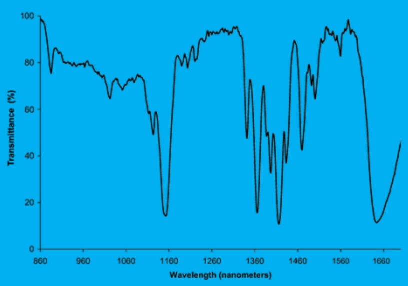
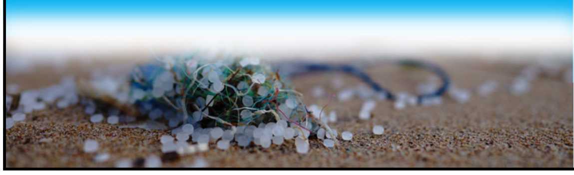
Quality assurance in qualitative analysis |worked example





Pancyprian Union of Chemists
Division of Quality Assurance
Eurachem Cyprus Committee

Eurachem
A focus for analytical chemistry in Europe

Nicosia, 12-13 March 2024

Ricardo Bettencout da Silva

Centro de Química Estrutural
Institute of Molecular Sciences
Faculdade de Ciências da Universidade de Lisboa

Ciências ULisboa
rjsilva@fc.ul.pt
https://mechem.rd.ciencias.ulisboa.pt/

Outline

Type of chemical analysis
Accreditation of qualitative analysis
Uncertainty of qualitative analysis result
Example| Identification of microplastics by micro-ATRFTIR

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

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 (…)**

Tests 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 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 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).

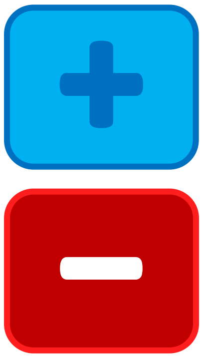
Result uncertainty

- **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:

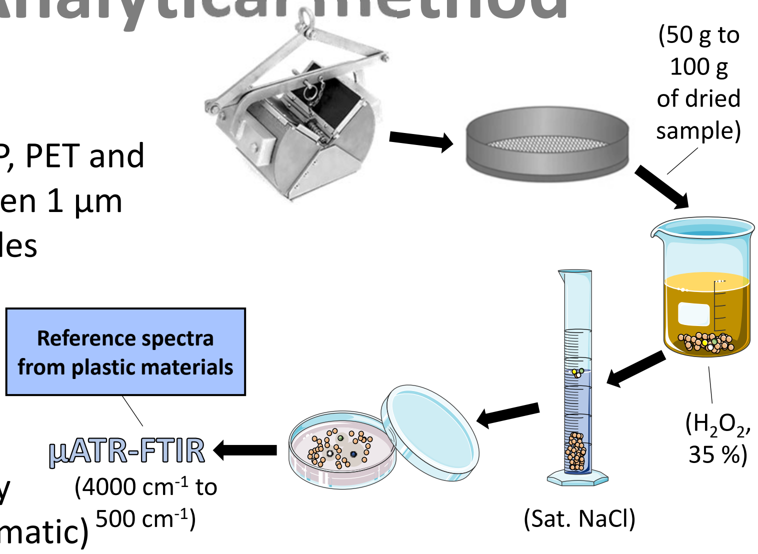
$$\frac{TP}{FP} = \frac{97.8\%}{0.33\%} = 296$$

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

Example| Analytical method

Scope: Identification of PE, PP, PET and PS particles, with sizes between 1 µm and 5 mm, in sediment samples

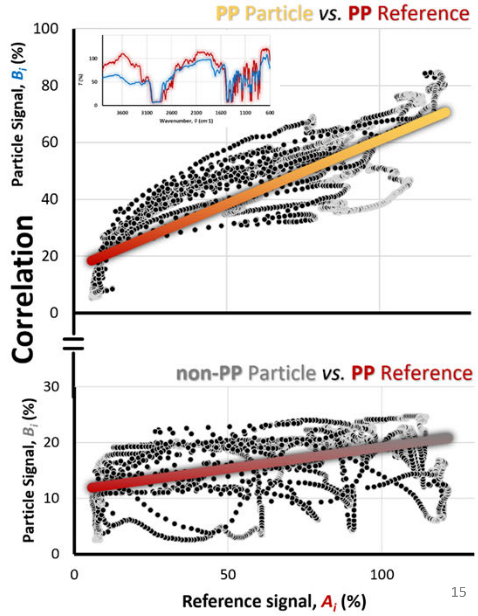
- Sediment collection
- Sieving
- Digestion
- Density separation
- Identification of particles by µATR-FTIR (manual or automatic)



PE – polyethylene; PP – polypropylene; PET – polyethylene terephthalate; PS – polystyrene.

Example | Analytical method

- Automatic µATR-FTIR identification: Involves assessing the match (correlation) between reference and particle spectra.
- Spectral comparison parameters:
  - » signal requirements
  - » wavenumber range
  - » signal processing
  - » Match algorithm
  - » target Match value



15

Example | Analytical method

Validation:

- Spectra processing:
  - » Identification reference: Plastic particles were identified manually

Attribution of characteristic bands of polypropylene, PP, particles using available reference spectra

	Wavenumber, $\tilde{\nu}$ (cm^{-1})									
	[3000-2800]			[1500-1450]	[1400-1350]	[1200-1150]	[1030-980]	[1000-940]	[850-800]	
	2950	2915	2838	1455	1377	1166	997	972	840	808
	2954.7	2910.1	2843.8	1451.8	1380.2	1167.1	997.4	972.6	841.1	808.3
Particle	υ(C-H)			δ(CH_2)	δ(CH_3)	υ(C-C) δ(CH)	δ(CH_3) δ(CH)	υ(C-C)	υ(C-CH_3)	υ(C-C) υ(C-CH)
PP#08/S3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#09/S3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#10/S3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#11/S3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#12/S3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#13/S3 II	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#14/S3 II	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PP#15/S3 II	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

16

Example| Analytical method

Validation:

- Spectra processing:

  (…)

  » Selection of a polymer type “X” (e.g. PP)

  [*All “X” particles are positive cases (+) and the others negative cases (-)*]

  » Exclusion of spectra with biofilm contamination and/or low band intensity

  » Comparison with reference spectrum using various “Match Methods”*

  *[*Match method - Combination of various comparison parameters*]

  » Collection of match values of positive and negative cases

  » Elimination of match values outliers

  (…)

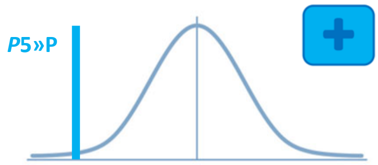
Example| Analytical method

Validation:

- Spectra processing:

  (…)

  » Estimation of the 5th percentile of the Match of positive cases, *P*5»P, after checking match normality.



$$P5»P = \bar{M} - s \cdot t^{\mathrm{one}}$$

$\bar{M}$ – Mean of match values

s – standard deviation of match values

t^{one} – t-distribution value for cumulative 5% probability and the degrees of freedom of $\bar{M}$ and s.

Example | Analytical method

Validation:

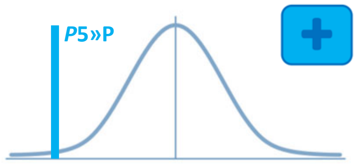
- Spectra processing:

  (…)

  » Estimation of the 5th percentile of the Match of positive cases, *P*5»P, after checking match normality.

  ◦ The *P*5»P is the minimum Match for a *TP* of 95%

  » Assuming the normal distribution of the Match of negative cases, it is estimated the probability (*FP*) of a negative case producing a Match ≥ *P*5»P



Example | Analytical method

Validation:

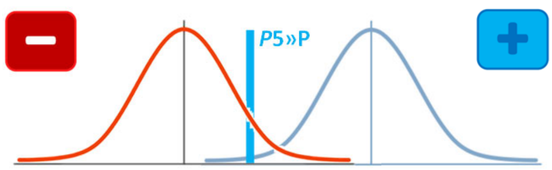
- Spectra processing:

  (…)

  » Estimation of the 5th percentile of the Match of positive cases, *P*5»P, after checking match normality.

  ◦ The *P*5»P is the minimum Match for a *TP* of 95%

  » Assuming the normal distribution of the Match of negative cases, it is estimated the probability (*FP*) of a negative case producing a Match ≥ *P*5»P



Example| Analytical method

Validation:

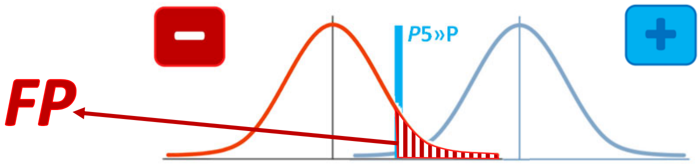
- Spectra processing:

  (…)

  » Estimation of the 5th percentile of the Match of positive cases, *P*5»P, after checking match normality.

  ◦ The *P*5»P is the minimum Match for a *TP* of 95%

  » Assuming the normal distribution of the Match of negative cases, it is estimated the probability (*FP*) of a negative case producing a Match ≥ *P*5»P



Example| Analytical method

Validation:

- Spectra processing:

  (…)

  » Estimation of the 5th percentile of the Match of positive cases, *P*5»P, after checking match normality.

  ◦ The *P*5»P is the minimum Match for a *TP* of 95%

  » Assuming the normal distribution of the Match of negative cases, it is estimated the probability (*FP*) of a negative case producing a Match ≥ *P*5»P

  » Calculation of *LR*(+) = *TP*/*FP*

  » Assessing if *LR*(+) ≥ 19 = 95%/5%

[Assessing the performance of the identification of other polymers]

Example| Analytical method

Validation:

- Spectra processing:
  (...)

Example| polypropylene identification:
Positive cases ($n = 86$):

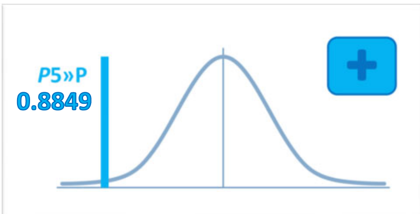
$$P5»P = \bar{M} - s \cdot t^{\text{one}}(95\%; n-1) \Leftrightarrow$$

$$\Leftrightarrow P5»P = 0.9232 - 0.023 \cdot 1.66 = 0.8849$$

$\bar{M}$ – Mean of match values
s – standard deviation of match values
$t^{\text{one}}(95\%; n-1)$ – t-distribution value for cumulative 5% probability and the degrees of freedom of $\bar{M}$ and s.

Example| Analytical method

Validation:



- Spectra processing:
  (...)

Example| polypropylene identification:
Positive cases ($n = 86$):

$$P5»P = \bar{M} - s \cdot t^{\text{one}}(95\%; n-1) \Leftrightarrow$$

$$\Leftrightarrow P5»P = 0.9232 - 0.023 \cdot 1.66 = 0.8849$$

P5»P = AVERAGE(#:#) – STDEV(#:#)* T.INV(0.95;86-1)

Example| Analytical method

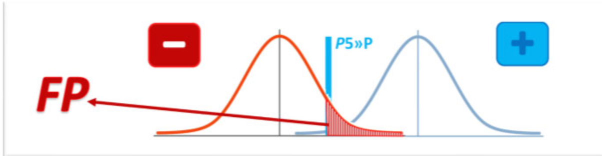
Validation:

- Spectra processing:

  (…)

Example| polypropylene identification:

Negative cases ($n = 203$):



$$\bar{M} + s \cdot t^{\text{one}}(\boldsymbol{FP}; 203 - 1) = 0.8849 = P5»P \Leftrightarrow$$

$$\Leftrightarrow 0.2310 + 0.1040 \cdot t^{\text{one}}(\boldsymbol{FP}; 203 - 1) = 0.8849$$

$$\boldsymbol{FP} = 9.67 \times 10^{-8}\%$$

Example| Analytical method

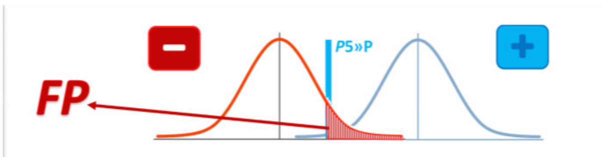
Validation:

- Spectra processing:

  (…)

Example| polypropylene identification:

Negative cases ($n = 203$):



$$\bar{M} + s \cdot t^{\text{one}}(\boldsymbol{FP}; 203 - 1) = 0.8849 = P5»P \Leftrightarrow$$

$$\Leftrightarrow 0.2310 + 0.1040 \cdot t^{\text{one}}(\boldsymbol{FP}; 203 - 1) = 0.8849$$

$$\boldsymbol{FP} = 9.67 \times 10^{-8}\%$$

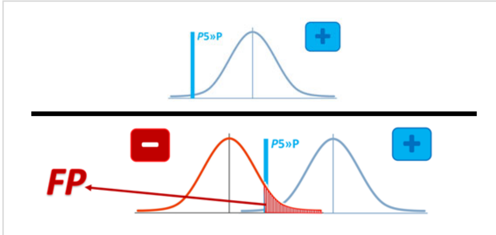
FP = 1 – T.DIST((P5»P–$\bar{M}$)/s;n–1;TRUE)

Example| Analytical method

Validation:

- Spectra processing:
  (…)

Example| polypropylene identification:
Likelihood ratio:

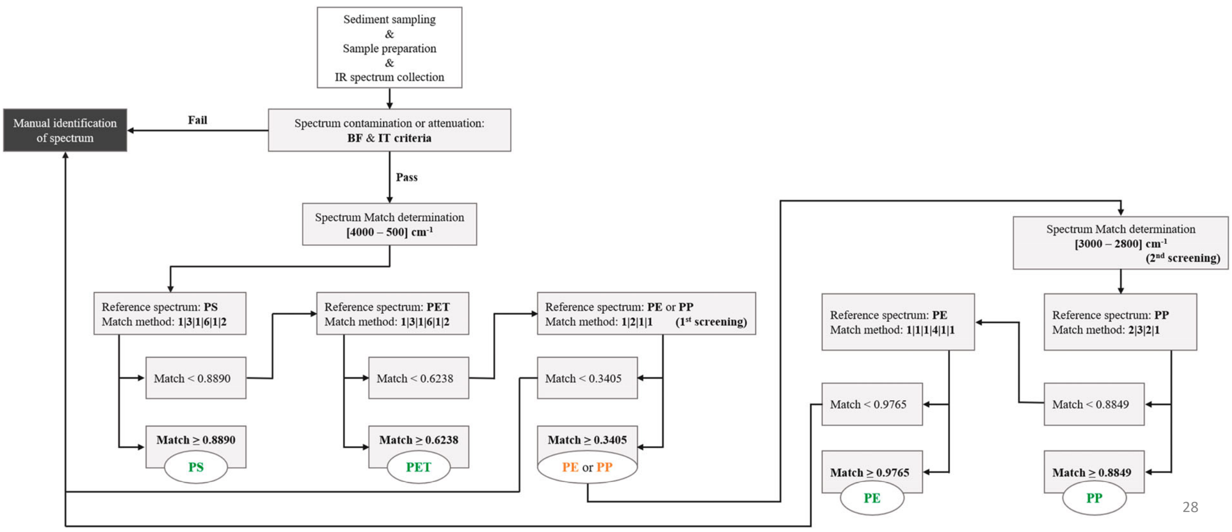


$$LR = \frac{TP}{FP} = \frac{95\%}{9.67 \times 10^{-8}\%} = 9.8 \times 10^{8}$$

A match above 0.8849 is a very strong evidence of PP polymer.

Example| Analytical method

Procedure overview:



Final remarks

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

Although not mandatory by laboratory accreditation, it is a very useful tool for laboratories

Pancyprian Union of Chemists
Division of Quality Assurance
Eurachem Cyprus Committee

Eurachem
A focus for analytical chemistry in Europe

Thanks for your attention

https://mechem.rd.ciencias.ulisboa.pt/