

Joint Research Centre (JRC)

Current approaches to determine limit of detection and limit of quantification



P. López, Sz. Szilágyi, D. Lerda, T. Wenzl

IRMM - Institute for Reference Materials and Measurements

Geel - Belgium

<http://irmm.jrc.ec.europa.eu/>

<http://www.jrc.ec.europa.eu/>

1. Introduction

- Legislative references
- Definitions
- Procedures

2. Practical example – GC/MS

- Ion to use for LOD: quantifier or qualifier?
- Comparison of procedures
- Calibration requirements

3. Summary: points of concern

INTRODUCTION

Regulation (EC) No 882/2004

LOD and LOQ are criteria to characterize the methods of analysis

Commission Regulation (EC) No 333/2007

LOD/LOQ definitions

Benzo[a]pyrene (BaP)

LOD = 0.3 $\mu\text{g}/\text{kg}$

LOQ = 0.9 $\mu\text{g}/\text{kg}$

Table 7
Performance criteria for methods of analysis for benzo(a)pyrene

Parameter	Value/Comment
Applicability	Foods specified in regulation (EC) No 1881/2006
LOD	Less than 0.3 $\mu\text{g}/\text{kg}$
LOQ	Less than 0.9 $\mu\text{g}/\text{kg}$
Precision	HORRATIO_r or HORRATIO_R values of less than 2
Recovery	50 to 120 %
Specificity	Free from matrix or spectral interferences, verification of positive detection

Commission Regulation (EC) No 1881/2006

Maximum levels of BaP ($\mu\text{g}/\text{kg}$ wet weight)

Oils and fats	2.0
Smoked meats (products)	5.0
Muscle meat of smoked fish (products)	5.0
Muscle meat of fish	2.0
Infant formula	1.0

Commission Regulation (EC) No 333/2007

LOD, smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The limit of detection is numerically equal to three times the standard deviation of the **mean of blank determinations** ($n > 20$)

IUPAC *(IUPAC: Orange Book, p. 5)*

The limit of detection, expressed as the concentration, c_L , or the quantity, q_L , is derived from the smallest measure, x_L , that can be detected with **reasonable certainty** for a given **analytical procedure**. The value of x_L is given by the equation

$$x_L = \bar{x}_{blank} + k * S_{blank}$$

Where \bar{x}_{blank} is the mean of the blank measures, S_{blank} is the standard deviation of the blank measures, and k is a numerical factor chosen according to the confidence level desired.

ISO 11843:1

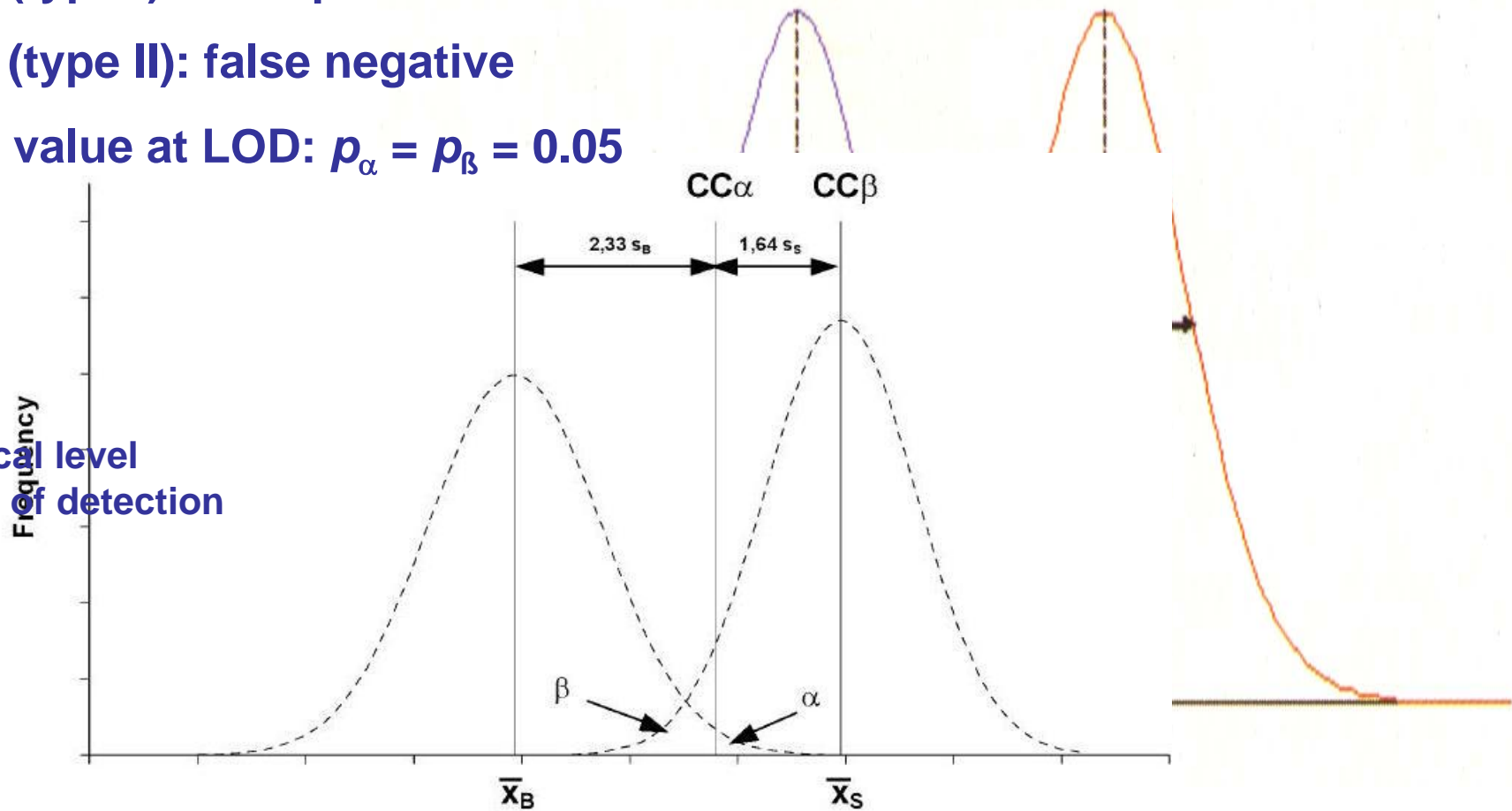
The true value of the net state variable, X , in the actual state that will lead with a **probability of $(1-\beta)$** to the conclusion that the system is not in the basic state

α -error (type I): false positive

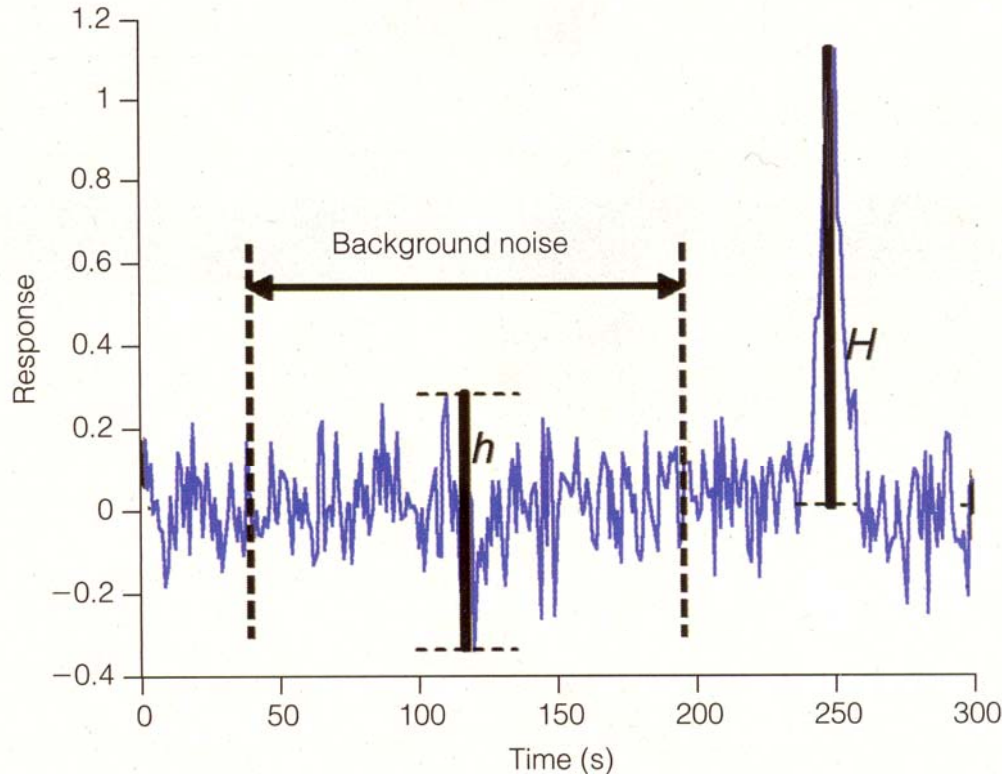
β -error (type II): false negative

Default value at LOD: $p_\alpha = p_\beta = 0.05$

L_C = critical level
 L_D = limit of detection



European Pharmacopeia



$$S/N = \frac{2H^*}{h}$$

Background noise shall be determined at position of peak from blank material
Width of noise area shall be 20 time peak width at half height

Figures from R. Boque and Y.V. Heyden, LCGC Europe, 2009 (2) 82-85

Commission Regulation (EC) No 333/2007

Limit of quantification (LOQ)

lowest content of the analyte which can be measured with **reasonable statistical certainty**. If both **accuracy and precision are constant over a concentration range** around the limit of detection, then the limit of quantification is numerically equal to six or 10 times the standard deviation of the mean of blank determinations ($n > 20$)

IUPAC

minimum quantifiable value (LQ)

analyte (true) value that will produce **estimates having a specified relative standard deviation (RSD)**, commonly 10%

Approaches for the estimation of LOD and LOQ

Calibration line method (ISO definition)

According to ISO 11843-2 or the German standard DIN 32645

From the residual standard deviation of the calibration line

Calculation from the 95% estimation interval of the calibration line

Blank procedures (IUPAC definition)

DIN 32645

$$LOD = 6 * \frac{s_{blank}}{b} \quad \begin{matrix} n=10 \\ \alpha=\beta=0.01 \end{matrix}$$

$$LOQ = 9 * \frac{s_{blank}}{b}$$

Regulation 333/2007

$$LOD = 3 * s_{blank} \quad \begin{matrix} n=20 \\ \alpha=\beta=0.05 \end{matrix}$$

$$LOQ = 6 * s_{blank}$$

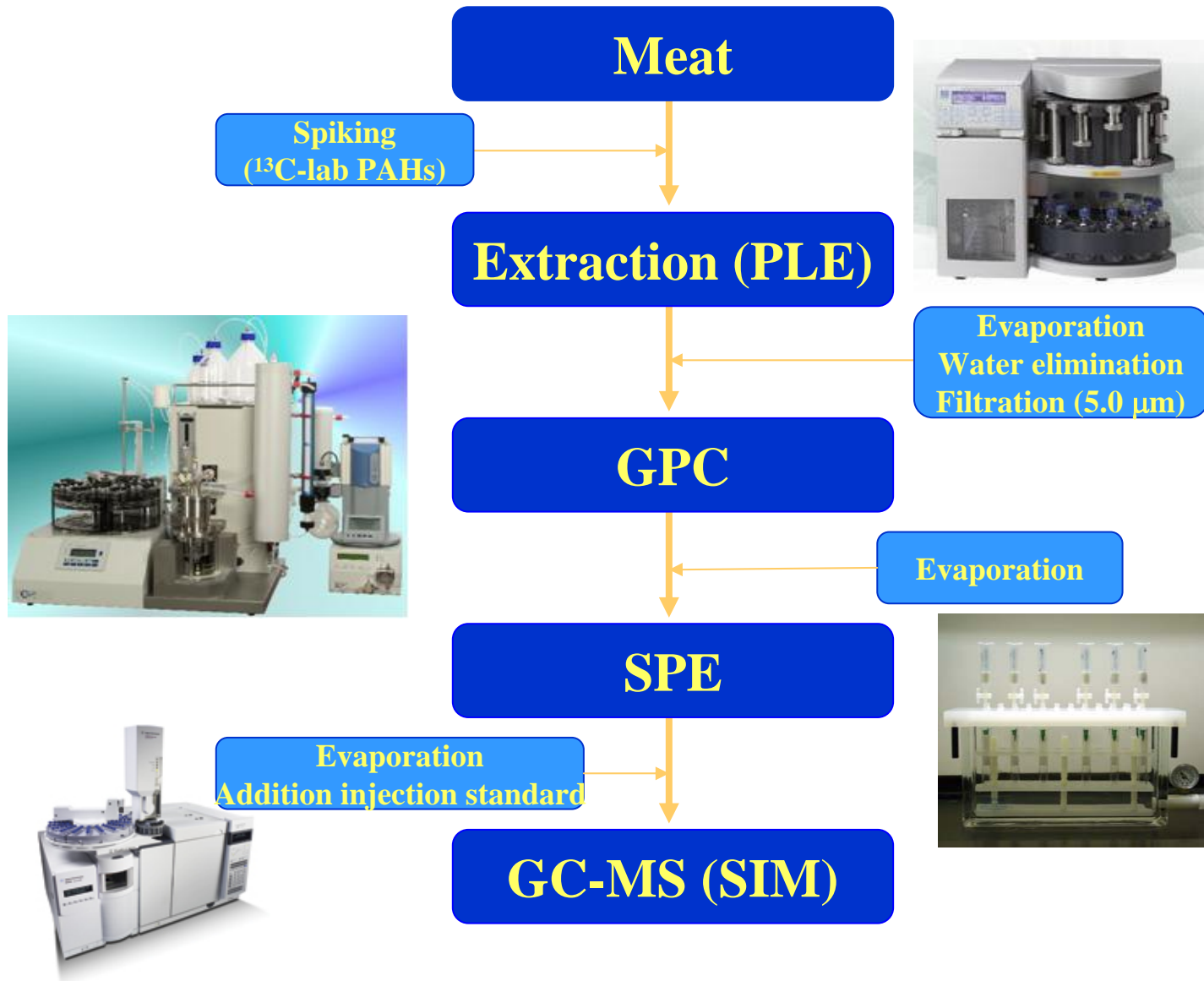
s_{blank} : standard deviation of the measurements at the blank level

b : slope of the calibration curve

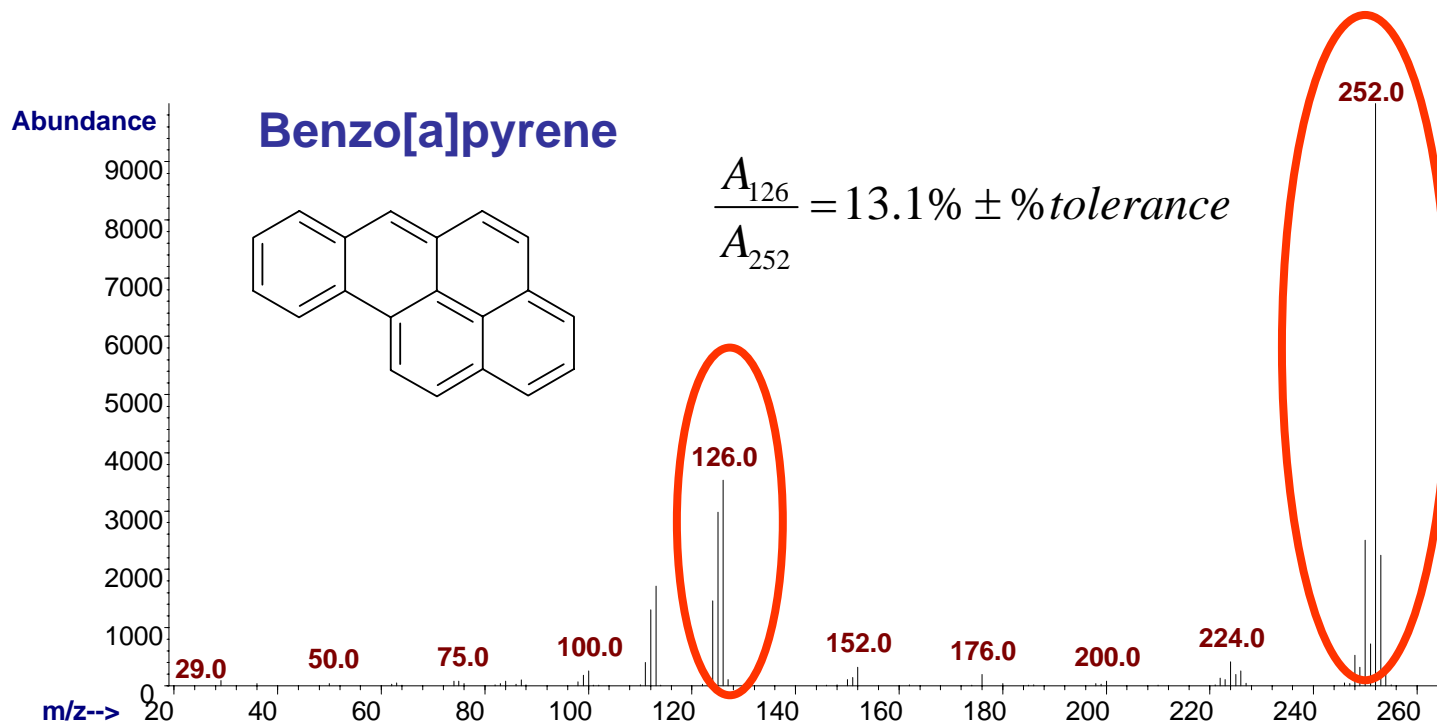
S/N ratio (European Pharmacopeia definition)

$$LOD = 3 \times S/N$$
$$LOQ = 10 \times S/N$$

PRACTICAL EXAMPLES



GC/MS in SIM (single ion monitoring)



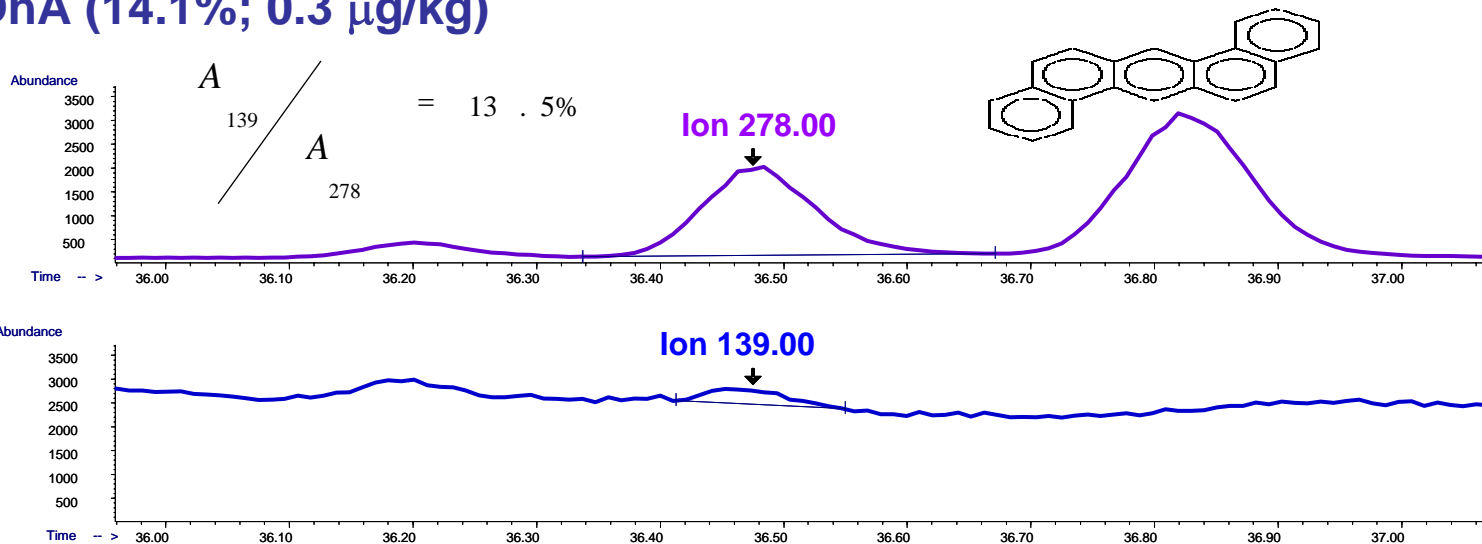
Guidance given by Commission Decision (EC) No 2002/657/EC

2.3.3.2. *Mass spectrometric detection*

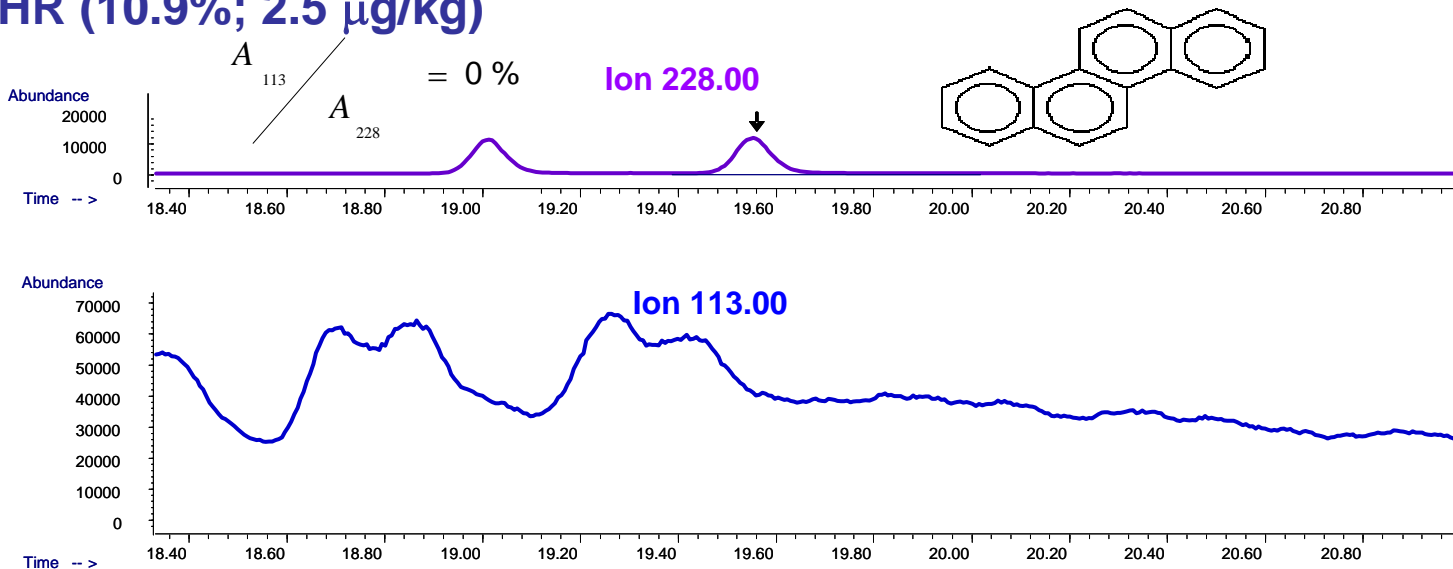
SIM: When mass spectrometric determination is performed by fragmentography, the molecular ion shall preferably be one of the selected diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and all their isotope ions). The selected diagnostic ions should not exclusively originate from the same part of the molecule. The signal-to-noise ratio for each diagnostic ion shall be $\geq 3:1$

Identification criterion has to be fulfilled

b) DhA (14.1%; 0.3 µg/kg)



c) CHR (10.9%; 2.5 µg/kg)



What if identification criterion is ignored?

$\mu\text{g/kg}$ DIN 32645		Quantifier		Qualifier	Combination	
		LOD	LOQ	LOD	LOD	LOQ
Benzo[a]anthracene	BaA	0.11	0.19	0.23	0.23	0.23
Chrysene	CHR	0.11	0.20	>2.45	-	-
Benzo[a]pyrene	BaP	0.15	0.25	0.28	0.28	0.28
Dibenzo[a,h]anthracene	DhA	0.12	0.22	0.24	0.24	0.24

too optimistic

Benzo[a]pyrene

S/N ratio	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
European Pharmacopeia	0.17	0.48

Blank procedure (IUPAC)	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
Blank standard deviation	0.15	0.47

Calibration line	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
ISO 11843:2	0.26	-
DIN 32645	0.15	0.25
Residuals	0.10	0.32
95% confidence interval	0.15	0.22

S/N ratio approach



Ease of implementation

Peak shape assumptions



Subjective evaluation: manual integration

Instability of the baseline over time

Blank procedures (blanks or fortified samples)





Inexistence of real blank matrices

Dependence on spiking concentration

LOD/LOQ $\mu\text{g}/\text{kg}$		Spiked blank at 0.3 $\mu\text{g}/\text{kg}$	Non-spiked blank samples
Benzo[a]anthracene	BaA	0.16 / 0.48	0.05 / 0.15
Cyclopenta[cd]pyrene	CPP	0.41 / 1.23	0.26 / 0.78
5-Methylchrysene	5MC	0.22 / 0.67	0.08 / 0.24
Dibenzo[a,h]pyrene	DIP	0.23 / 0.69	0.06 / 0.20

Calibration line methods

-  More rigorous approach
More accurate estimations of LOD/LOQ
-  Computationally and experimentally more complex

Comparison DIN 32645 vs ISO 11843-2

	Matrix	Linearity	Homoscedascity	Heteroscedascity	Replicates
DIN 32645	X	X	X		<i>I, J, L</i>
ISO 11843-2	X	X	X	X	<i>I, J, K, L</i>

I: Reference states used in calibration (3-5)

J: Number of preparations for each reference state (J=2)

K: Number of preparations for the actual state

L: Number of repeated measurements per preparation

Calibration curve and blanks should be prepared in matrix

LOD/LOQ ($\mu\text{g}/\text{kg}$)	Matrix	Solvent
DIN 32645	0.15 / 0.25	0.08 / 0.15
Blank procedure	0.15 / 0.47	0.01 / 0.04

Individual preparations / pooled matrix extract

LOD/LOQ ($\mu\text{g}/\text{kg}$)	Individual	Pooled extract
DIN 32645	0.15 / 0.25	0.06 / 0.20

Calibration range close to LOD/LOQ values

LOD/LOQ ($\mu\text{g}/\text{kg}$)	(0 - 2.5 $\mu\text{g}/\text{kg}$)	(2 – 15 $\mu\text{g}/\text{kg}$)
DIN 32645	0.15 / 0.25	0.77 / 1.24

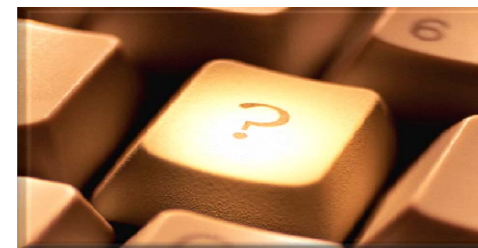
SUMMARY

- **LODs/LOQs: Differences due to estimation methodology**
methodology SHOULD be mentioned in the report
- **LODs/LOQs MUST be determined in MATRIX**
and as INDIVIDUAL PREPARATIONS
- **LODs/LOQs are likely to change over time**
Method has to be UNDER CONTROL
- **Variability of blank signal frequently underestimated**
more than 1 sample
more than 1 replicate
- **LODs/LOQs should be verified in praxis**
- **Reporting LOD/LOQs: NEVER rounded down**

➤ Importance of LOD/LOQs:

- ❑ Fit for purpose: method feasibility to reach maximum levels of PAHs (BaP) in food ($1 \mu\text{g}/\text{kg}$)
- ❑ Risk assessment: estimation of exposure data

Thank you very much for your attention



Joint Research Centre (JRC)

**Comparison of different approaches to estimate
the limit of detection and limit of quantification
of the 15+1 EU priority polycyclic aromatic hydrocarbons
(PAHs) in meat products**



P. López, Sz. Szilágyi, D. Lerda, T. Wenzl

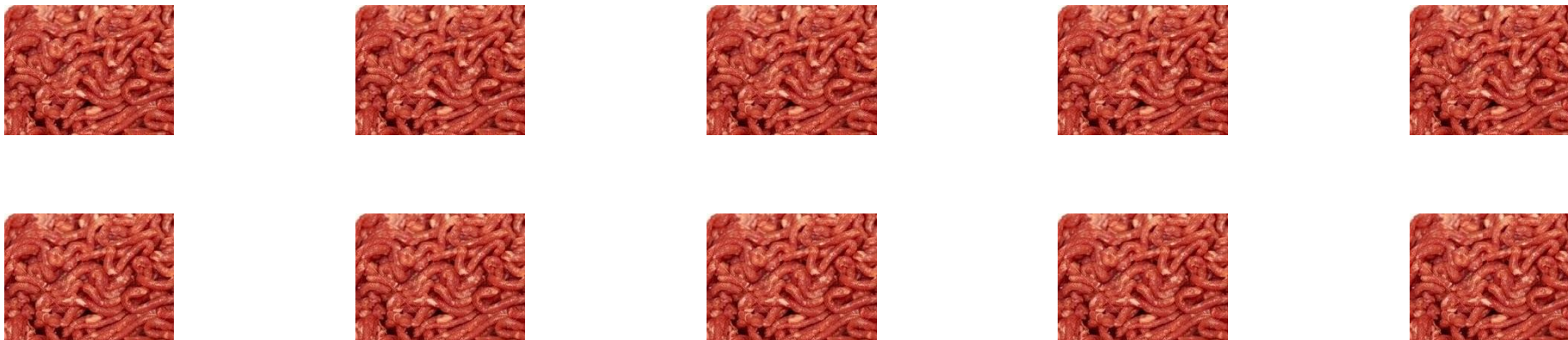
IRMM - Institute for Reference Materials and Measurements

Geel - Belgium

<http://irmm.jrc.ec.europa.eu/>

<http://www.jrc.ec.europa.eu/>

Blank procedures



5g meat (n = 10 samples)

200 μ L 35 ng/mL IS PAHs \Rightarrow 1.4 μ g/kg

200 μ L 5.6 ng/mL PAHs \Rightarrow 0.3 μ g/kg

ASE + GPC + SPE + GC/MS 1 injection/preparation

Data treatment: DIN 32645 (software MVA)

Calibration procedures (5 g meat per preparation)

Level 0



Level 1



Level 2



Level 3



Level 4



Level 5



200 μ L	0	1	2	3	4	5
IS PAHs	1.4 μ g/kg	1.4 μ g/kg	1.4 μ g/kg	1.4 μ g/kg	1.4 μ g/kg	1.4 μ g/kg
PAHs	0 μ g/kg	0.3 μ g/kg	1.0 μ g/kg	1.6 μ g/kg	2.0 μ g/kg	2.5 μ g/kg

2 individual preparations per calibration level

ASE + GPC + SPE + GC/MS 2 injection/preparation

Data treatment: Excel macro (ISO 11843:2), Validata® (DIN 32645)

S/N ratio

Preparations used for the calibration approach

ChemStation software (Agilent)

```

Signal Used: Mass 302; data.ms

Signal region: 43.19 to 43.25 min; height: 282
Noise region : 42.63 to 42.85 min; Max noise 167.0, Min noise 152.0

Calculations                                     Value
-----
Noise Points used                               26
Average noise = (sum of noise)/points           159.2
Corrected Signal = height/Average noise        122.8
Pk-pk noise = Max noise/Min noise              15.0
Pk-pk S/N = Corrected signal/Pk-pk noise      8.2
RMS noise = Sqrt(sum(square(noise-avg noise))/points) 4.0
RMS S/N = Corrected signal/RMS noise          30.4
  
```

LOD (S/N = 3)
LOQ (S/N = 10)

