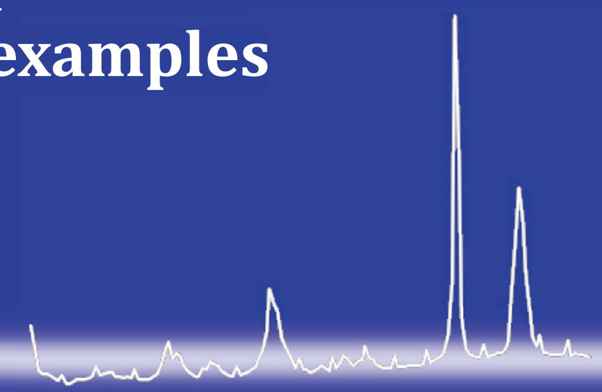
Evaluation of the uncertainty of qualitative analysis:

Relevance & practical examples



Eurachem

A focus for analytical chemistry in Europe

Bucharest, 26-27 May 2024

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Outline

Type of chemical analysis and its requirements

Accreditation of qualitative analysis

Uncertainty of qualitative analysis results

Example 1| Setting retention time criteria

Example 2| Identification of microplastics by micro-Raman

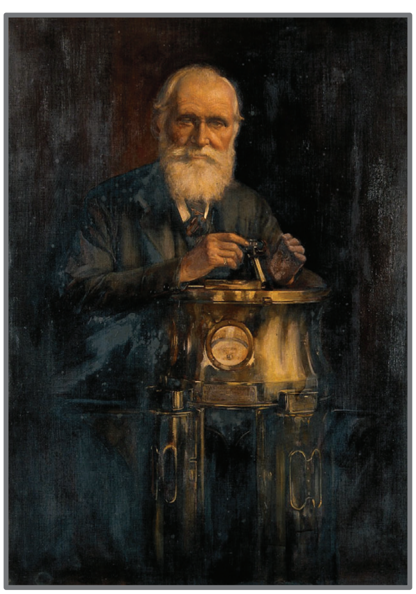
Types of chemical analysis

- **Quantifications – Measurements [1]**
- **Qualitative analysis – Examinations [2]**

1. JCGM, International Vocabulary of Metrology (VIM) – JCGM 200, BIPM, 2012 (bipm.org)
2. G. Nordin, R. Dybkaer, U. Forsum, X. Fuentes-Arderiu, F. Pontet, Vocabulary on nominal property, examination, and related concepts for clinical laboratory sciences (IFCC-IUPAC Recommendations 2017), Pure Appl. Chem. 2018; 90(5): 913–935 (www.degruyter.com)

Types of chemical analysis

- **Quantifications are important…but**



with known Uncertainty

or examine

" When you cannot measure, your knowledge is meager and unsatisfactory"

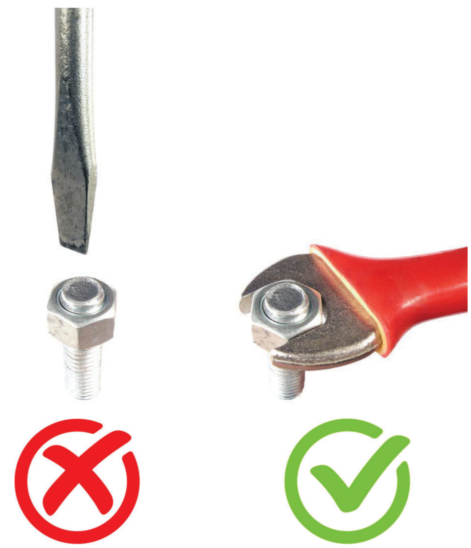
Lord Kelvin

Types of chemical analysis

Chemical analyses are only fit for the intended use if:

- **Based on adequate references**
- **Affected by an adequate uncertainty**

(the uncertainty should be reported or,
at least, considered in results interpretation)



Types of chemical analysis

Analytical methods are valid if:

- **Applicable to an adequate diversity of analysed items**
- **Able to produce fit for purpose results (…)**

Test quality should be checked through an adequate quality control

International Accreditation

Measurements:

Laboratories should be able to report measurement uncertainty and take it into account in the result interpretation

Qualitative Analysis:

Laboratories are not asked to evaluate results uncertainty but should prove produced results are fit for purpose *(classical validation)* [3]

3. ILAC, ILAC Guidelines for Measurement Uncertainty in Testing (ILAC G17:01), Silverwater: ILAC, 2021

Eurachem/CITAC Guidance

Guidance on the assessment of the performance and uncertainty of qualitative analysis was developed due to:

- **Relevance of qualitative analysis**
- **Lack of references to help analysts in deciding if these analyses are fit for the intended use**

» The quantification of the uncertainty is more relevant when a high rate of false results is expected.

4. Eurachem/CITAC Guide: Assessment of performance and uncertainty in qualitative chemical analysis. First Edition, Eurachem (2021).

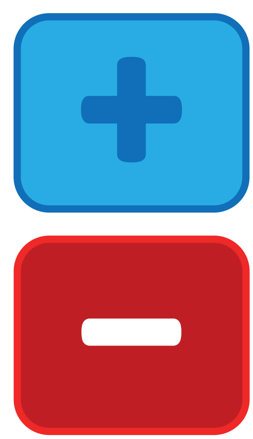
Result uncertainty

- **Measurement: Coverage interval**
- **Qualitative analysis: Metric that expresses the chance of correct or incorrect classification (probability, likelihood, odds, etc.).**
  - » **Metrics quality depends on the number and diversity of studied cases**
  - » **Uncertainty allows identifying cases where improvements or caution is needed**

Performance quantification

Results are labelled as "positive" or "negative".

Rates of true and false results can be quantified relative to the relevant type of case



Performance quantification

Contingency table:

		Case		
		Positive (*pc*)	**Negative (*nc*)**	**Results totals**
Result	**Positive (*p*)**	*tp*	*fp*	*p*
	Negative (*n*)	*fn*	*tn*	*n*
	Case totals	*pc*	*nc*	

True positive rate = $TP = tp/pc$

False positive rate = $FP = fp/nc$

Performance quantification

Contingency table:

		Case		
		Positive (*pc*)	**Negative (*nc*)**	**Results totals**
Result	**Positive (*p*)**	$tp = 228$	$fp = 1$	$p = 229$
	Negative (*n*)	$fn = 5$	$tn = 300$	$n = 305$
	Case totals	$pc = 233$	$nc = 301$	

True positive rate = $TP = tp/pc = 228/233 = 97.8\ \%$

False positive rate = $FP = fp/nc = 1/301 = 0.33\ \%$

Performance quantification

Likelihood ratio:

$$\frac{TP}{FP}$$

Performance quantification

Likelihood ratio:

$$\frac{TP}{FP} = \frac{97.8\%}{0.33\%} = 296$$

If positive and negative cases are equally likely, this can be interpreted as a positive case is 296 times more likely to be true than false.

Performance quantification

Probability of results correctness, *PP*:

$$PP = \frac{P(+)TP}{P(+)TP + P(-)FP}$$

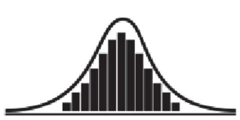
where $P(+)$ and $P(-)$ express the prevalence of positive and negative cases.

$$PP = \frac{25\% \, 97.8\%}{25\% \, 97.8\% + 75\% \, 0.33\%} = 99\%$$

Example 1| Setting retention time criteria

In chromatographic methods of analysis, retention time is used for analyte identification.

» Assuming retention time estimated under repeatability conditions is normally distributed:



Determination of Benzene in air by GC-FID (NIOSH 1501)

	Retention time (min)	
	Replicate 1	Replicate 2
Date	x_1	x_2
05/02/2025	3.01645	3.01624
12/02/2025	3.01448	3.01438
19/02/2025	3.01624	3.01599
(...)	(...)	(...)
02/04/2025	3.01516	3.01524
09/04/2025	3.01449	3.01473
16/04/2025	3.01479	3.01482

5. NIOSH 1501, Hydrocarbons, Aromatic, 2003

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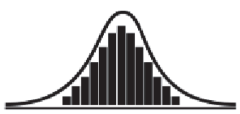


Determination of Benzene in air by GC-FID (NIOSH 1501)

	Retention time (min)		
	Replicate 1	Replicate 2	Range
Date	x_1	x_2	$\lvert x_1 - x_2 \rvert$
05/02/2025	3.01645	3.01624	0.00021
12/02/2025	3.01448	3.01438	1E-04
19/02/2025	3.01624	3.01599	0.00025
(...)	(...)	(...)	(...)
02/04/2025	3.01516	3.01524	8×10^{-5}
09/04/2025	3.01449	3.01473	0.00024
16/04/2025	3.01479	3.01482	3×10^{-5}
		Mean range, A:	0.000165

5. NIOSH 1501, Hydrocarbons, Aromatic, 2003

Example 1| Setting retention time criteria

In chromatographic methods of analysis, retention time is used for analyte identification.

» Assuming retention time estimated under repeatability conditions is normally distributed:

Determination of Benzene in air by GC-FID (NIOSH 1501)

Mean range, A:	0.000165
Maximum range of duplicate results [2.8(A/1.128)]:	0.00041

Date	20/05/2025

	Retention time (min)
Standard	3.01470
Sample 23/05	3.01457
Range	0.00013

Peak size difference < 5

Since 0.0013 < 0.00041, analyte presence is indicated

5. NIOSH 1501, Hydrocarbons, Aromatic, 2003

Example 1| Setting retention time criteria

In chromatographic methods of analysis, retention time is used for analyte identification.

» Assuming retention time estimated under repeatability conditions is normally distributed:

Determination of Benzene in air by GC-FID (NIOSH 1501)

Maximum range of duplicate results [2.8(A/1.128)]:	0.00041

➔ Chromatographic identification with a:

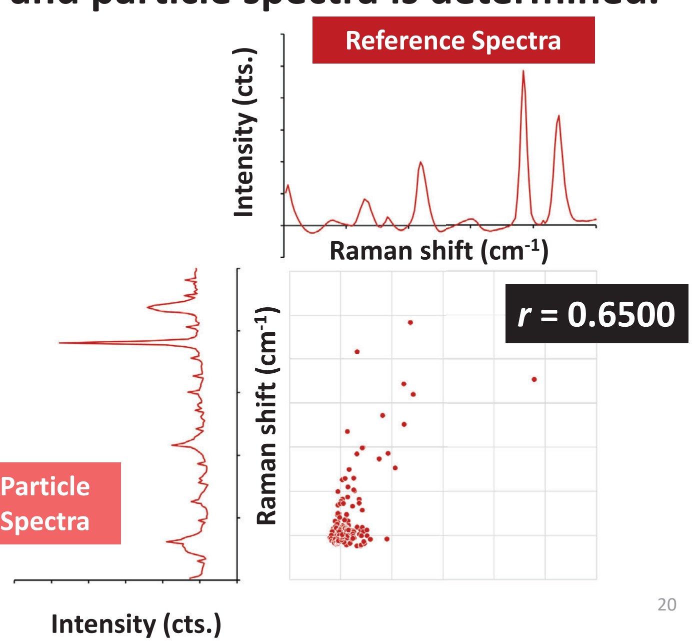
TP = 95%

5. NIOSH 1501, Hydrocarbons, Aromatic, 2003

Example 2| Microplastics identification

In the automatic identification of PET microplastics by micro-Raman, the correlation or match between reference and particle spectra is determined.

» Different correlation algorithms can be used:
- **original signal or its differentiation;**
- **unweighted or weighted correlation coefficient (e.g. Pearson, *r*, or Spearman)**



Example 2| Microplastics identification

In the automatic identification of PET microplastics by micro-Raman, the correlation or match between reference and particle spectra is determined.

Identification method validation:

(1) Selection of a polymer type (e.g. PET)
(2) Collection of a reference micro-Raman spectrum
(3) Collection of spectra from microplastics
(4) Manual identification of particles
(5) Separation of positive and negative cases (e.g. PET and non-PE)
(…)

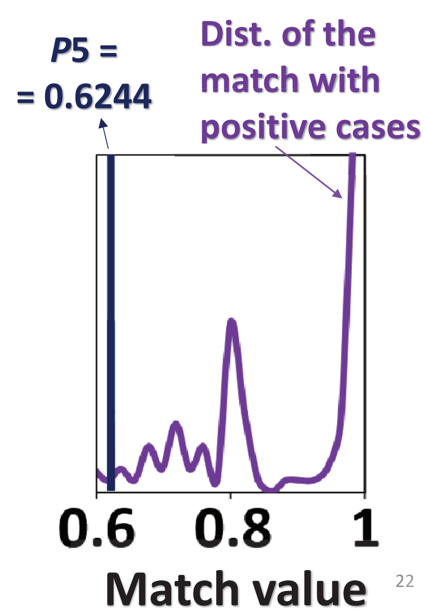
Example 2| Microplastics identification

In the automatic identification of PET microplastics by micro-Raman, the correlation or match between reference and particle spectra is determined.

Identification method validation:

(…)
(6) Determination of the match between reference and positive cases & the respective 5th percentile (*P*5)

***P*5 is the minimum match for identifications with a *TP* = 95%**



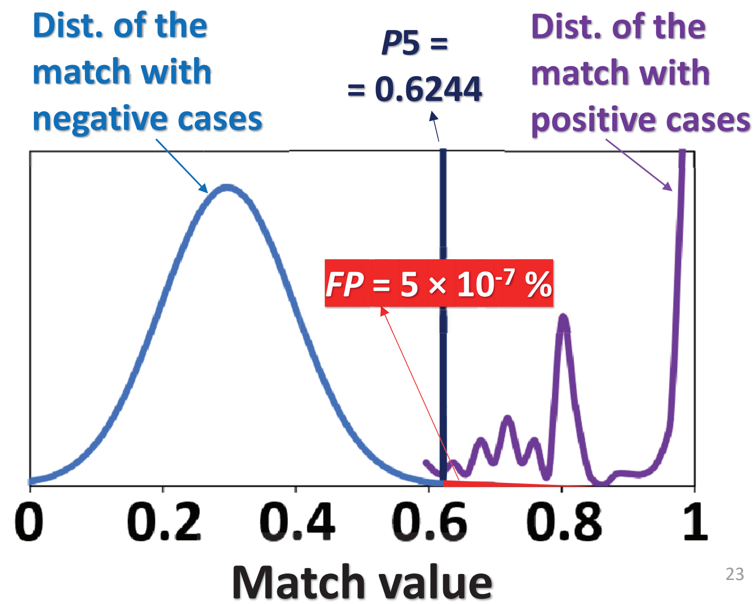
Example 2| Microplastics identification

In the automatic identification of PET microplastics by micro-Raman, the correlation or match between reference and particle spectra is determined.

Identification method validation:

(…)

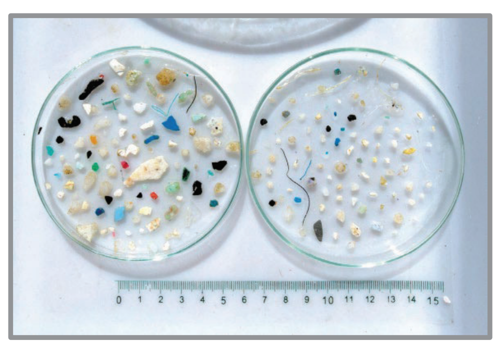
(7) Determination of the match between reference and negative cases & the probability that a negative case produces a match with the reference above *P*5, *FP*.



Example 2| Microplastics identification

In the automatic identification of PET microplastics by micro-Raman, the correlation or match between reference and particle spectra is determined.

Microplastics identification with a *TP* = 95% and *FP* <= 5% are valid for environmental analysis since, typically, many particles need to be identified in each environmental sample.



Final remarks

- The quantification of qualitative analysis performance or uncertainty allows an objective method validation

- Although the evaluation of the uncertainty of qualitative analysis is not mandatory by laboratory accreditation, it is a handy tool for laboratories

Thanks for your attention

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