Approach towards reliable analyses

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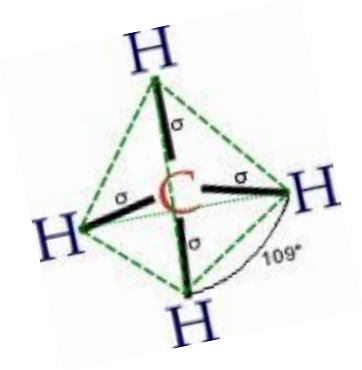
There are several approaches …..

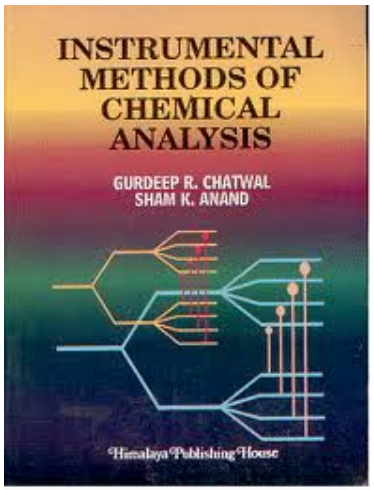
… such as crystal ball questioning

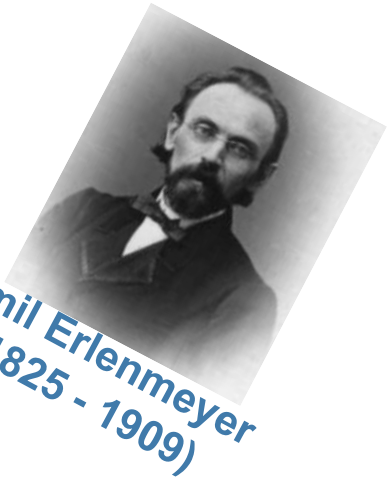
…

… such as reading coffee grounds ….

… for reliable analysis … also scientific ones:







Le Chatelier's principle of chemical equilibrium:

$$H_2O_{(l)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + OH^-_{(aq)}$$

Taking a closer look.

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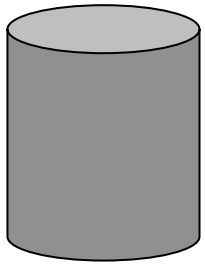
2. Sample Homogenisation

4. Instrumental Analysis



1. Sample entrance

3. Sample extraction and clean-up

Getting the whole context

Costs / Prices

Time limitations

Complexity of samples

Constraints

versus

Competent Analysts

Modern equipment

Accuracy

Investments

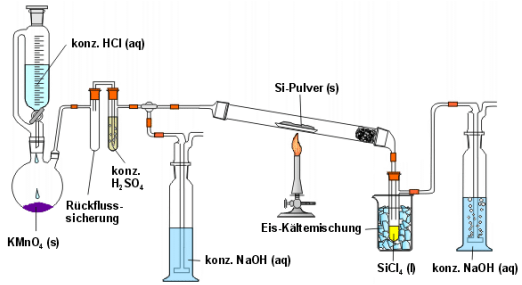
Characteristics of organic samples: Residues at low concentration levels

Requires

- *Correct identification of residues (no false negative findings)*
- *Correct quantification of residues*
- *No false positive findings of residues*

Challenges in analysing organic samples

Appropriate analytical methods established?



- ➔ Multi-Methods (e.g. QuEChERS)
- ➔ Group-specific Methods (e.g. Dithiocarbamates)
- ➔ Single-Residue Methods (e.g. Ethephon,Glyphosate)
- ➔ *More? Depends on the types of matrices analysed.*

Limit of Quantification

BNN guideline value (practical “0”-level: 0,01 mg/kg)

➔ *Possible for all matrix/analyte combinations offered?*

or

??

Laboratory performances

Results of competence tests / ringtests

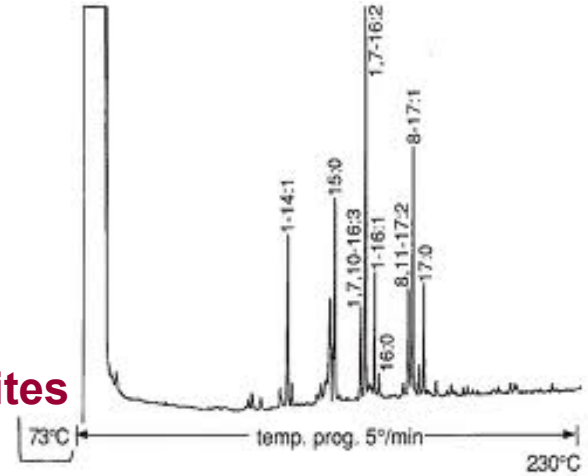
➔ *Design of tests appropriate for the special organic question (low concentration levels)?*

Challenges in analysing organic samples

Qualified operators

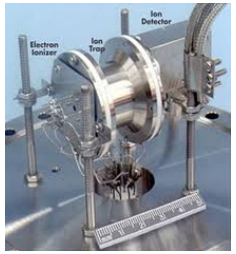
- Is the peak a pesticide peak?
  Or is it just a peak caused by matrix interferences?
- Shift in retention time – the peak comes earlier
  or later than expected!

→ **Experience and accuracy are crucial pre-requisites for reliable analysis**



Appropriate Laboratory equipment

Today: GC-MS/**MS** and LC/MS/MS



Competent evaluation of results

Experiences with individual matrix characteristics:
Case by case evaluation of results, as…..

Conclusions

Pesticides	Yes	No
No	(**NO** Application of pesticides)	Application of pesticides (already degraded → no residues)
Yes	Contaminations (not to be avoided)	Conventional products (396/2005)

Taking a closer look.

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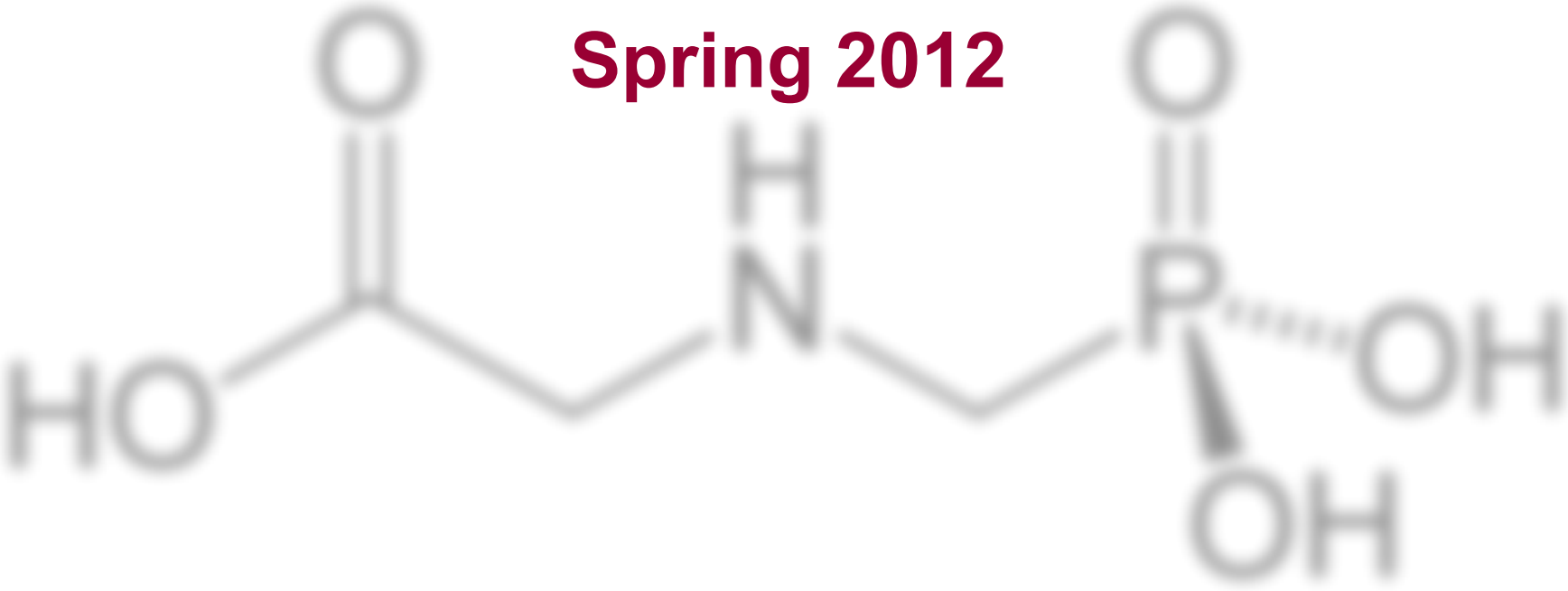
Glyphosate Method Ring Test in Lentils and Soybeans

Spring 2012



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VBP/ Bionext

But first:

A small lesson in *reading* and *understanding*

Ring Test results

Assessment of of Ring Tests

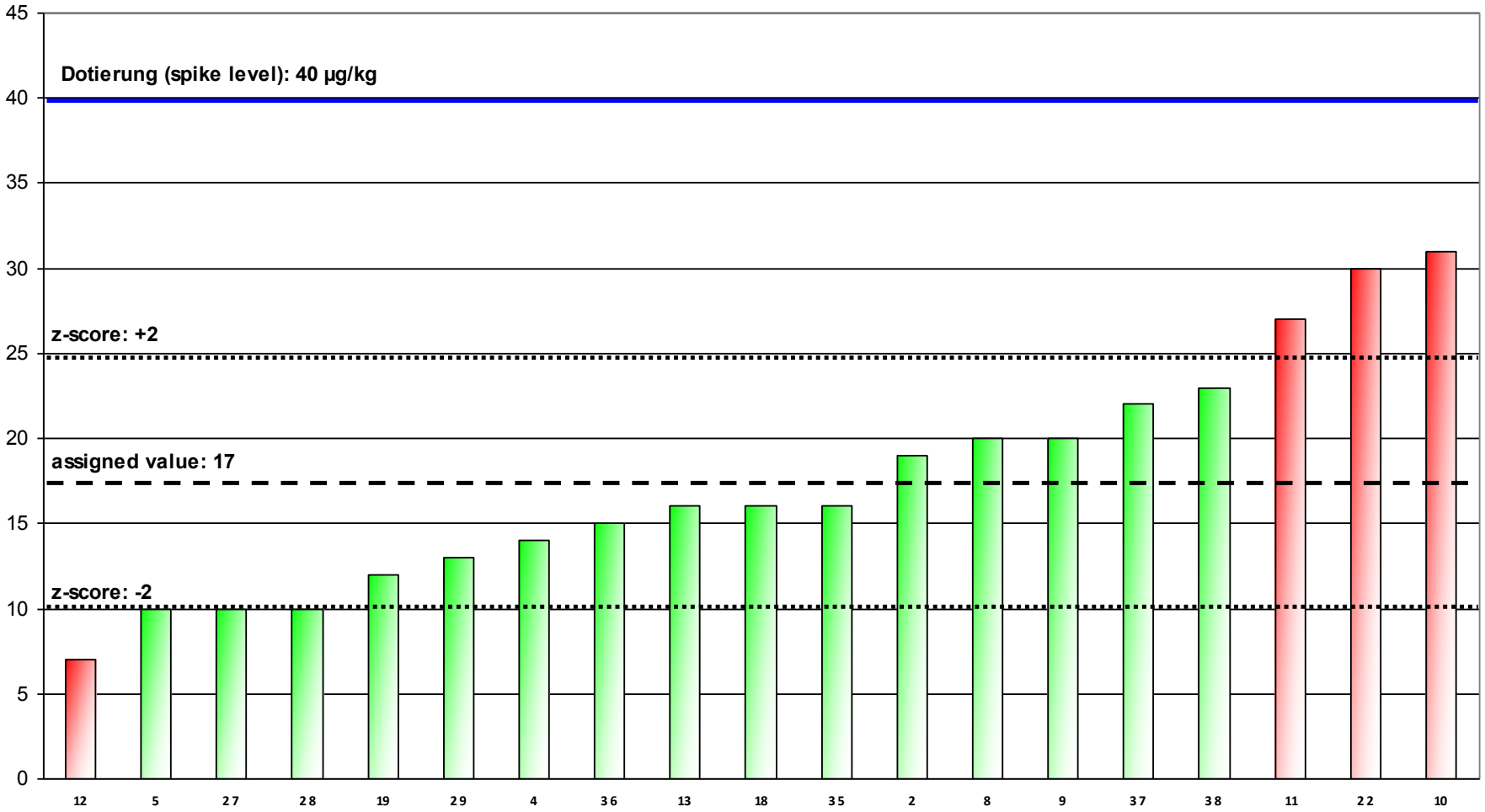
The evaluation of most of the Ring Test providers is based on the **comparison of the single participant's result with the average of all participants** ('assigned value' / z-score model):

Average!

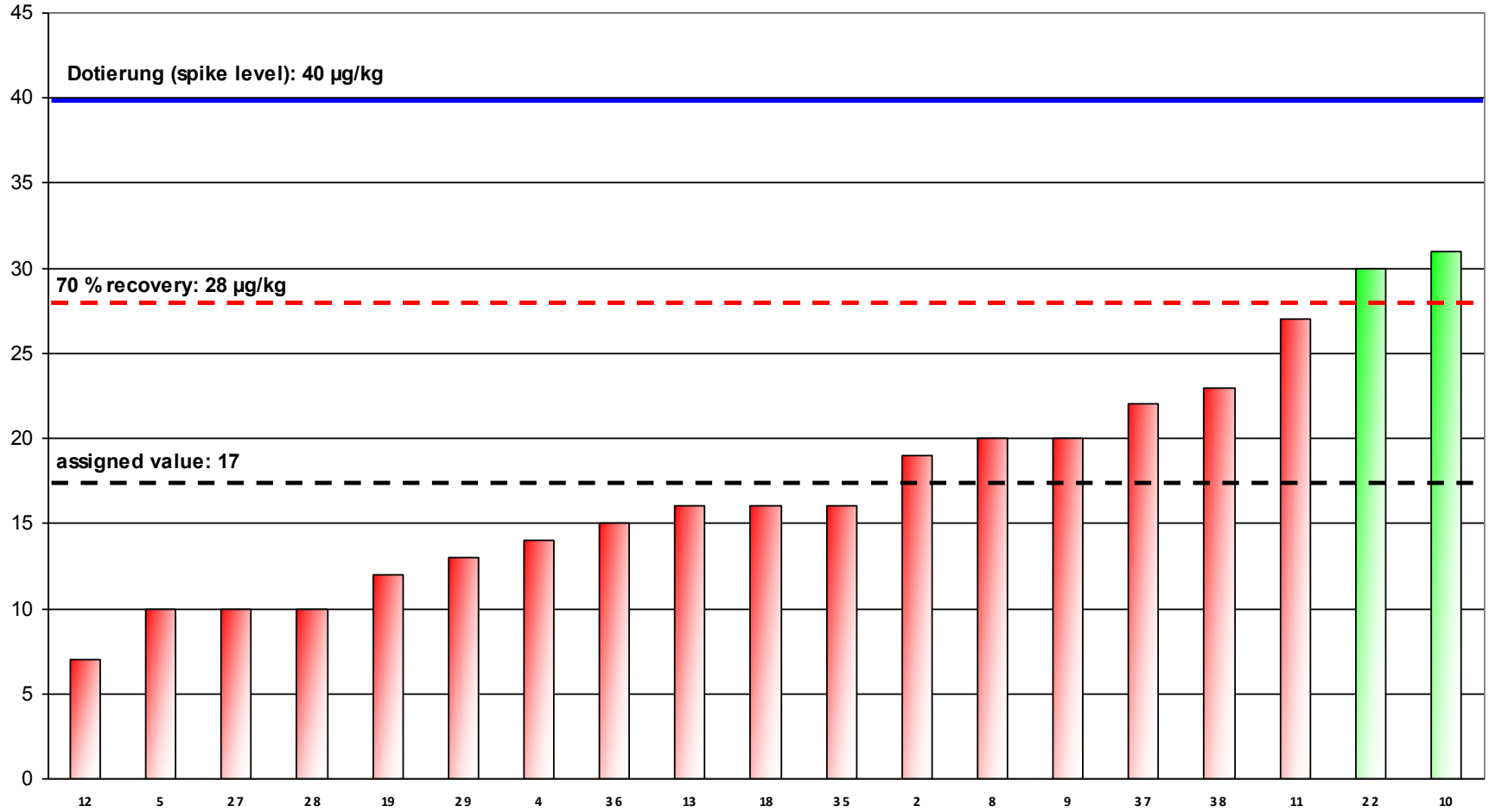
The closer the participant's result to the laboratory average ('assigned value') the better its performance is considered.

Average!

Results of Pymetrozin (Samples A)



Results of Pymetrozin (Samples A)



Design of the Glyphosate Test Material (1 of 2)

Test Material	Spiked level
1) Lentils* low *level	
Glyphosate	25 µg/kg
2) Lentils* incurred *residues	
Glyphosate	2,03 mg/kg (average of participants)
3) Soybeans* low *level	
Glyphosate	55 µg/kg
4) Soybeans* high *level	
Glyphosate	2,25 mg/kg

Design of the Glyphosate Test Material (2 of 2)

- Homogenised Test Materials with spiked and incurred Glyphosate residues

- Two different matrices at two different concentration levels
  ➔ thus 4 different Test Materials were provided:

two Lentil products with Glyphosate
spiked Test Material at a lower concentration level (ppb),
incurred Test Material at a higher concentration level (ppm);

two Soybean products with Glyphosate
spiked Test Material at a lower concentration level (ppb),
spiked Test Material at a higher concentration level (ppm).

Assessment of recoveries of spiked levels (Trueness criteria) 1 of 2

Test Material	spiked level	number of satisfactory results (70-120% of the spiked level)	total number of participants	satisfactory (%)
Lentils* low *level				
Glyphosate	25 µg/kg	11	15	**73**
Soybeans* low *level				
Glyphosate	55 µg/kg	10	15	**67**
Soybeans* high *level				
Glyphosate	2,25 mg/kg	10	15	**67**

Assessment of recoveries of spiked levels (Trueness criteria) 2 of 2

- The most challenging Test Material:
  - Soybean Material at low and at high Glyphosate concentration levels.
  - The different concentration levels (high and low) do not have a significant impact on the analytical performances.

- Results outside 70-120% recovery of the spiked level:
  - tendency towards higher results

All in all, there is not a big difference in the analytical performance between Soybeans and lentils when assessing the results according to the trueness criterion.

≈

Assessment of z-scores (Comparability criteria) 1 of 2

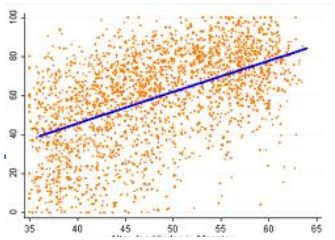
Test Material	Assigned value	Spiked level	No. of satisfactory results: -2 < |z| > +2	total No. of participants	Satis-factory (%)
Lentils* low *level					
Glyphosate	26 µg/kg	25 µg/kg	12	15	**80**
Lentils* incurred *residues					
Glyphosate	2,03 mg/kg	---	12	15	**80**
Soybeans* low *level					
Glyphosate	56 µg/kg	55 µg/kg	12	15	**80**
Soybeans* high *level					
Glyphosate	2,49 mg/kg	2,25 mg/kg	15	15	**100**

Assessment of z-scores (Comparability criteria) 2 of 2

- The score of satisfying results is in general higher
- The best results of comparability show **soybeans at high concentration level** (100% showed satisfying results).

- Comparing the assigned values with the spiked levels: **high compliance with assigned and spiked value at Test Materials (Soybeans and Lentils) at low concentration levels of Glyphosate**.
- **Soybean Test Material** at high concentration level shows a **slight tendency towards higher levels** (assigned value) when comparing with the spiked level.

- All 4 Test Materials show **higher deviations** than statistically expected. As a conclusion, the results **vary more** than expected.



Overall results of the Glyphosate Method Ring Test

Criteria	number of satisfactory participants	total number of participants	**satisfactory (%)**
Correctly identified AND reported satisfactory results for Glyphosate **related to the trueness criterion** (recovery of the spiked level) in 3 spiked Test Materials	5	15	**33**
Correctly identified AND reported satisfactory results for Glyphosate **related to the comparability criterion** (z-score) in all 4 Test Materials (spiked and incurred)	8	15	**53**

"Quaternary Ammonium Compounds" (QAC)

in vegetable (banana, basil)

and animal products (milk, quark, meat)

August 2012

Assessment of recoveries of spiked levels (Trueness criteria) 1 of 2

Test Material	spiked level [mg/kg]	No. of satisfactory results (70-120% of the spiked level)	total number of participants	satisfactory (%)
Banana purée				
DDAC (C-10)	0,15	8	10	**80**
Basil purée				
DDAC (C-10)	2,3	8	10	**80**
BAC (C-12)	0,15	9	10	**90**
Milk (liquid)				
DDAC (C-10)	0,03	8	10	**80**
BAC (C-12)	0,22	9	10	**90**
BAC (C-14)	0,11	10	10	**100**
BAC (C-16)	0,05	9	10	**90**
Quark purée				
BAC (C-12)	Incurred residues --- Incurred residues --- Incurred residues			
BAC (C-14)	Incurred residues --- Incurred residues --- Incurred residues			
Meat purée				
BAC (C-12)	Incurred residues --- Incurred residues --- Incurred residues			
BAC (C-14)	Incurred residues --- Incurred residues --- Incurred residues			
BAC (C-16)	Incurred residues --- Incurred residues --- Incurred residues			

Assessment of recoveries of spiked levels (Trueness criteria) 2 of 2

- The overall performance is **quite satisfying**.
  In general 80-100% of the participating laboratories showed results between 70 and 120% recovery of the spiked levels.

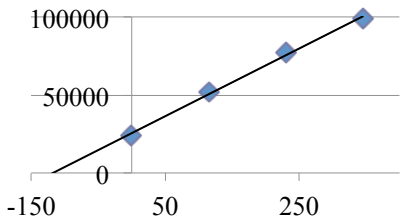
- The most challenging QAC is **the DDAC (C-10) at low (banana and milk) and high concentration levels (basil)**. 20% of the participants showed dissatisfying results (which still is a satisfying result).

Assessment of z-scores (Comparability criteria) 1 of 2

Test Material	Assigned value (robust mean)	Spiked level	No. of satisfactory results: z-score ≤ |±z|	total No. of participants	satisfactory (%)
Banana purée					
DDAC (C-10)	130 µg/kg	150 µg/kg	10	***10***	**100**
Basil purée					
DDAC (C-10)	1,81 mg/kg	2,3 mg/kg	10	**10**	**100**
BAC (C-12)	130 µg/kg	150 µg/kg	10	**10**	**100**
Milk (liquid)					
DDAC (C-10)	30 µg/kg	30 µg/kg	9	**10**	**90**
BAC (C-12)	219 µg/kg	220 µg/kg	10	**10**	**100**
BAC (C-14)	104 µg/kg	110 µg/kg	10	**10**	**100**
BAC (C-16)	51 µg/kg	50 µg/kg	9	**10**	**90**
Quark purée					
BAC (C-12)	39 µg/kg	Incurred	9	**9**	**100**
BAC (C-14)	27 µg/kg	Incurred	9	**9**	**100**
Meat purée					
BAC (C-12)	700 µg/kg	Incurred	9	**9**	**100**
BAC (C-14)	227 µg/kg	Incurred	9	**9**	**100**
BAC (C-16)	18 µg/kg	Incurred	7	**9**	**78**

Assessment of z-scores (Comparability criteria) 2 of 2

- The results are in general **very satisfying**.
- **9 out of 11 QAV/matrix combinations show 100% satisfying results.**
- The "worst" results are related to **BAC C-16 in Meat Test Material** (78% satisfying results).
- All 5 Test Materials show **lower deviations** than statistically expected. As a conclusion, the results **vary LESS than expected**.



QAC - Method Ring Test

Participants:

➔ The six relana® members

➔ 5 state laboratories of Germany

relana® ???

reliable analyses

relana®- Quality criteria (selection)

- **Annual lab audit** with detailed expert audit report including appropriate recommendations for necessary measures.
- Special focus is on the activities and the scope of **validations** in the employed test procedures.
- Check of the routine lab performance by repeated submissions of **undercover samples** (in 2012: cherry tomatoes and table grapes).
- Participation in **relana® method tests** for progress in the development of analytical possibilities. ➔ e.g. **Glyphosate, QAC**
- Check of quality in **sample preparation** and **homogenisation** by means of real samples.
- Exchange and discussion of analytical questions and information provided by relana® as a **platform and network** for the participants.

relana®- Requirements of quantification

- Matrix-matched calibration is essential
- Multi-level calibration (minimum: 3 levels)
- LC-MS/MS: nearly always suppression effects caused by matrix
- LC-MS/MS: the reference for calibration has to be the same matrix like the sample
- GC-MS/MS: Suppression AND amplification of signals are possible
- Confirmation analysis: Always by standard addition

Ringtests vs Routine Samples

Routine Samples

- unannounced arrival

- short turn-around times (48 hours or even less)

- the kind and level of pesticide residues are not known

Ringtests vs Routine Samples

Routine Samples

- blank material is not provided

- the analysis is performed on a routine basis (no “special” care)

- the particular result is regarded in isolation (no statistics are applied)

New approach: relana® tests with undercover samples

Design of relana® undercover samples

- shorter turn-around-times (“urgent” thus 24-48 hours)

- the scope of possible pesticides is undefined

- sample material may be „unprocessed“, like e.g. table grapes, cherry tomatoes etc. ➔ unsuspicious for the lab

- Consideration of “assigned value” OR recovery of spiking level

- spiked pesticides and their levels are chosen according to their findings in reality

New approach: relana® tests with undercover samples

Design of the performance assessment

- One crucial aim of the new approach is the consideration of the clients' perspectives:
- What is in the sample?
- How reliable are the results?
- Is the interpretation of the results correct (MRL, toxicological evaluation, Organic guidelines,…)?
- Fast turn-around-times.

relana®

relana® represents a **quality circle of laboratories** in the field of **residue and contaminant analytics** for labs, which have a commitment for particularly **high standards** in relation to their services.

Living analytical competence:

"Not just during an audit or on paper - but day to day in routine."

For more information:
www.relana-online.com

We hope you feel like …

…and not like him !

Thank you