

## LC-MS Method Validation On-line course

[https://sisu.ut.ee/lcms\\_method\\_validation/](https://sisu.ut.ee/lcms_method_validation/)



# What are the issues encountered in the real world?

## Lessons learned from an online validation course



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University of Tartu

[sisu.ut.ee/lcms\\_method\\_validation/](https://sisu.ut.ee/lcms_method_validation/)

**VALIDATION OF LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC-MS) METHODS**

This course is offered as a MOOC (Massive Open Online Course) during December 16, 2025 - February 27, 2026. The course is open for registration. To register, please visit the [course registration page](#).

**LC-MS Method Validation**

**Introduction**

**Course introduction**  
<http://www.utv.ee/naita?id=23245>  
<https://www.youtube.com/watch?v=jbdABPhPdLY>

**SHORT DESCRIPTION OF THE COURSE**

This course – *LC-MS Method Validation* – is a practice-oriented on-line course on validation of analytical methods, specifically using LC-MS as technique. The course introduces the main concepts and mathematical apparatus of validation, covers the most important method performance parameters and ways of estimating them. The course is largely based on the published two-part tutorial review:

- Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part I. A. Kruse, R. Rebane, K. Kipper, M.-L. Oidekop, H. Evard, K. Herodes, P. Raviio, I. Leito. *Anal. Chim. Acta* **2015**, 870, 29-44
- Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part II. A. Kruse, R. Rebane, K. Kipper, M.-L. Oidekop, H. Evard, K. Herodes, P. Raviio, I. Leito. *Anal. Chim. Acta* **2015**, 870, 8-28

# Outline

- Overview of the **LC-MS Method Validation web course**
  - [https://sisu.ut.ee/lcms\\_method\\_validation/](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** Forum posts
    - **336 participant feedback submissions** Feedback
    - Living memory of teachers
- 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?

1  
2  
3  
4  
5  
6  
7  
8  
9  
10

**Guidelines?**

**Guidelines for the Validation of Analytical Methods**  
 Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002.  
 © 2002 IUPAC  
 INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY  
 ANALYTICAL, APPLIED, CLINICAL, INORGANIC, AND  
 PHYSICAL CHEMISTRY DIVISIONS  
 INTERDIVISIONAL WORKING PARTY FOR HARMONIZATION OF  
 QUALITY ASSURANCE SCHEMES FOR ANALYTICAL LABORATORIES<sup>a</sup>  
**HARMONIZED GUIDELINES FOR SINGLE-  
 LABORATORY VALIDATION OF METHODS OF  
 ANALYSIS**  
**(IUPAC Technical Report)**  
 Resulting from the Symposium on Harmonization of Quality Assurance Systems for Analytical  
 Laboratories, Budapest, Hungary, 4–5 November 1999, held under the sponsorship of IUPAC, ISO,  
 and AOAC International  
 Prepared for publication by  
 MICHAEL THOMPSON<sup>1</sup>, STEPHEN L. R. ELLISON<sup>2</sup>, AND ROGER WOOD<sup>3,4</sup>  
<sup>1</sup>Department of Chemistry, Birkbeck College (University of London), London WC1H 0PP, UK;  
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<sup>3</sup>Agency, c/o Institute of Food Research, Norwich Research Park, Colney, Norwich,  
 NR4 7UA, UK  
<sup>4</sup>1997–2000 was as follows:  
 Members: K. Berglund (Norway); Carmen Casteja (Spain);  
 J. H. J. van den Hul (Netherlands); S. Coates (AOAC Int., USA); T. De Bievre  
 (Honey (Hungary); D. G. Holcombe (UK); P. T. Holland (New  
 Zealand); K. Krich (Germany); E. A. Mair (Belgium); C. Niess De  
 Vries (Belgium); M. Thompson (UK); M. J. Vermeir

**Guideline in Validation of  
 Alternative Proprietary Chemical Methods**  
 NordVal Protocol Document 9  
 NordVal Protocol No. 2, Approved 26 May 2010  
 Contents  
 DEFINITIONS ..... 2  
 PART 1: VALIDATION AND EVALUATION OF QUALITATIVE PROPRIETARY  
 METHODS ..... 5  
 A. METHOD COMPARISON STUDY ..... 5  
 B. INTERMEDIATE STUDY ..... 9  
 C. INTERPRETATION ..... 9  
 PART 2: VALIDATION AND EVALUATION OF QUANTITATIVE PROPRIETARY  
 METHODS ..... 10  
 A. METHOD COMPARISON STUDY ..... 10  
 B. INTERMEDIATE STUDY ..... 14  
 REFERENCES ..... 15

**The Fitness for Purpose of  
 Analytical Methods**  
 A Laboratory Guide to Method Validation and Related Topics  
 Eurachem  
 A focus for analytical chemistry in Europe  
 Second

**Guidance for Industry  
 Bioanalytical Method Validation**  
 Additional copies are available from:  
 Office of Communications  
 Division of Drug Information, FDA, Room 2201  
 Center for Drug Evaluation and Research  
 10903 New Hampshire Ave., Silver Spring, MD 20993  
 Phone: 301-796-3400, Fax: 301-847-3714  
[druginfo@fda.gov](mailto:druginfo@fda.gov)  
<http://www.fda.gov/Drugs/Guidance/Compliance/Informational/GuidanceforIndustry.htm>  
 and/or  
 Communications Staff, HFT-11  
 Center for Veterinary Medicine  
 Food and Drug Administration  
 7519 Standish Place, Rockville, MD 20855  
 (703) 240-2760/2760  
<http://www.fda.gov/CDER/CenterforVeterinaryMedicine/Informational/GuidanceforIndustry.htm>  
 U.S. Department of Health and Human Services  
 Food and Drug Administration  
 Center for Drug Evaluation and Research (CDER)  
 Center for Veterinary Medicine (CVM)  
 September 2013  
 Biopharmaceutics  
 Revision 1

**ICH HARMONISED TRIPARTITE GUIDELINE  
 REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN  
 USE**  
**ICH HARMONISED TRIPARTITE GUIDELINE  
 VALIDATION OF ANALYTICAL PROCEDURES:  
 TEXT AND METHODOLOGY  
 Q2(R1)**  
 Current Step 4 version  
 Parent Guideline dated 27 October 1994  
 Parent Guideline on Methodology dated 6 November 1996  
 (Complementary Guideline on Methodology incorporated in November 2005)  
 This Guideline has been developed by the appropriate ICH Expert Working Group and  
 has been subject to consultation by the regulatory parties, in accordance with the ICH  
 Process. At Step 4 of the Process the final draft is recommended for adoption to the  
 regulatory bodies of the European Union, Japan and USA.

**COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE  
 (CHMP)**  
**DRAFT**  
**GUIDELINE ON VALIDATION OF BIOANALYTICAL METHODS**  
 September 2009  
 19 November 2009  
 31 May 2010  
**DRAFT AGREED BY THE EFFICACY WORKING PARTY**  
**ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION**  
**END OF CONSULTATION (DEADLINE FOR COMMENTS)**  
 Comments should be provided using the template to [EDP@ema.europa.eu](mailto:EDP@ema.europa.eu)  
**KEYWORDS** CHMP, EMA, Guideline, validation, bioanalytical method, analyses

11.12.2025

workshop on LC-MS metho

# *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

Validation of liquid chromatography x +

sisu.ut.ee/lcms\_method\_validation/

## VALIDATION OF LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC-MS) METHODS

Course introduction

1. Validation: General
2. Selectivity and identity confirmation
3. Linearity of signal, linear range, sensitivity
4. Precision
5. Trueness
6. Precision and trueness: some additional aspects
7. Accuracy
8. Stability
9. LoD and LoQ
10. Ruggedness, robustness
11. Reporting

Acknowledgements

Questions and answers about precision, trueness and accuracy topics

References

Glossary

What our participants say?

This course is offered as a MOOC (Massive Open Online Course) during December 16, 2025 – February 27, 2026.  
The course is open for registration. To register, please visit the course registration page.

**LC-MS Method Validation**

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<https://www.youtube.com/watch?v=jbdA8PnPdLY>

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- Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II. K. Herodes, P. Ravo, I. Leito. *Anal. Chim. Acta* **2015**, 870, 8–28

[sisu.ut.ee/lcms\\_method\\_validation/](http://sisu.ut.ee/lcms_method_validation/)

# *Features of the web course*

[sisu.ut.ee/lcms\\_method\\_validation/](https://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<sup>n</sup> 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/](https://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

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



Course: LC-MS Method Validation

moodle.ut.ee/course/view.php?id=3714

TARTU ÜLIKOOL

LC-MS Method Validation (P2AV.TK.829)

Dashboard / P2AV.TK.829

Completion Progress

Overview of students

ESTONIAN COURSE QUALITY LABEL 2020

Activities

- BigBlueButton
- Feedback
- Forums
- Quizzes
- Resources

Administration

- Course administration
  - Settings
  - Course completion
  - Users
  - Filters
  - Reports
  - Grades
  - Gradebook setup
  - Badges
  - Question bank
  - Legacy course files
  - LTI External tools
  - Accessibility toolkit
  - Switch role to...
  - Course reuse
  - Reset
  - Reminders

Calendar

December 2025

Mo Tu We Th Fri Sat Su

1 2 3 4 5 6 7

8 9 10 11 12 13 14

15 16 17 18 19 20 21

22 23 24 25 26 27 28

29 30 31

Course calendar

Import or export calendars

Technical problems

If you have such technical problem, that is hindering your studies (something is not opening etc.), then you can send an e-mail directly to [eoqe@ut.ee](mailto:eoqe@ut.ee) with the problem description, to speed up the resolving process. Please add also a screenshot and the link of the page, where the problem occurred.

Online users

1 online user (last 10 minutes)

Ivo Leito

Hello and welcome to the course LC-MS Method Validation!

This course is offered as online course in Moodle environment during **16.12.2025 to 27.02.2026**.

During the online course period the online materials are supplemented by discussion forums and possibility to ask questions from the teachers, as well as by tests and exercises that will be graded (and will jointly determine the final grade).

The students who successfully pass the course will get **certificate from University of Tartu**. A digital certificate of completion is free of charge. Please read the technical requirements for downloading and opening the digitally verified document [here](#).

A certificate of completion on paper can be requested for a fee of 61 euros (incl. all taxes). The basis of payment for the certificate on paper is the invoice issued by the university. The certificate will be sent out after the receipt of payment. Please inform Esta Pilt ([esta.pilt@ut.ee](mailto:esta.pilt@ut.ee)) if you would like to get certificate on paper and send her your correct postal address.

**NBI** Certificate will be send to you at the end of the course.

**NBI** We cannot provide both, paper, as well as digital certificate. So when you order a paper certificate, then you cannot have the digital one and opposite.

**NBI** If you notice that your name in Moodle is different than in your passport, please inform [esta.pilt@ut.ee](mailto:esta.pilt@ut.ee).

Course introduction

News

General questions

This forum is for general questions that do not fall under the topics in the sections.

Technical problems

This forum is for letting the organizers know of technical problems.

ValChrom related questions

This forum is for asking about ValChrom related questions.

BBB classes

Pre-questionnaire

Useful references

# 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/](https://sisu.ut.ee/lcms_method_validation/)**

# *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

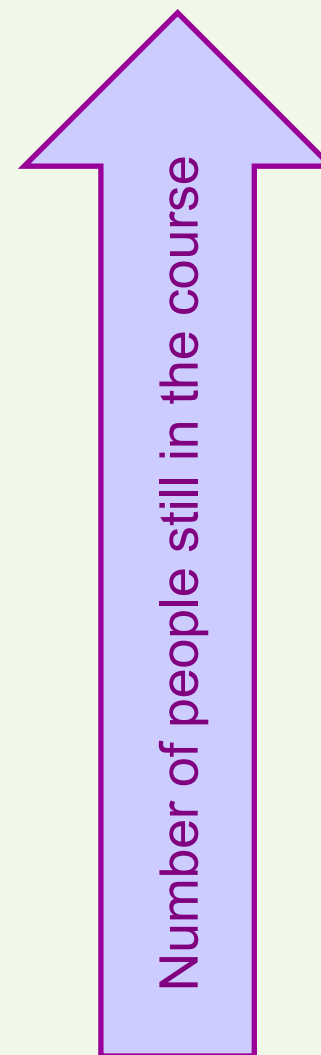
# Forums: Caveats 1 - 3

- **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



# 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



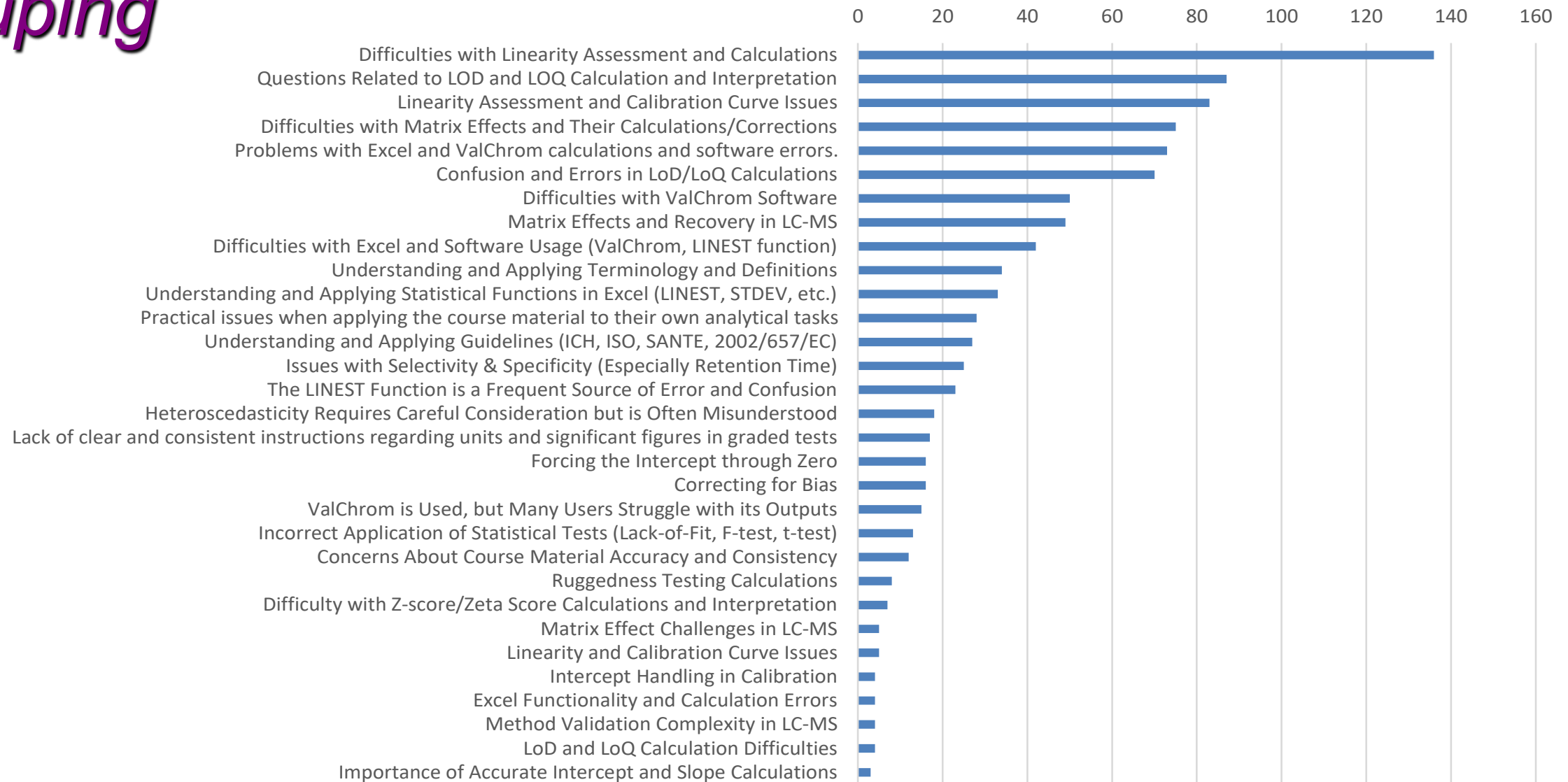
This **does influence** the relative abundance of the topics in **posts** should take this into account

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

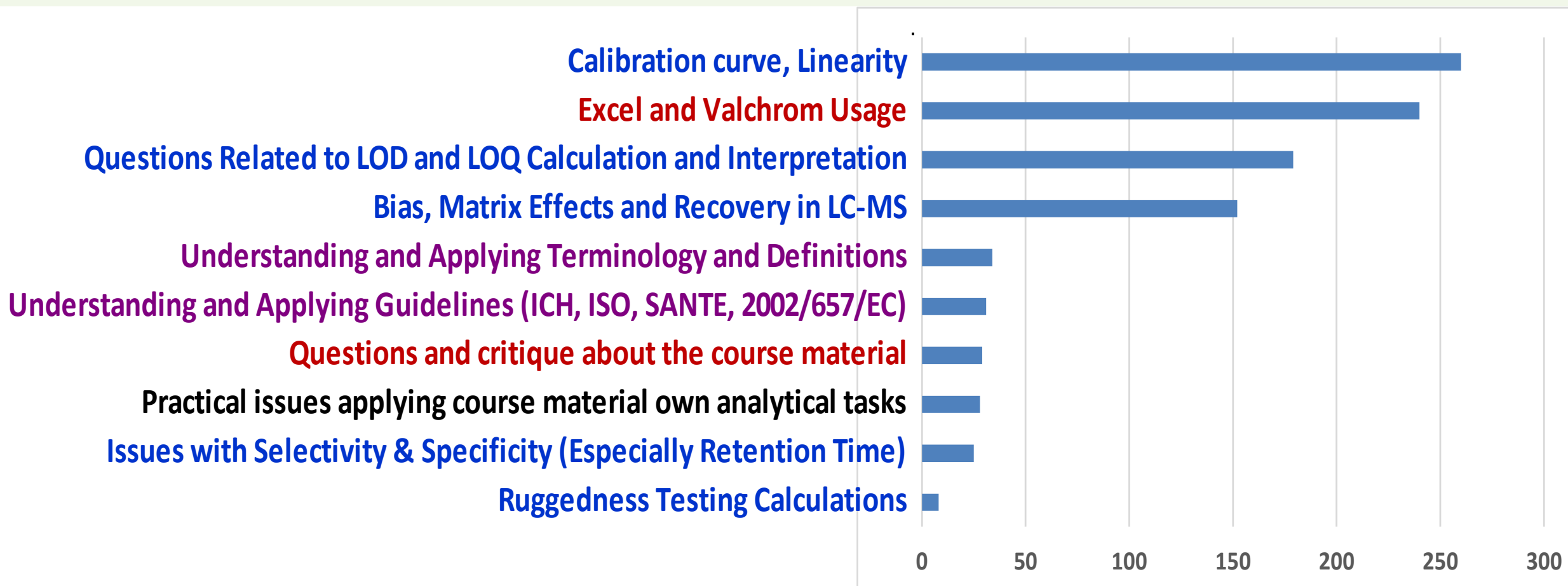
# Forum posts: initial AI-generated grouping

What practitioners need?

Forum posts

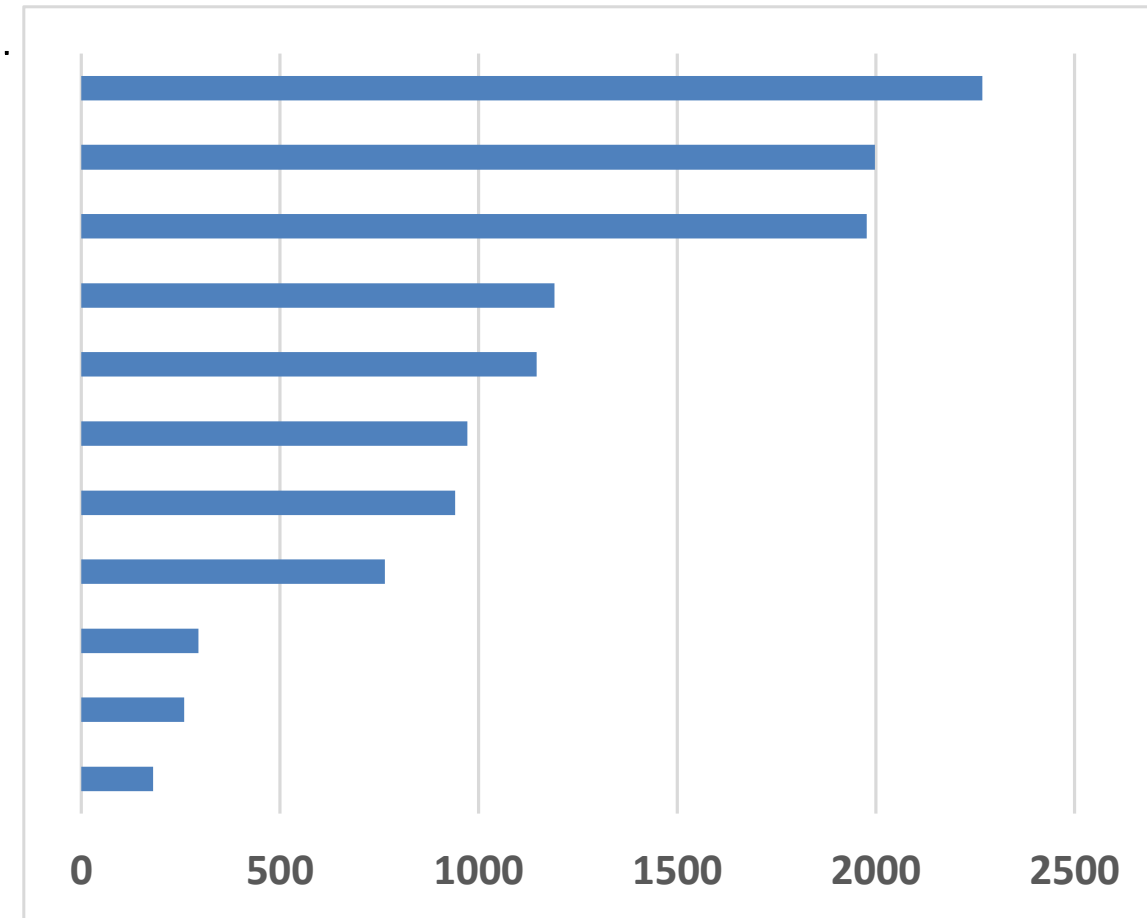


# Forum posts: groups aggregated topic-wise by human



# Forum posts: word/phrase counts

LoD, LoQ, CC $\alpha$ , CC $\beta$ , 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?

## 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

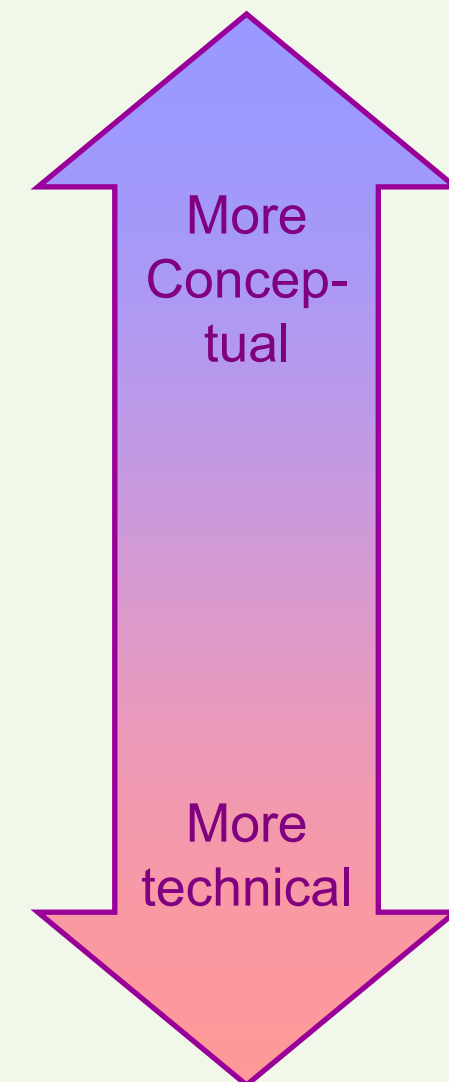
- Number of calibration levels,  $R^2$  as linearity indicator, what is acceptable linearity, dilution plots in matrix effect calculations...

## 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?*

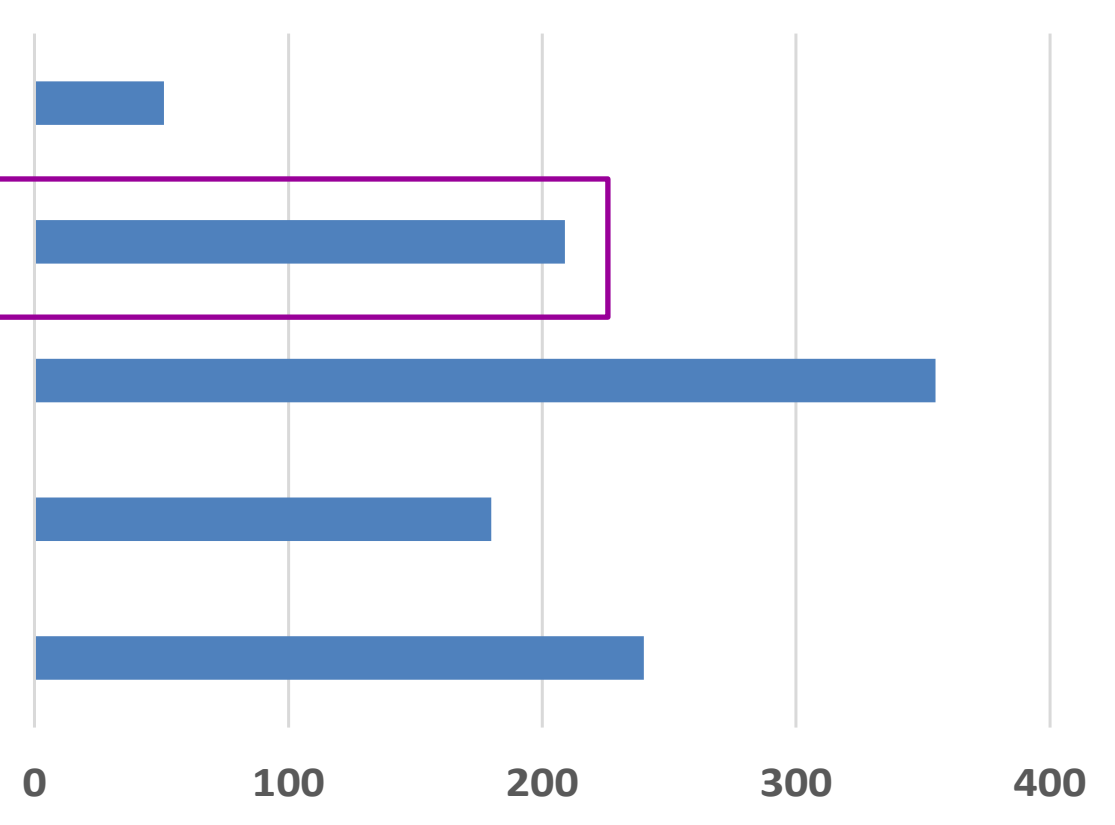
**1. Conceptual/Fundamental**

**2. Analytical chemistry**

**3. Mathematical/Data analysis**

**4. Carrying out calculations**

**5. Problems with software**



# Course feedback: Questions

What practitioners need?

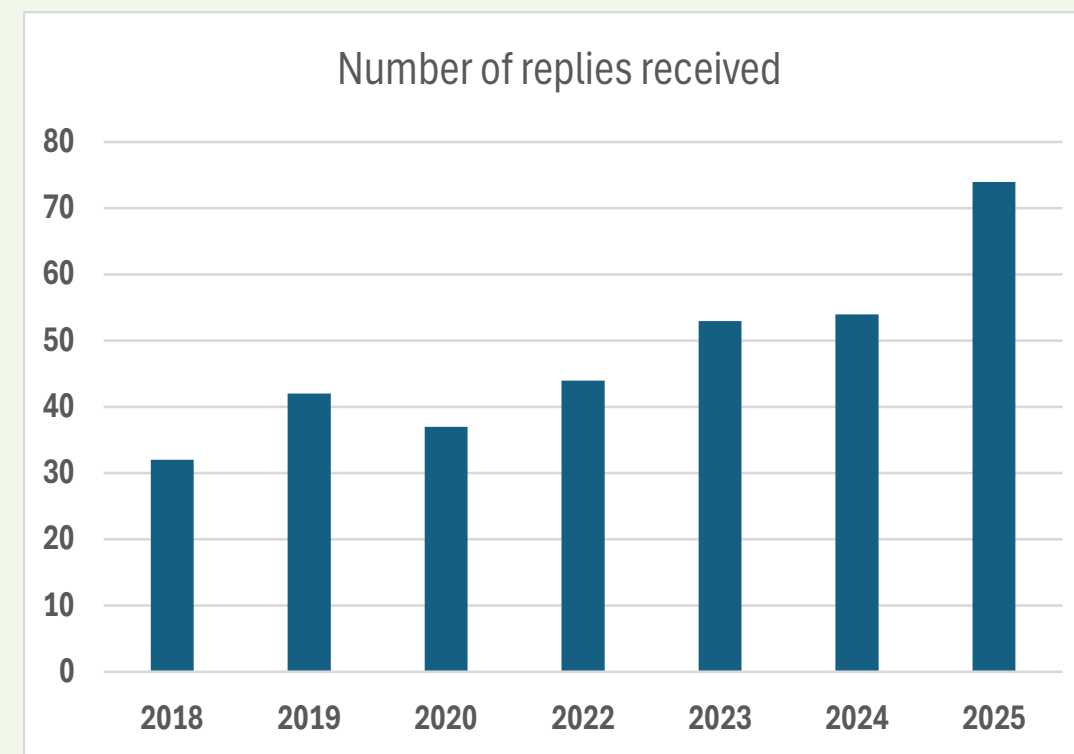
Feedback

- 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](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

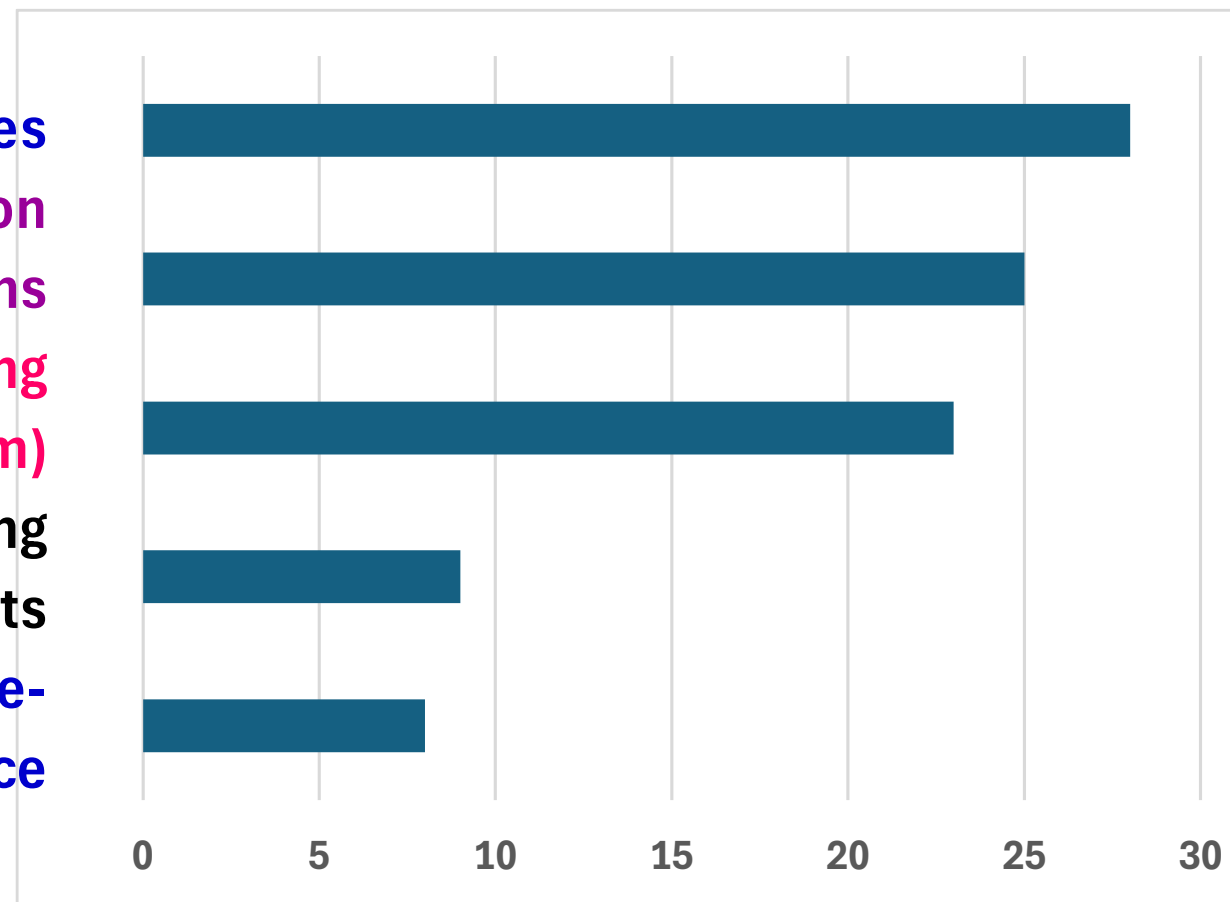


# Recurring suggestions

What practitioners need?

Feedback

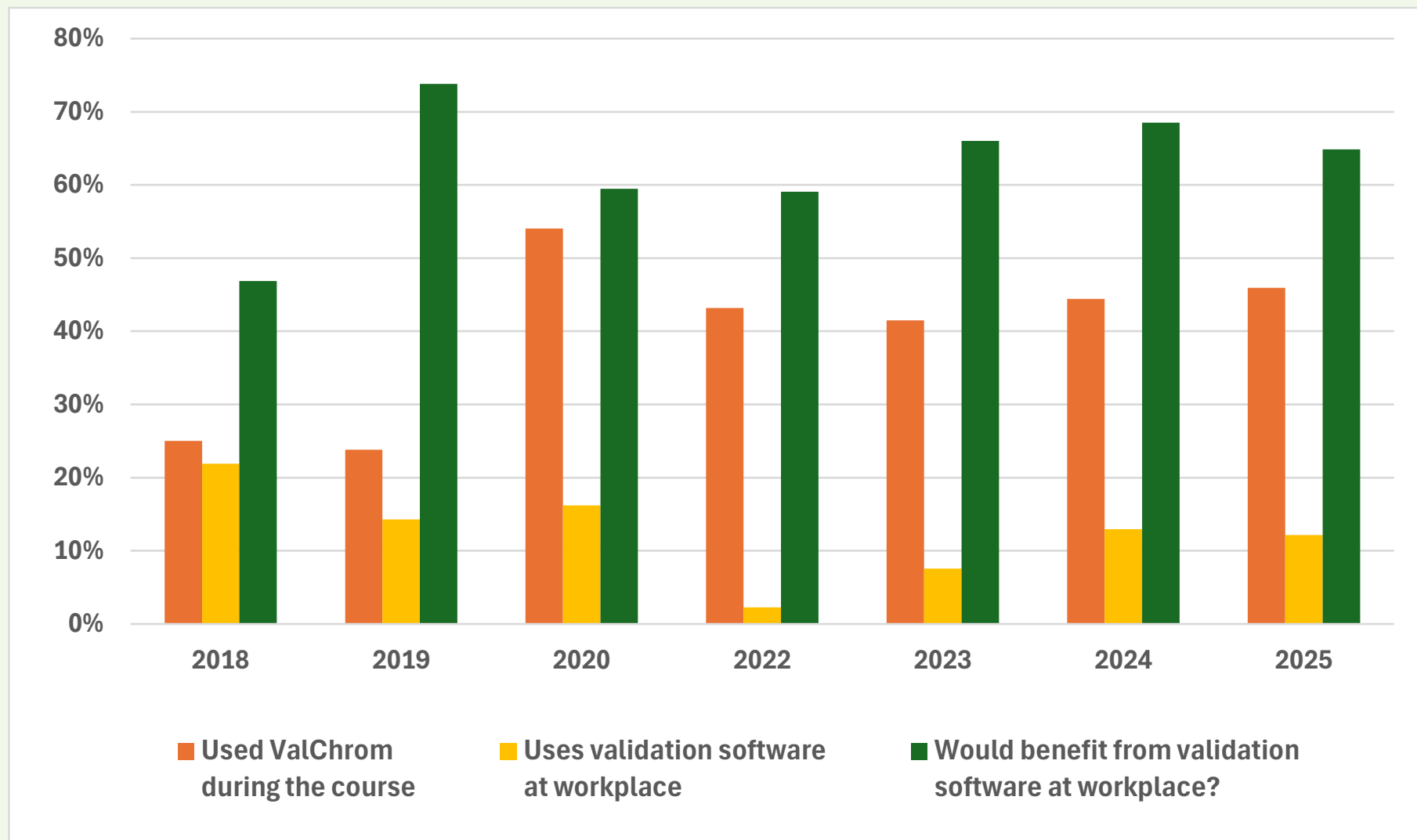
**More real-life examples**  
**More exercises, help and information on  
mathematics and calculations**  
**More help with software (including  
ValChrom)**  
**Live discussions with teachers and sharing  
experience between participants**  
**Keep up to date and improve guideline-  
related advice**



*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*

Feedback





# *1. Practitioners 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/CC $\beta$ , CC $\alpha$ , 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

This course is part of:



Co-funded by  
the European Union

(Excellence in Analytical Chemistry, <https://www.analyticalchemistry.eu/>)

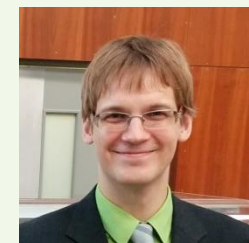
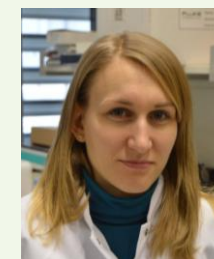
Supported by:



**AKKI**

Analüütilise keemia  
kvaliteedi infrastruktuur

(Estonian Center of Analytical Chemistry, TARISTU24-TK15, <https://www.akkiee>)





# *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
...	

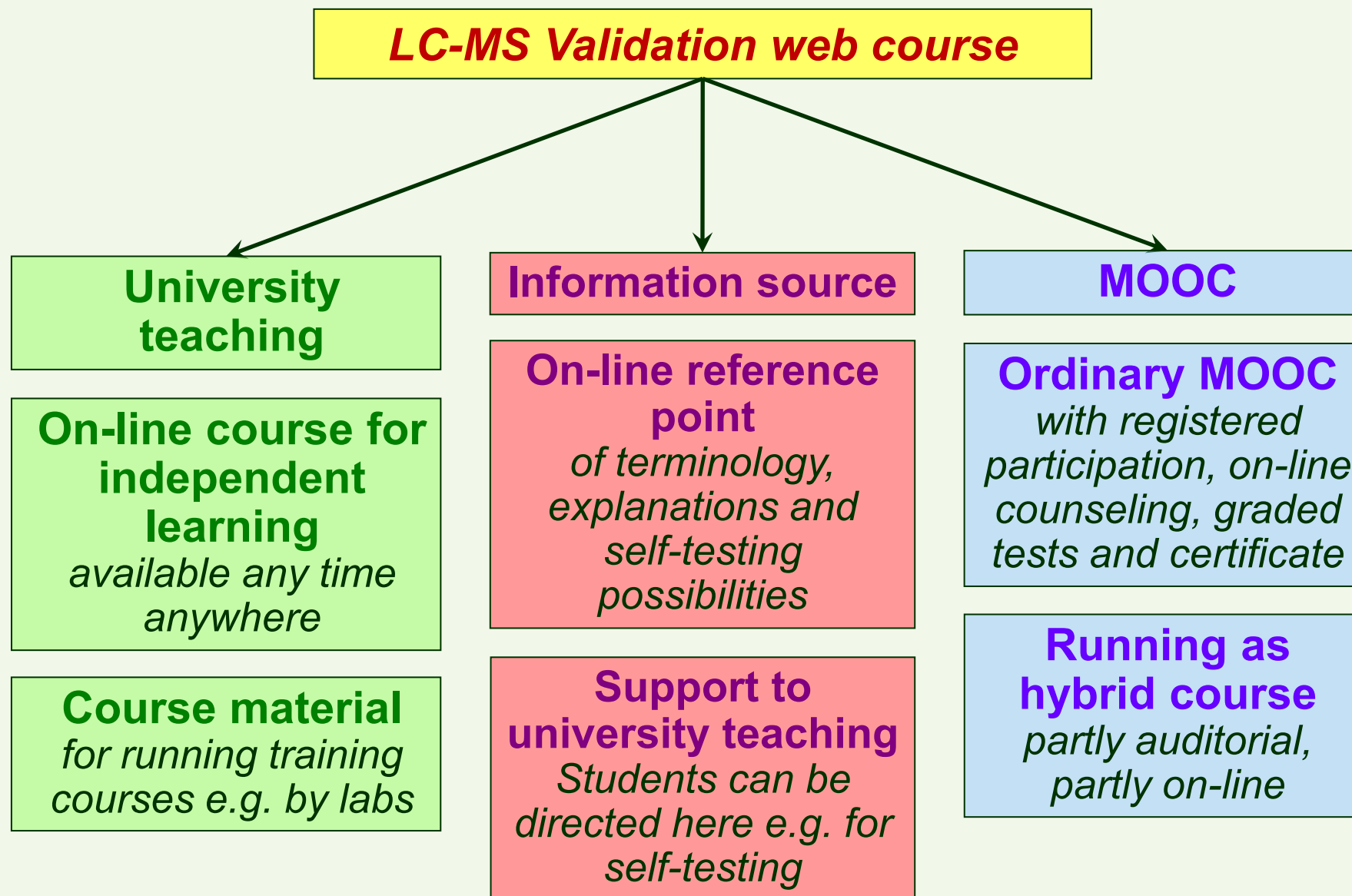
Italy	54
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**137 countries overall**



# 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 <sup>a</sup>	Medium	Medium	Medium
Costs of running the course <sup>a</sup>	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