38th International Symposium on Halogenated Persistent Organic Pollutants (POPs)
Thermo User Meeting - DFS Magnetic Sector GC-HRMS

Krakow, August 26 – 31, 2018

Estimation of LOQ for the Analysis of Persistent Organic Pollutants, in particular PCDD/Fs and PCBs

Alexander Schaechtele[1], Johannes Haedrich[1], Roberta Ceci[2], Gianfranco Diletti[2], Gauthier Eppe[3], Alwyn Fernandes[4], Jerry Hart[5], Helge Hove[6], Leondios Leondiadis[7], Philippe Marchand[8], Wolfgang Moche[9], Georges Scholl[3], Giampiero Scortichini[10], Yves Tondeur[11], Stefan van Leeuwen[12], Irene Vasileiadou[7], Rainer Malisch[1]

1 European Union Reference Laboratory (EURL) for Dioxins and PCBs / Halogenated POPs in Feed and Food, Freiburg, Germany
2 Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy
3 Centre de Recherche Analytique et Technologique (CART) University of Liège, Liège, Belgium
4 FERA Science Ltd, York, UK
5 Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK
6 The National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway
7 National Center for Scientific Research (NCSR) "Demokritos", Athens, Greece
8 Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, France
9 Umweltbundesamt, Vienna, Austria
10 Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy
11 Independent expert, USA
12 RIKILT -Wageningen University and Research, Wageningen, NL

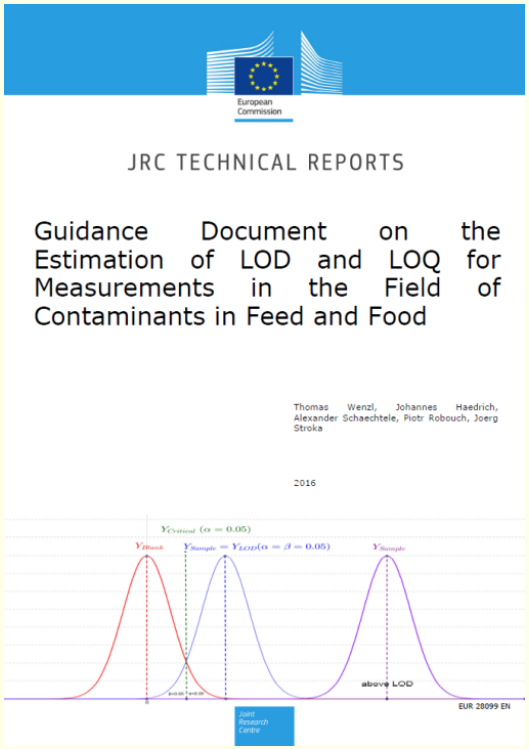
Guidance Document

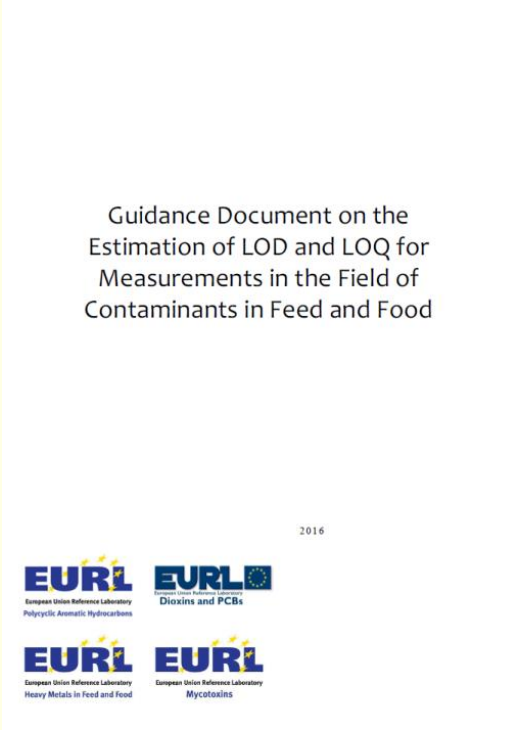
- **Guidance** on determination of **LOD/LOQ** of the analytical methods for the determination of individual substances in the field of **contaminants** in **feed** and **food** required by EU Commission

- Joint guidance document of **4 EURLs** working in the field of contaminants
  - EURL for PAHs
  - EURL for Heavy metals
  - EURL for Mycotoxins
  - **EURL for PCDD/Fs and PCBs (since 2018: EURL for halogenated POPs)**

- Coordination by EURLs at the Joint Research Centre (JRC)

Guidance Document





Available online:

European Commission > Food Safety > Food > Animal Feed

- https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_document_lod_en.pdf

European Commission > Food Safety > Food > Chemical safety > Contaminants > Sampling and Analysis

- https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-lod-loq.pdf

Concepts

Two different concepts for estimation of **LOD** and/or **LOQ** in official feed and food analysis corresponding to the different requirements

- **Heavy metals**, **PAHs** and **Mycotoxins**
  - Estimation procedure based on blank (matrix) samples,
  - Alternative: Calibration model using spiked blank (matrix) samples

- **POPs**, in particular **PCDD/Fs** and **PCBs** using isotope-dilution mass spectrometry
  - Signal-to-noise ratios (S/N)
  - Calibration experiments

  taking into account procedural blank samples

Concepts

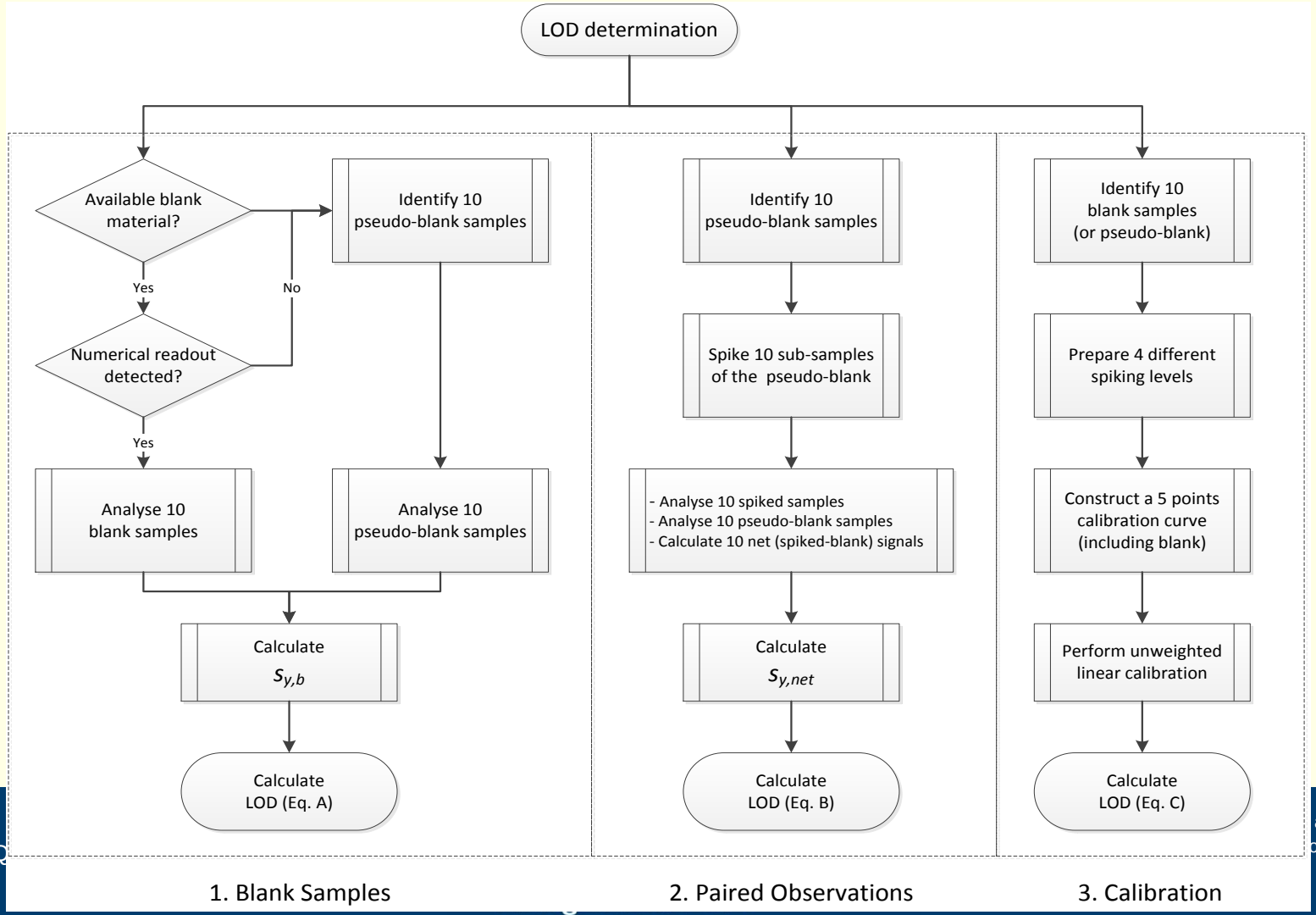
Approaches for the estimation of **LOD/LOQ** described for the different fields of application

	Signal-to-noise ratio	Blank (matrix) samples	Procedural blanks	Calibration (spiked blank samples)
Heavy metals		x		x
Mycotoxins		x		x
PAHs		x		x
PCDD/Fs and PCBs (LOQ)	x		x	x

Estimation of LOD

Heavy metals, Mycotoxins, PAHs

Flow chart for estimation of **LOD**, applied in the fields of **heavy metal**, **mycotoxin**, and **PAH** analysis



PCDD/Fs and PCBs

using isotope-dilution mass spectrometry

State Institute for Chemical and
Veterinary Analysis of Food
CVUA Freiburg

EU regulations

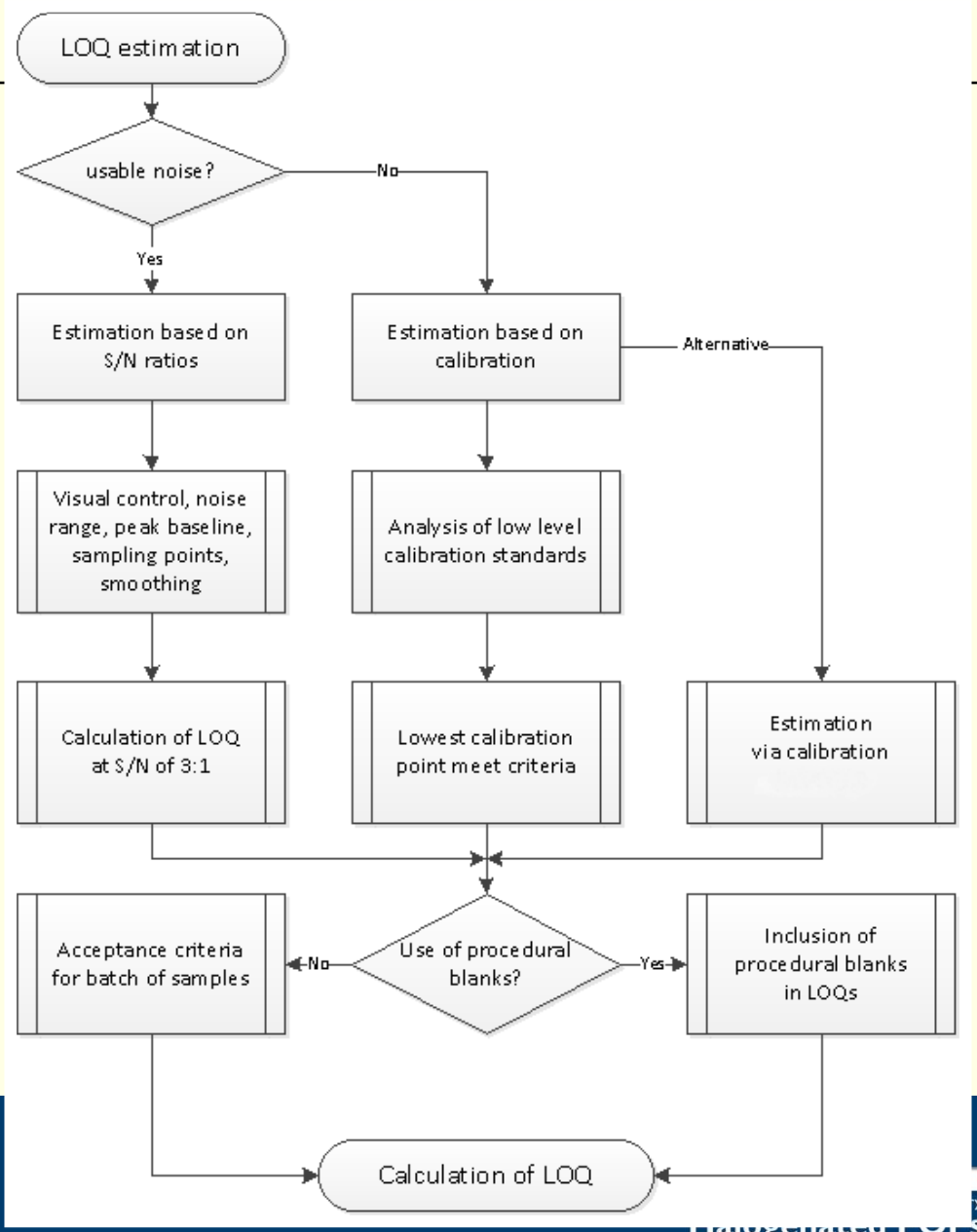
Analytical criteria defined in Commission Regulation (EU) 2017/644 and (EC) No 152/2009:

- Specific **LOQ** of an individual congener in a sample:
  - Lowest content of the analyte that can be measured with reasonable statistical certainty, fulfilling the identification criteria
  - The **limit of quantification** of an individual congener may be identified as
    - (a) the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with a **S/N** (signal / noise) **ratio** of **3:1** for the less intensive raw data signal;
  - or, if for technical reasons the signal-to-noise calculation does not provide reliable results,
    - (b) the **lowest concentration point** on a calibration curve that gives an **acceptable** (≤ 30 %) and **consistent** (measured at least at the start and at the end of an analytical series of samples) **deviation** to the average relative response factor calculated for all points on the calibration curve in each series of samples*.

* LOQ calculated from lowest calibration point taking into account recovery and sample intake.

Estimation of LOQ

PCDD/Fs and PCBs



Flow chart for **LOQ estimation** in the field of **PCDD/Fs** and **PCBs** using isotope dilution mass spectrometry

Estimation of LOQ
S/N

- **Congener-based LOQ** for each congener within each sample
  - Concentration producing an instrumental response on **two different diagnostic ion mass traces**.
  - Related to e.g. …
    - day-to-day method performance, related to efficiency of extraction, clean-up
    - changes in sensitivity of the detection system and background noise levels

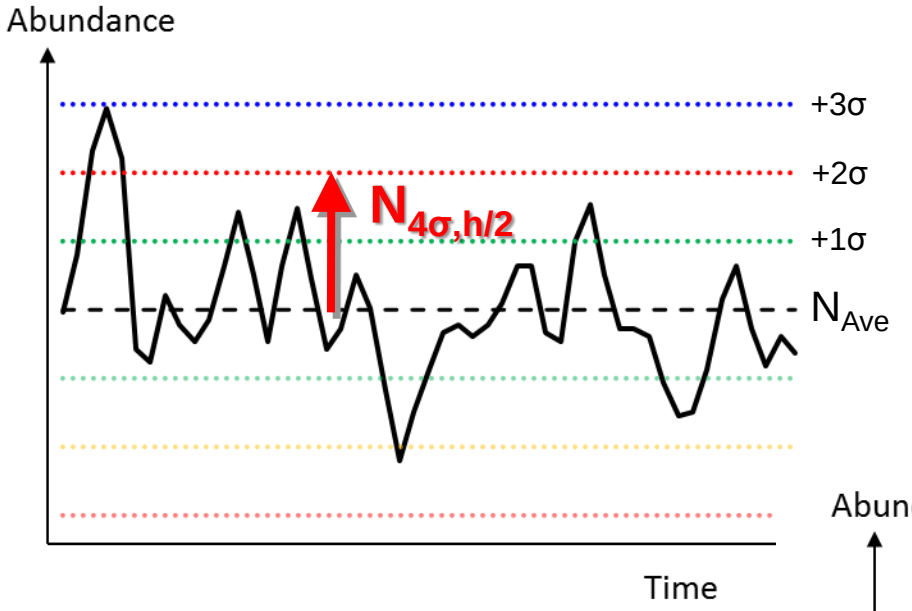
LOQ calculated as the concentration corresponding to a signal (S), which is **3 times** the noise height **N**

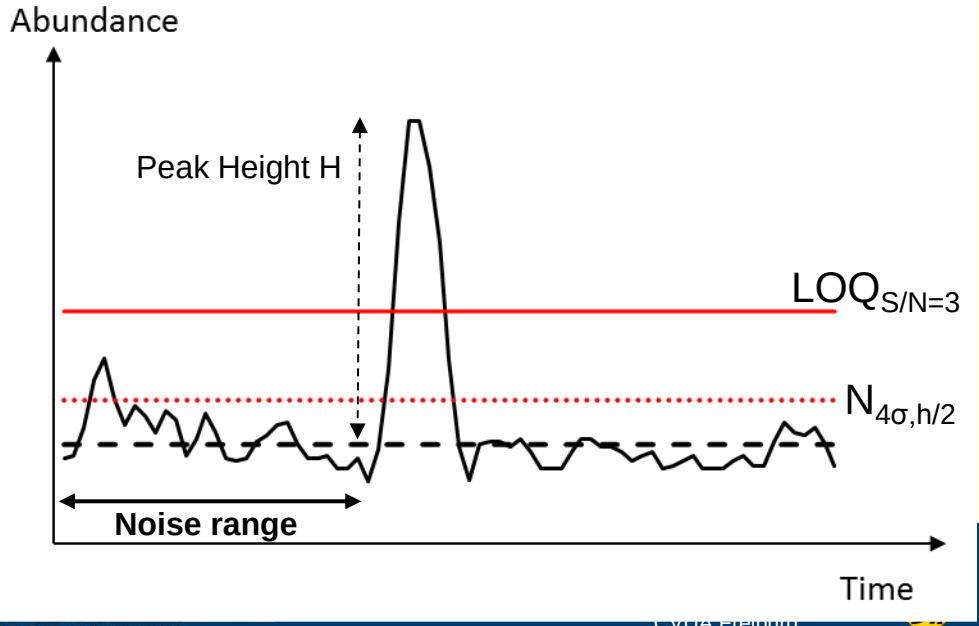
$$LOQ_{S/N=3} = 3 \cdot N = 6 \cdot \sigma_{noise}$$

σ_{noise}: Standard deviation of the baseline noise

N: Noise height measured from the baseline: $N = 2 \cdot \sigma_{noise}$

Noise


Abundance
+3σ
+2σ
+1σ
N_{Ave}
$N_{4\sigma,h/2}$
Time


Abundance
Peak Height H
$LOQ_{S/N=3}$
$N_{4\sigma,h/2}$
Noise range
Time

Additional procedures
S/N

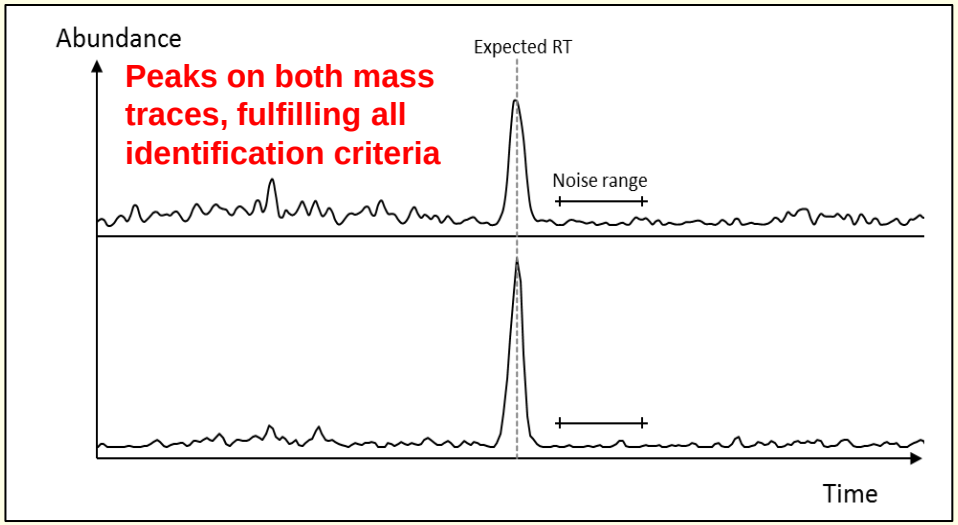
- **Visual check** of chromatogram for the presence of signals and noise levels
- Selection of the **noise range**
- Setting of the **peak baseline**
- Checking of the number of **sampling points**
- Use of area/height **transformation factor**
- Application of **smoothing** procedures
- Application of **software-based S/N calculations**

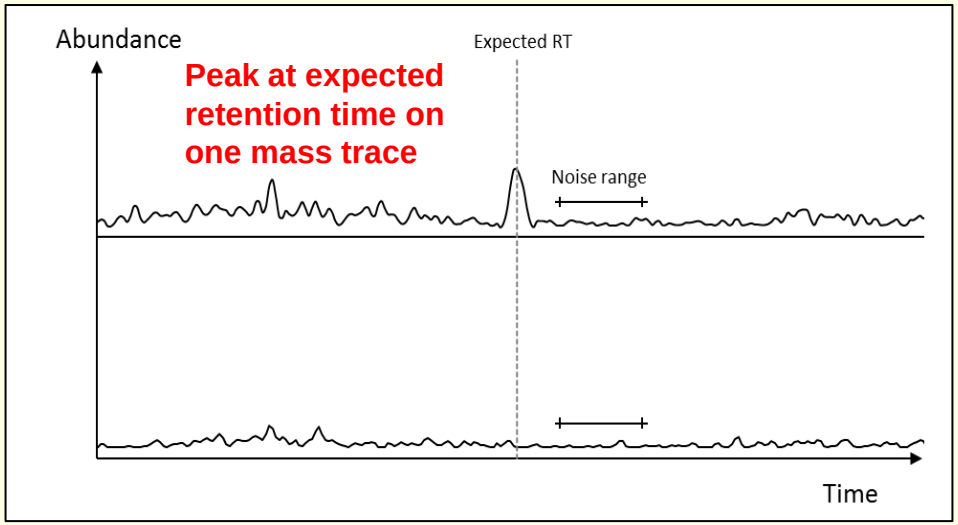
Specific identification criteria apply:

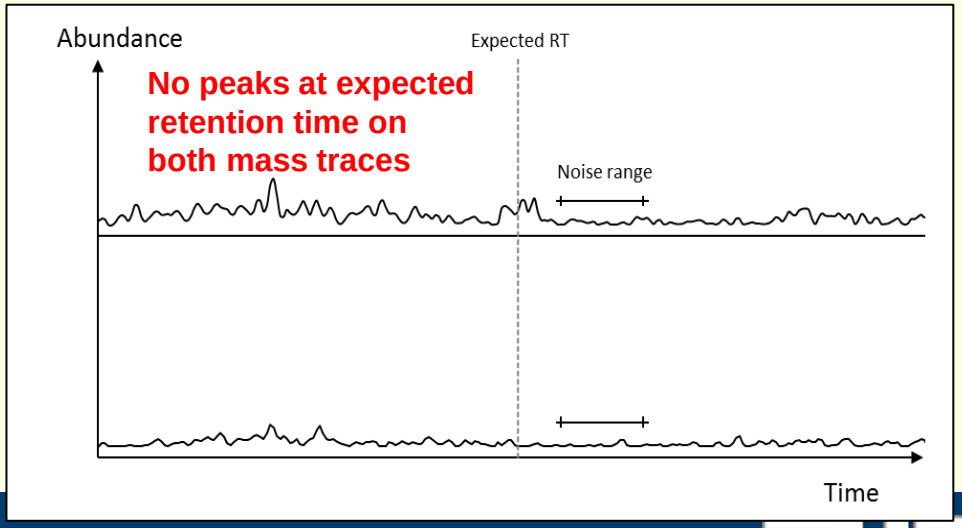
- Requirements for **relative ion intensities** at and above the LOQ must be met.
- LOQ calculations are performed on **both diagnostic ions**. The respective **higher LOQ** value is used.

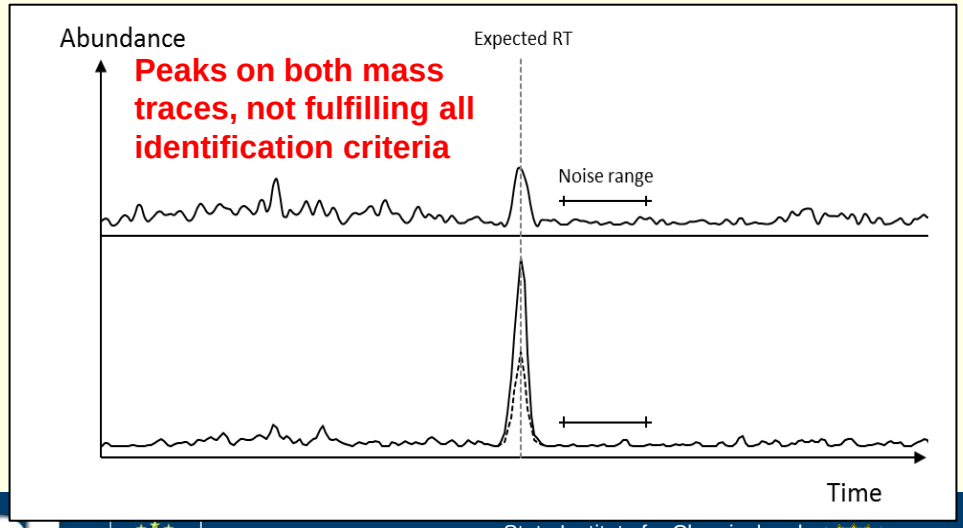
Visual control of Noise Range

S/N









Estimation of LOQ
Calibration Standards

- **Noise level too small** to perform a reliable signal-to-noise ratio calculation, no noise level is measurable at all
- Estimation of LOQ from **calibration standards** provided that **matrix effects** and **interferences** caused by the test sample do **not contribute** to variability and bias of the analytical results.
- Otherwise, matrix calibration is necessary.

The calibration range defines the working range of the analytical method. However, the instrument-LOQs (iLOQ) may be even below this range. These iLOQs may then be approximated by measuring standard solutions with concentrations below the working range, followed by checking compliance with legal requirements.

Estimation of LOQ
Calibration Standards

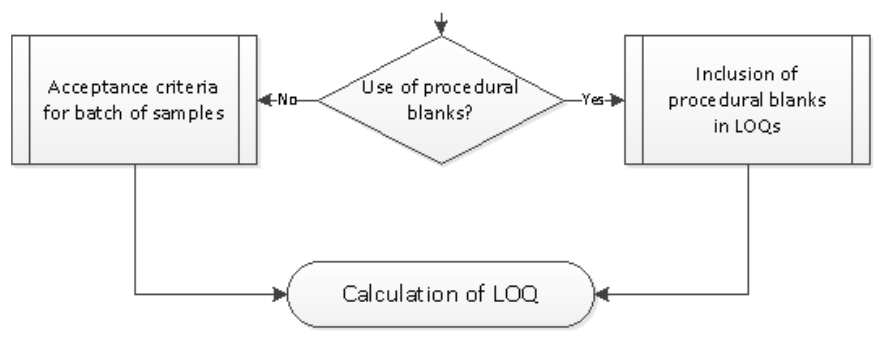
- **Congener-based LOQs** equal the **lowest standard concentration** meeting the analytical criteria in EU regulations:
  - **Retention time window** (for all monitored ions),
  - **Relative ion intensities** (≤ 15 %),
  - **Acceptable** and **consistent deviation** (≤ 30 %, measured at least at the start and at the end of sample series) from the **average relative response factor** calculated at all points of the calibration curve.

  Consideration of **sample intake**, **final extract volume**, and the **recovery** of the internal standard.

Laboratories may use alternative approaches provided that identification and quantification criteria specified in Commission Regulations (EU) No 152/2009 and 2017/644 are fulfilled.

Use of Procedural Blanks

Analysis with **every batch** of samples providing information on **method performance**, such as effects/interferences from the chemical measurement process



Two options:

- Monitoring in **QC charts**:
  - **Check** for **acceptance** of a batch
- Consideration in **LOQ estimation**:
  - If calculated **LOQs** or **measured analyte contents** of procedural blanks are **higher** than analyte contents in test samples of the same batch, values estimated/measured in the **procedural blanks** are applied as **LOQs**
  - If the **estimated/measured values** of procedural blanks are **lower** than the values of test samples, the **values of the test samples** are used for TEQ calculations.

Estimation of LOQs for Sum Parameters

- The **LOQ** associated with a **WHO-TEQ sum parameter** must not exceed the respective target limit of quantification (target-LOQ)
- Target-LOQ ≈ **1/5th** of the **maximum level**

- <u>Practical approach:</u>
  - Calculation of **WHO-TEQ values** for the **procedural blank** representative for the respective series of samples
  - For **non-quantifiable congeners LOQ** is used for TEQ calculation.

  ➢ WHO-TEQ values of the procedural blank used as LOQs, representing the laboratory's contribution to blank signals

We wish to acknowledge the scientific contributions of Thomas Wenzl (EURL for Polycyclic Aromatic Hydrocarbons), Piotr Robouch (EURL for Heavy Metals in Feed and Food) and Joerg Stroka (EURL for Mycotoxins) and the networks of National Reference Laboratories. We would also like to thank the European Commission for the financial support of the work of the EURL for Dioxins and PCBs in Feed and Food, Freiburg, Germany.

Thank you very much for your attention !

Contact: eurl-dioxin@cvuafr.bwl.de