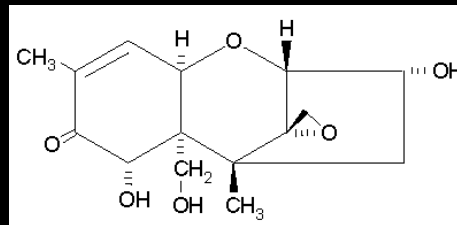




Quality and reliability of mycotoxin analysis



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Mycotoxins analysis



Variability and complexity of matrices

➡ various protocols of analysis

Trace analysis : small contents (ppb = $\mu\text{g}/\text{kg}$; ppt = ng/kg)

➡ Efforts on limits of detection and/or quantification

Particular physico-chemical Properties

. low molecular weight

(ex. PAT : 154 g/mol – OTA : 403 g/mol)

. various and limited spectroscopic properties

. stability

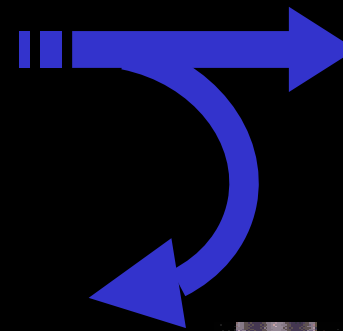
General principle of mycotoxins analysis



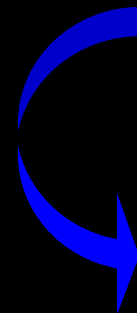
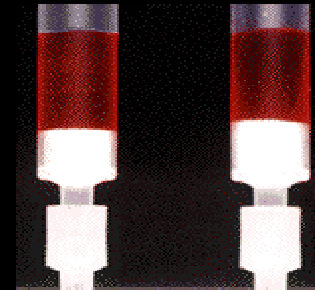
SAMPLE PREPARATION
mixing/grinding/straining



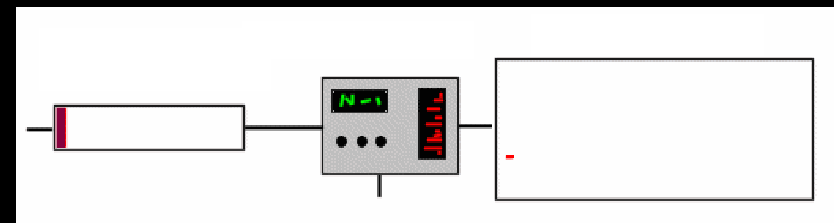
EXTRACTION



**EXTRACTION
PURIFICATION
CONCENTRATION**



**ANALYSIS : SEPARATION &
DETECTION**



SCREENING-DETECTION
(qualitative: ELISA, TLC)

DETERMINATION

(quantitative: TLC, LC, GC)

CONFIRMATION

(identification: GC/LC-MS)



QUALITY & RELIABILITY

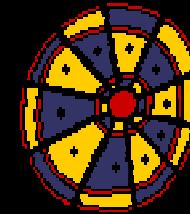
Why is it essential to have reliable analytical results ?

A result :

accurate

reproducible

→→ *Comparable*



- Equal or as close as possible to the true value
- Almost same results for regulatory or any official purposes or in case of dispute
- Comparability of results coming from a laboratory network

What is the origin of the uncertainty of a result ?

- **Sample:**
 - representativity
 - homogeneity
 - preparation (matrix interferences)
- **Laboratory :**
 - environment
 - equipment
 - training level of the analyst
 - method of analysis
- **Uncertainty of the measure**
- **Systematic variability**

RELIABILITY and QUALITY

- **sampling**
- **quality assurance for the analysis of mycotoxins**
- **validation and standardisation of analytical methods**
- **result expression**

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Importance of the sampling for mycotoxin analysis 1/2



A large sampling plan is necessary to minimisation of the consumer risk

RELIABILITY and QUALITY

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Importance of the laboratory quality assurance

International Standard ISO 17025

« General Requirements for the Competence of Testing and Calibration Laboratories »

recognized as the basic document for accreditation of laboratories including chemical analysis

The 5 keys of lab accreditation :

- Instrumentation
- Materials, products and reagents
- Methods
- Laboratory facilities
- Laboratory staff

Quality assurance

- ☒ Use of **validated methods or standards**
- ☒ Use of **internal control figures (CRMs)**
- ☒ Satisfactory results in **proficiency tests**
- ☒ **Accreditation as a recognition**

What type of method can be used ?

Reference : recommended by a reference laboratory

Official : recognised by an official body and/or organism

Standardised : usually validated by an interlaboratory exercise, written in a standardised format and adopted by a standardization body (AOAC, IUPAC, CEN...)

Validated : intra-laboratory or inter-laboratories

Published : characterised, and/or peer reviewed

An analytical method should be well characterised and validated in house, but also through an interlaboratory trial

Analyte standards & Reference materials : essential tools for the analysis of mycotoxin

The use of stds & RM provides the most accurate means for assessing the reliability and reproducibility of the data obtained

① Standards of mycotoxins

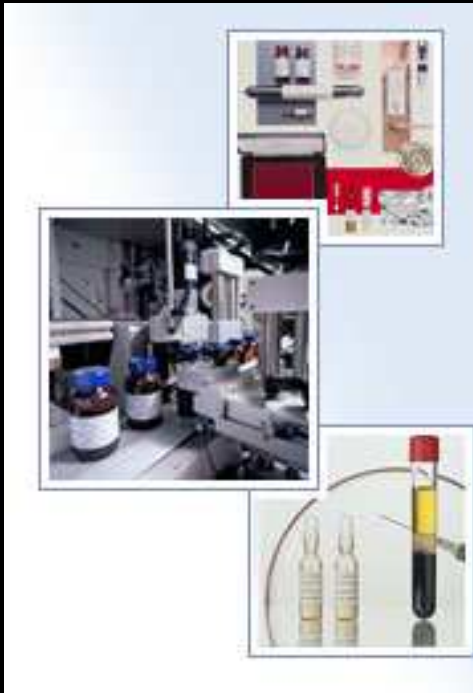


- Commercial availability for the major toxins
- 98 % purity in freeze dried film or powder, or in organic aqueous solution
- Necessity of checking both concentration (quantity) and purity (quality) of std stock solutions by spectrophotometry

- Establishment of calibration curve,
- check linearity of the method

② Certified Reference Materials

Institute for Reference Materials and Measurements, Brussels (EC)



CRM are materials containing a certified mycotoxin content.

Used as external criteria for quality assurance

CRM are key tools to show traceability in analytical work in comparing the measured value to the certified value

>>> trueness

Manufacturing of CRMs is a long & expensive process >>> very limited choice of matrix-mycotoxin combination

Substance	BCR-262 Defatted peanut meal (blank)	BCR-264 Defatted peanut meal (high level)	BCR-375 Compound feed (very low level blank)	BCR-376 Compound feed (low level)
Aflatoxin B1	Mass fraction (µg/kg) < 3	Mass fraction (µg/kg) 208 ± 13	Mass fraction (µg/kg) < 1	Mass fraction (µg/kg) 0.3 ± 0.5

Availability: Sachets sealed under vacuum containing about 50 g (BCR-375 and BCR-376), about 100 g (BCR-262) and about 150 g (BCR-284) of finely ground defatted peanut meal.

	Description	Aflatoxin M ₁ (µg/kg)
ERM-BD282	Whole milk powder (zero level)	< 0.02
ERM-BD283	Whole milk powder (low level)	± 0.018
ERM-BD284	Whole milk powder (high level)	± 0.06

Availability: The materials are provided in units of 30 g in amber glass bottles filled and sealed under nitrogen.

Substance	BCR-401 Peanut butter (low level)
Aflatoxin B1	Mass fraction (µg/kg) < 0.2
Aflatoxin B2	< 0.1
Aflatoxin G1	< 0.1
Aflatoxin G2	< 0.1
Total	< 0.5

Availability: The samples are supplied in units of about 50 g in metal cans sealed under nitrogen.

Substance	BCR-377 Maize Flour (very low level blank)	BCR-378 Maize Flour (medium level)	BCR-396 Wheat Flour (very low level blank)
DON	Mass fraction (mg/kg) < 0.05	Mass fraction (mg/kg) 0.43 ± 0.04	Mass fraction (mg/kg) < 0.05

Availability: Sachets sealed under vacuum containing about 150 g of sealed finely ground flour.

Substance	BCR-471 Wheat (blank)
Ochratoxin A	<u>Mass fraction</u> (µg/kg) < 0.6

Availability: Units of about 55 g in foil-laminate pouches sealed under vacuum.

Substance	ERM-BC716 Maize	ERM-BC717 Maize
ZON	<u>Mass fraction</u> (µg/kg) < 5	<u>Mass fraction</u> (µg/kg) 83 ± 9

Availability: ERM-BC716 and ERM-BC717 are supplied in sachets containing at least 60 g.

For more information about CRM :

IRMM *Institute for Reference Materials and Measurements*

<http://www.irmm.jrc.be/html/homepage.htm>

NIST *National Institute of Standard and Technology*

<http://www.nist.gov/>

Crucial need for having another method to obtain other RMs

>>> materials distributed in the course of **proficiency testing**

>>> **mycotoxin contents are ascertained (not certified)**

Proficiency testing : an interlaboratory exercise

1/2

Main purpose : study the performance of laboratories

It is a predictable tool for external quality assurance and a mean of monitoring quality control

Raw data from laboratories are analysed according to appropriate statistical tests

Lab data are checked for trueness and inter-laboratory reproducibility

Laboratories which do not pass the statistical tests should carry out corrective actions to improve their analytical competency (to keep their agreement)

Proficiency testing : an interlaboratory exercise

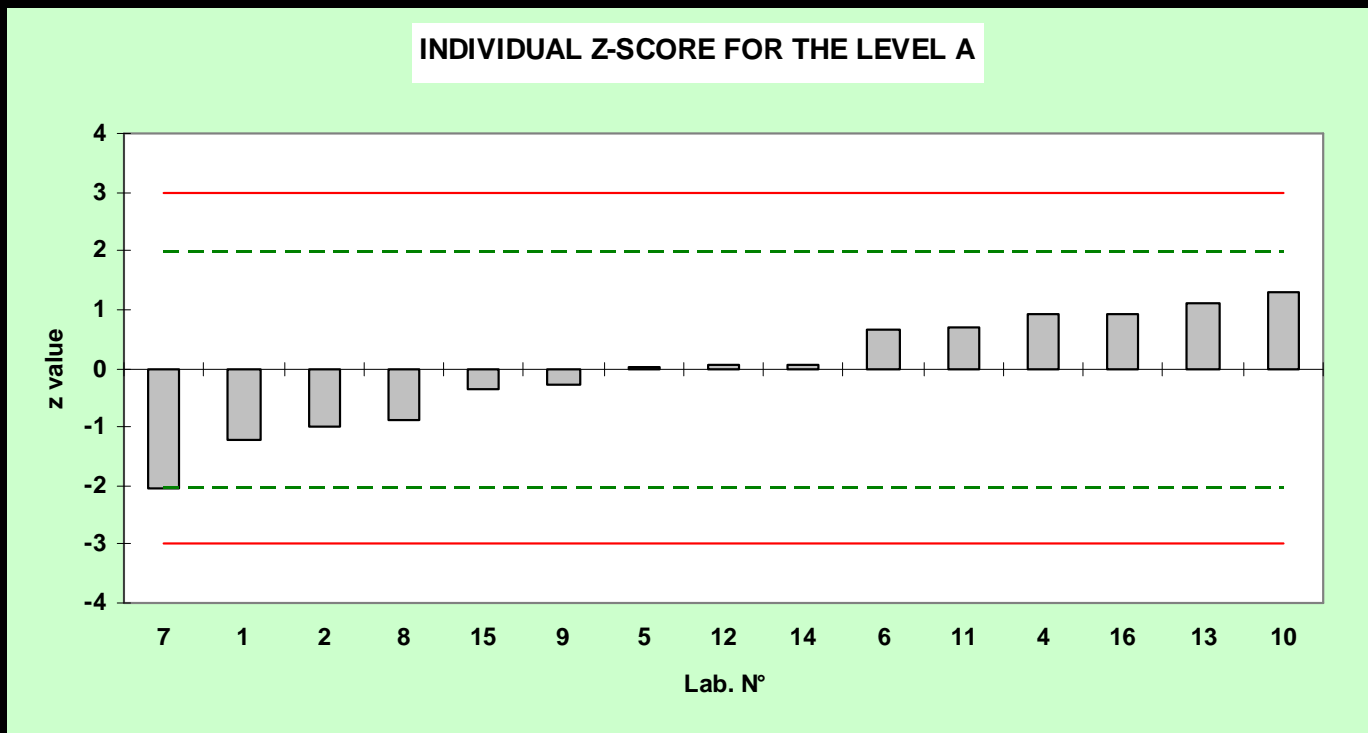
2/2

$$\text{z-score: } z = (m_{\text{lab}} - M_{\text{network}}) / SD_R$$

$|z| \leq 2$ \Rightarrow **satisfying**

$2 < |z| \leq 3$ \Rightarrow **doubtful**

$3 < |z|$ \Rightarrow **non satisfying**



Proficiency testing for mycotoxin analysis

Proficiency testings suppliers are :

- Reference laboratories (NRL or CRL)
- Specialized organisms :
 - BIPEA (France)
 - FAPAS (UK) « Food Analysis Performance Assessment Scheme »

<http://www.fapas.com/>

Aflatoxins B & G &/or total in various matrix types

Aflatoxin M1 (in milk or milk powder)

Patulin (in apple juice or puree)

OTA in various matrix types

Fusarium toxins (FB1 & FB2, DON, ZON) in various matrix types

RELIABILITY and QUALITY

- sampling
- quality assurance for the analysis of mycotoxins
- **validation and standardisation of analytical methods**
- result expression

Validation of methods

Why is it necessary ?

In order to prove that the method is acceptable for its intended purpose

In order to prove its performances

In general, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, detection limit, quantification limit, and robustness.

How a method can be validated ?

- Single-laboratory validation

HARMONIZED GUIDELINES FOR SINGLE LABORATORY VALIDATION OF METHODS OF ANALYSIS (IUPAC Technical Report)

Pure Appl. Chem., Vol. 74, No. 5, pp. 835-855, 2002.

- **linearity, LOD, LOQ : necessary to have standard solutions**
- **recovery rate : spiked samples**
- **intra lab trueness : CRM**
- **repeatability**
- **uncertainty measure**
- **« fitness-for-purpose »**

- Inter-laboratories validation

ISO 5725-1-1994: ACCURACY (trueness and precision) OF MEASUREMENT METHODS AND RESULTS

> precision (repeatability + reproducibility), inter lab trueness

↪ if possible, at least 12 participants

↪ several sample analysis: homogeneity should be proved (ANOVA)

↪ inter-lab analysis

↪ statistical data analysis

↪ if the performances are satisfying :

↪ validation document of the standardized method

↪ publication (eventually)

CRITERIA of ANALYTICAL METHODS of MYCOTOXINS

Recommended by CEN/TC 275 WG 5 Biotoxins (CR 13505 : 1999)

Recovery rates **50 - 120 % depending on the type of toxins and level**

For example : AFM1 from 10-50 ng/l - recovery : 60-120%

AFM1 > 50 ng/l - recovery : 70-110%

Reproducibility **< 20 - < 60 % depending on the type of toxins and level**

Repetability **< 15 - < 40 % depending on the type of toxins and level**

Limit of Quantification **the lowest validated level**

RELIABILITY and QUALITY

- sampling
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Which analytical result ?

Analytical protocol

RAW RESULT

Recovery Rate

CORRECTED RESULT

Uncertainty

RESULT and M U

Use of recovery rate

Working document IUPAC-ISO-AOAC

$$\text{Tx \%} = R_{\text{measured}} / R_{\text{theoretical}} \times 100$$

[Food analysis : Tx = 60-130 %

depending on matrix interferences and analytical procedure complexity]

>>> Various use according countries

- Disturb Comparison of results of analysis between labs from countries with different rules
- Which consequences by comparing corrected result to MLs?

Expression of uncertainty

U = uncertainty (CGMA/AFNOR: FD X 07-021)

U = 2 S_{Rintra} intra lab reproducibility

U = 2 S_{Rinter} inter labs validation

U = 2 S_{Rapt} proficiency testing

U = 2 S_{RHor} Horwitz value
(can be used for most of contaminants >ppb)

$$\mathbf{RSD_R \% = 2(1 - 0,5\log_{10}C)}$$

Uncertainty consequences

$$\text{RSD}_R \% = 2^{(1 - 0,5\log_{10}C)}$$

C > 1 g	variability = 1 à 2 %
C = ppm	variability ≈ 10-20 %
C = ppb ou ppt	variability ≈ 15-40 %

Should the « uncertainty » be taken into account for the interpretation of the results ?

Discussion initiated at *Codex Alimentarius*

Thank you !