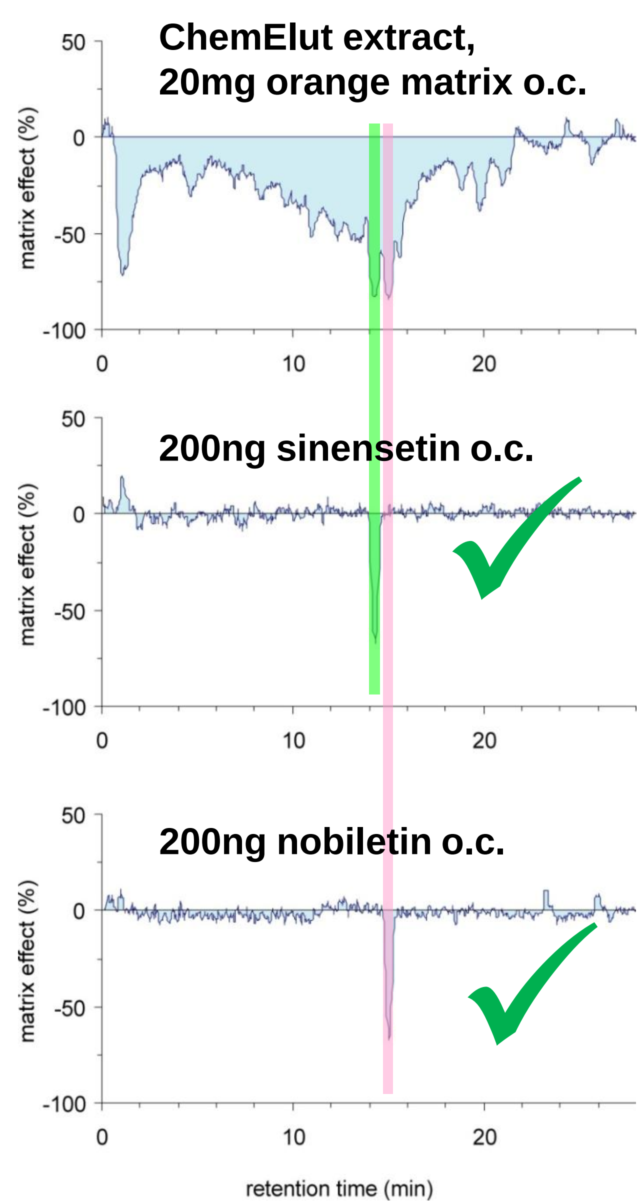
Polymethoxy flavones are responsible for signal suppressions in extracts of orange *(Peak #3 and Peak #4)*

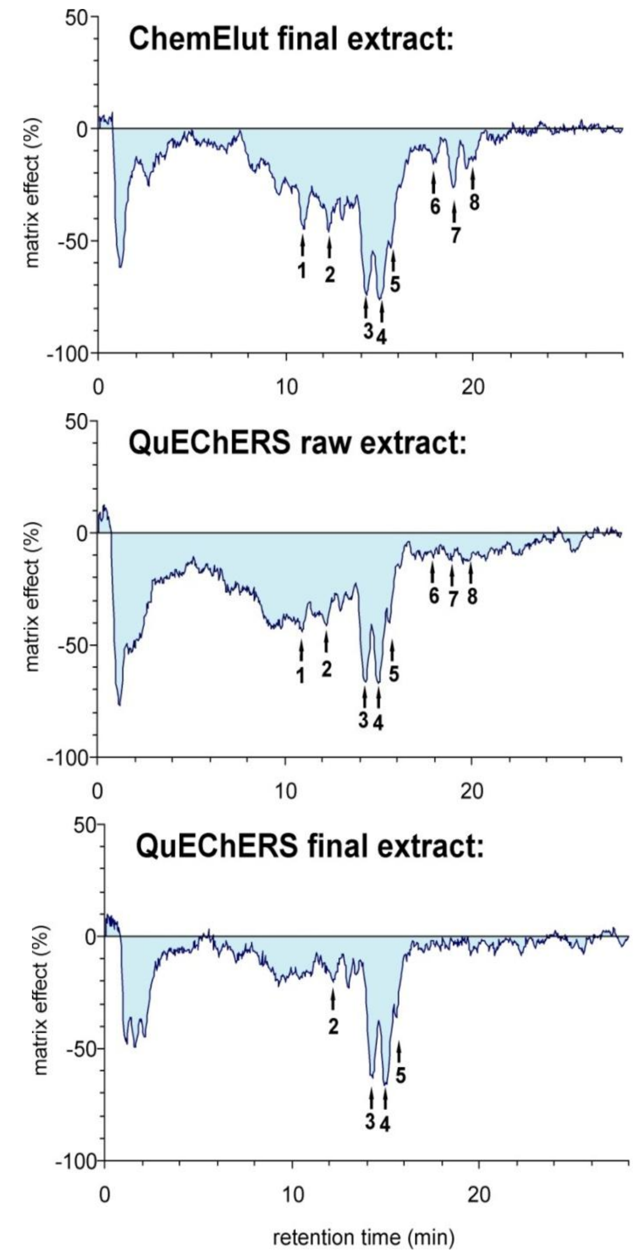


R = H **Sinensetin**, ($C_{20}H_{20}O_7$)
3',4',5,6,7-pentamethoxy flavone,
neutral mass 372.1209amu

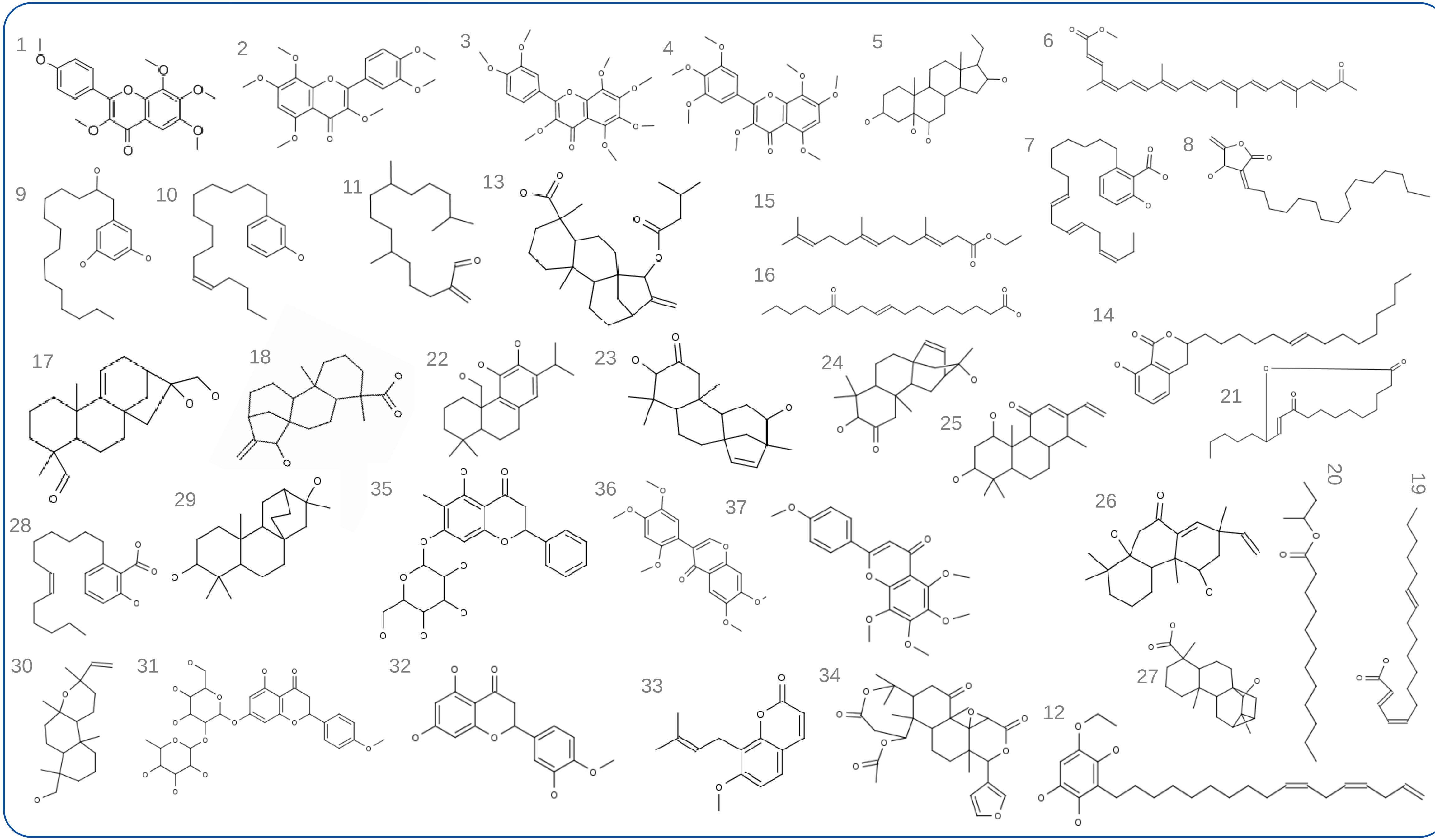
R = OCH_3 **Nobiletin**, ($C_{21}H_{22}O_8$)
3',4',5,6,7,8-hexamethoxy flavone,
neutral mass 402.1315amu

both detected as $[M+H]^+$, $[M+Na]^+$, $[2M+Na]^+$, typical citrus flavonoids, expected conc. 10ppm





Further candidates for matrix effects in extracts of orange and wheat flour (hits from DFC database)



Typical strategies to react on matrix effects in LC-ESI-MS

Minimization (reduction of that matrix amount, which reaches the ESI source simultaneously with the analyte) by:

- improvement of clean-up
- optimization of chromatographic separation
- dilution of the final extract

Compensation (standards in solvent only for screening) by:

- calibration with matrix-matched standards
- calibration with internal stable isotope labeled standards
- standard addition technique

Demonstration of absence of matrix effects by:

- comparison of matrix-matched standards with standards in solvent
- permanent infusion of analytes after HPLC column (CHOI, 1999)

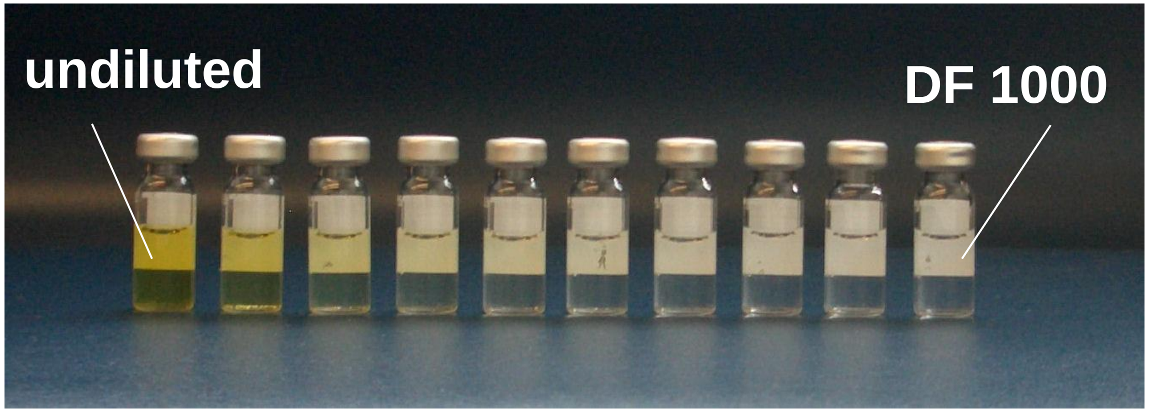
“Dilute-and-shoot“ –

How much dilution is needed?

Experimental

- Determination of matrix effects for **dilution series of QuEChERS extracts**
  - **4 matrixes**: orange *(high acid content)*, avocado *(high oil content)*, rocket *(high water content)*, black tea *(difficult)*
  - preparation of matrix-matched standards postextraction spiked with **39 pesticides** at a level of 100ppb and of solvent standards in corresponding concentrations

    acephate, aldicarb, atrazine, azoxystrobin, bifenthrin, bitertanol, butylate, carbaryl, chlorpyrifos, chlorthiamid, cyromazine, deltamethrin, diazinon, diuron, EPTC, fluazifop-butyl, flurochloridone, hexaconazole, imazalil, isoproturon, kresoxim-methyl, malathion, MCPA-butotyl, metazachlor, methamidophos, monolinuron, myclobutanil, oxydemeton-methyl, penconazole, pirimicarb, pirimiphos-methyl, profenofos, propachlor, propaquizafop, pyrazophos, simetryn, tau-fluvalinate, terbutryn, triazophos
  - **10 dilution factors** (DF) per series: DF 1/undiluted, 2, 5, 10, 20, 50, 100, 200, 500 and 1000



Dilution series of an orange extract

Experimental

- **Instrumentation:**

  Agilent 1200 LC series coupled by a TurboV ESI source with an AB Sciex QTrap 5500 mass spectrometer

- **Column:**

  Aqua 5µ C18 125Å, 50 mm, 2 mm ID with Aqua 10µ C18 125Å, 4 mm, 2 mm ID precolumn (Phenomenex®)

- **Eluent A:**

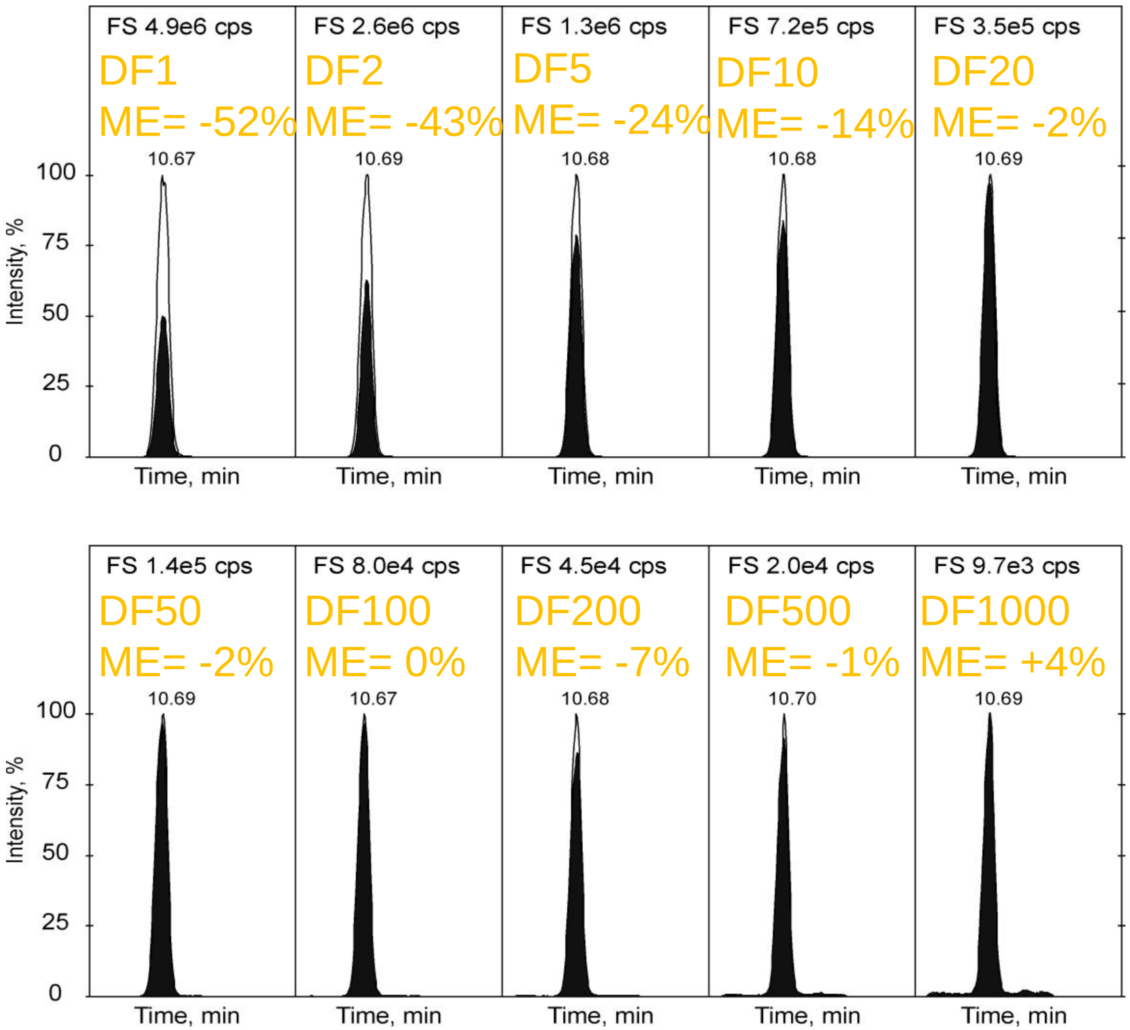
  MeOH/H_2O (2:8; v:v) + 5 mmol $HCOONH_4$/L

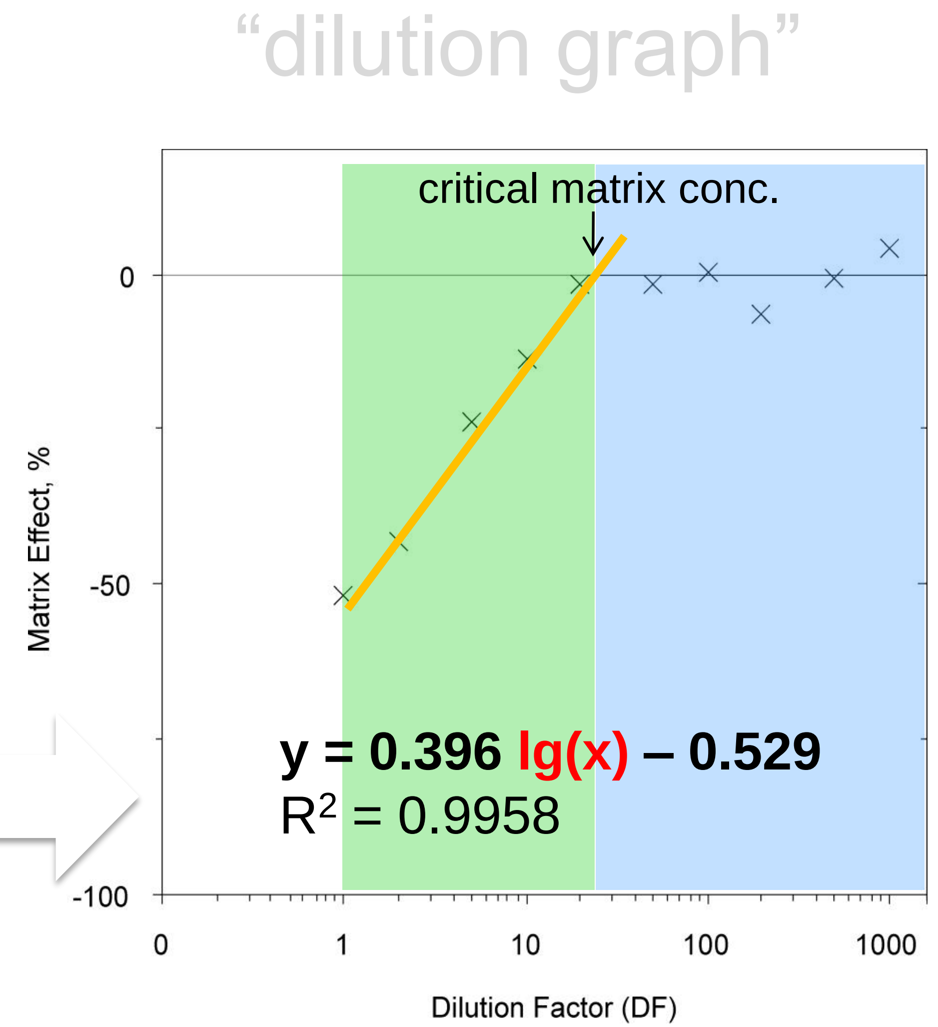
  Eluent B:

  MeOH/H_2O (9:1; v:v) + 5 mmol $HCOONH_4$/L

- **Flow rate:** 200 µL/min
- **Injection volume:** 12 µL
- **Capillary voltage:** 5.5 kV
- **Source temp:** 400°C

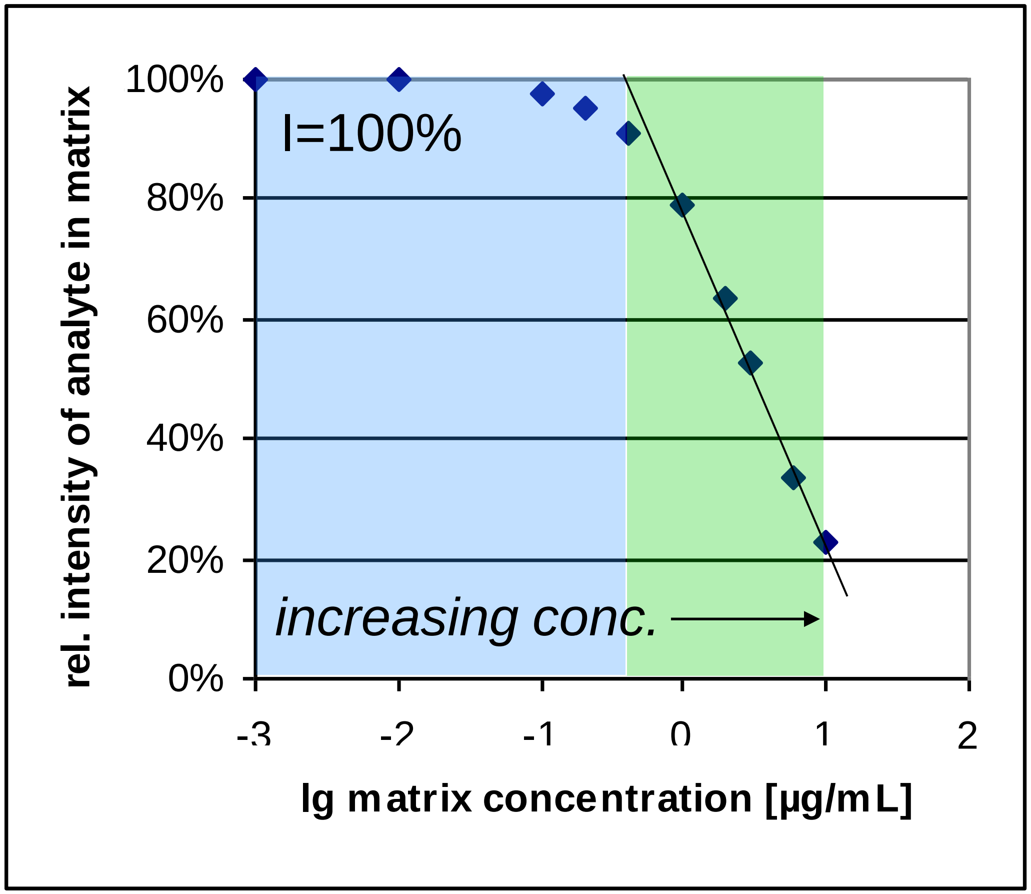
1st example: “dilution graph“ of diuron obtained with a QuEChERS extract of orange



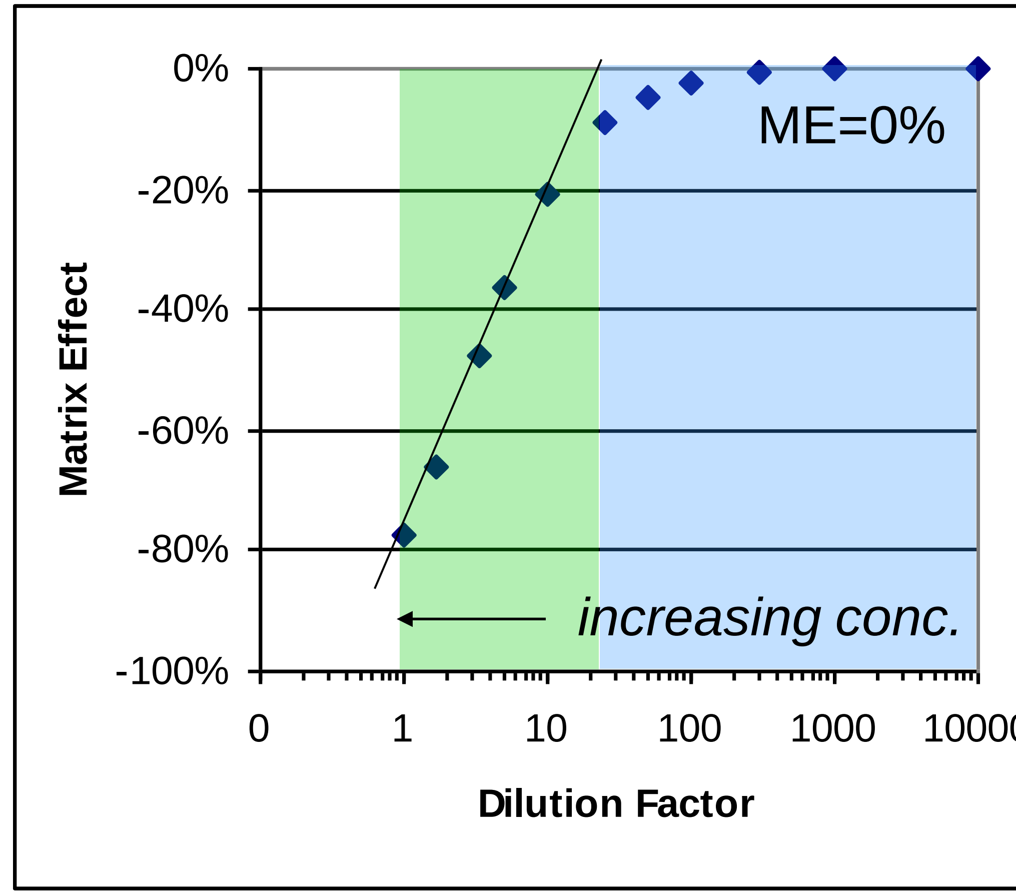


From influence of matrix concentration on analyte's response to influence of dilution factor on matrix effect

Theoret. graph based on a **model of Enke**



Dilution graph



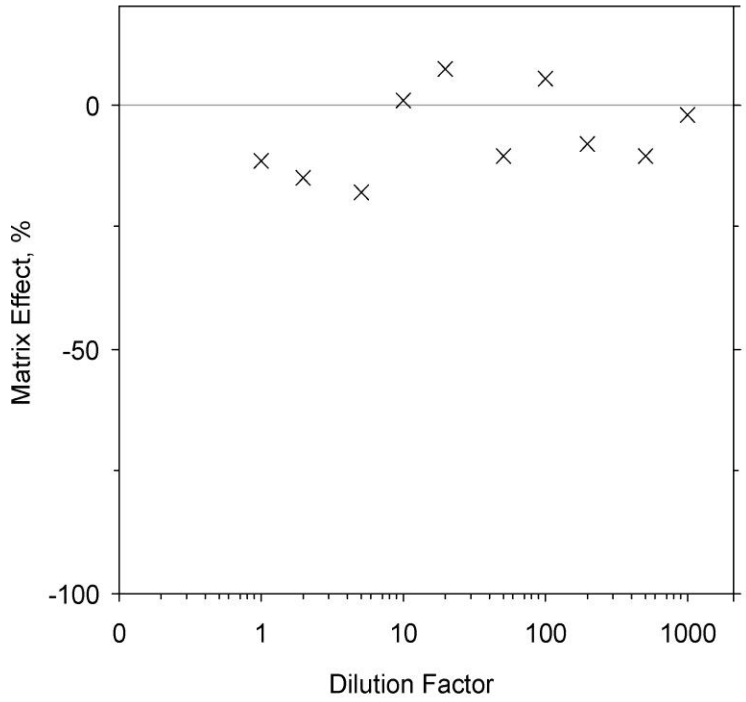
electrolyte:	analyte:	matrix:
c_E = 5 mmol/L	c_A = 0.003…300ng/mL	c_M =0.0001…10µg/mL
K_E = 1	K_A = 1000	K_M = 1000

(reference: Enke Ch. G., Anal. Chem. 69 (1997) 4885-4893)

Further examples: Categories of dilution graphs

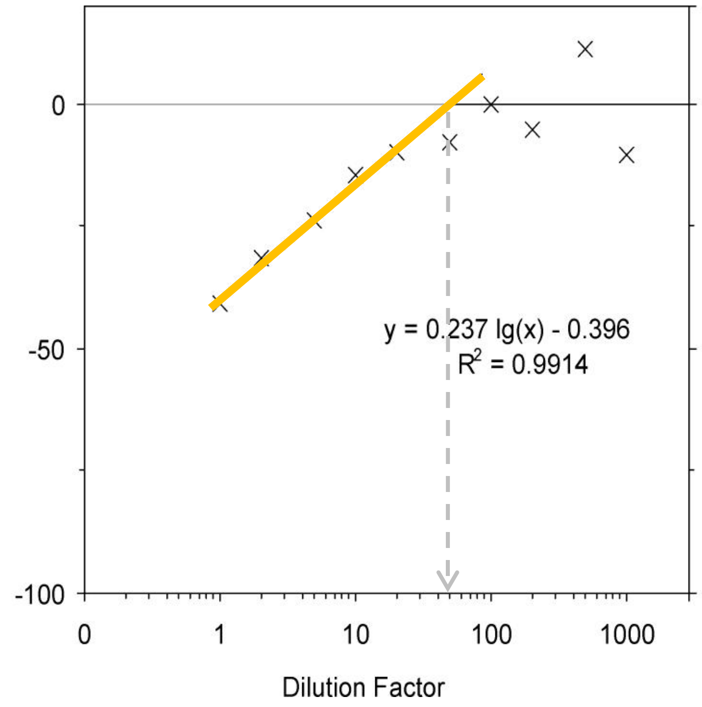
<u>in total 117 evaluable dilution graphs</u>

metazachlor
in avocado extract



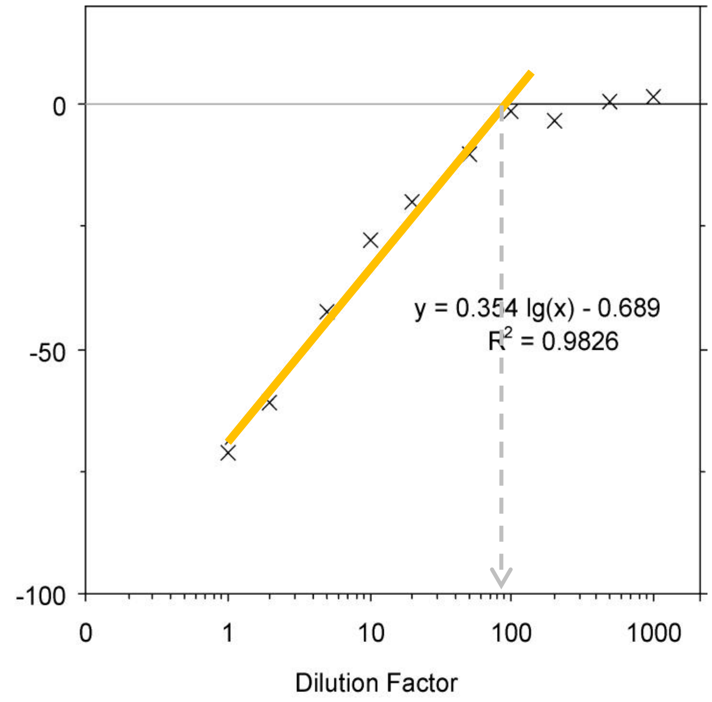
- **no matrix effect**
  recovery varies between 80%-120%, in 20% of analyte / matrix comb.

fluazifop-butyl
in black tea extract



- **small signal suppression**
  60-80% recovery with the undiluted extract, in 14% of analyte / matrix comb.

kresoxim-methyl
in rocket extract



- **significant signal suppr.**
  <60% recovery with the undiluted extract, in 66% of analyte / matrix comb.

Benefit of different dilution factors

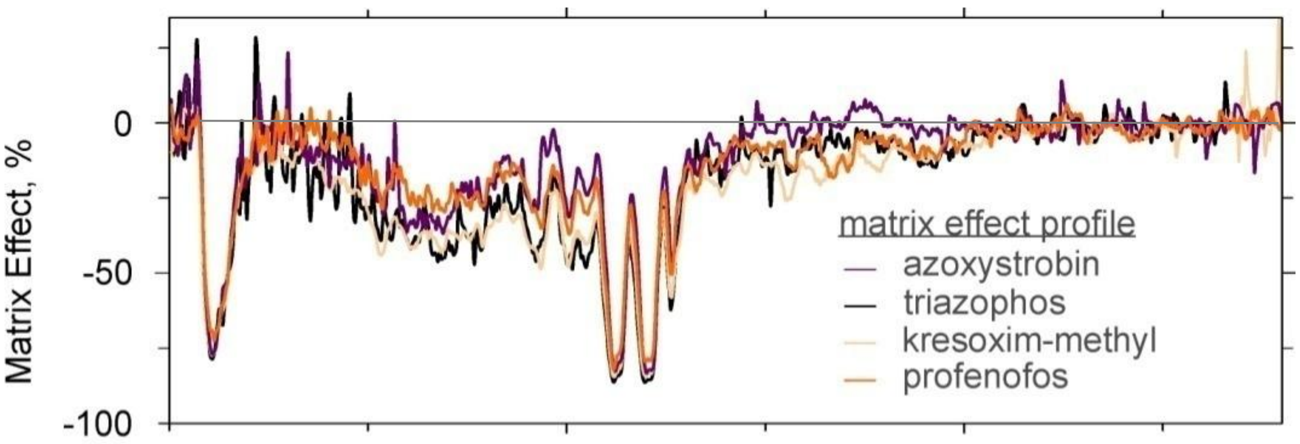
Percentage of pesticides free of matrix effects after extract dilution:

dilution factor	orange	avocado	rocket	tea
undiluted	38%	31%	3%	4%
2	38%	31%	3%	4%
5	38%	31%	3%	4%
10	38%	31%	7%	4%
20	50%	41%	17%	4%
50	78%	72%	37%	58%
100	81%	79%	77%	77%
200	91%	93%	100%	100%
500	94%	97%	100%	100%
1000	97%	100%	100%	100%

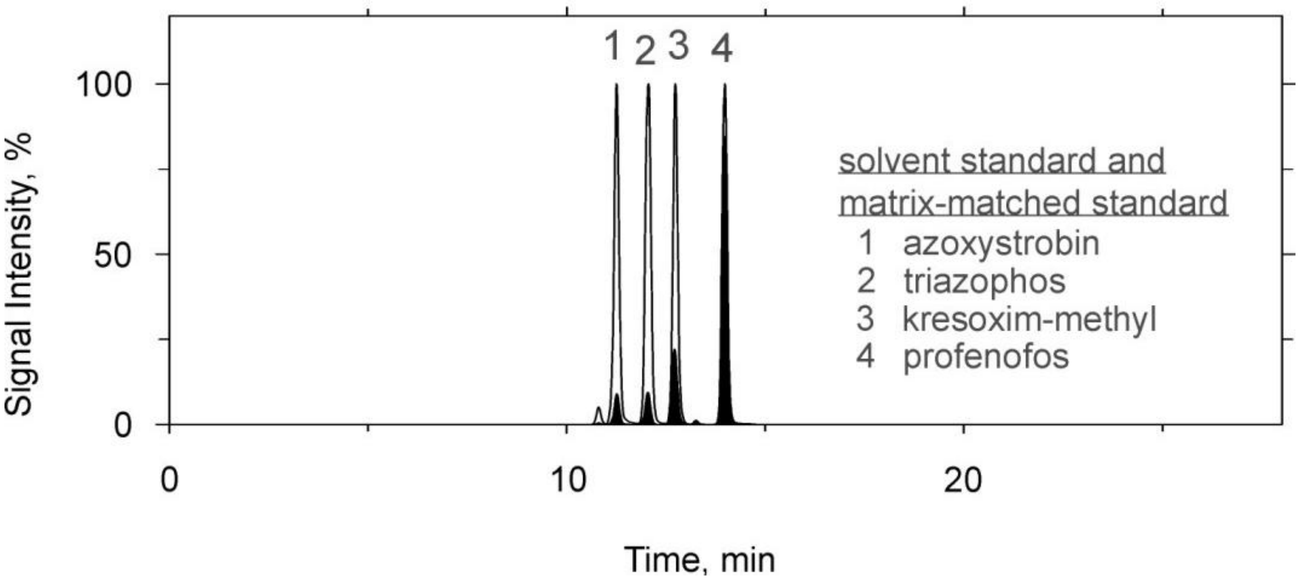
QuEChERS orange extract: **high dilution factors (DF) required** for *flurochloridone* (DF of 323), *azoxystrobin* (DF of 821) and *triazophos* (DF of 1508)

Matrix effect profiles help to identify critical and uncritical regions in a chromatogram

Permanent postcolumn infusion:



Conventional matrix effect determination:



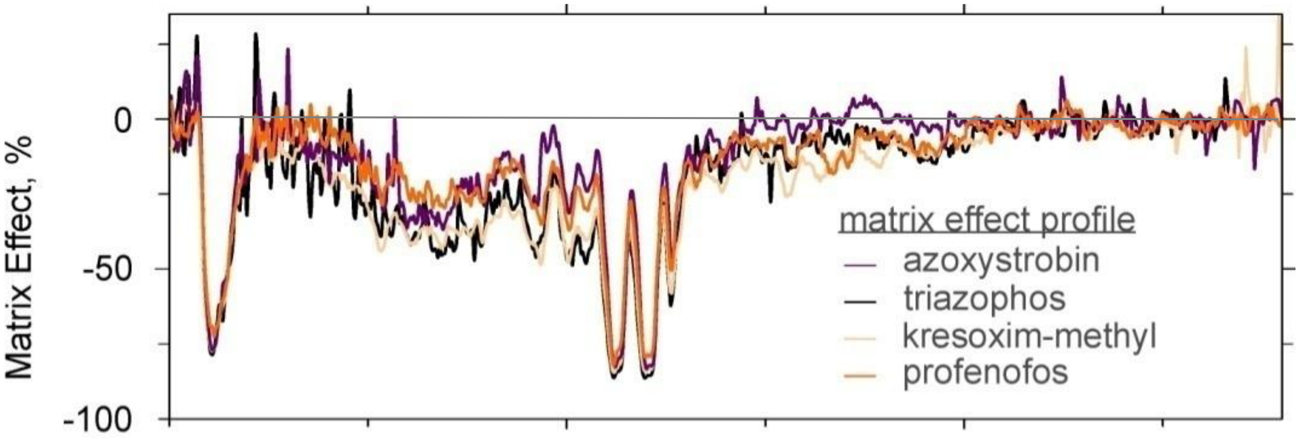
"The perfectionist"

Matrix Effects *(convent.)*	**Required DF for no ME**	**n**
between -29% and -40%	17-142 (5x >100)	15
between -40% and -50%	8-81	16
between -50% and -60%	12-193	23
between -60% and -70%	29-127	19
between -70% and -80%	32-149	10
of -80% and stronger	47-1511 (2x <100)	10

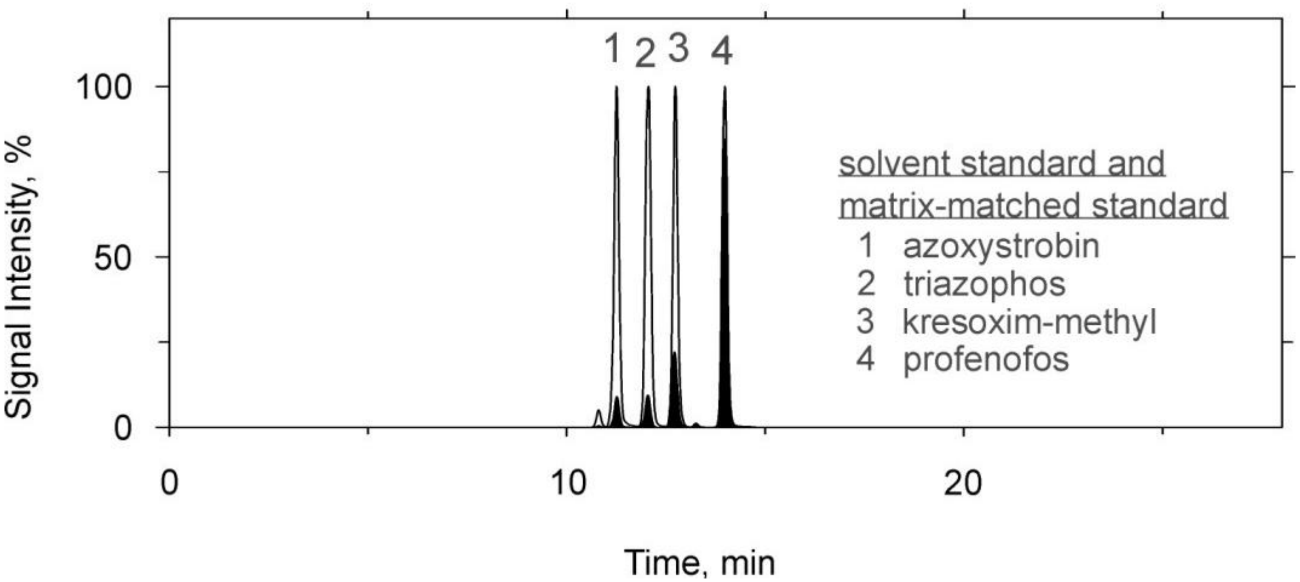
n – number of relevant analyte / matrix comb.

Matrix effect profiles help to identify critical and uncritical regions in a chromatogram

Permanent postcolumn infusion:



Conventional matrix effect determination:



"The practitionist"

Matrix Effects *(convent.)*	**Required DF for ME of ≤ -20%**	**n**
between -29% and -40%	3-10	15
between -40% and -50%	4-10	16
between -50% and -60%	5-20	23
between -60% and -70%	10-31	19
between -70% and -80%	13-40	10
of -80% and stronger	20-419	10

n – number of relevant analyte / matrix comb.

Handling of matrix effects in pesticide residue analysis

1. measuring of a matrix effect profile

2. for regions in the chromatogram

   - with absence of matrix effects *(circa 20% of cases)*

     ⇒ no correction of analytical results needed

   - with signal suppressions up to 80%, i.e. 20% analyte recovery, *(circa 70% of cases)*

     ⇒ dilute a final QuEChERS extract:

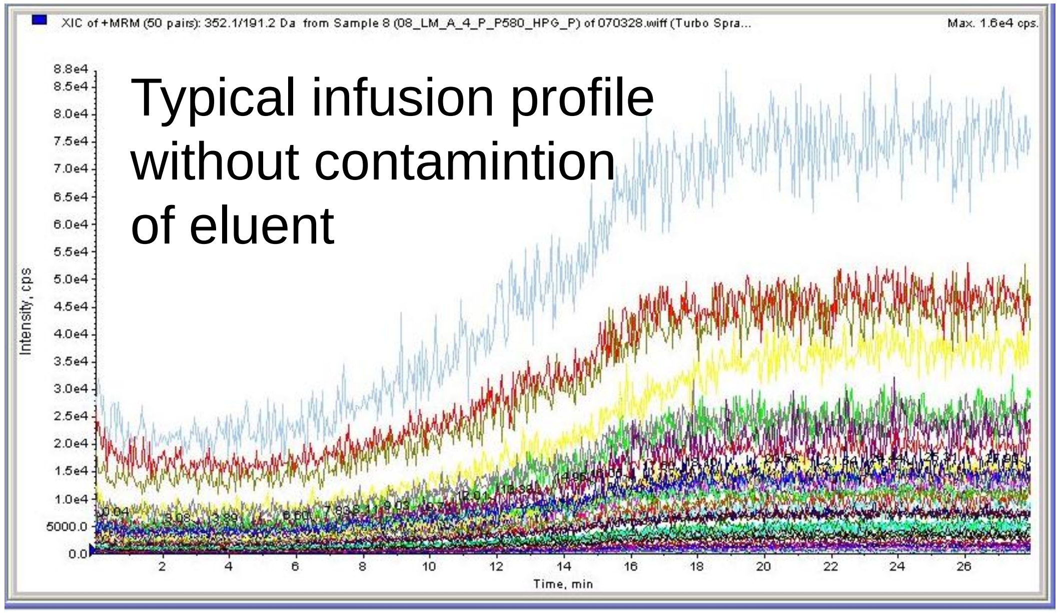
     10-fold dilution for matrix effects up to -50%,
     20-fold dilution for matrix effects up to -60%
     30-fold dilution for matrix effects up to -70%
     40-fold dilution for matrix effects up to -80%

   - with signal suppressions stronger 80% *(<10% of cases)*

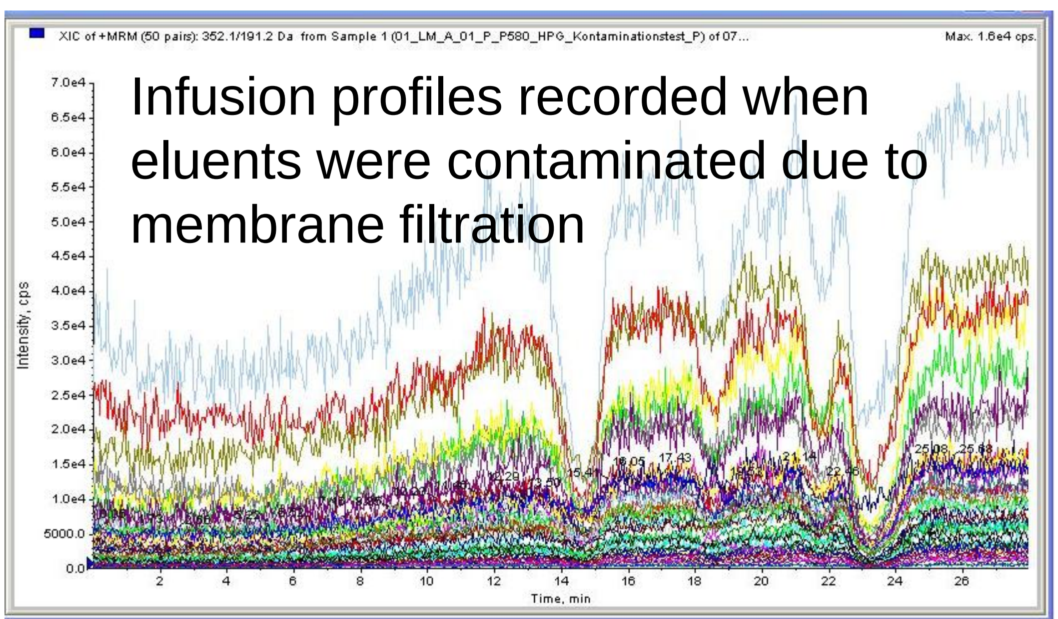
     ⇒ compensation method needed *(e.g. standard addition)*

Permanent postcolumn infusion to detect unexpected suppressions not caused by matrix

A



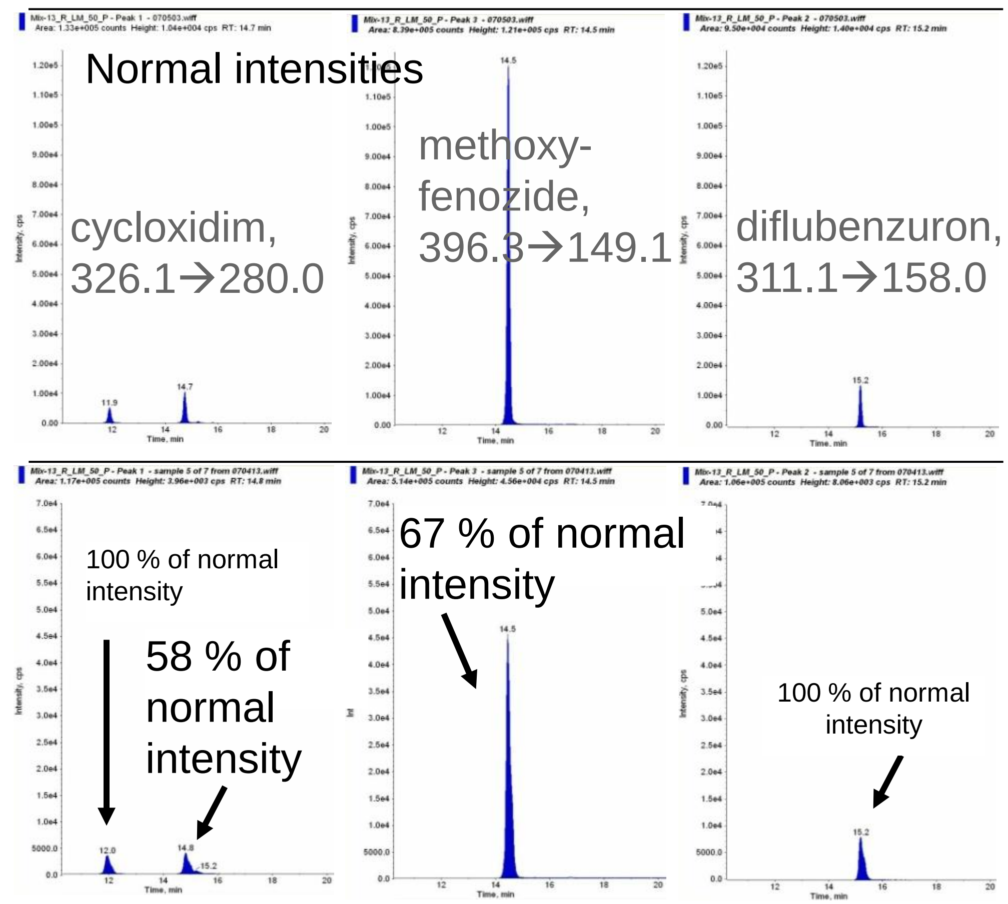
B



Chromatograms of instrument check mixture

A

B



Infusion profiles allow to check the absence of matrix effects over the entire length of chromatographic runs

BfR

Summary

⊖ Hardly a chance to selectively remove the food ingredients which cause matrix effects from final extracts by a better clean-up

⊕ **Possibility to monitor matrix effects over entire length of chromatographic runs by simply infusing analyte standards permanently postcolumn**

⊕ **No longer randomly occurring matrix effects with permanent postcolumn infusion**

⊕ **Deeper understanding of matrix effects in ESI-MS:**

- Matrix effects occur due to competition for charges.
- Matrix effects depend mainly on retention time, i.e. on the co-eluting matrix component. Other analyte properties have only a minor influence.
- After a critical concentration is exceeded matrix effects increase logarithmically with the matrix concentration.

⊕ **Possibility to minimize matrix effects in a simple way by dilutions of final extracts**. Depending on the strength of signal suppressions dilution factors between 10 and 40 are appropriate. Need of laborious compensation methods like standard addition only in case of suppressions stronger than 80%.

Risiken erkennen – Gesundheit schützen

FEDERAL INSTITUTE FOR RISK ASSESSMENT

Thank you for your attention!

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