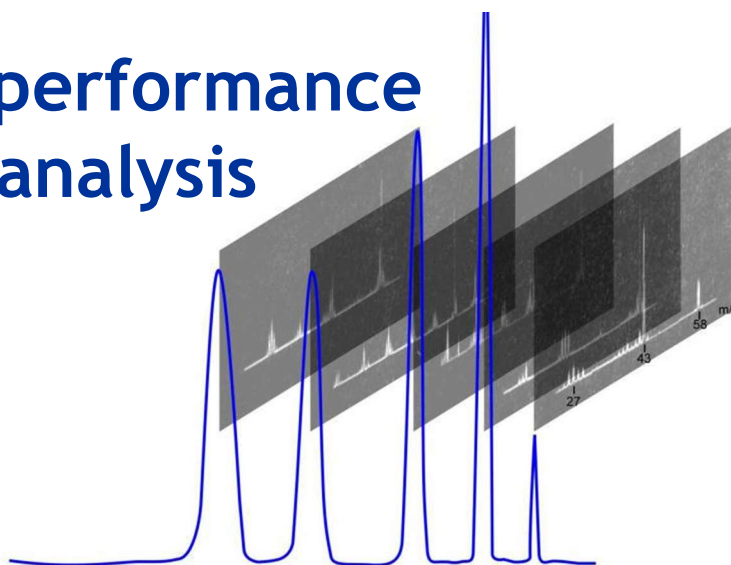


Ciências
ULisboa

Assessing the performance of qualitative analysis

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ME|Chem: <http://mechem.rd.ciencias.ulisboa.pt>



1



Outline

- Types of chemical analysis
- Eurachem CITAC/Guide
- Challenges that need to be faced
- Examples of performance assessments
- Final remarks

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2

Types of chemical analysis

Chemical analysis can be:

- Qualitative - Nominal property [Examinations]
- Quantitative - Quantifiable property [Measurements]

[Many measurements are performed after challenging qualitative analysis]

JCGM 200, International Vocabulary of Metrology - Basic and general concepts and associated terms (VIM), 3rd edition, 2012.

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News on qualitative analysis

Regularly, the community faces cases of false positive or false negative qualitative analysis results ...

The image displays three overlapping screenshots. On the left is a CBS News article titled 'Home kits to...'. In the center is a news article titled 'Testosterone, Carbon Isotope' with a sub-headline 'The New York Times broke the story today that the test champion Floyd Landis's blood was not from a natural and how reliable is the test?'. On the right is a journal article from Clinical Chemistry titled 'False-Positive Rates for the Qualitative Analysis of Urine Benzodiazepines and Metabolites with the Reformulated Abbott Multigent™ Reagents' by Gifford Lum, Barry Mushlin, and Linda Farney. The journal article includes an abstract and a 'To the Editor' section.

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News on qualitative analysis

Regularly, the community faces cases of false positive or false negative results.



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5

Eurachem/CITAC Guide

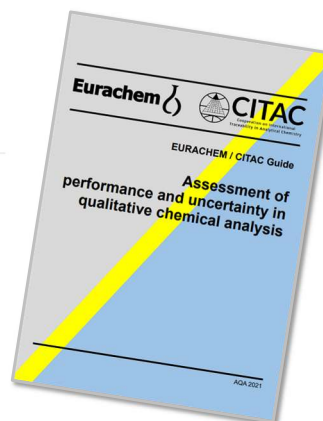
There is a need to improve the performance of qualitative analysis in chemistry...

Availability

The guide may be downloaded from this website at no cost.

- > Download the Guide in Czech 📄 (PDF, 1.4 Mb) (Published 2022-12-20)
- > Download the Guide in English 📄 (PDF, 1.8 Mb) (Published* 2021-11-17)
- > Download the Guide in Farsi 📄 (PDF, 2.3 Mb) (Published 2024-04-04)
- > Download the Guide in German 📄 (PDF, 1.4 Mb) (Published 2024-04-04)
- > Download the Guide in Russian 📄 (PDF, 2.6 Mb) (Published 2024-06-18)

* First published 2021-11-11. Publication dates above are dates of file publication on this website.



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

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6

Challenges that need to be faced

Qualitative analysis performance can be described by a contingency table

		Cases		Total results
		Positives (pc)	Negatives (nc)	
Results	Positives (p)	tp	fp	p
	Negatives (n)	fn	tn	n
Total cases		pc	nc	

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7

Challenges that need to be faced

Qualitative analysis performance can be described by a contingency table: Table feed with experimental data

		Cases		Total results
		Positives (pc)	Negatives (nc)	
Results	Positives (p)	$tp = 228$	$fp = 1$	$p = 229$
	Negatives (n)	$fn = 5$	$tn = 300$	$n = 305$
Total cases		$pc = 233$	$nc = 301$	

$TP = 97.8\% \rightarrow$ Sensitivity

$TN = tn/nc = 300/301 = 99.7\% \rightarrow$ Specificity

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8

Challenges that need to be faced

...but, in order to produce a reliable estimate of an *FP* of 1%, how many analyses of negative cases need to be performed?



Challenges that need to be faced

...but, in order to produce a reliable estimate of an *FP* of 1%, how many analyses of negative cases need to be performed?

Table 4. The minimum number of analyses to find one or more false (positive or negative) result(s).

False result rate	Confidence level	
	95 %	99 %
0.5 %	598	919
1 %	299	459
5 %	59	90

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

Challenges that need to be faced

...and, how diverse should be the negative cases...

a: No V mark



b: Full V, low



c: Broken V with yellow tip



d: Marginal mark



e: Redspot



f: Red leaflet



g: Red midrib



h: Red leaf



i: Red fleck



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11

Challenges that need to be faced

In many analytical fields, highly selective methods such as GC-MS-MS are used...



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12

Challenges that need to be faced

In many analytical fields, highly selective methods such as GC-MS-MS are used...

How many tests do we need to perform to assess the performance of GC-MS-MS analysis?



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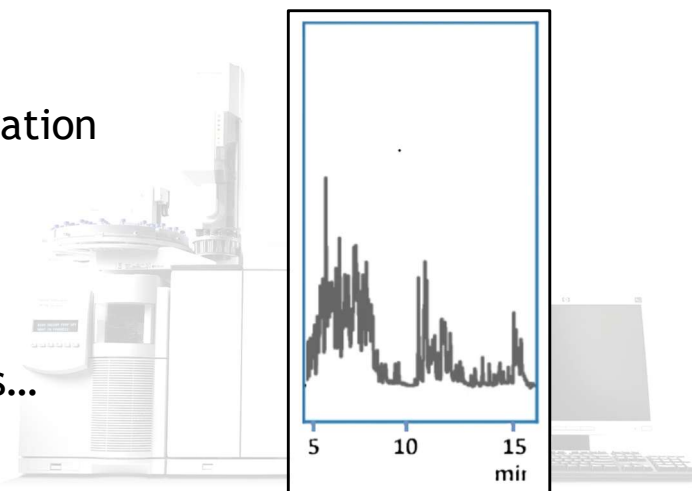
13

Challenges that need to be faced

In many analytical fields, highly selective methods such as GC-MS-MS are used...

But, as analyte concentration decreases and matrix complexity increases...

the selectivity decreases...



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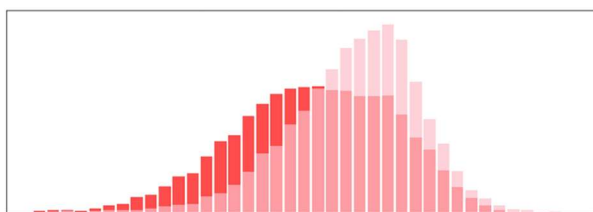
14

Challenges that need to be faced

Possible solution

Do not assess performance exclusively from experimentation
(...)

Experimental data & Modelling



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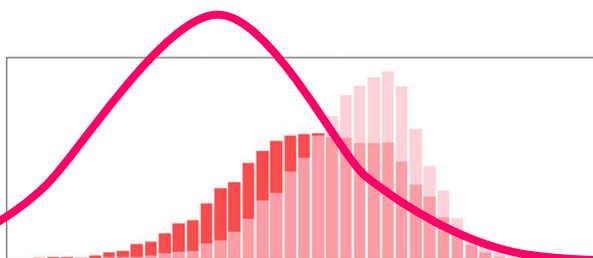
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15

Challenges that need to be faced

Possible solution

Do not assess performance exclusively from experimentation
(...) Modelling is powerful but can be misleading....



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16

Challenges that need to be faced

What can we do?

1st: Set statistically sound identification criteria that establishes the *TP*

2nd: Define sound models of negative responses to estimate *FP*

3rd: Assess if the *TP* and *FP* are fit for purpose.

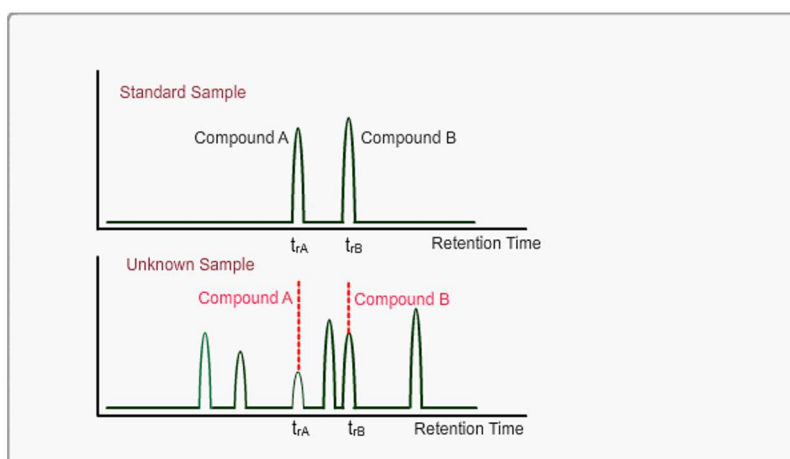


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17

Example - Using retention time in GC



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18

Example - Using retention time in GC

(1) Estimate the standard deviation of retention time repeatability, $s_{R(tr)}$, from retention time replicates:

Day	Rep.1	Rep.2	Rep.3	Rep.4	Rep.5
05/02/2024	13.723	13.555	13.790		
08/02/2024	13.607	13.593	13.685	13.589	
11/02/2024	14.074	14.134	13.958	13.870	
17/02/2024	16.040	16.241	15.965		
24/02/2024	14.164	14.170	14.329	14.096	
27/02/2024	14.238	14.157	14.205	14.364	14.314
05/03/2024	15.645	15.199	15.373	15.785	15.700

s	n	n-1	s ² *(n-1)
0.1211	3	2	0.02931
0.0450	4	3	0.00608
0.1180	4	3	0.04177
0.1427	3	2	0.04073
0.0987	4	3	0.02923
0.0833	5	4	0.02775
0.2454	5	4	0.24082
Sum	21		0.41570

$$s_{tr(r)} = \sqrt{\frac{\sum_{i=1}^p (n_i - 1) s_{tr(i)}^2}{\sum_{i=1}^p (n_i - 1)}}$$

$$s_{tr(r)} = \sqrt{\frac{B}{A}} = 0.246 \text{ min}$$

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19

Example - Using retention time in GC

(2) Identification criteria for unknown peaks:

$$|t_{r(std)} - t_{r(unk)}| < 3\sqrt{2}s_{tr(r)}$$

$t_{r(std)}$ - Analyte

$t_{r(unk)}$ - Retent

Some people use a fixed 0.1 min tolerance for retention time, but this should be assessed for the *TP* (...)

Identification with a *TP* of 99%.

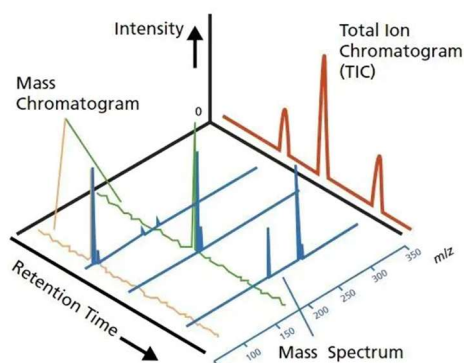
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20

Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...



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21

Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...

Abundance value records...

		Analyte: PAVA							
		1	2	3	4	5	6	7	8
		Fragments (m/z)							
Seq. ord.	#	ID	Date	RT (min)	298	195	151	137	
1	1	P1402-3-D	14/02/23	17.724	188824	59768	77972	53728	
2	2	P1402-3-D	14/02/23	17.731	163712	70901	95580	67836	
3	3	P1602-1-D	16/02/23	17.728	57752	24504	35216	24592	
4	4	P1602-2-D	16/02/23	17.724	69376	29616	41728	30256	
5	5	P1702-D	17/02/23	17.728	30568	12741	18568	13902	
6	6	P1702-1-D	17/02/23	17.727	36168	15120	21856	160256	
7	7	P1702-2-D	17/02/23	17.724	41888	18888	27304	188224	
8	8	P2002-D	20/02/23	17.724	36160	15463	22688	162688	
9	9	P2002-1-D	20/02/23	17.724	52808	23152	33072	228032	
10	10	P2002-2-D	20/02/23	17.724	46504	19024	26872	197376	
11	11	P2202-1-D	22/02/23	17.717	17464	7589	10467	83296	
12	12	P2202-2-D	22/02/23	17.717	23632	10567	16163	113160	
13	13	P2202-3-D	22/02/23	17.728	24312	9595	14915	108192	
14	14	P2302-1-D	23/02/23	17.712	25472	11634	16760	121496	
15	15	P2402-1-D	24/02/23	17.728	40088	16104	26388	184576	
16	16	P2402-2-D	24/02/23	17.728	33744	14706	19576	147904	
17	17	P1303-1-D	13/03/23	17.694	20376	8849	13229	102080	
18	18	P1303-2-D	13/03/23	17.694	16744	7147	10704	79696	
19	19	P1403-1-D	14/03/23	17.686	28216	12137	18016	128296	
20	20	P1403-2-D	14/03/23	17.686	41486	17194	24304	143872	

R. Bettencourt da Silva, P. Salgueiro, Talanta 282 (2025) 127061

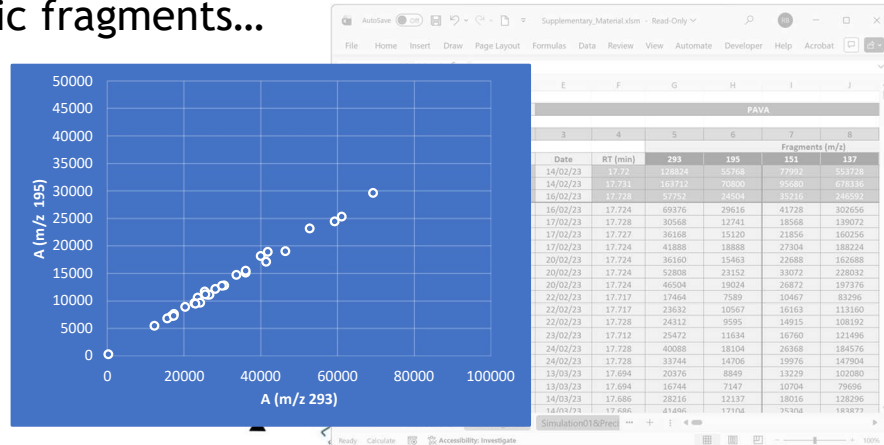
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Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...



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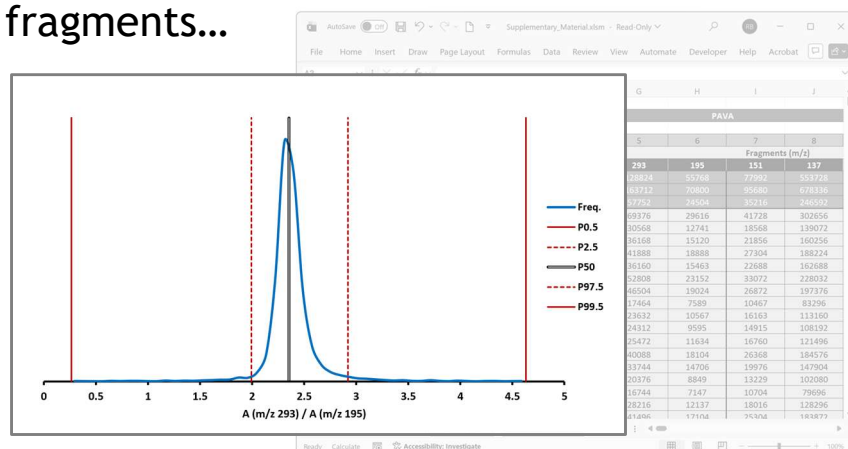
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Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...

Abundance ratio confidence limits [for $TP = 99\%$]



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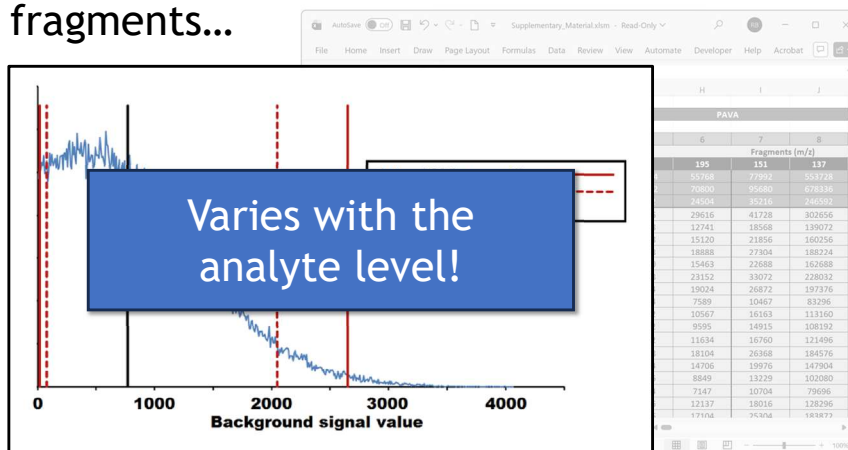
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24

Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...

FP
simulation
[chance of
blank signal
meeting
identification
criteria]



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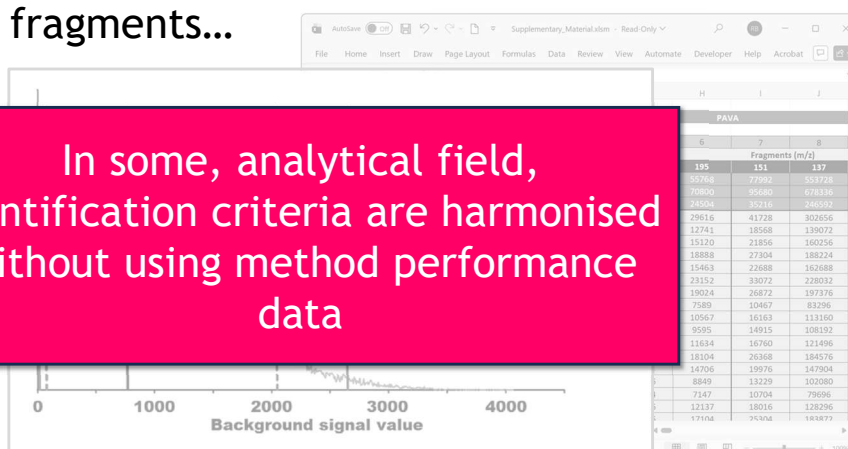
25

Example - Using by MS

Trace levels of compounds are identified by low-resolution mass spectrometry by taking the abundance ratio of characteristic fragments...

FP
simulation
[chance
blank sign
meets
identification
criteria]

In some, analytical field,
identification criteria are harmonised
without using method performance
data



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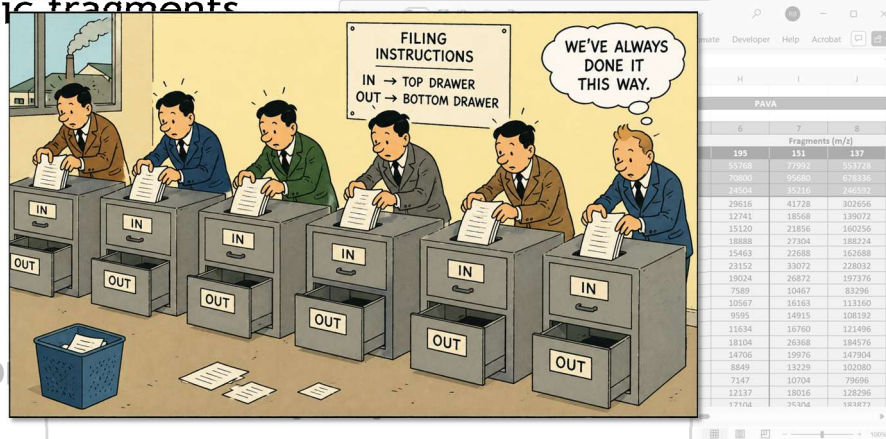
Example - Using by MS

Trace levels
mass spectro
characteristic fragments

Harmonisation of inconsistencies!

resolution
o of

FP
simulation
[chance
blank signal
meets
identification
criteria]



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27

Final remarks

There is a need to improve the way the performance of qualitative analysis is assessed in routine analysis...

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28

Final remarks

There is a need to improve the way the performance of qualitative analysis is assessed in routine analysis...

Tools for performing this assessment are available...

Final remarks

There is a need to improve the way the performance of qualitative analysis is assessed in routine analysis...

Tools for performing this assessment are available...

We would need more attention from the international accreditation on this issue!



Thank you for
your attention!

