Detection and elimination strategies of matrix effects in quantitative multi-target LC-ESI-MS/MS analysis

David Steiner, Michael Sulyok, Leonardo Mariño Repizo, Lidija Kenjerić, Rudolf Krska

MultiCoop Training School, Prague, 28th November 2018

COMET Competence Centers for Excellent Technologies

FFG Austrian Research Promotion Agency

bmwfw Bundesministerium für Wissenschaft, Forschung und Wirtschaft

bmvit Bundesministerium

Forschung LAND OBERÖSTERREICH

wirtschaftsagentur wien Ein Fonds der Stadt Wien

Background

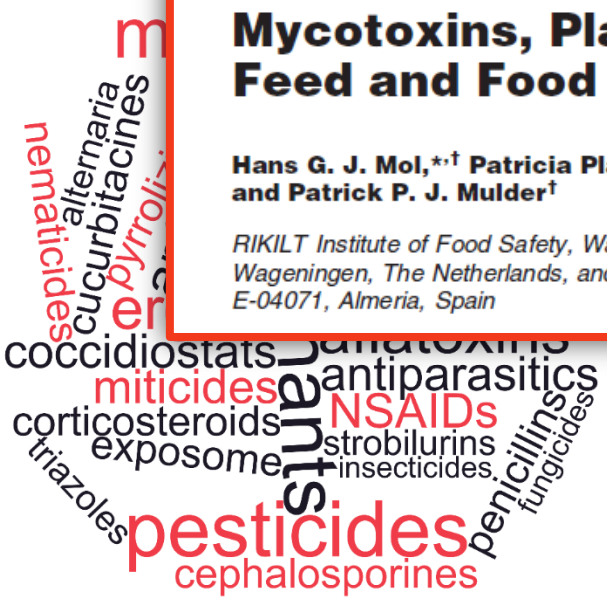
- routine laboratory analysis is undergoing a noticable change
- LC-TQMS, 2D-LC-MS/MS, LC-HRMS instrumental approaches gain traction
- singl

Anal. Chem. **2008**, *80*, 9450–9459

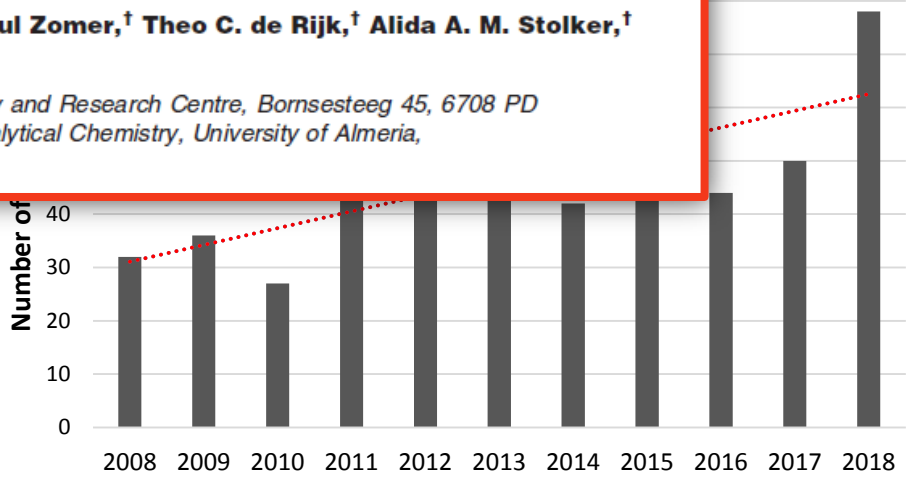
Toward a Generic Extraction Method for Simultaneous Determination of Pesticides, Mycotoxins, Plant Toxins, and Veterinary Drugs in Feed and Food Matrixes

Hans G. J. Mol,*,† Patricia Plaza-Bolaños,‡ Paul Zomer,† Theo C. de Rijk,† Alida A. M. Stolker,† and Patrick P. J. Mulder†

RIKILT Institute of Food Safety, Wageningen University and Research Centre, Bornsesteeg 45, 6708 PD Wageningen, The Netherlands, and Department of Analytical Chemistry, University of Almeria, E-04071, Almeria, Spain

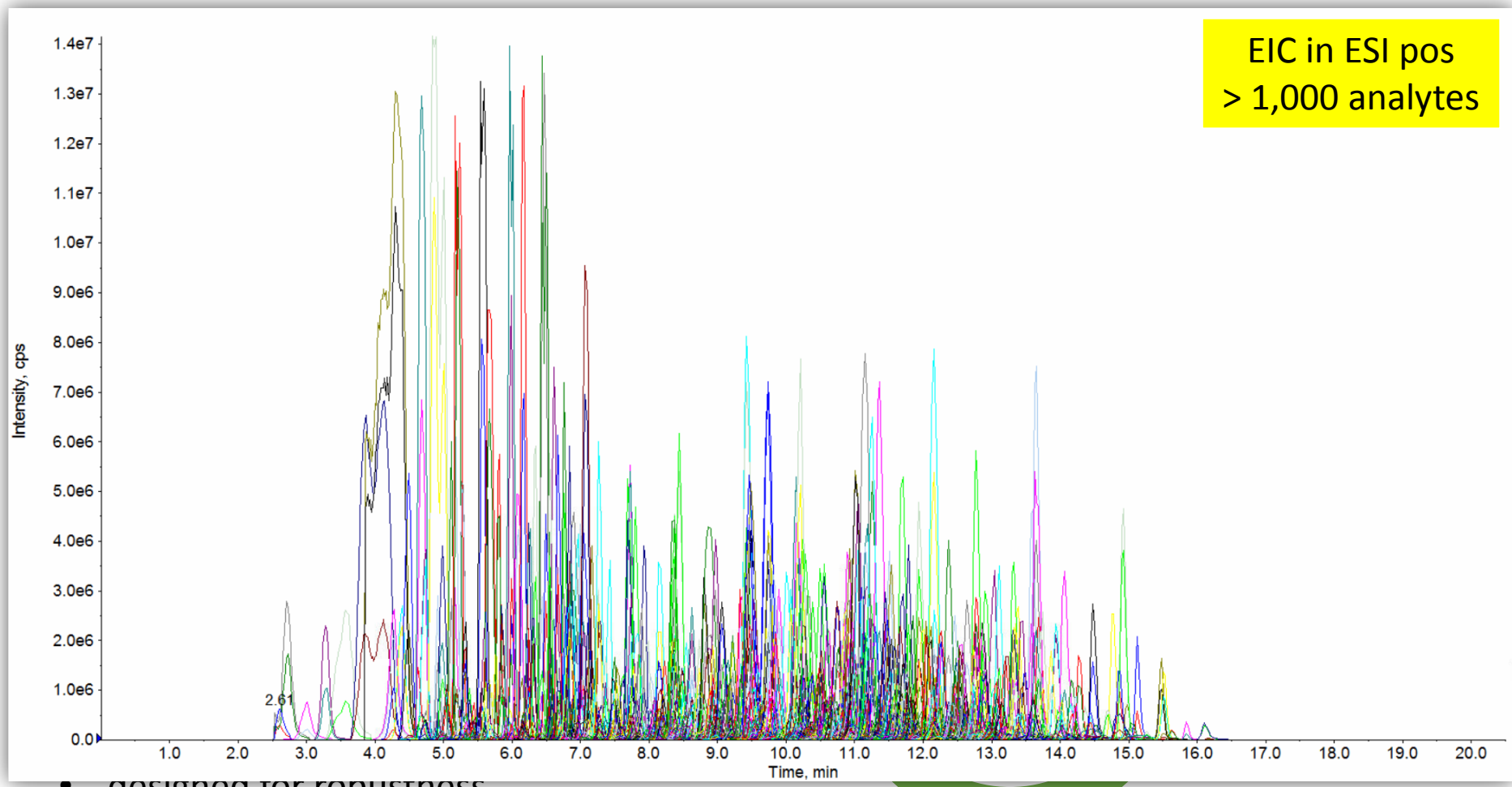


2008 and 2018



Analysis Scheme

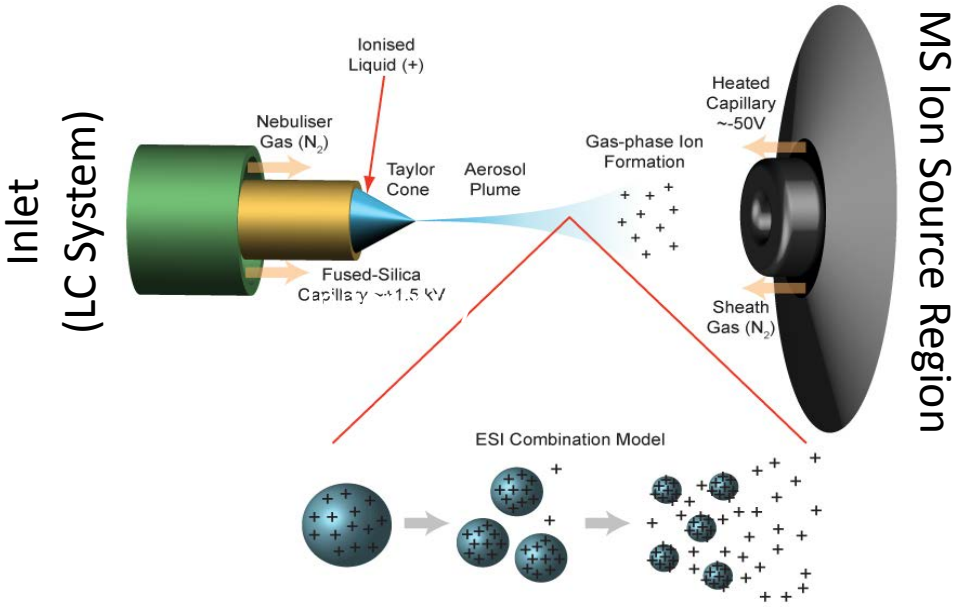
Phenomenex Gemini C18



- designed for robustness

ESI and Ion Source Overview

*Electrospray Ionisation



http://www.lamondlab.com/MSResource/LCMS/MassSpectrometry/electrosprayIonisation.php

$$SSE = \frac{\text{Area of post extraction spike}}{\text{Area of neat standard}}$$

$$RSD\ of\ SSE > 20\%$$

Matrix Effect (ME)

combined effect of all components of the sample other than the analyte

absolute ME

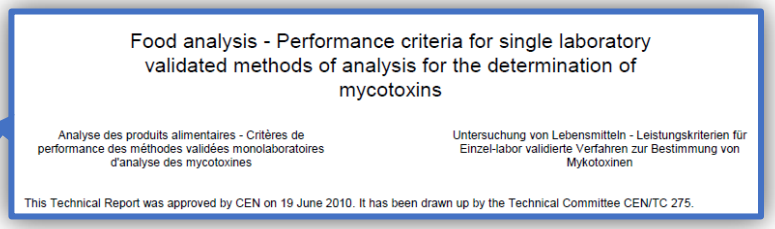
increase or decrease in response between solvent standard and spiked pretreated sample

relative ME

differences in response, accuracy and/or precision between different batches of the same matrix

Legislation & Performance Criteria

- EC 32/2002 Undesirable substances
- EC 576/2006 Mycotoxins in feed
- EC 1881/2006 Mycotoxins
- EC 396/2005 Pesticides
- EC 37/2010 Pharmacologicals

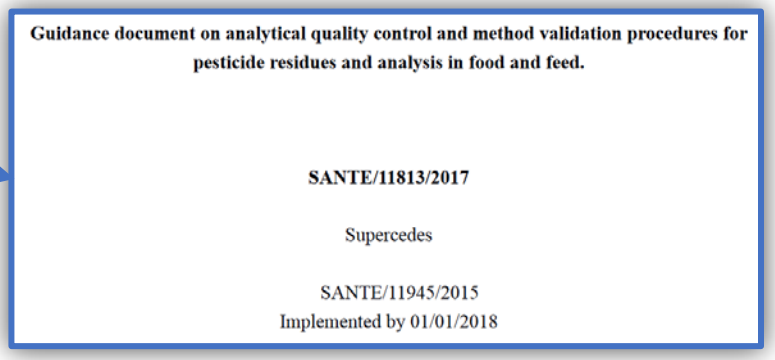

Food analysis - Performance criteria for single laboratory validated methods of analysis for the determination of mycotoxins

Analyse des produits alimentaires - Critères de performance des méthodes validées monolaboratoires d'analyse des mycotoxines

Untersuchung von Lebensmitteln - Leistungskriterien für Einzel-labor validierte Verfahren zur Bestimmung von Mykotoxinen

This Technical Report was approved by CEN on 19 June 2010. It has been drawn up by the Technical Committee CEN/TC 275.

in case of more than 20% signal suppression or enhancement, MEs need to be addressed in calibration


Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.

SANTE/11813/2017

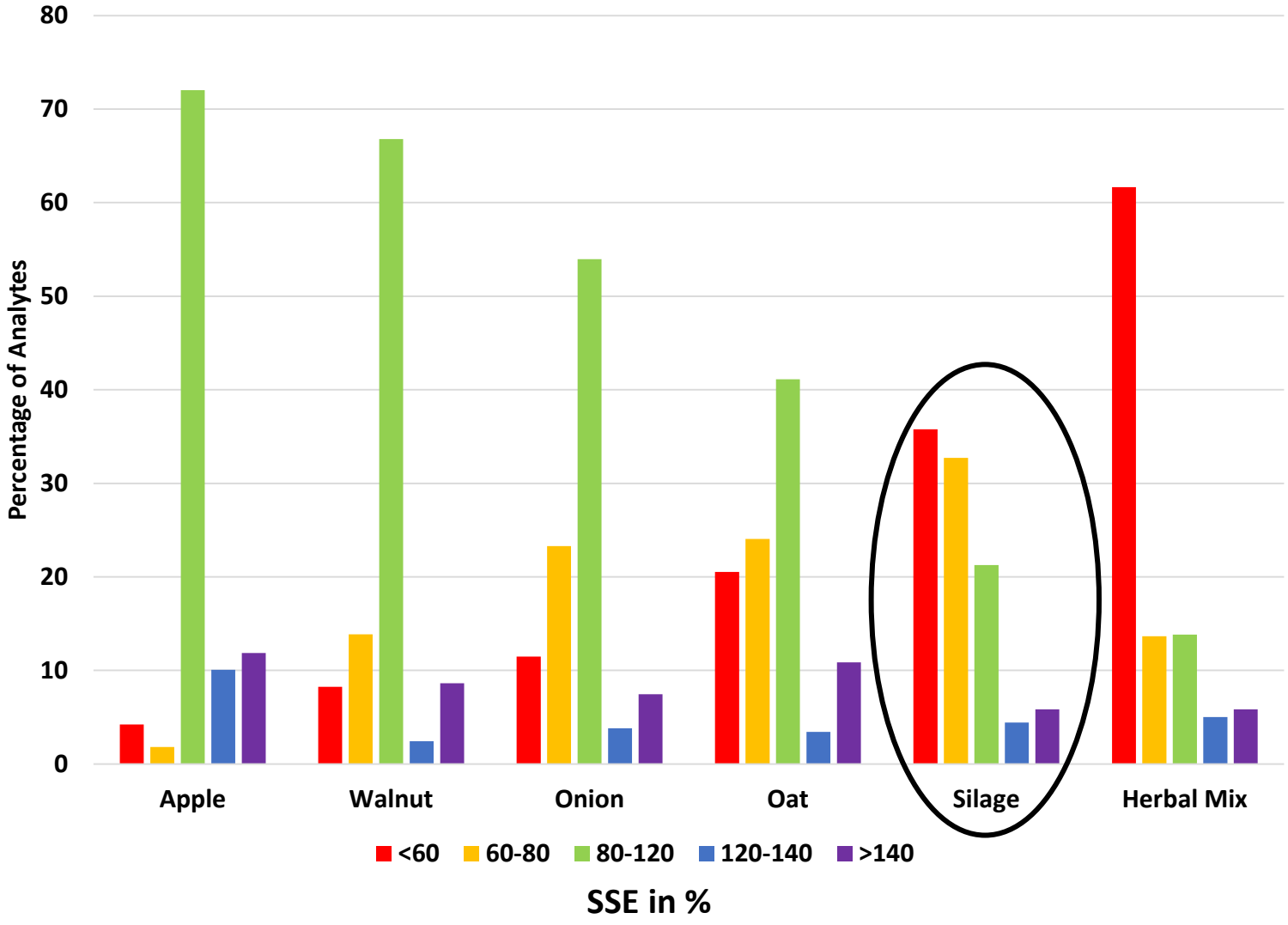
Supercedes

SANTE/11945/2015
Implemented by 01/01/2018

Evaluation of Matrix Effects

- comprehensive evaluation of **SSE% in 6 different matrices** from different food & feed commodities
- effects were evaluated for **50 mycotoxins**
- <u>matrix effect potential from:</u>
  - carbohydrates & dietary fibre
  - lipids & peptids
  - polar co-eluting substances
  - ionic species
  - interferents with similar chemical structure

Evaluation of Matrix Effects



Matrix Effects in Animal Feed

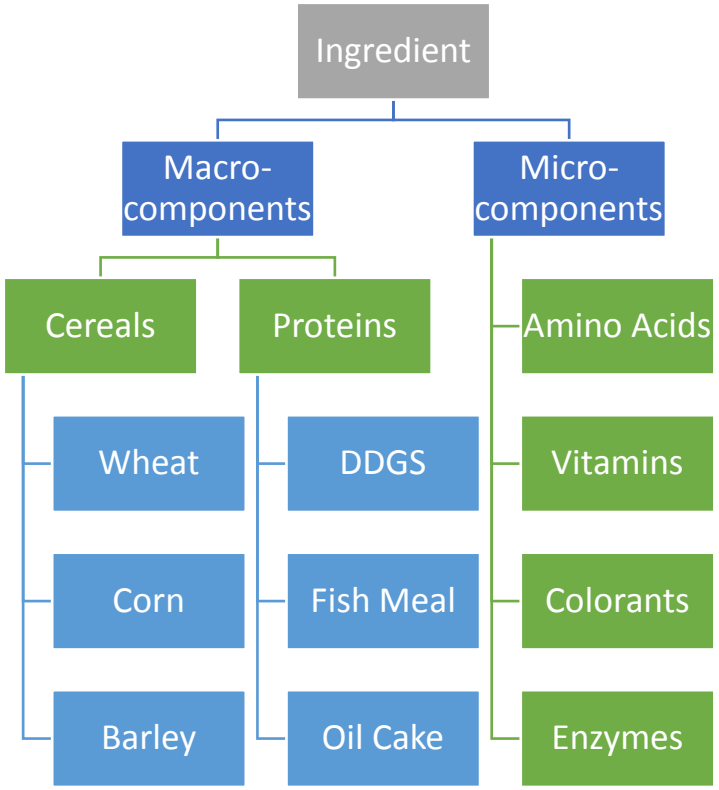
Animal Feed – Validation

DAkkS Deutsche Akkreditierungsstelle

Status quo

Validation data must be maintained for each feed group on at least one of the listed sample matrices

feed group	characteristics
forage crops	↑ water
fruit marc	↑ acidicty
extraction cake	↑ sugar, ↓ water
oilseeds	↑ fat, ↓↓ water
grains	↓ water, ↓ fat, ↑ fibre
legumes	↓ water, ↑ protein



Matrix effect → (± 20%)

Repeatibility → RSDr ≤ 20%

variation on global compound feed market → compositional uncertainty

Complex Feed Matrices

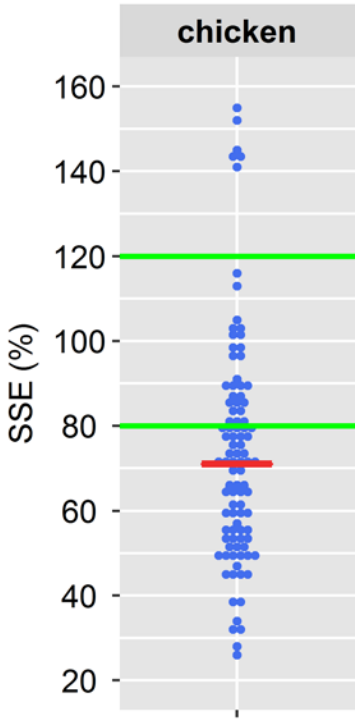
28 compound feed samples

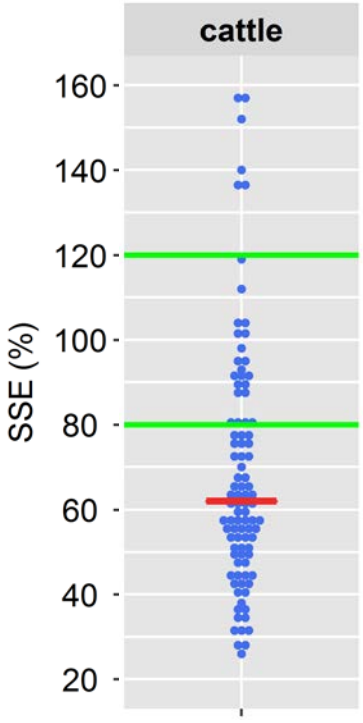
chicken, pig, fish, cattle

7 different lots of each sample type

evaluation of SSE, EE, RA

Absolute matrix effects





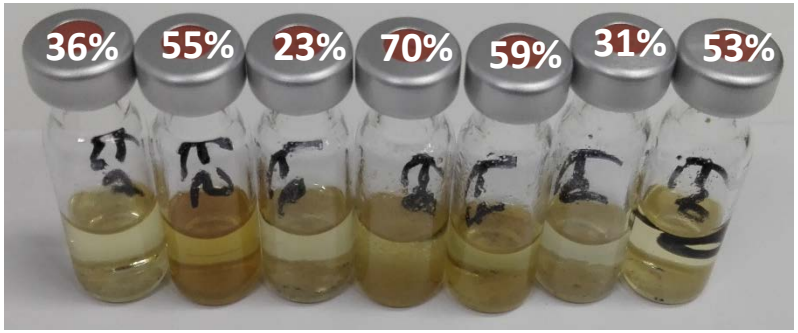
Relative Matrix Effects

Frequency of analytes affected by intra matrix variation

chicken < pig < fish < cattle

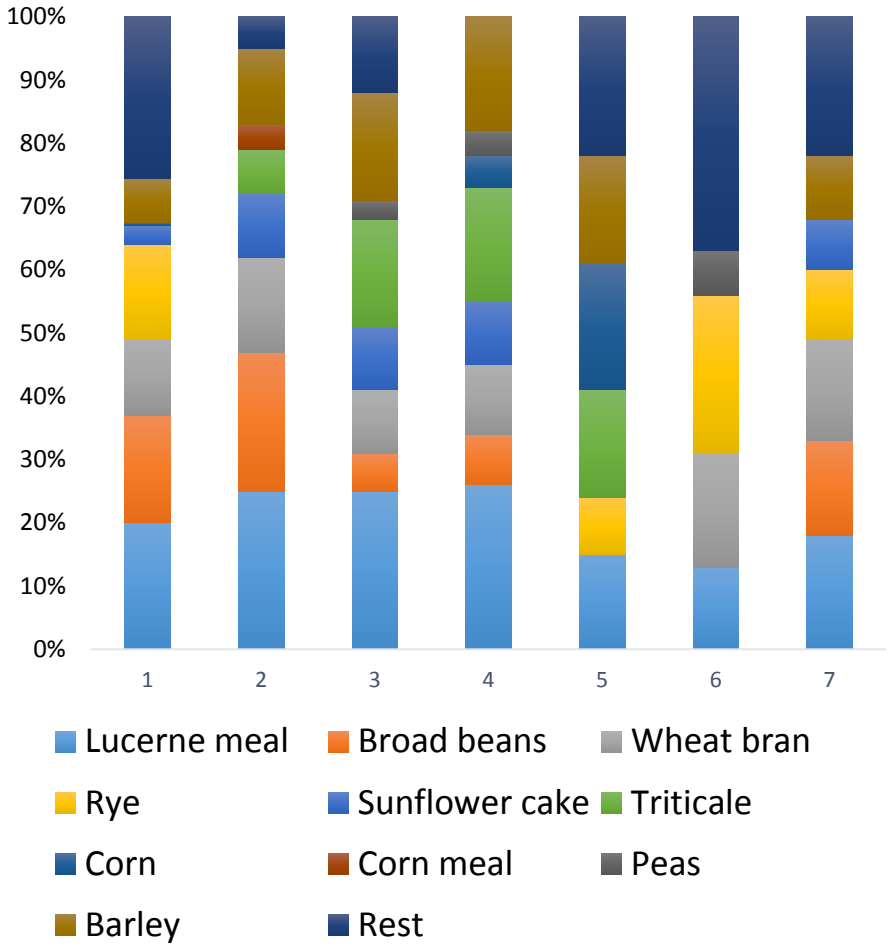
9% 20 % 24 % 32 %

Signal suppressions for Zearalenone in cattle feed



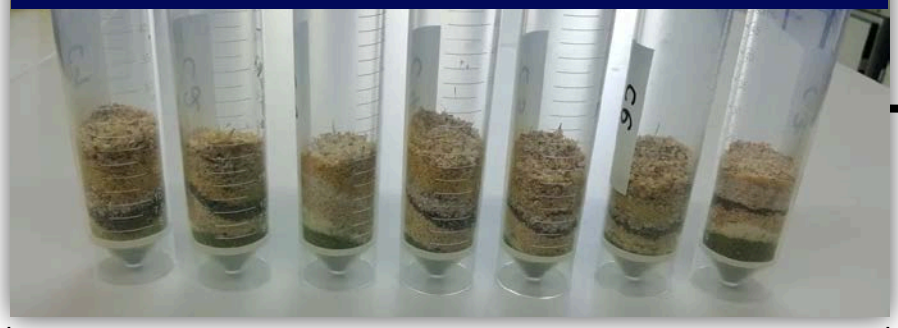
- no uniformity regarding the composition
- compositional source of uncertainty up to 35 %

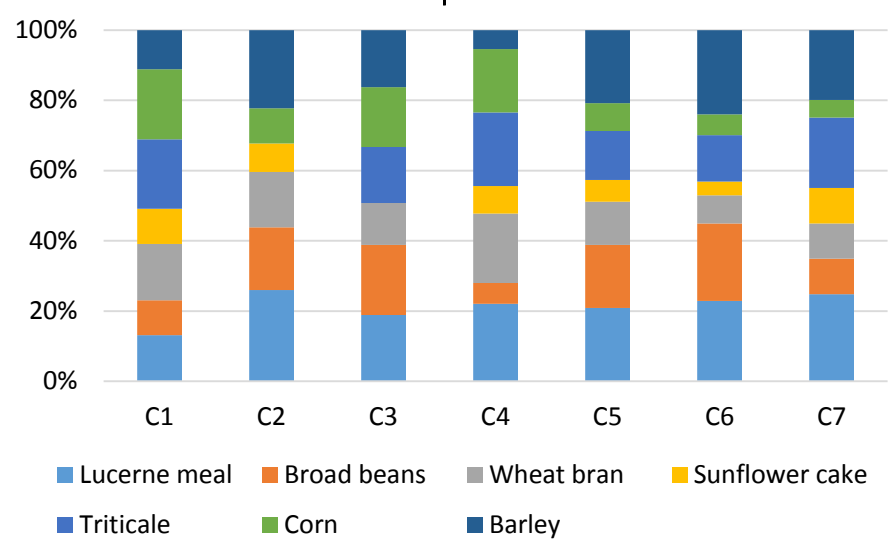
Composition of 7 compound cattle feed formulas



Feed Model Matrices

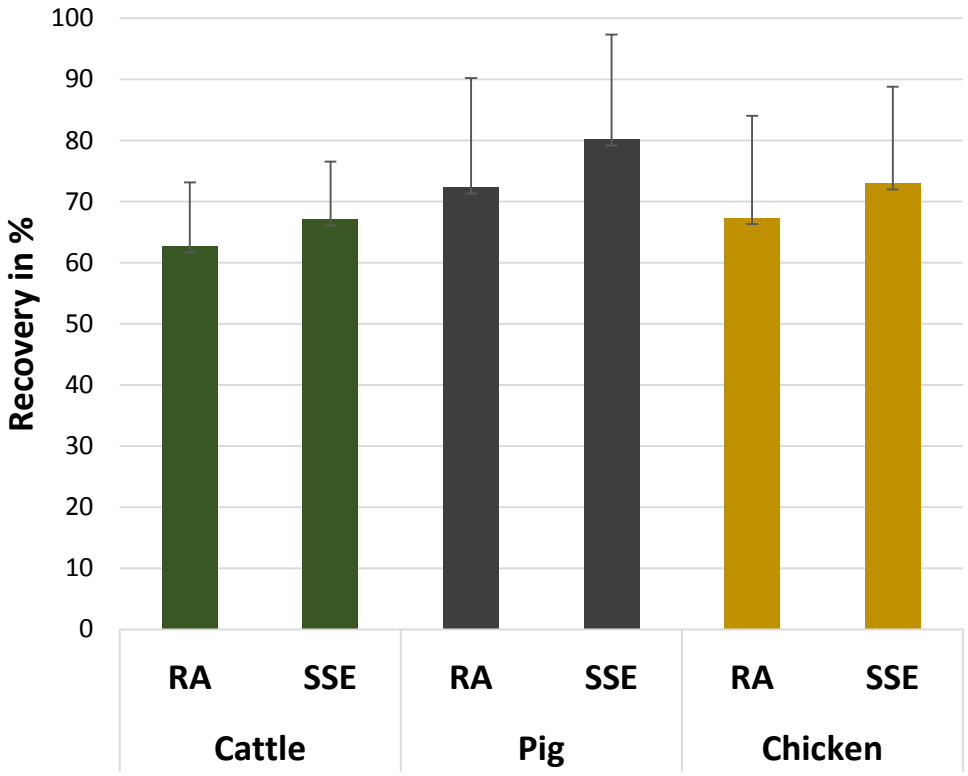
Cattle Feed



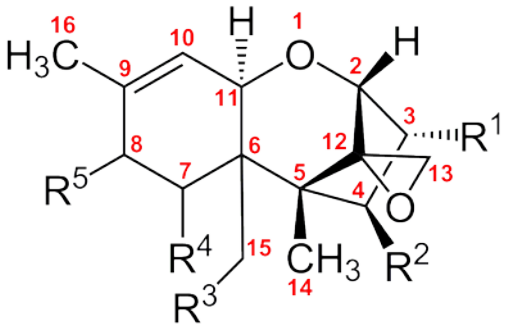


- no compositional uncertainties
- use of blank single feed ingredients
- simulation of intra-matrix variation

Results – Model Matrices
Fusarium Metabolites



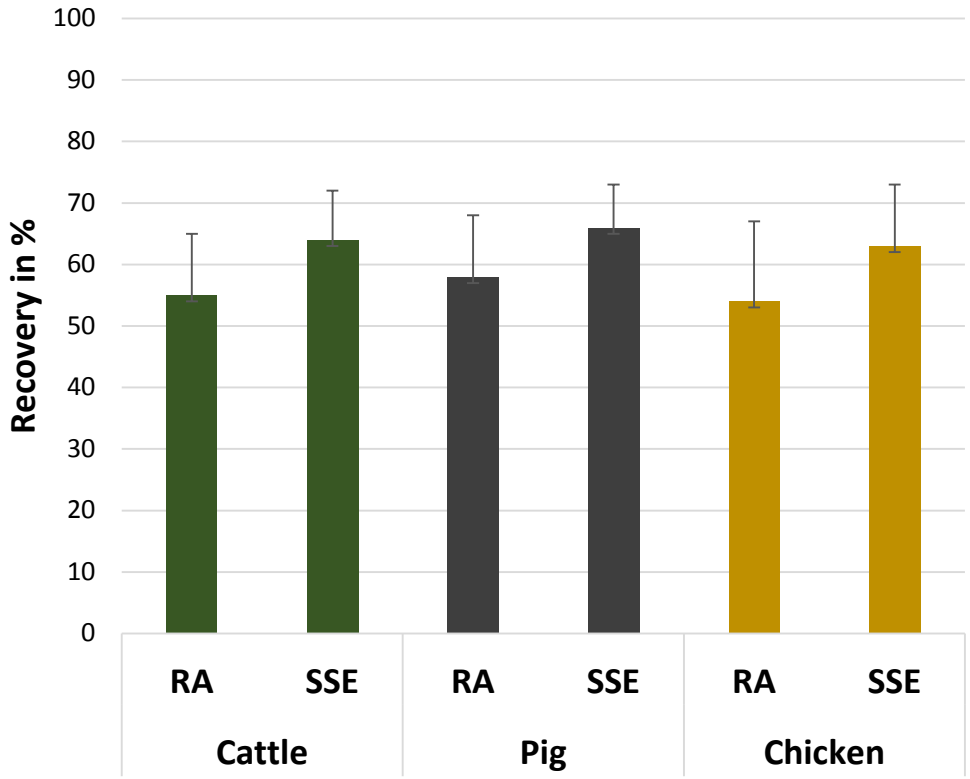
- absolute matrix effects: SSE → 20 – 33 %
- relative matrix effects: RSD → 9 – 18 %



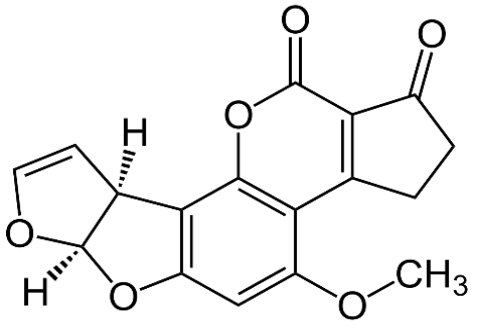
Trichothecenes

- 15-Acetyldeoxynivalenol
- 3-Acetyldeoxynivalenol
- Diacetoxyscirpenol
- Fusarenon X
- HT-2 Toxin
- Monoacetoxyscirpenol
- Neosolaniol
- Nivalenol
- T-2 Toxin

Results – Model Matrices
Aspergillus Metabolites



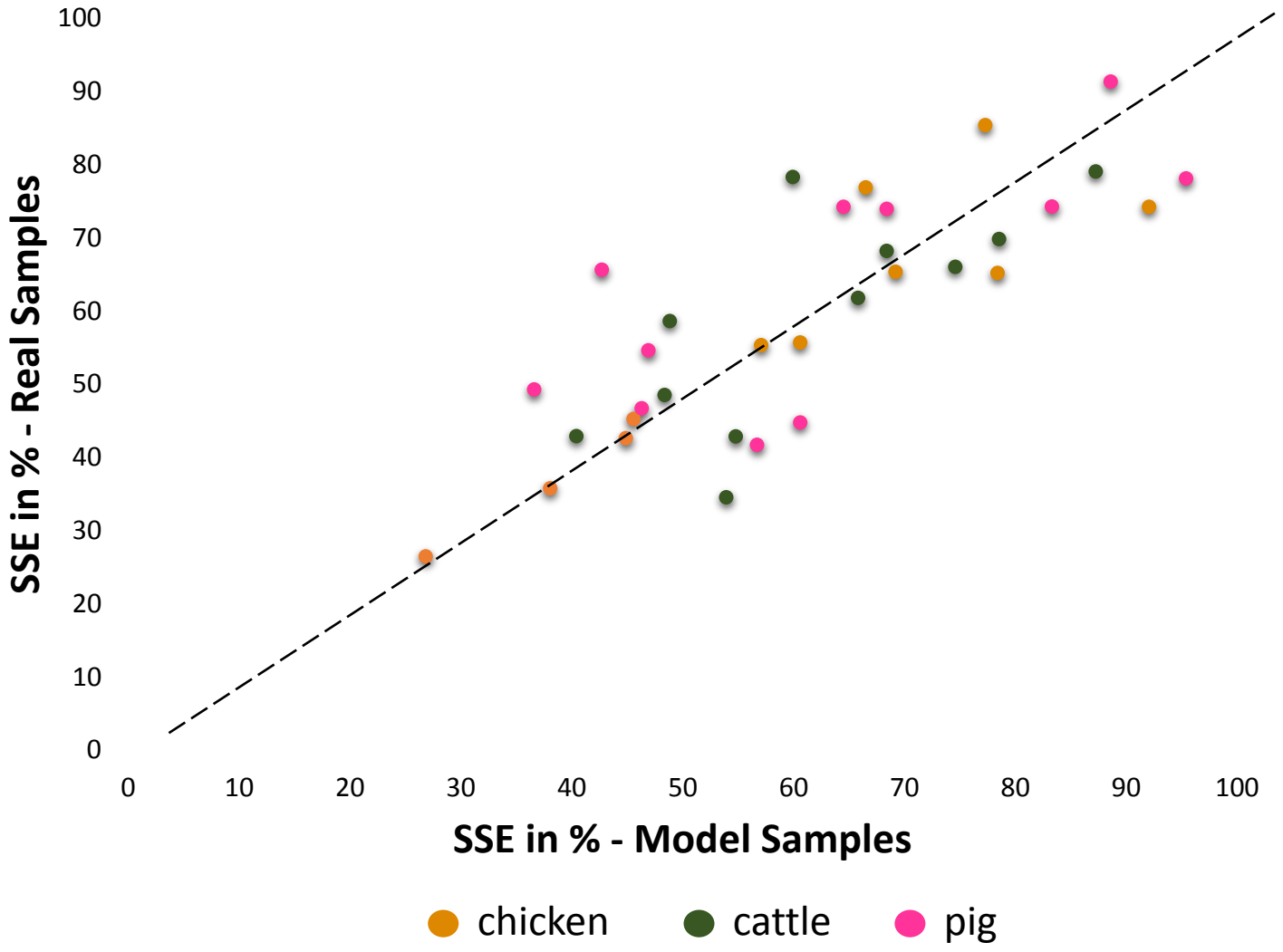
- absolute matrix effects: SSE → 34 – 37 %
- relative matrix effects: RSD → 7 – 13 %



Aflatoxin B1

- Aflatoxin B1
- Aflatoxin B2
- Aflatoxin G1
- Aflatoxin G2
- Aflatoxin M1
- Averantin
- Averufin
- Sterigmatocystin
- Versicolorin A

Real Sample vs. Model Matrix



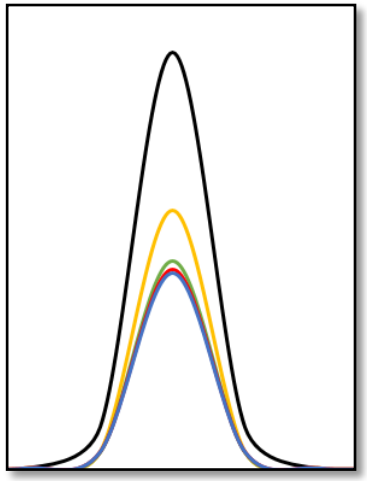
Prediction of Matrix Effects

Corn

Soy

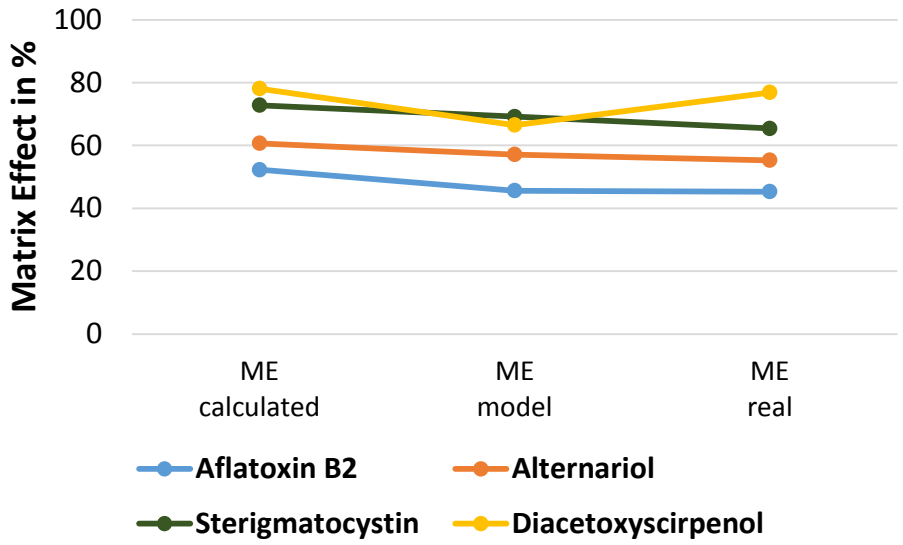
DDGS

Rapeseed



$$ME = 100 - \sum n \ (p * F_{SSE})$$

ME	matrix effect
n	number of single feed ingredients
p	percentage SSE contribution
F_{SSE}	signal suppression/enhancement factor

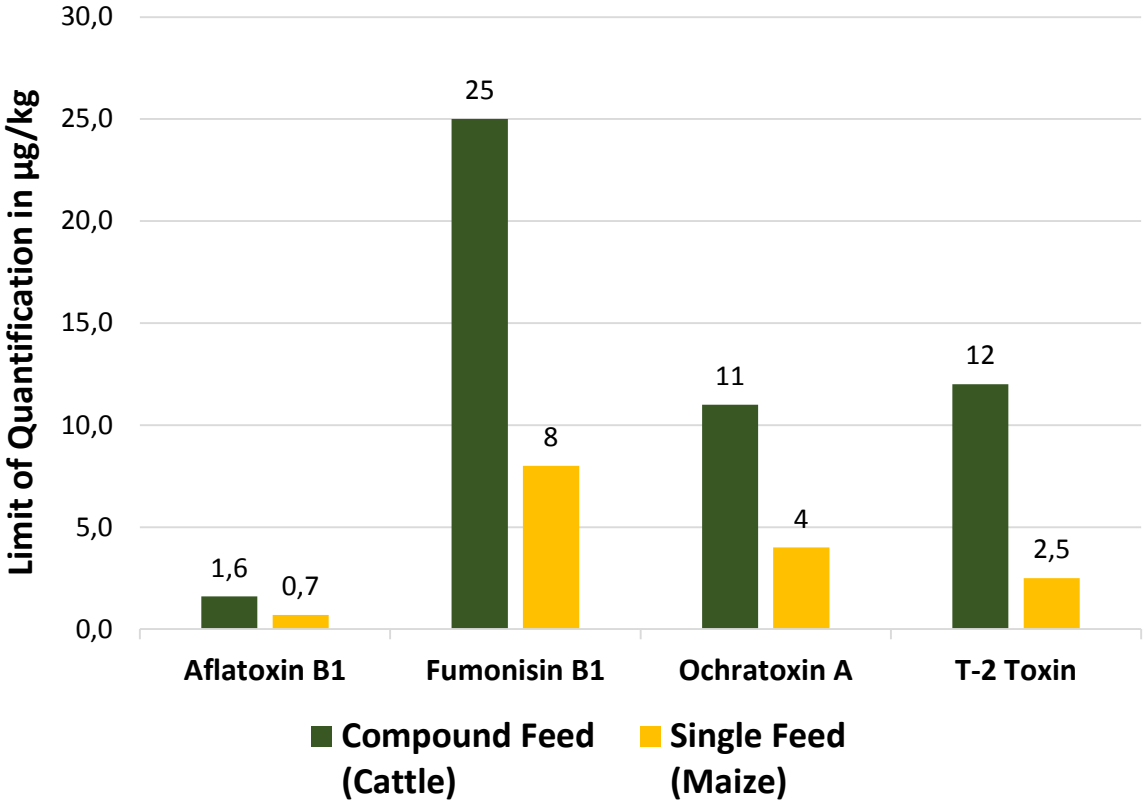


Method Performance

Eurachem

$$s_0' = \frac{s_0}{\sqrt{n}}$$

s_0 is the estimated standard deviation of *m* single result
s'_0 is the standard deviation used for calculating LOD and LOQ
n is the number of replicate observations avaraged when reporting results



Directive 2002/32/EG	
Analyte	**ML in µg/kg**
Aflatoxin B1	5
Fumonisin B1	5000
Ochratoxin A	50

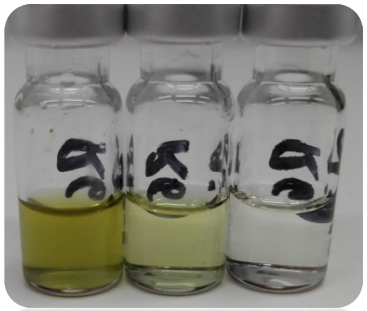
more realistic estimation of the methods performance by using self designed compound feed formulas

Matrix Reduction Strategies

cattle feed

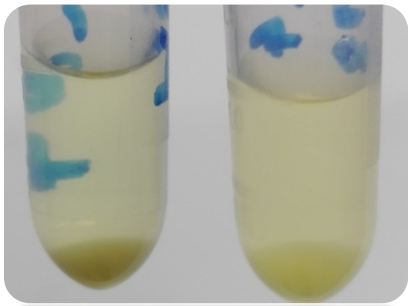
dilution

dilution of extract
(1:10 and 1:100)



QuEChERS

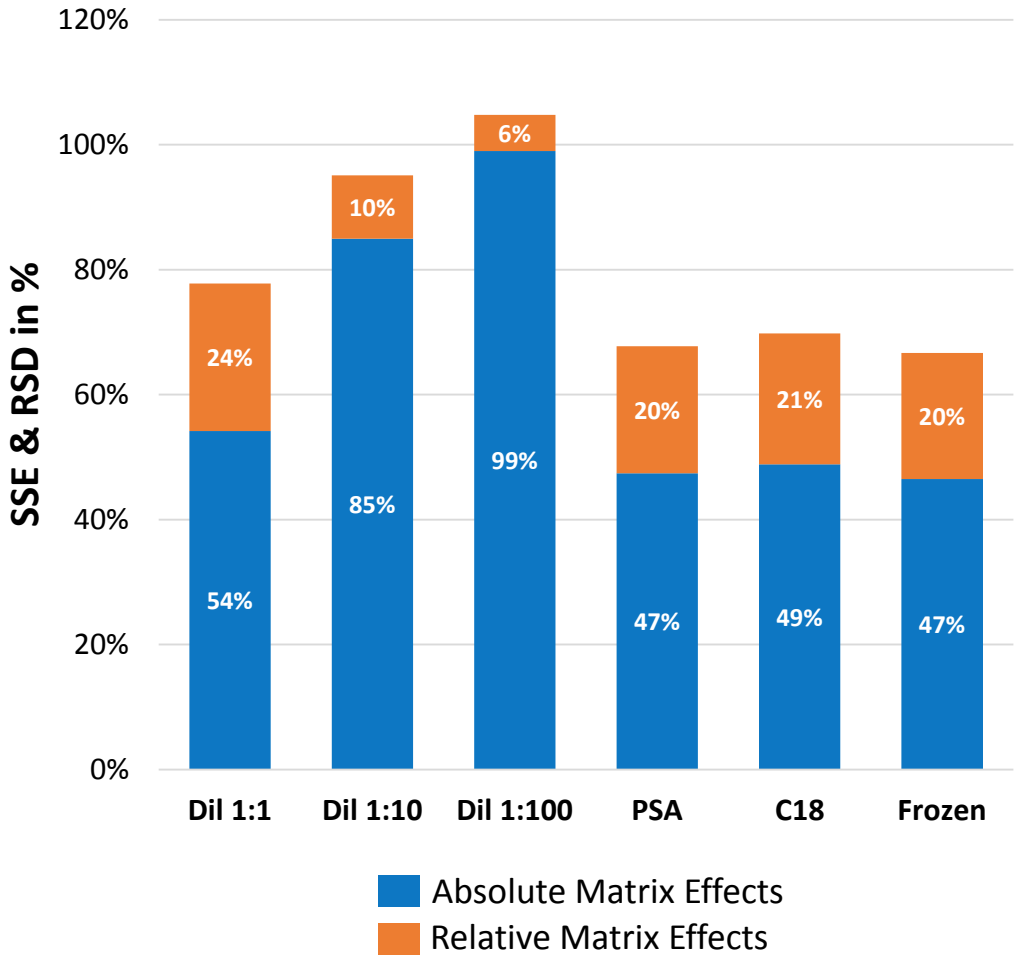
anhydrous $MgSO_4$ and NaCl
+ C18 (Discovery DSC-18)
+ PSA (PSA-Silica Sigma)



deep freezing

anhydrous $MgSO_4$ and NaCl
+ freezing of extract
at -20° C for 16h

Reduction Efficiency



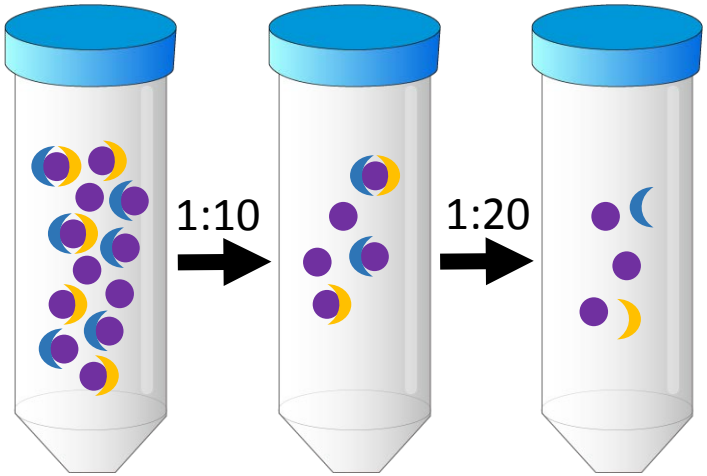
- dilution of extract reduces both, relative and absolute matrix effects
- **tenfold dilution steps → reduction of relative matrix effects by a factor of 2**
- matrix reduction with QuEChERS extraction followed by an unspecific clean up (PSA, C18) is less efficient
- loss of Fumonisins during PSA step

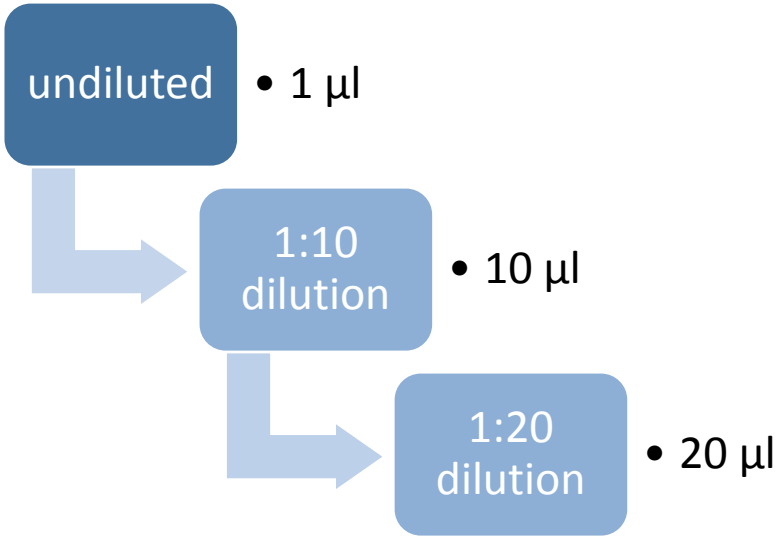
Dilute & Shoot on trial

"Simple dilution of the samples proved to be an efficient and fast way to reduce the signal suppressing/enhancing matrix effects provided by the matrices."

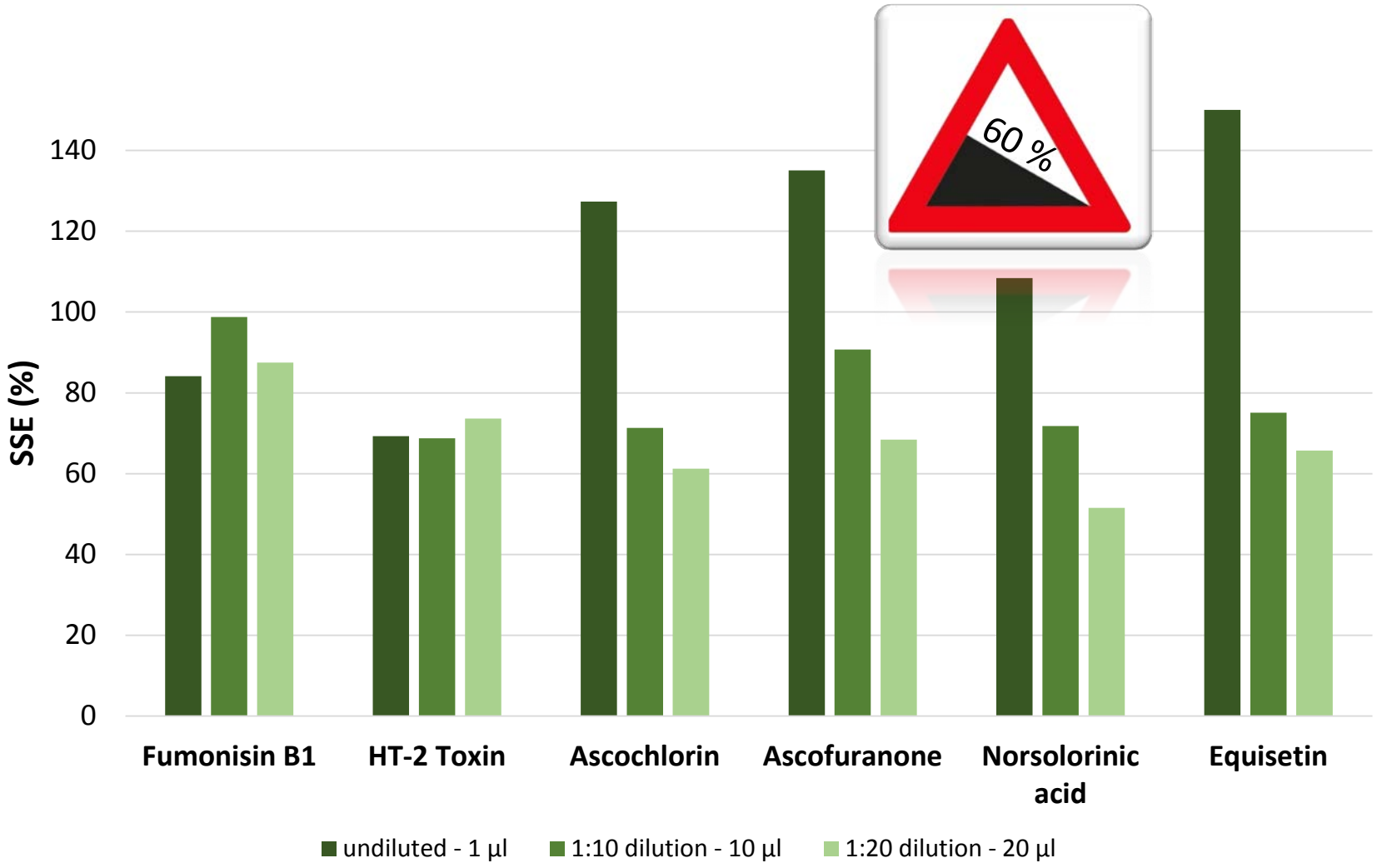
[Eilfeld and Czapiewski 2013]

8 different herbal mix samples



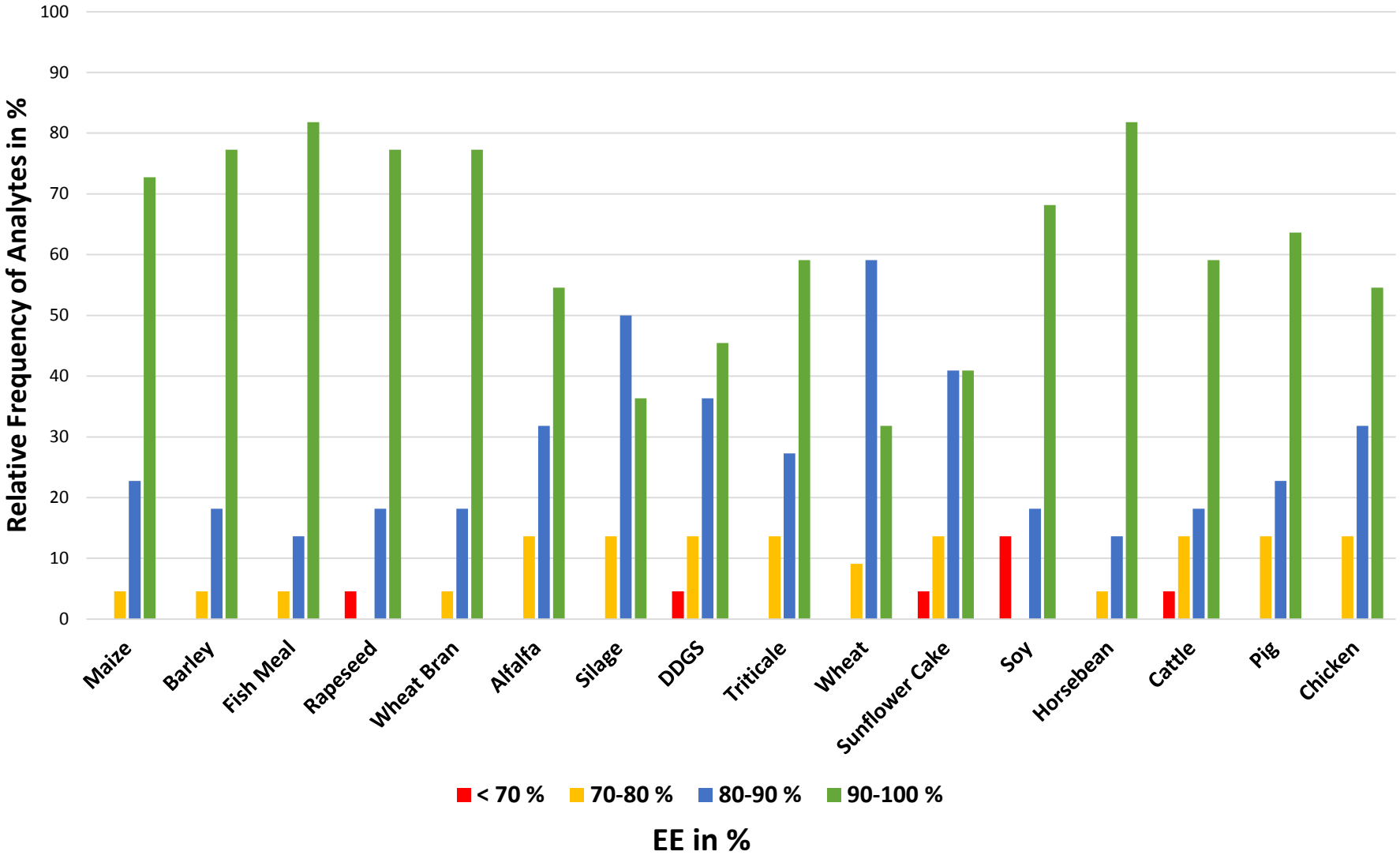


„Matrix Protectants“



What do high absolute and relative matrix effects mean? Is the method fit for purpose?

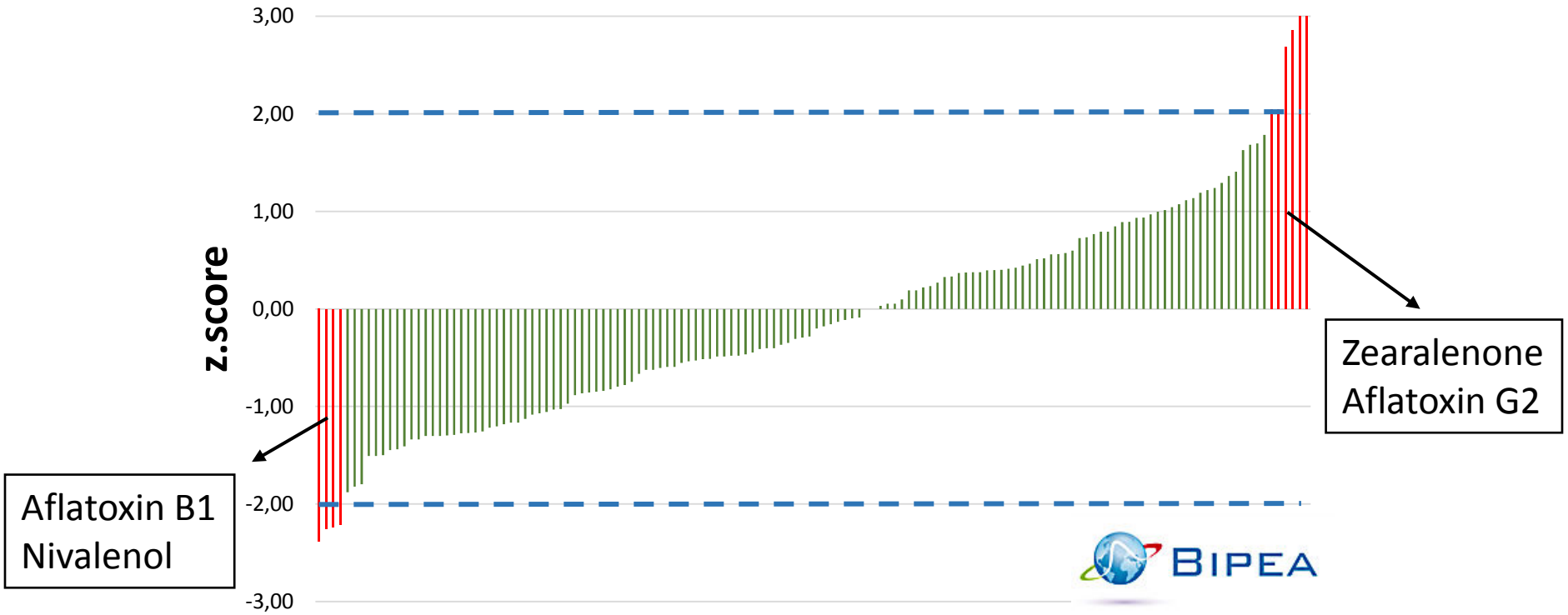
Extraction Efficiencies



Proficiency Tests

- 140 PT results for regulated mycotoxins in animal feed obtained within 8 years
- >93 % of submitted results in the satisfactory range

Compilaton of z-scores obtained by our method in routine proficiency testing (BIPEA)



Summary

- matrix effects (ME) are a major limitating factor for LC-ESI-MS/MS multi-class methods

- dilution of extracts is a straightforward solution for a decisive reduction of ME
  - degree of dilution has to be considered in terms of sensitivity and protective mechanisms

- complex matrices like animal feedstuff represents an additional challenge in terms of relative matrix effects

- validation scheme should take intra-matrix variation into account
  - feed model matrices solves the compositional uncertainty
  - better estimation for method performance

Acknowledgement

Gerd Schatzmayr

Andreas Gschaider
Zoltan Balla

Eduard Schneeberger

Leonardo Mariño Repizo

Lidija Kenjerić

Rudolf Krska, Michael Sulyok

COMET
Competence Centers for Excellent Technologies

FFG Austrian Research Promotion Agency
bmwfw Bundesministerium für Wissenschaft, Forschung und Wirtschaft
bmvit Bundesministerium
Forschung Land Oberösterreich
wirtschaftsagentur wien Ein Fonds der Stadt Wien