EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

Mycotoxins
The Criteria Approach for Analysis

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Future Needs

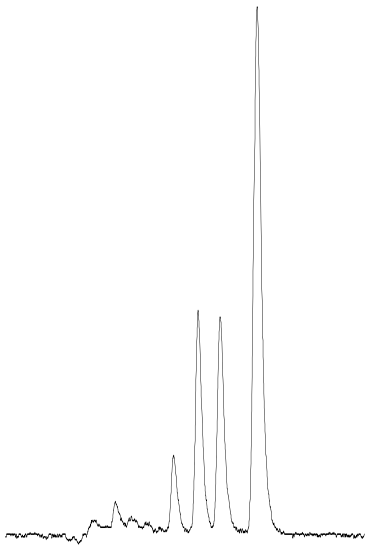
- **Number of test samples will increase**
  - Need for reliable “multi-methods”
  - Need for reliable simple + rapid methods
  - **Need for “workable” sampling schemes**

…that are all “Fit-for-purpose”.

Requirements on a Method

- User friendly (acceptance by lab technicians)
- Environmental safe (e.g. Montreal Protocol)
- As simple as possible (instrumentation)
- <u>Method performance must be known</u>

The Situation in Food Control



Result = f(sampling, sample preparation & analysis)

Background

- **Treaty establishing the European Community:**

  – Articles 94 and 95 (ex-100 and ex 100a):

    - Commission proposing directives for the approximation of such laws, regulations or administrative provisions of the Member States that **effect the establishment or function of the common market**

  AND

    - Considering **scientific facts, consumer health and safety** will be taken into account at a high level regarded for the harmonisation of the common market.

Relevant EU Directives and Regulations

- **Directive 85/591** (laying down the framework for sampling and analysis methods on community level)
- **Directive 89/397** (general principles for the official control of foodstuffs)
- **Directive 93/99** (supplement to 89/397/EEC)
- **Directive 98/53** (sampling and analysis methods)
- **Regulation 466/2001** (maximum levels for mycotoxins)
- **Regulation 882/2004** (on official controls to ensure compliance with food and feed law)

Legislative Approaches for Methodology

- **Explicitly specified analytical method**
  - e.g. Mercury, humidity or phosphate in animal feed (93/28/EG + 71/393/EEC)
  - e.g. Purity of food additives (81/712/EEC)
- **Quasi horizontal method specifications**
  - e.g. Thyreostatica in and hormone residues in milk (93/256/EEC)
- **Horizontal specifications ('Performance based')**
  - Generic specifications for methods intended for monitoring (**85/591/EEC**)
  - Certain contaminants (mycotoxins) in food (**98/53/EEC**)

Requirements - Directive 85/591

- **Specific** to the analyte
- **Accuracy** (Trueness & Precision)
- **Precision** (RSD_r and RSD_R from collabor. trial)
- **Limit of detection** and **quantification**
- **Sensitivity**
- **Applicability**

Requirements - Directive 85/591

- **Precision parameters must be obtained according to an internationally accepted protocol:**
  - ISO
  - CEN
  - AOAC
  - IUPAC

Directive 98/53

- **Applicability:**
  - **Sampling** and **analysis methods** intended for the '***official control***' of certain contaminants in foodstuffs (mycotoxins)

- **General requirements:**
  - Method must comply (whenever possible) with Council Directive 85/591/EEC

- **Specific requirements:**
  - When no specific method is prescribed at Community level, method must meet certain criteria:
  - max **RSD_r** and **RSD_R** and **recovery limits** for methods to be used.

Origin of Parameters

- **Repeatability and Reproducibility (RSD_r and RSD_R) are derived from the Horwitz equation:**
  - **Recommended value**: as given by the **Horwitz equation**
  - Maximum value: 2 x value given by the Horwitz equation
    - W Horwitz (1982) Anal Chem 54, 67A-76A

- Recovery limits **are in accordance to the limits given in the** CEN Report 13505 **about criteria of analytical methods for mycotoxins**

EU Legislation for Mycotoxins

- **Regulations** setting Legislative Limits
- **Directives** on Sampling and Methods

4.3. *Specific requirements*

Where no specific methods for the determination of patulin in foodstuffs are prescribed at Community level, laboratories may select any method provided the selected method meets the following criteria:

Performance characteristics for patulin

Level µg/kg	Patulin		
	RSD_r %	RSD_R %	Recovery %
< 20	≤ 30	≤ 40	50 to 120
20-50	≤ 20	≤ 30	70 to 105
> 50	≤ 15	≤ 25	75 to 105

The detection limits of the methods used are not stated as the precision values are given at the concentrations of interest.

The precision values are calculated from the Horwitz equation:

$$RSD_R = 2^{(1-0,5\log C)}$$

where:

— RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions $[(s_R/\bar{x}) \times 100]$.

— C is the concentration ratio (i.e. 1 = 100g/100g, 0,001 = 1,000 mg/kg)

This is a generalised precision equation, which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

EU Legislation for Mycotoxins

4. **Method of analysis to be used by the laboratory and laboratory control requirements**

4.1. *Definitions*

A number of the most commonly used definitions that the laboratory will be required to use are given below.

The most commonly quoted precision parameters are repeatability and reproducibility.

r = Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i.e. same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95 %) and hence $r = 2{,}8 \times s_r$.

s_r = Standard deviation, calculated from results generated under repeatability conditions.

RSD_r = Relative standard deviation, calculated from results generated under repeatability conditions $[(s_r/\bar{x}) \times 100]$, where $\bar{x}$ is the average of results over all laboratories and samples.

R = Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e. on identical material obtained by operators in different laboratories, using the standardised test method) may be expected to lie within a certain probability (typically 95 %); $R = 2{,}8 \times s_R$.

s_R = Standard deviation, calculated from results under reproducibility conditions.

RSD_R = Relative standard deviation calculated from results generated under reproducibility conditions $[(s_R/\bar{x}) \times 100]$.

CEN TC257/WG5

- **WG5 selects and elaborates methods of analysis for mycotoxins that become European Standards.**

- **These Standards can be used for monitoring according to legal limits.**

CEN Technical Report CR 13505

(Criteria of analytical methods of mycotoxins)

- **Definitions for performance parameters** (ISO 5725)

- **Performance characteristics values** RSD_r, RSD_R and recovery according to state-of-the-art knowledge and experince (publications from collaborative trial studies in the time from 1974 resp. 1985 – 1997)

  – Aflatoxins, OTA, Patulin, Fumonisins, DON, NIV, Zearalenone, HT-2 and T-2 toxin.

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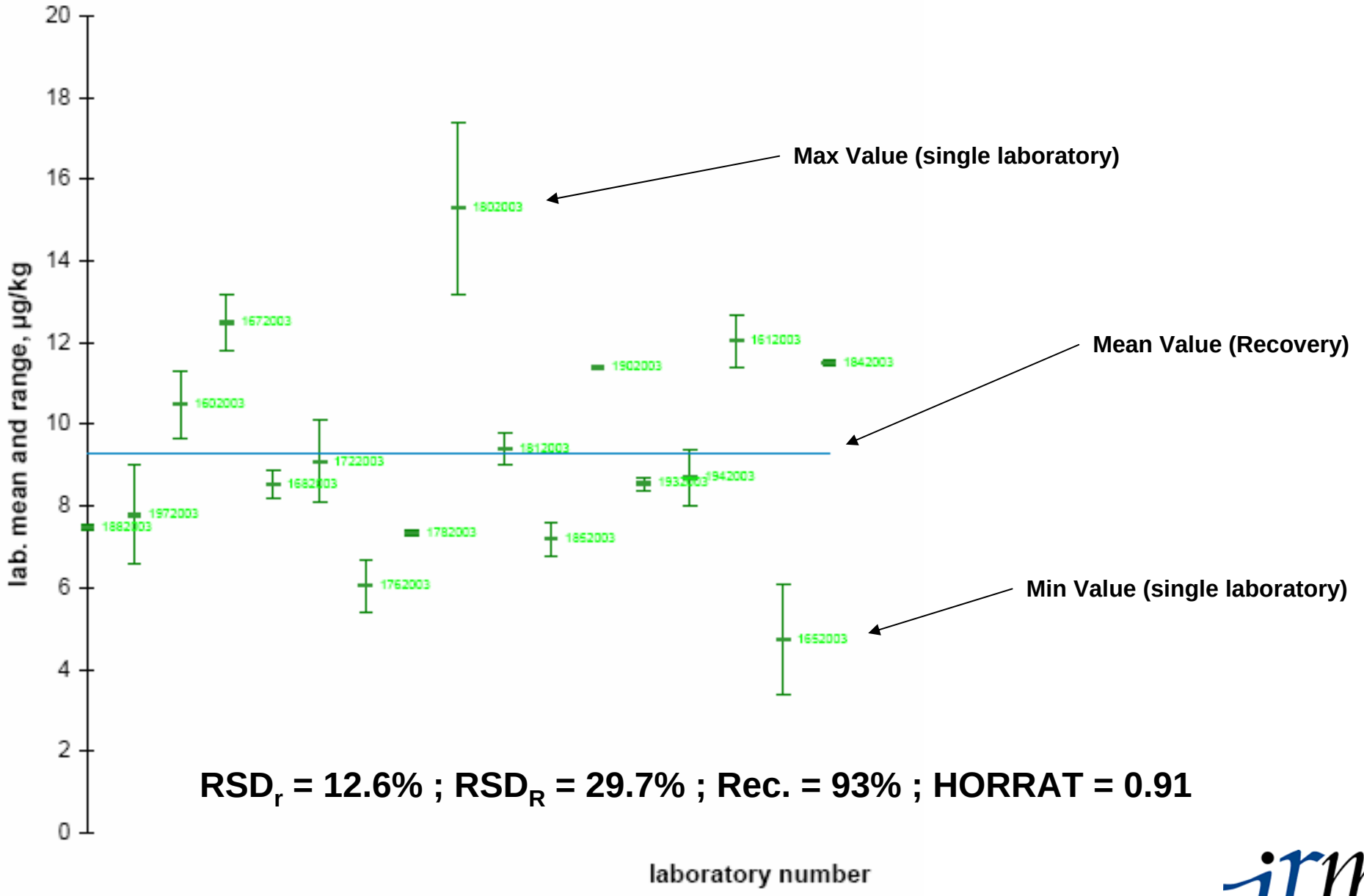
Data from a Collaborative Trial



RSD_r = 12.6% ; RSD_R = 29.7% ; Rec. = 93% ; HORRAT = 0.91

“Fit-for-purpose”?

- Analyte
- Matrix
- Concentration
- **Performance**

- Time and Materials
- Location and Availability!!!

"Fitness-for-purpose" in EU Legislation

- **Background: Limited number of fully validated methods.**
- **Solution: Uncertainty function to specify maximum levels of uncertainty.**

$$Uf = \sqrt{(LOD/2)^2 + (\alpha \times C)^2}$$

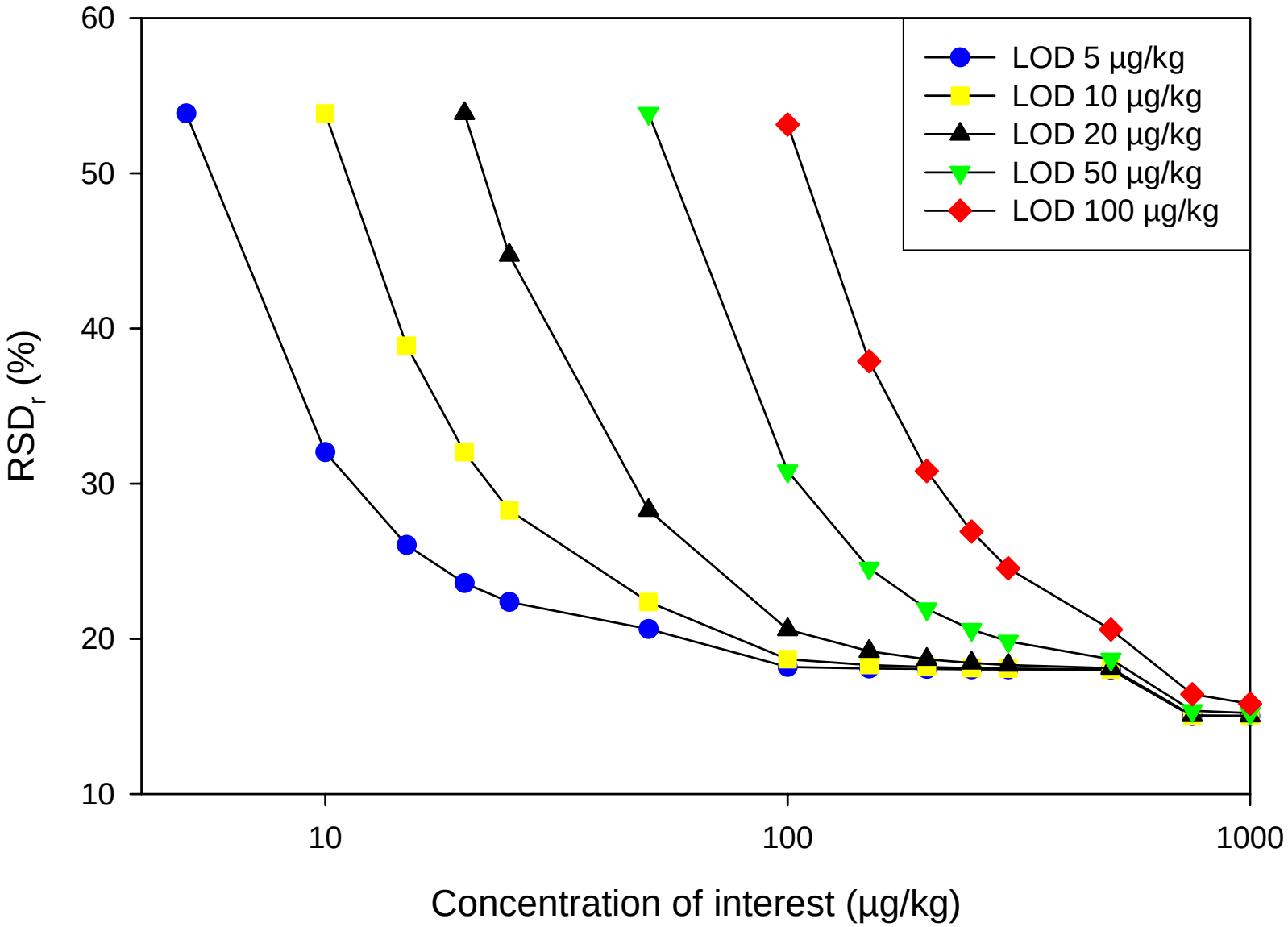
Uf = max. standard uncertainty (µg/kg)

LOD = Limit of Detection (µg/kg)

α = constant (0.1 – 0.2) depending on the concentration (C)

C = concentration of interest (µg/kg)

“Fitness-for-purpose” Graph



Thank you!