NRL experiences on quantification strategies

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Main responsibility

NRL for pesticide residues (FV, CF, AO, SRM)

Development over the years ...

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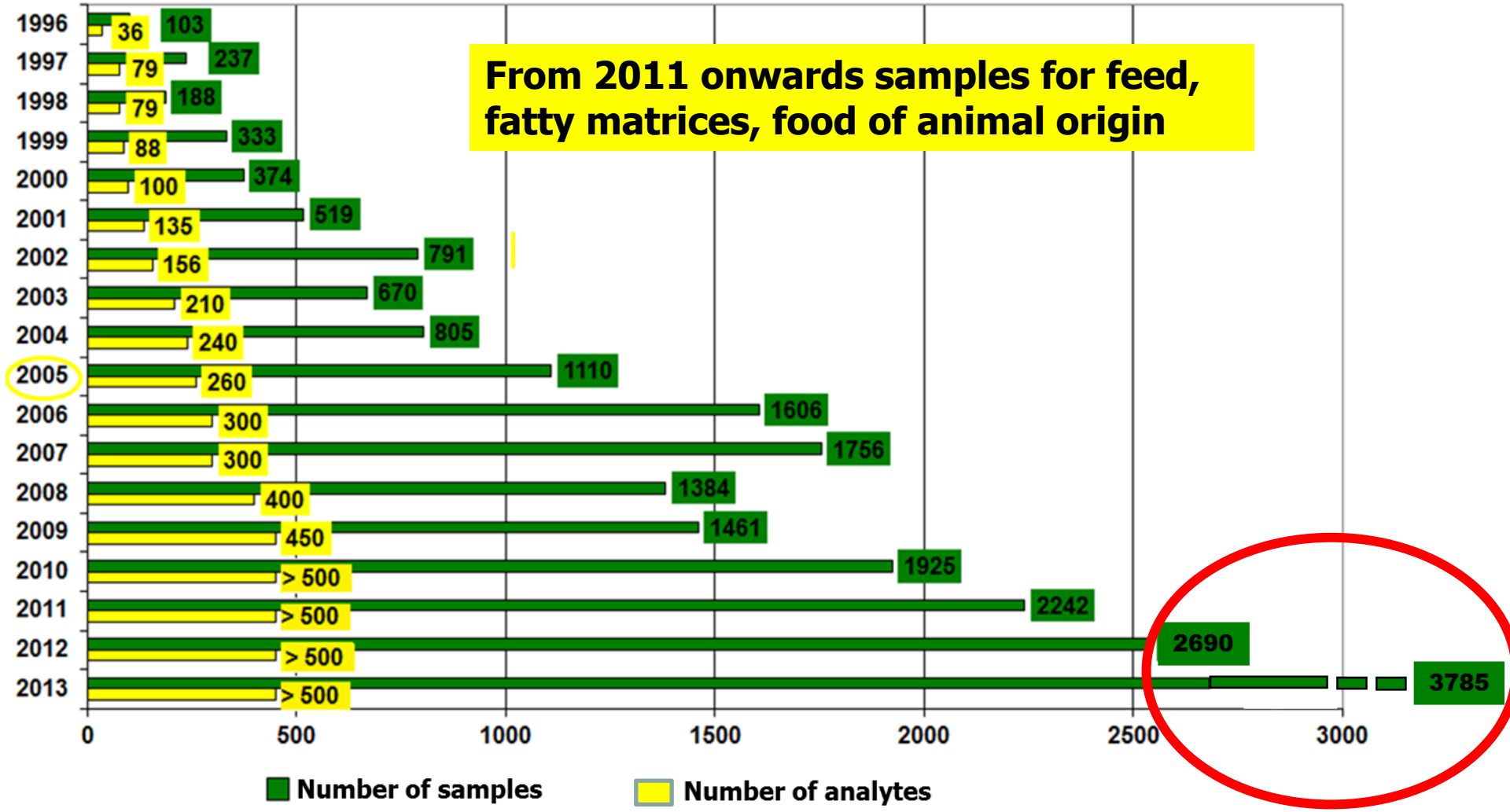


~ 2350 samples

QuEChERs
Multi residue method
(in routine since 2006)

QuPPe
Dithiocarbamates (CS2)
Inorganic Bromide
QACs
Organotin compounds
Problematic MRMs
Captan/Folpet/Chlorothalonil/Captafol
…

Routine analysis 2013

~ 350 samples

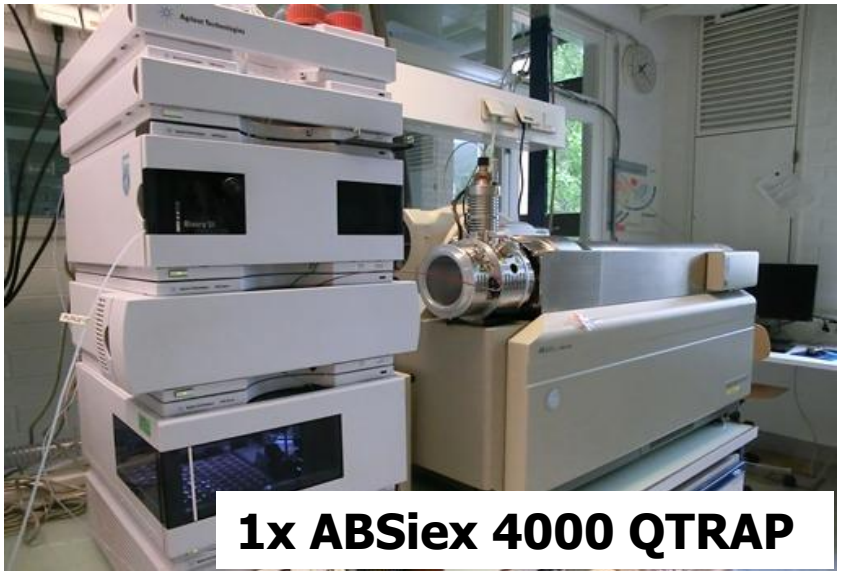
Modif. QuEChERs
Multi residue method
(in routine since 2013)

~ 1050 samples

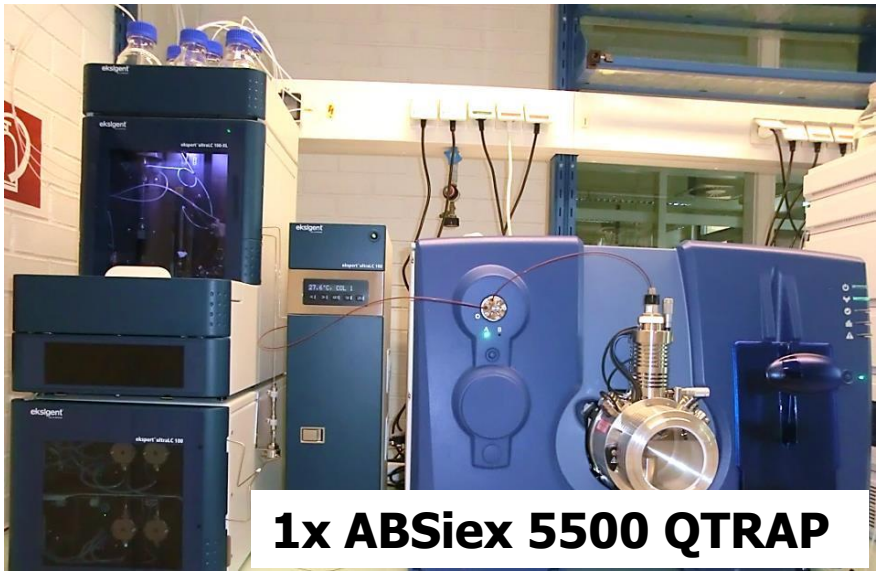
NRCP (Dir. 96/23)
(Modif. QuEChERs)
(in routine since 2013)

Instrumentation for MRM methods

2x GC- MS/MS Agilent 7000 QQQ
1x GC-MSD 6890 (NCI),
1x APGC (7890, Xevo TQ MS)



1x ABSiex 4000 QTRAP



1x ABSiex 5500 QTRAP

Different approaches for Routine analysis in FV samples

I. Routine samples (MACP and National Monitoring Programme)
   - ☞ Quite frequent findings

II. Matrices with high fat content (plant oils, oil seed)
   - ☞ method requirements

III. Low frequency matrices, Import controls (Reg 669/2009)
   - ☞ no blank matrix, time pressure

IV. baby and infant food
   - ☞ low/no expectation of findings

I. Routine samples (MACP and NMP)

☞ Quite frequent findings

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Grouping commodities for batches (fruits, vegetables, cereals, …)

Min 1x QC sample (spiked @ LOQ) for recovery check
4-point calibration (10 – 200 ppb) – bracketing
Drift check – repeated injection of QC-sample (Start, End)
Only procedural IS to check for extraction – no recovery correction
CRMs – quarterly checked
Check old vs. new standard solutions/mixtures

QC for all analytes

No need for repeated analysis

Exception

Residues beyond calibration range – repeated analysis dilution of the samples

Values at MRL level and/or poor recovery – repeated analysis with SA approach as method of choice

GC-MS/MS

Matrix-Matched Calibration (grapes blank matrix for FV or cereals)

in case of MRL exceedance – confirmation via SA approach and/or different ionisation mode (NCI, APGC)

LC-MS/MS ESI pos. mode

solvent Calibration – dilution in water (1:11) diminishes matrix effects
Confirmation via SA approach

LC-MS/MS ESI neg. mode

single point matrix calibration at LOQ level (semiquantitative screening)

Reason:

Compounds require Matrix-matched calibration – no need for full calibration curve as residues are quite seldom

Once residues observed – SA approach

(in 2014: > 30x Dithianon, 4x 2,4-D; 1xDichlorprop, 1x MCPA,

II. Fatty matrices (plant oils, oil seed)

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☞ method requirements

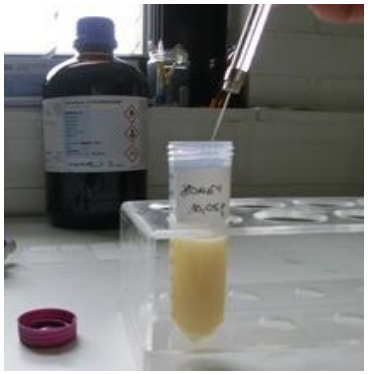
MATRIX-MATCHED CALIBRATION

Spiking of blank matrix
after extraction
= recovery

VS.

PROCEDURAL MATRIX CALIBRATION

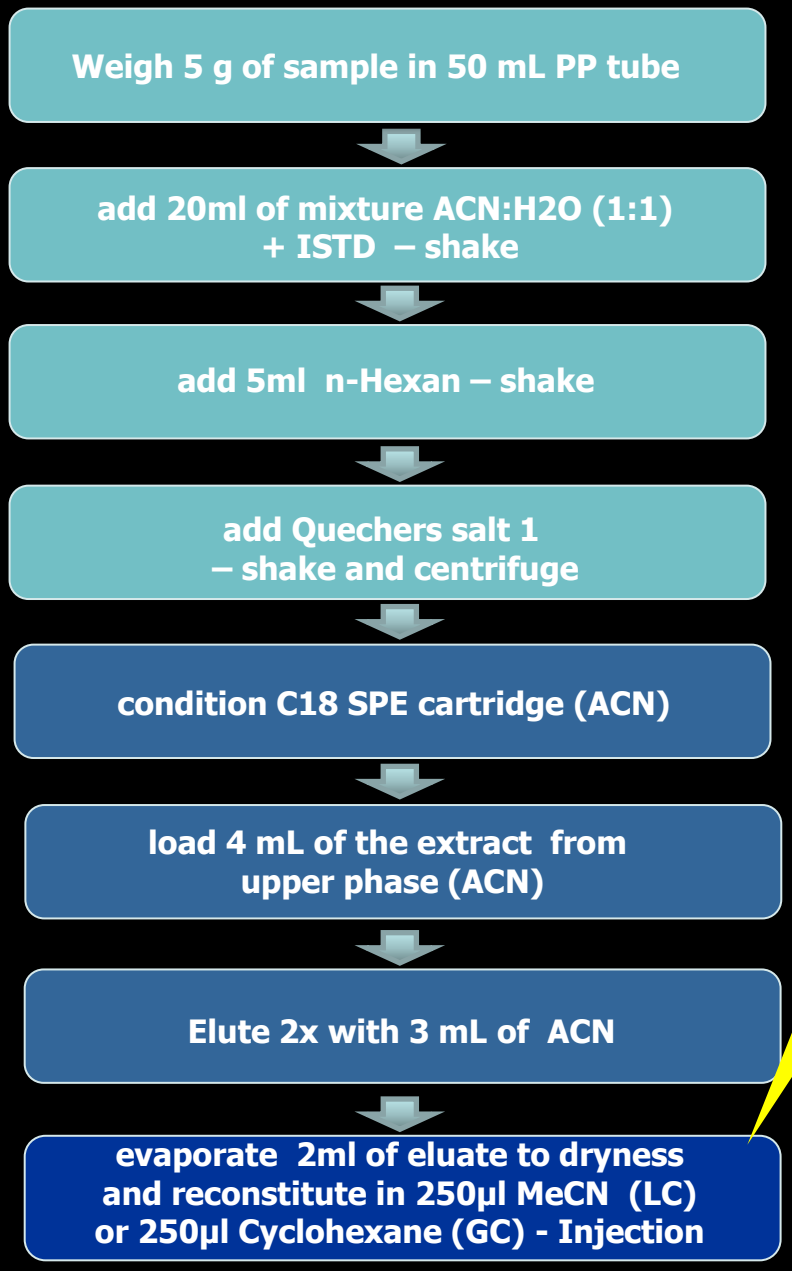
Spiking of blank matrix
before extraction
= automatically
recovery corrected
„apparent recovery“



- **Practical reasons (concentrating of the final extract)**
- **Compensates for matrix effects of variable magnitude**

Method QuEChERs Combi
QuEChERs + EURL FV (2012-M6*)

EURL-FV (2012-M6) | Validation Data of 127 Pesticides Using a Multiresidue Method by LC-MS/MS and GC-MS/MS in Olive Oil



Procedural Standard Calibration

Spiking of blank matrix
or sample
at RL (0,01 mg/kg)
before extraction

= automatically
recovery corrected

If positive findings – **SA approach !!!**

III. Low frequency matrices, Import controls (Reg 669/2009)

☞ no blank matrix, time pressure

No blank material and no validation data available

Import Controls – finished within same working day (08:00 – 16:00)

Procedural calibration (full recovery correction):

Spiking of sample at 10-30 ppb (preferably LOQ-Level) – qualitative screening at least one calibration point to check for recovery

In case of positive findings – **SA approach**

Modif. Quechers with LLE*

Weigh 2,5 g of sample in 50 mL PP tube

Add 20 mL of mixture MeCN/water (1:1)
Shake in automatic axial extractor for 10min

add QuEChERs salt 1
and shake 1min by hand

Centrifuge for 5 min at 4000 rpm

clean-up with Quechers salt2
[MgSO4/C18/PSA (100/50/75 mg/mL)]

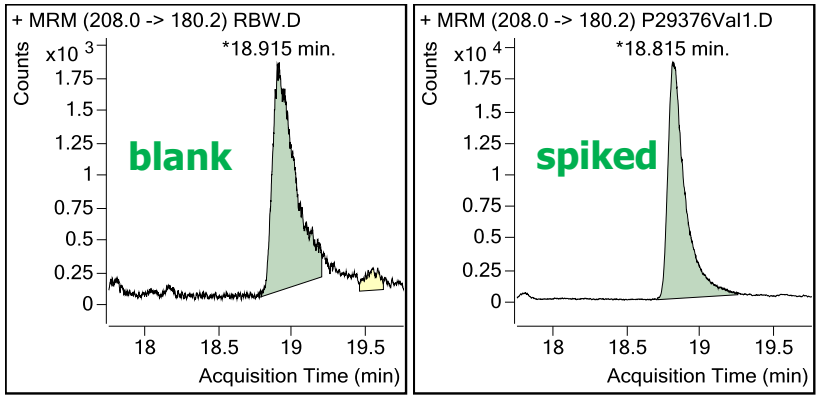
Centrifuge for 5 min at 4000 rpm

LLE (liquid-liquid-extraction) with n-Hexane

Injection of 12.5µl (MMI, solvent vent mode)
into GC-MS/MS
MSHP5msUi 30mx0,25mmx0,25µm

Antraquinone in green tea

Validation in tea matrix @ Spiking level 0,011mg/kg
Mean Recovery 80%



Sensitivity quite O.K.

hardly any tea matrix without traces of Anthraquinone available (and matrix effect differs a lot)

Quantification via SA approach !!

* Cajka T. et al., Analytica Chimica Acta Volume 743, 19 September 2012, Pages 51–60

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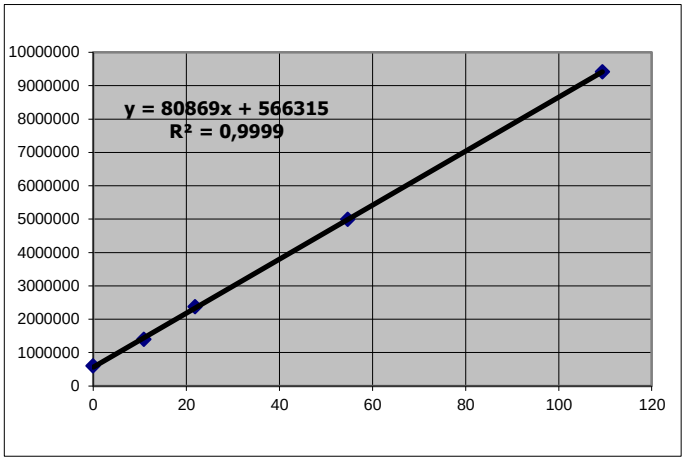
MMI: Solvent vent mode

Antraquinone in green tea

SA approach



7.00 ng/ml
Result 0.028 mg/kg

* Cajka T. et al., Analytica Chimica Acta Volume 743, 19 September 2012, Pages 51–60

IV. Baby and infant food/formulae

☞ residues not expected

Spiking of sample @ default MRL of 0,01 mg/kg resp. Specific MRLs (Dir 141/2006)

Annex 8
PESTICIDES WHICH SHALL NOT BE USED IN AGRICULTURAL PRODUCTION INTENDED FOR THE PRODUCTION OF INFANT FORMULAE AND FOLLOW ON FORMULAE
Table 1 and 2

Annex 9
SPECIFIC MAXIMUM RESIDUE LEVELS OF PESTICIDES OR METABOLITES OF PESTICIDES IN INFANT FORMULAE AND FOLLOW-ON FORMULAE

Number of lowered MRLs – different spiking solution for this type of matrix (not analysed within the same batch of conventional/organic products)

Standard addition approach

Useful approach for confirmation (MRL violation) or if blank matrix is not available

Spiking of sample vs. extract

SANCO/12571/2013

C25 "increasing amounts of the standard analyte are added to the other test portions immediately **prior to extraction**"

C26 Addition of at least two known quantities of analyte to aliquots of the sample extract, e.g. prior to injection, is another form of standard addition, but in this case adjustment is only for possible injection errors and matrix effects, but not for recovery losses.

SA prior vs. after extraction

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Proficiency test in apple púree (2013)

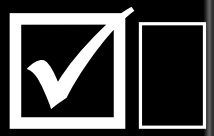
Analyte	Spiking of sample (mg/kg)	Spiking of extract (mg/kg)
Fenhexamide	0,0352	0,0349
Iprodione	0,0103	0,0109
Pyraclostrobin	0,0118	0,0129

As long as extraction efficiency not compromised (e.g. pH-dependency) and mean recovery over a wide range of matrices acceptable both approaches deliver similar results

Summary

FV samples – quite frequent findings

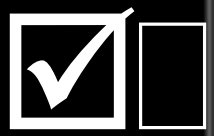
conventional approach more useful (multiple-level calibration, recovery determination, check for drift)



For rare matrices or urgent cases:

First of all screening of samples – (PMC in same type of matrix or 1-point calibration) - aim is to assure LOQ level (not detected)

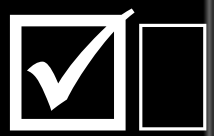
(Prerequisite – parameters within validated scope; extraction conditions known)



Practical approach using procedural matrix calibration (PM-Cal) in same type of matrix – very effective way of "targeted screening" (at LOQ)

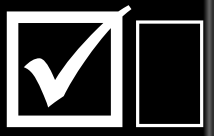
in products with low frequency of detection

(organic products, baby formulae, food of animal origin)



All cases of MRL exceedances or health concerns

Confirmation of critical results via SA approach and/or different ionisation techniques (GC-MSD NCI, APGC)



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... THANK YOU

for your attention ...