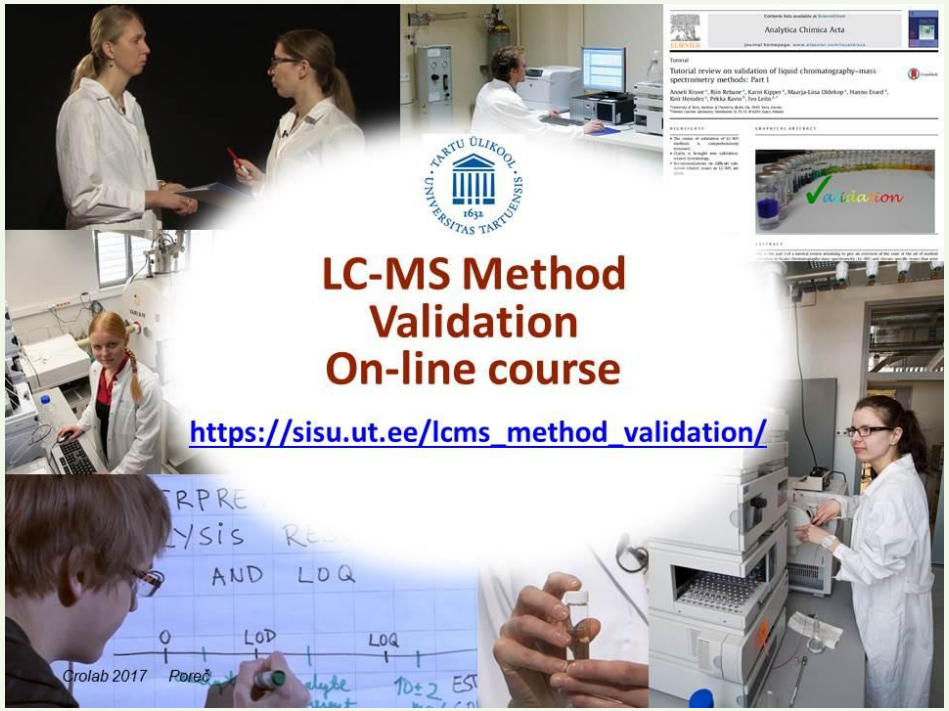
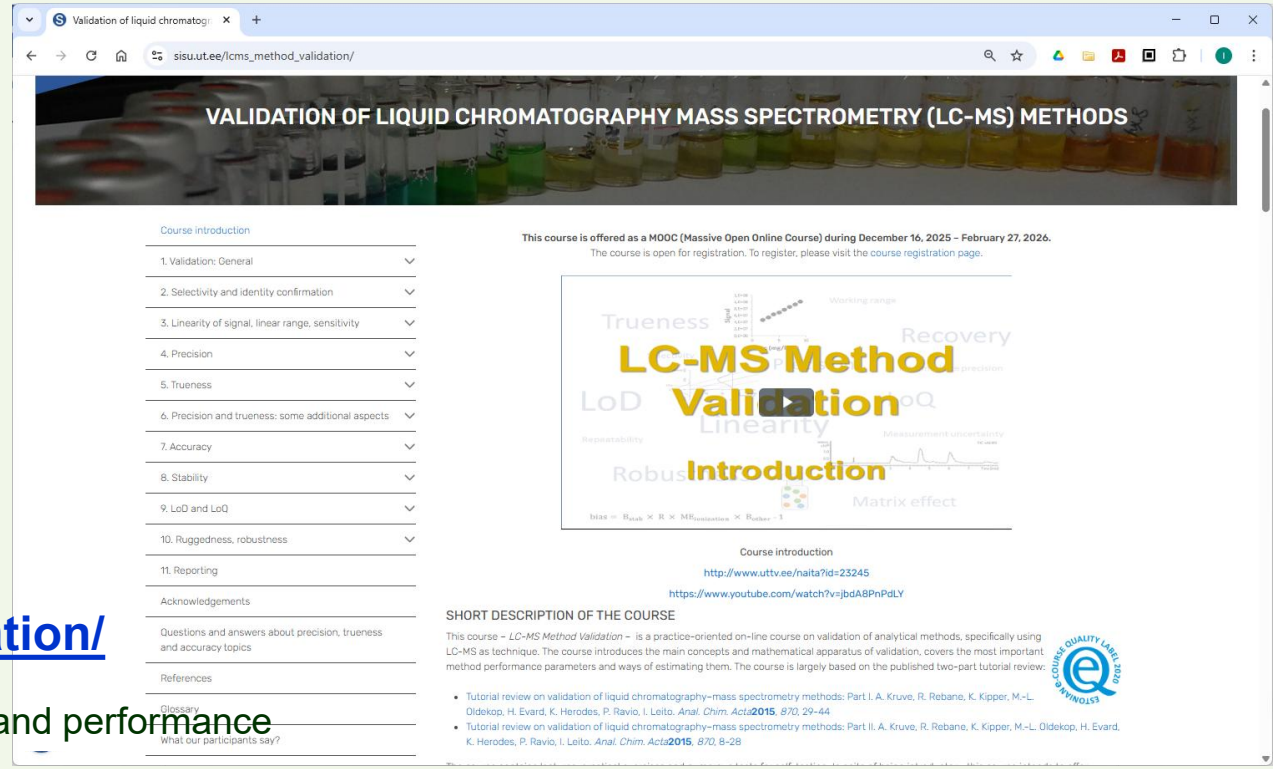


What are the issues encountered in the real world?

Lessons learned form an online validation course



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sisu.ut.ee/lcms_method_validation/

Outline

- Overview of the **LC-MS Method Validation web course**
  - https://sisu.ut.ee/lcms_method_validation/

Web course

- What are the issues in „real life“?

What practitioners need?

  - Analysis of
    - **2640 course forum posts**
    - **336 participant feedback submissions**
    - Living memory of teachers

Forum posts

Feedback

- Generalisations and **lessons learned**

Lessons learned

LC-MS as technique

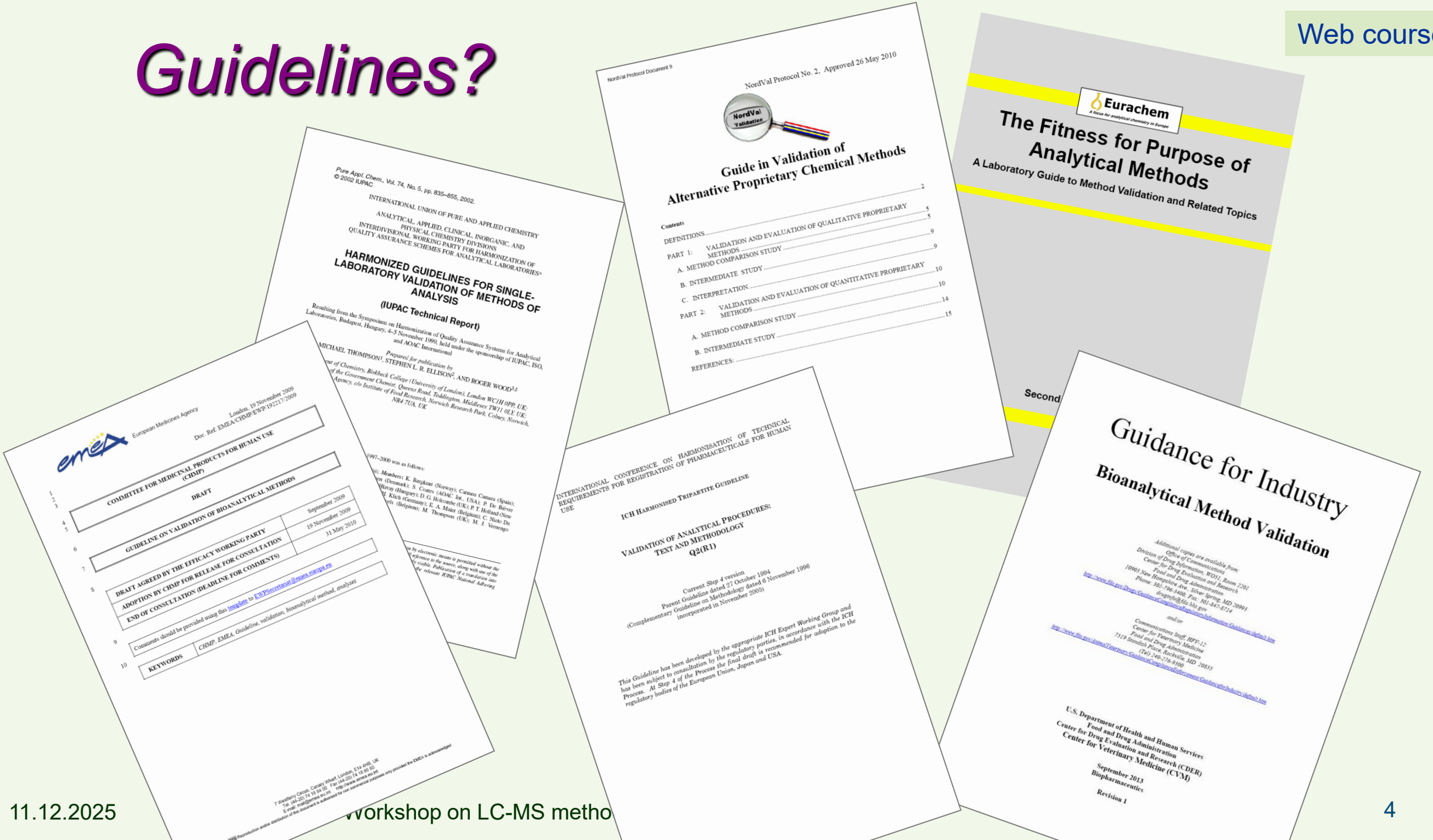
- No 1 technique for determination of **low levels** of organics in **difficult matrices**
  - Biomedical, environmental, „-omics“, …
- LC-MS: **many adjustable parameters**
  - In LC
  - In MS

Checking that the method performs as required is not trivial!

Validation is BIG in LC-MS!

Guidelines?

Web course



Validation guidelines

- Guidelines are useful, but ...
  - Sometimes very **general**
    - How many replicates? Which spiking levels? How many days? ...
  - Sometimes **different** recommendations
  - Usually **LC-MS is not specifically addressed**
    - Except e.g. 2021/808/EC, SANTE
  - Sometimes **advanced calculations** are required

Validation in LC-MS is not easy!

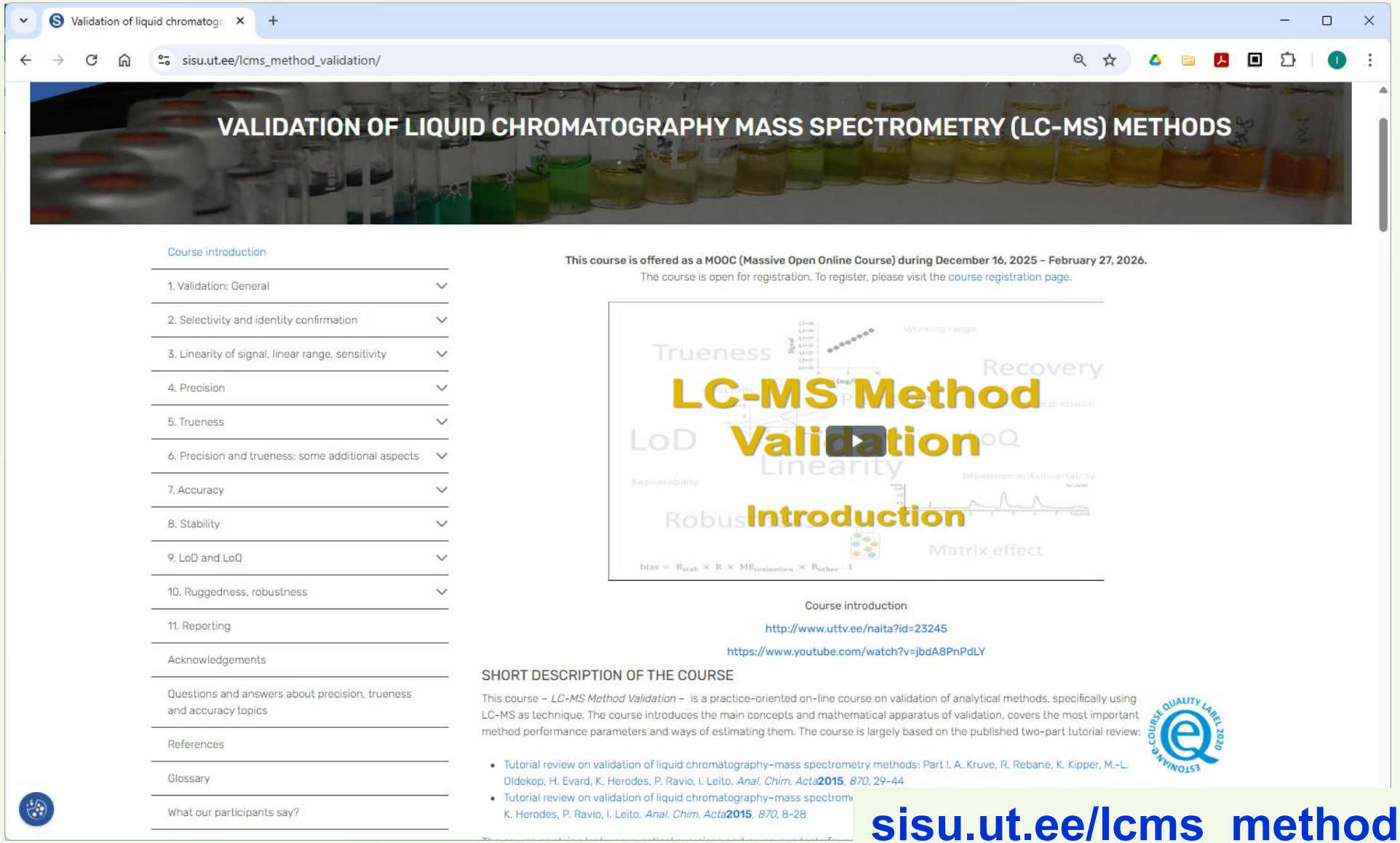
Practitioners need help!

Our goal with the course

- Web-based teaching material for
  - **Independent** learning
  - Knowledge applicable **in real-life situations**
  - On-line **reference point** of explanations of concepts and approaches
  - Offering as Massive Open Online Courses (**MOOCs**)
  - **Support for auditorial teaching** at UT
  - **Promoting** our analytical chemistry education

On-line course: LC/MS Method Validation

Web course



sisu.ut.ee/lcms_method_validation/

Features of the web course

sisu.ut.ee/lcms_method_validation/

- **Diverse teaching materials**
  - Lecture videos, discussion videos
  - Calculation videos, example calculation files
  - Self-tests and graded tests

- **Main validation guidelines** are reviewed and compared
  - With every performance characteristic (parameter)
- **Recommendations** are given how to determine performance characteristics
  - Synthesis from guidelines and our experience
- Specific **LC-MS issues**
  - Ionization, matrix effects, MS^n selectivity, ...
- **General workflow** of LC-MS method validation is presented

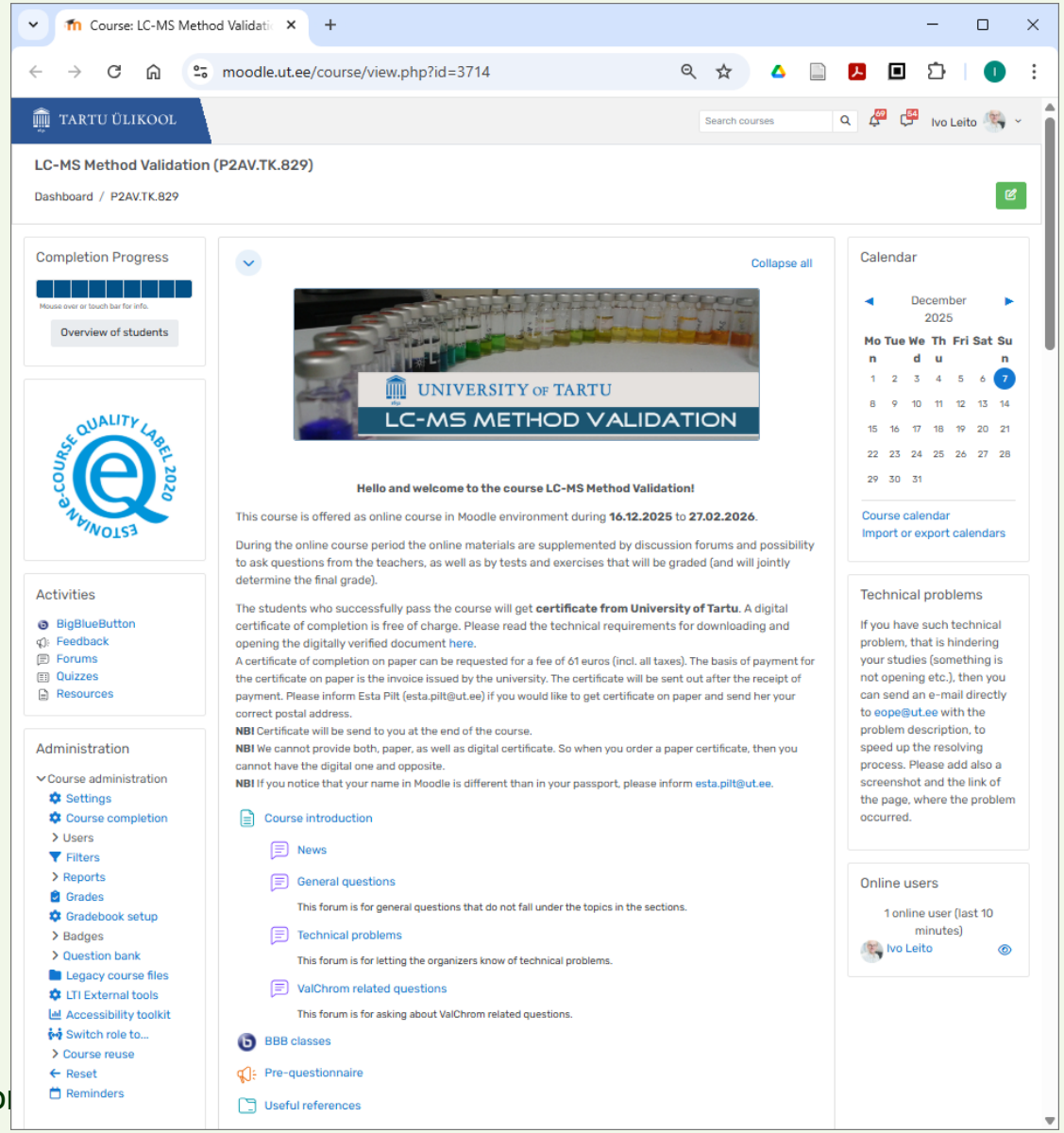
Features of the web course

- Two ways of learning:
  - **Independently**: Materials are freely available 24/7 at sisu.ut.ee/lcms_method_validation/
  - As **registered participant**:
    - Once a year (usually Nov-Feb)
    - Participation is free of charge

Way of working and support offered for registered participants

Web course

- **Moodle** platform
  - Progress monitoring
  - Graded tests
    - basis for issuing **course completion certificate**
  - Forums
    - **Ask questions** from teachers
- ValChrom
  - Online **validation software**



Web course

LC-MS Validation course completion statistics

LC-MS Method Validation	2017	2018	2019	2020	2021	2022	2023	2024	2025	Total
Registered participants	303	424	426	515	791	850	903	1014	1029	6255
Successfully finished	168	159	125	161	221	209	218	311	299	1871
Active participants	224	236	227	267	338	380	376	508	509	3065
Successfully finished %	55%	38%	29%	31%	28%	25%	24%	31%	29%	30%
Participated %	74%	56%	53%	52%	43%	45%	42%	50%	49%	49%
Successfully finished % (active participants)	75%	67%	55%	60%	65%	55%	58%	61%	59%	61%
Number of countries	61	71	70	77	86	97	104	109	99	137

Next edition: will take place Dec 16, 2025 – Feb 27, 2026

(right now: 800+ registered)

Registration is open until 15.12.25 at: **sisu.ut.ee/lcms_method_validation/**

What practitioners need?

Data sources and analysis

- **Sources:**
  - 2640 Forum posts from 2016 to 2024
    - Both participants and teachers
  - 336 submissions of course feedback from 2016 to 2024
  - Living memory of teachers

- **Analysis:**
  - Forum posts:
    - AI-powered classification and grouping
    - Word and phrase counting
    - Defining categories and sorting
  - Feedback:
    - Analysis of feedback by human

Forums: Caveats 1 - 3

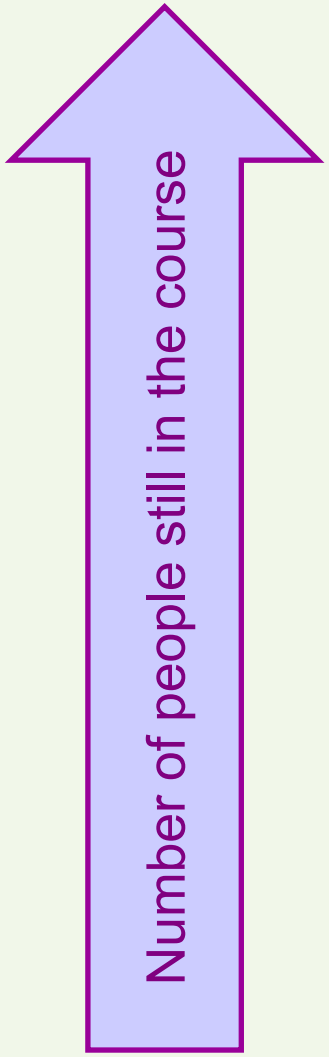
- **The AI-based analysis is crude**
  - AI's limited abilities
  - Out of the 2640 posts only 986 are reasonably grouped
    - A lot of those that were left out are organisational („where is my certificate?“, „could we prolong the course?“, „I got it now, thank you!“...)
- **Some words and phrases are by nature more frequent**
  - The word „linear“ is also related with LoD
  - It is easier to write „LoD“ than „matrix effect“
- **The groups are not orthogonal**
  - The same post can address several topics
    - E.g. calculating LoD and using Excel for doing that

What practitioners need?

Forum posts

Caveat 4

- **Order of topics in the course:**
  - General
  - Selectivity, identity
  - Linearity, linear range, sensitivity
  - Precision
  - Trueness
  - Precision, trueness, accuracy
  - Stability
  - LoD, LoQ
  - Ruggedness, robustness

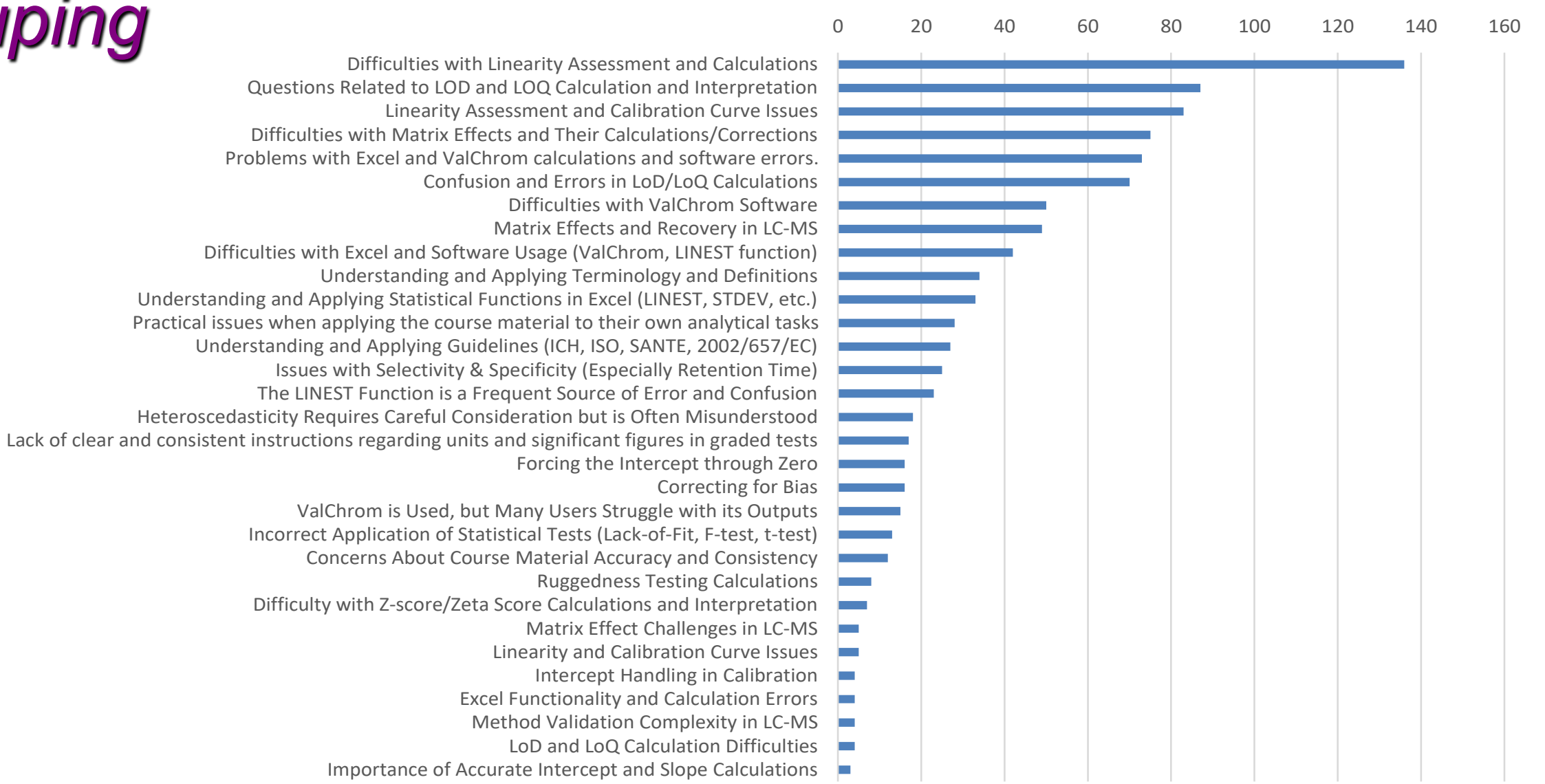


This **does influence** the relative abundance of the topics in **posts** should take this into account

(This **does not** influence the relative abundance of topics in **course feedback**)

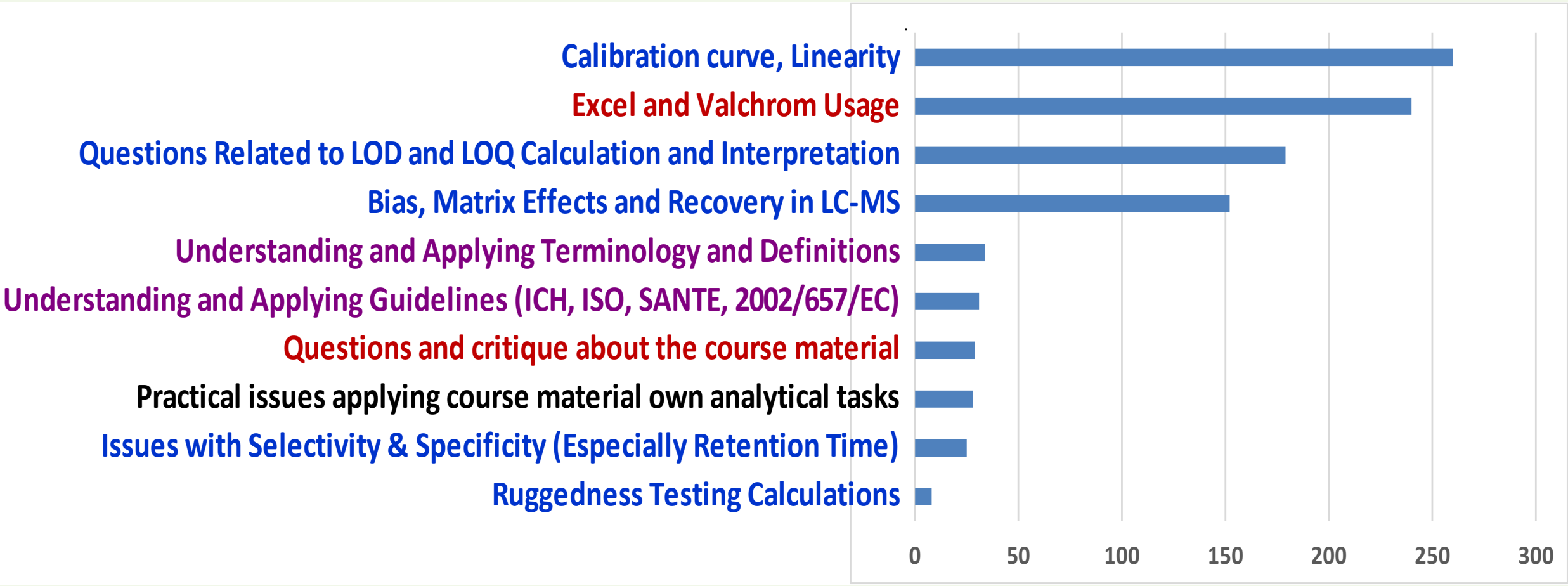
Forum posts: initial AI-generated grouping

What practitioners need?



Forum posts: groups aggregated topic-wise by human

Forum posts



Forum posts: word/phrase counts

LoD, LoQ, CCα, CCβ, detection limit

linearity, linear, sensitivity, residuals

trueness, accuracy, bias, matrix effect, recovery

precision, standard deviation, repeatability

selectivity, identity, identification

uncertainty, uncertainties, error

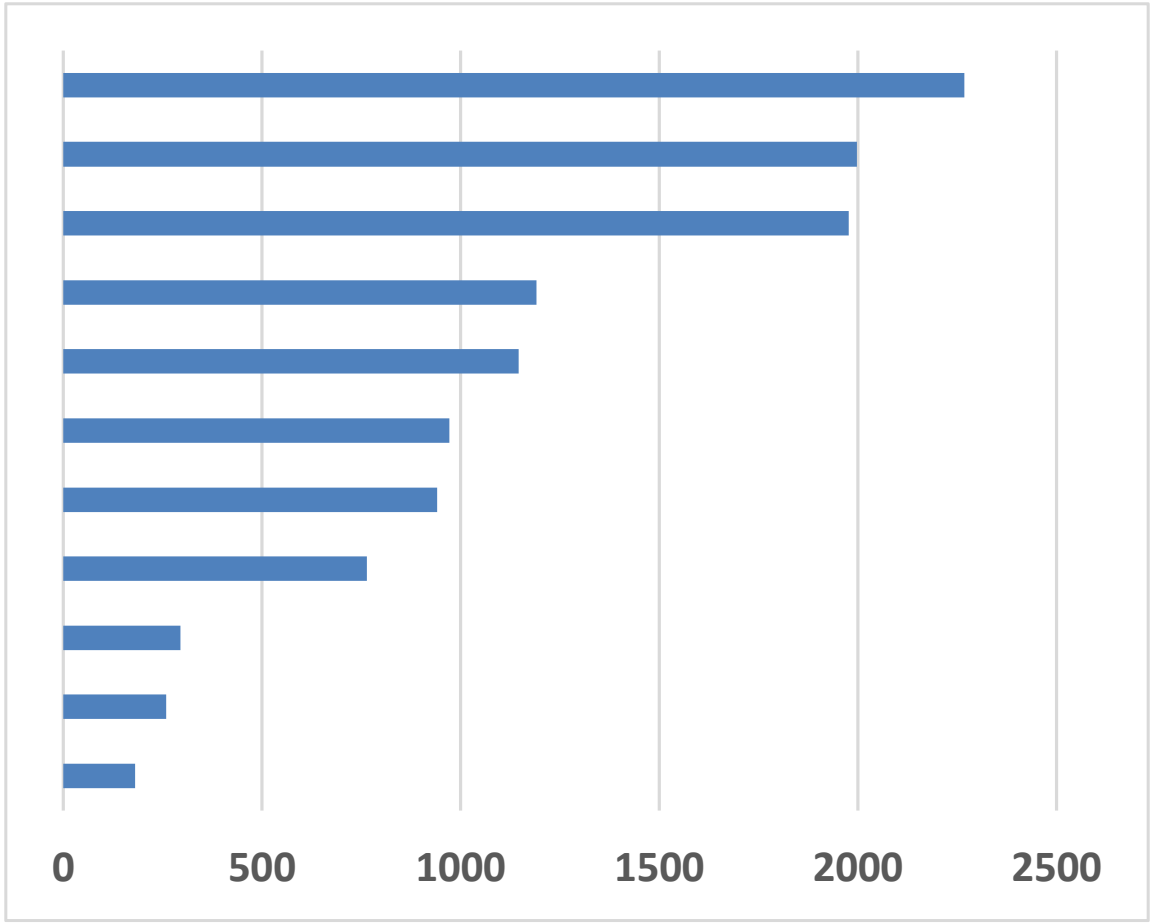
ValChrom, Excel, software, spreadsheet

calibration

guidelines, 2002/657/EC, sante, ICH, FDA, EMA

stability

ruggedness, robustness

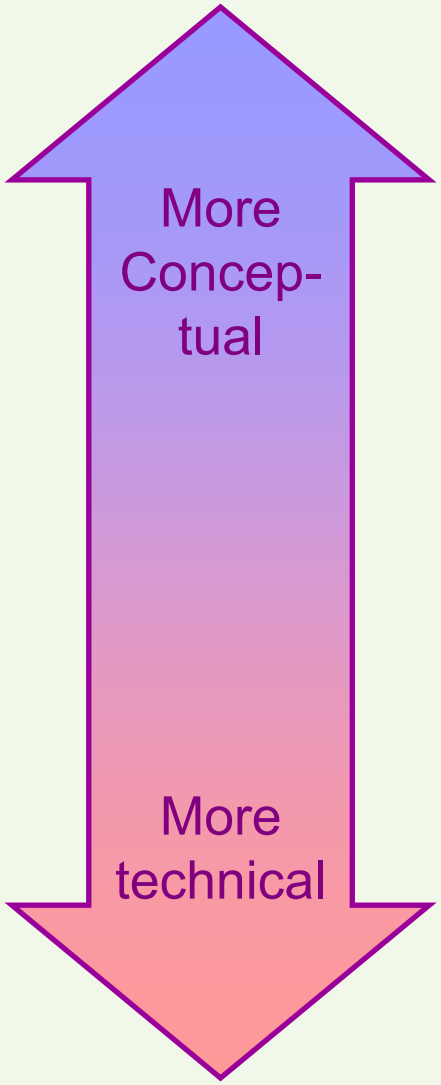


Issues: technical or conceptual?

What practitioners need?

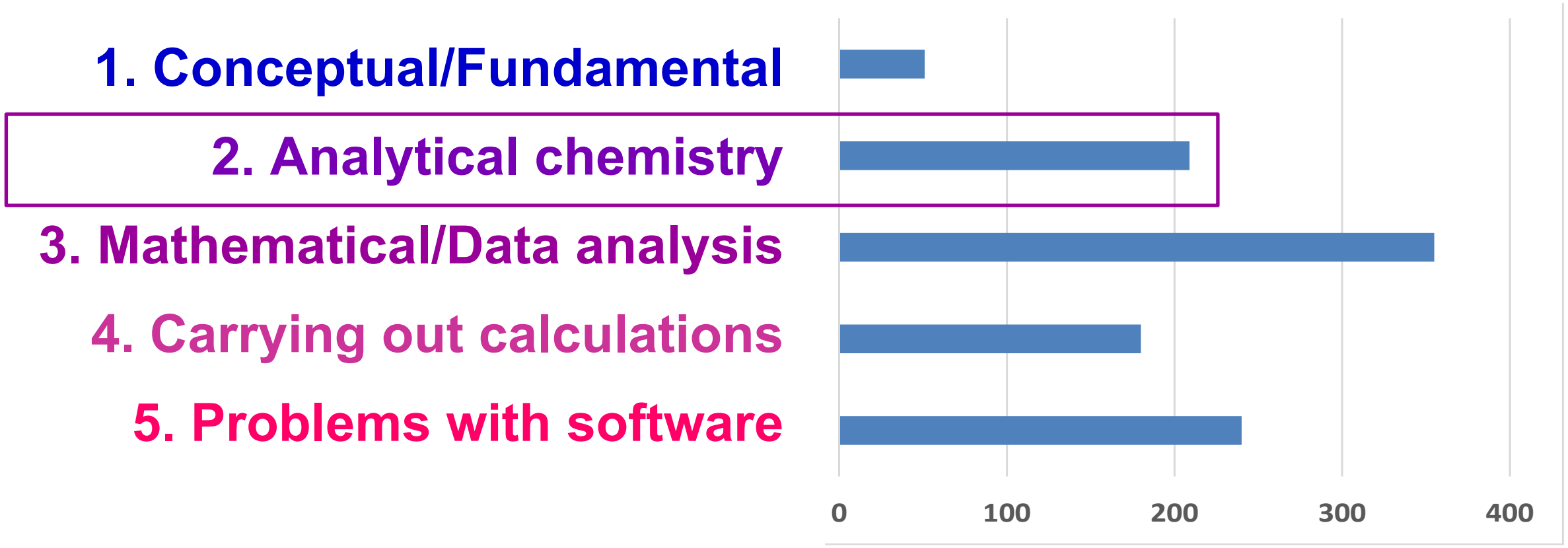
Forum posts

1. **Conceptual/Fundamental**
   - Reference value vs true value, fundamental meaning of some characteristic...
2. **Analytical chemistry**
   - MS and LC related issues, Finding suitable control sample, Ensuring matrix match, Choosing internal standard, ...
   - Also interpreting the requirements of guidelines
3. **Mathematical/Data analysis**
   - Number of calibration levels, R^2 as linearity indicator, what is acceptable linearity, dilution plots in matrix effect calculations...
4. **Carrying out calculations**
   - Which equation to use, suitability of data, correctness of equations, ...
5. **Problems with software**
   - „How do I use this function?“, „Why do I get this error?“, ...



Issues: technical or conceptual?

Forum posts



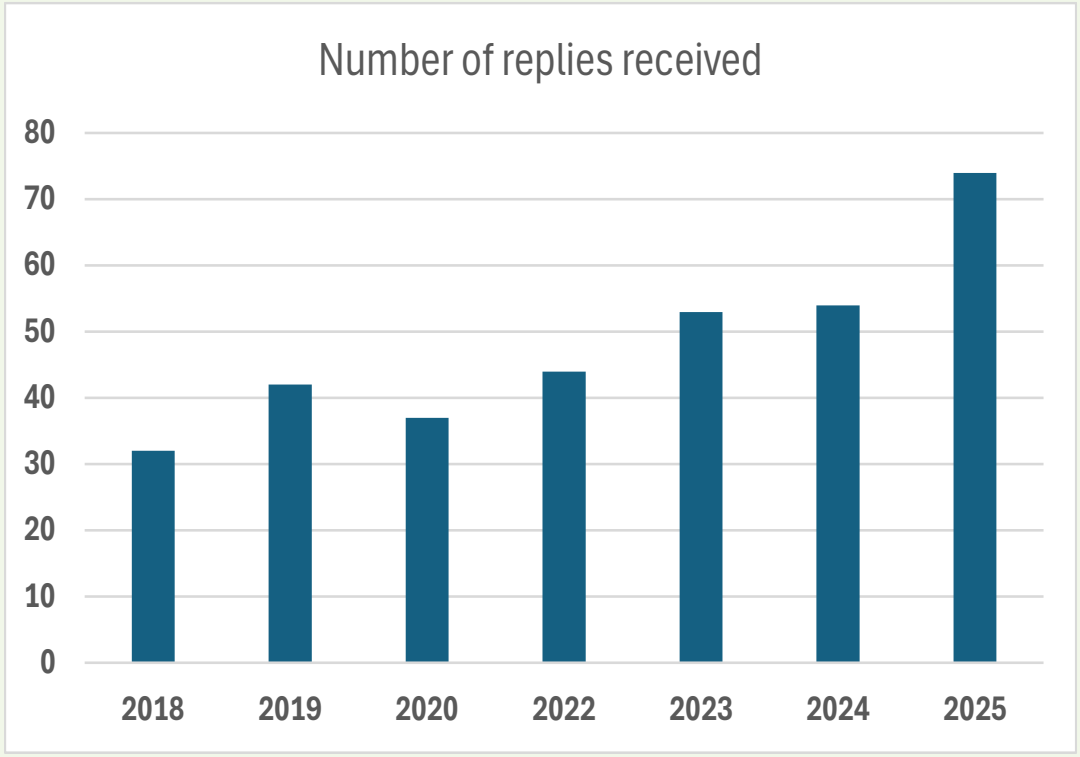
Course feedback: Questions

- What did you like most about this course?
- **What did you like the least about this course?**
- How did you like the structure of this course?
- How would you rate the course materials (videos, textual materials, self-tests)?
- Were the course materials (https://sisu.ut.ee/lcms_method_validation) were sufficient for successful passing of the course?
- Give your opinion about the self-tests and graded tests by marking the statements with which you agree.
- Was the explanatory feedback of the self-tests helpful?
- Was the information about the grading system sufficient?
- Were sufficient explanations about the organisation of the course given in the course?
- Was the support from the teachers during the course sufficient?
- **During the course, did you use ValChrom validation software?**
- **You are welcome to comment the previous answer.**
- **Does your institution use any validation software?**
- **Would your institution benefit from validation software (ValChrom or other)?**
- **Please add your comments on validation software (ValChrom or other).**
- Did you have technical problems during this course?
- Did you get new knowledge during this course?
- Do you think that the knowledge and skills obtained during the course will be helpful for you in your everyday work and/or future career?
- Did the course fulfill your expectations?
- Would you recommend this course to your friend or colleague?
- What was your final grade of the course?
- If it was F (i.e. you did not complete the course), then what was the reason for this?
- **What suggestions do you have for teachers and for possible changes in the course?**

Some questions have been omitted for clarity

Course feedback: statistics of replies

- **336 replies** analysed
  - 7 editions
- Overall reply rate: **13%**
  - Active participants only
- Mostly successful participants replied
  - Replies are positively biased



Recurring suggestions

What practitioners need?

Feedback

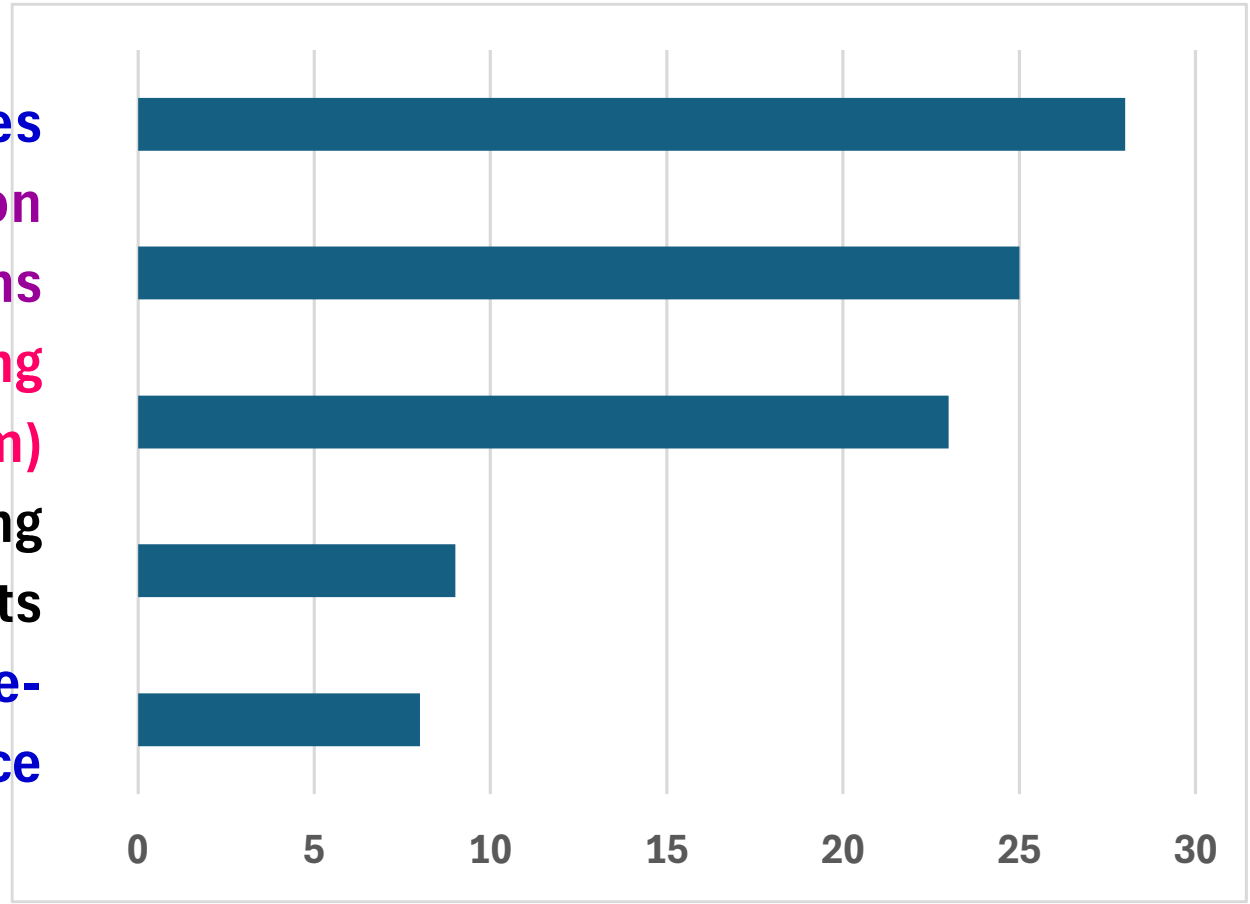
More real-life examples

More exercises, help and information on mathematics and calculations

More help with software (including ValChrom)

Live discussions with teachers and sharing experience between participants

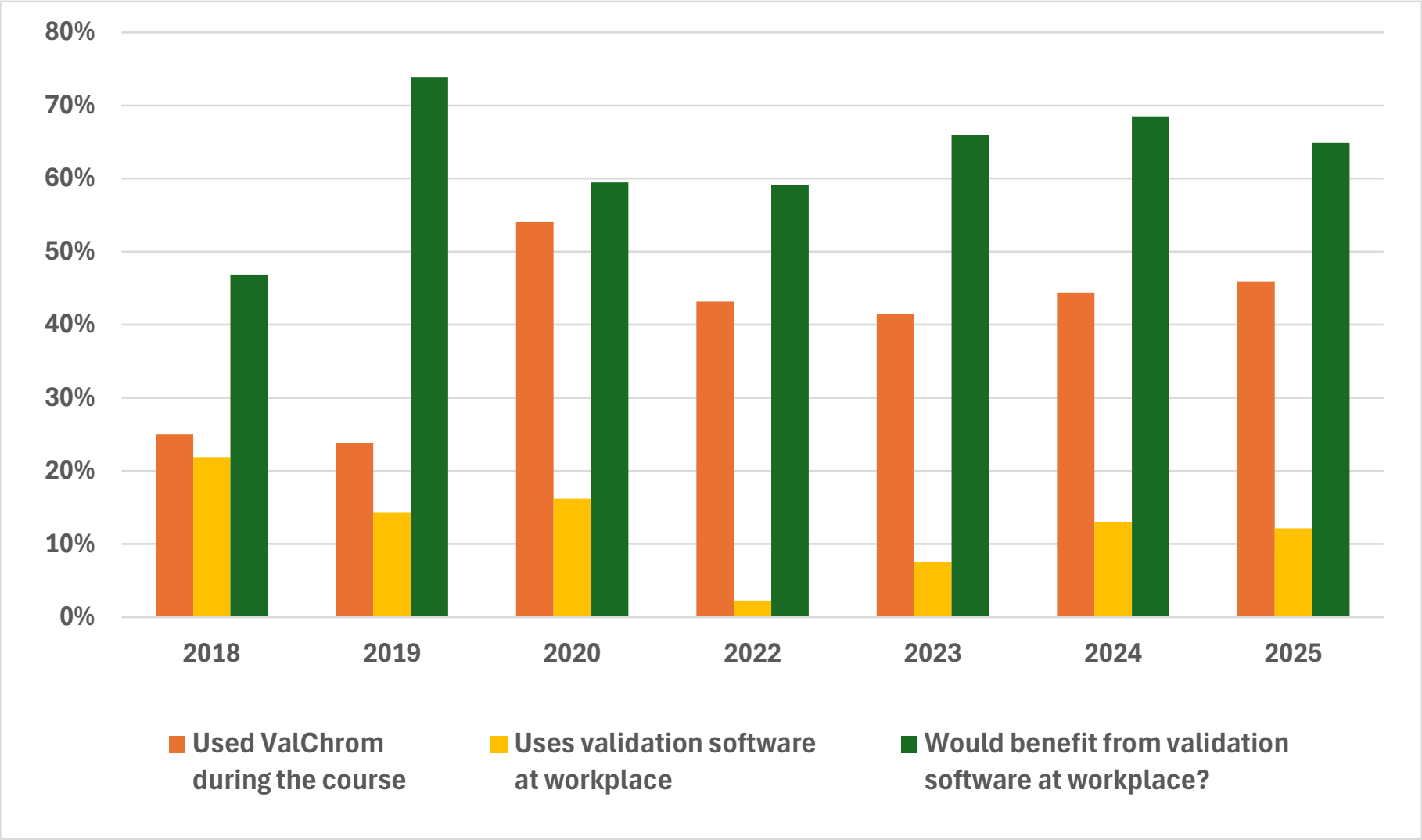
Keep up to date and improve guideline-related advice



Course-organisational suggestions have been omitted

(quality of presentations, language and audio, extent and speed of feedback, number of attempts in self-tests, length of course period, course certificate, etc)

Course feedback: Validation software



1. Practiotioners have highly practical issues and wishes

- Participants are most interested in
  - More **practical examples**
  - How to **calculate** specific characteristics
  - Everything related to **software**

- Technical questions dominate

2. Validation is often seen a “checklist of calculations“ exercise

- Many practitioners struggle with
  - formulas
  - spreadsheets

- **Mathematics** (as **opposed to chemistry**) tends to consume a lot of effort

- This takes away resource that otherwise could be used for „real chemistry“

3. There seems to be correlation between mathematical complexity and degree of attention

- Receive **most** attention:
  - LoD/CCβ, CCα, LoQ, linearity
  - also matrix effects

- Receive **less** attention:
  - Selectivity, precision

- Receives **surprisingly little** attention:
  - Ruggedness/robustness

4. Dedicated validation software is not much used but is seen as a big hope

- **Software with step-by-step guidance** is most appreciated
- Important: the software has to be user-friendly
  - ValChrom was both criticised and praised

Big thanks to the course team!

Core team

Koit Herodes, Irja Helm, Riin Rebane,
Maarja-Liisa Oldekop, Asko Laaniste
Karin Kipper, Hanno Evard, Anneli Kruve

Video, web design, IT, admin

Enno Kaasik, Triin Marandi, Lehti Pilt,
Esta Pilt, Toomas Petersell

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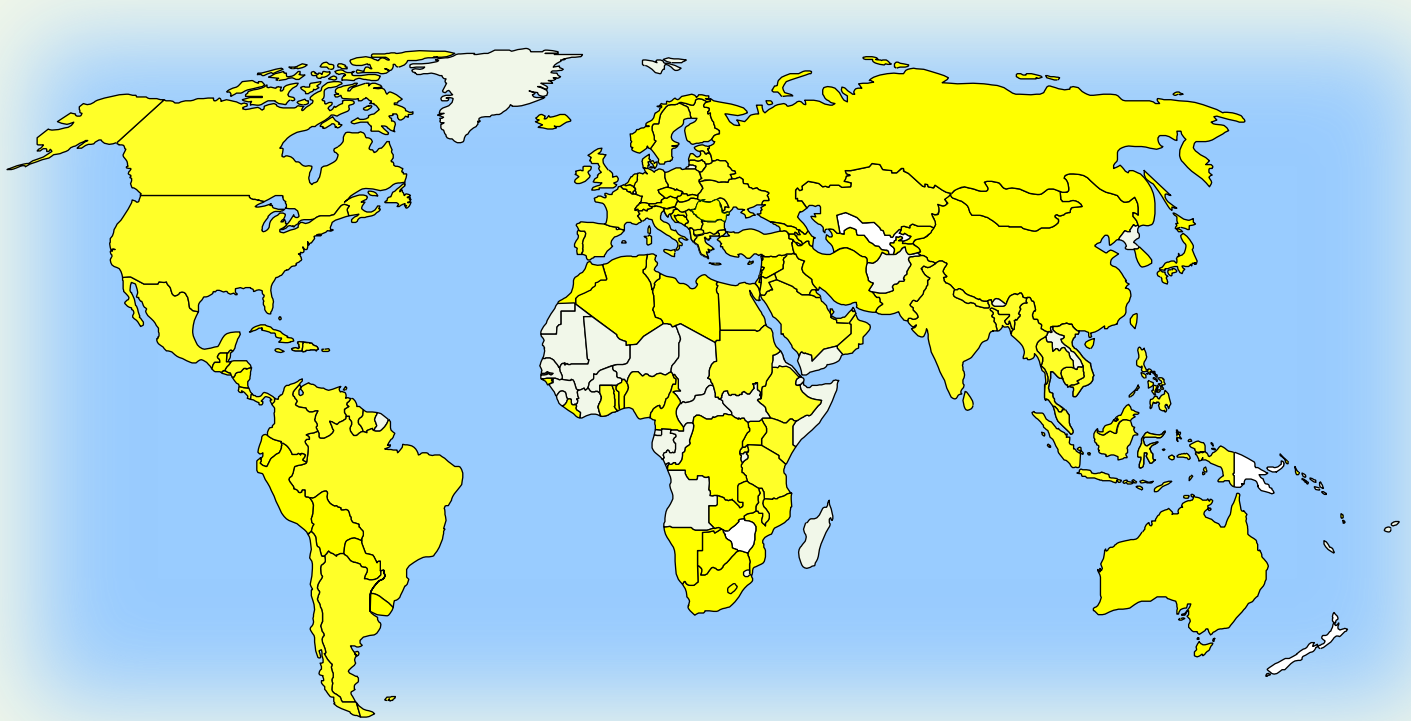
(Estonian Center of Analytical Chemistry, TARISTU24-TK15, https://www.akki.ee)

Many thanks for your attention!

Top countries by number of registered participants:

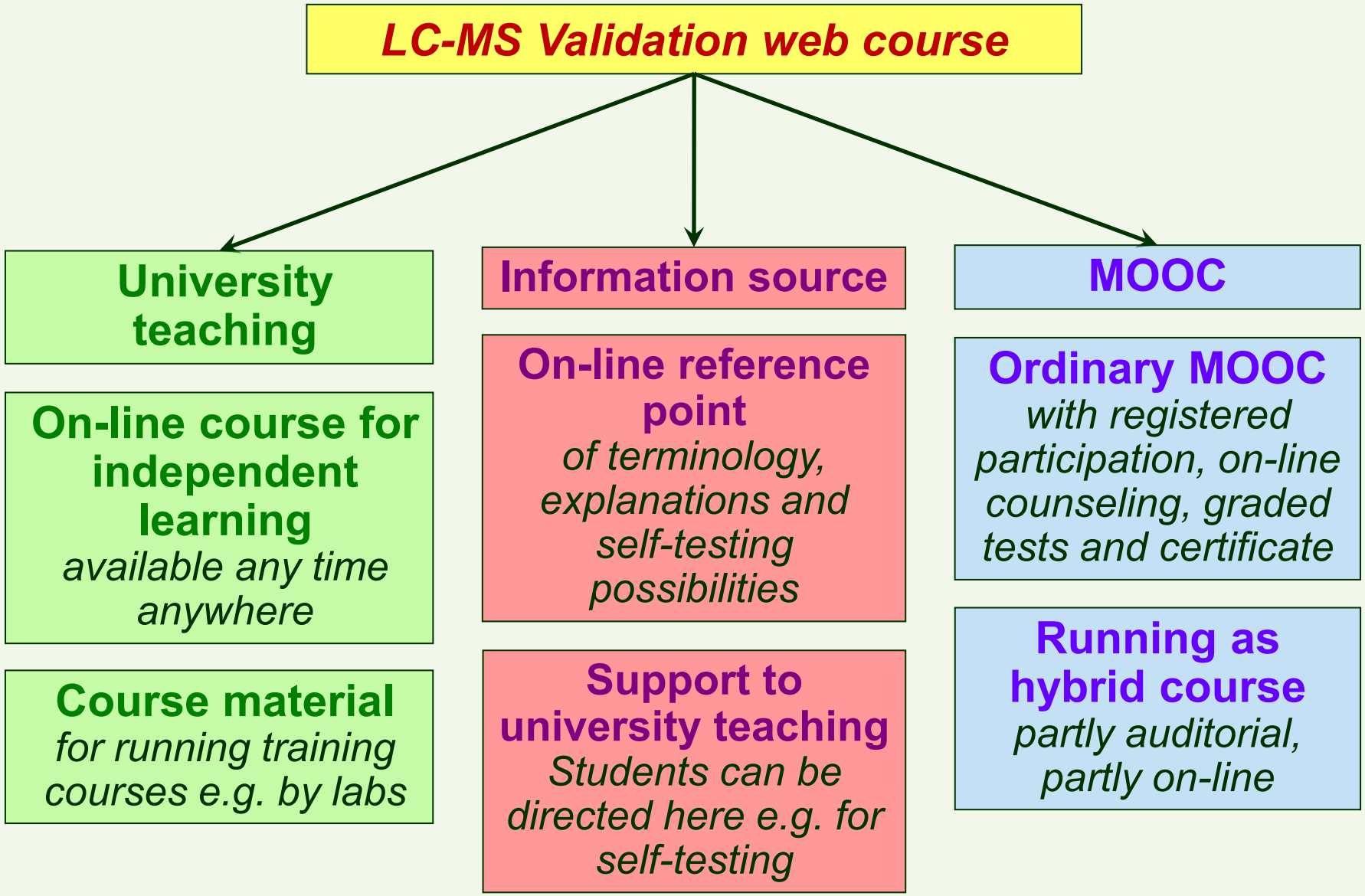
Philippines	565
Poland	404
Brasil	349
Estonia	347
Egypt	312
Spain	190
India	185
Costa Rica	161
Serbia	135
Columbia	124
…	
Italy	54



137 countries overall

MOOCs vs „traditional“ teaching

Aspect	Conventional university course	Practitioner training (short) course	MOOC
Interaction between students and teachers	Direct	Direct	Remote
Possibility to deliver the course simultaneously to many participants	Low	Low	High
Level of self-discipline needed from participants	Average	Average	High
Time constraints, time to “digest” the knowledge	Not a problem	Serious time constraints	Not a problem
Possibility of independent homework	Possible	Usually impossible	Possible
Possibility of hands-on problem-solving	Possible	Possible (within the time constraints)	Possible
Possibility of teamwork	Possible	Possible (within the time constraints)	Not easy
Possibility of experimental work	Easy	Possible, but not easy	Not possible
Possibility of working with participants of uneven level or preparation	Difficult but doable	Difficult	Possible
Possibilities of meaningful assessment of obtained knowledge	Wide possibilities	Difficult	Possible
Danger of cheating during knowledge assessment	Can be made low	Can be made low	Can be high
Costs of setting up the course[a]	Medium	Medium	Medium
Costs of running the course[a]	High	High	Low
Travel and accommodation costs	Can be high	Can be high	None



I. Leito, I. Helm, L. Jalukse. Using MOOCs for teaching analytical chemistry: experience at University of Tartu. *Anal. Bioanal. Chem*. **2015**, *407*, 1277–1281