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LC-MS METHOD VALIDATION IN SCIENTIFIC RESEARCH: IT'S TIME TO HARMONIZE AND EXEMPLIFY

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ADVANCES AND CHALLENGE IN LC/MS (1/2)

✔ Over the past 25 years, liquid chromatography-mass spectrometry (LC-MS) has transitioned from a scientific novelty to a widely used technique, increasingly employed in routine field laboratories.

✔ Compared to traditional chromatography (which uses a unidimensional detector like UV absorbance), LC-MS provides enhanced selectivity and identity confirmation by measuring the mass-to-charge ratio of ions or capturing MS data for the entire chromatogram, often generating three-dimensional datasets.

→ LC-MS systems are complex, requiring numerous parameters to be set at or near optimal levels to achieve the desired performance.

→ When developing an LC-MS-based analytical method, its performance must be thoroughly evaluated and consistently monitored.

ADVANCES AND CHALLENGE IN LC/MS (2/2)

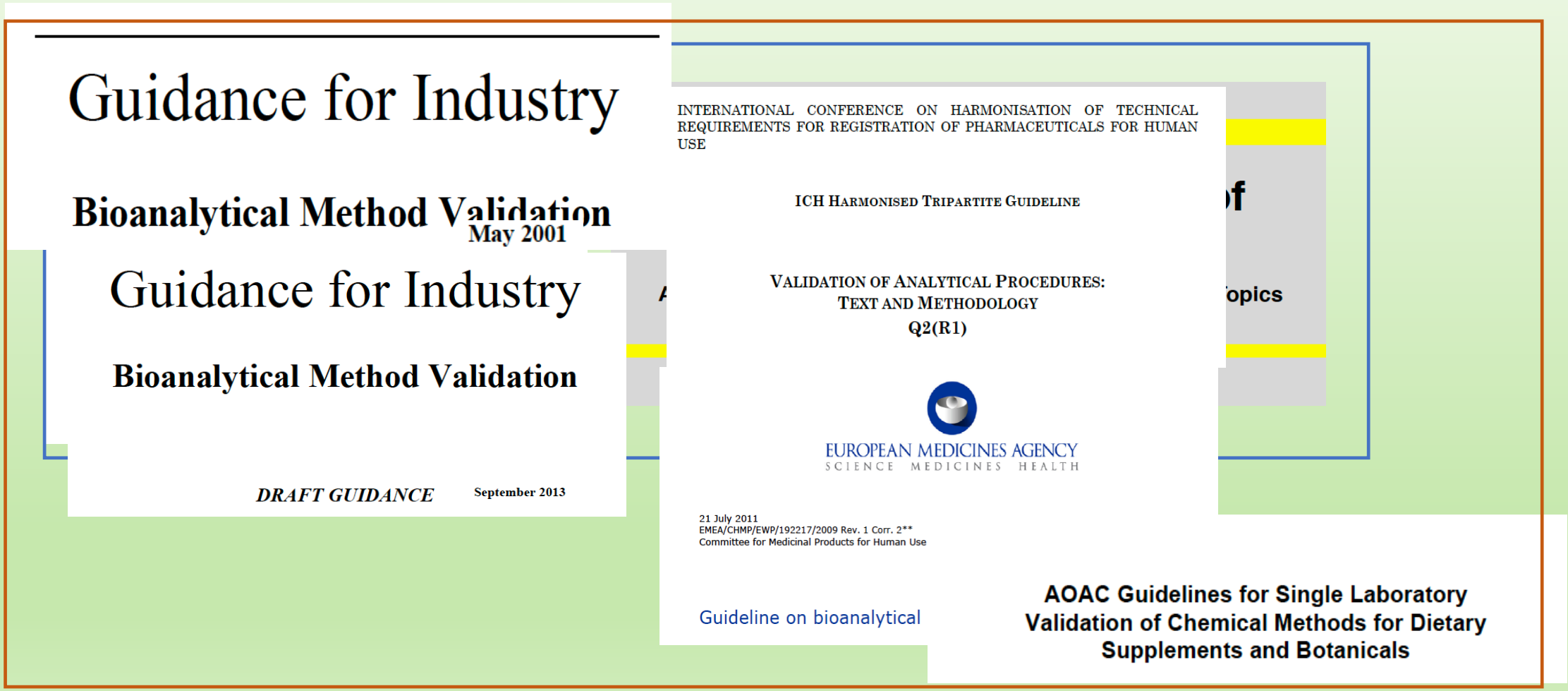
- Method validation is a crucial step in chemical analysis, essential for ensuring reliable results.

- Methods based on LC–MS are notorious for their complexity

- In addition to its inherent necessity, method validation is increasingly mandated by regulations governing laboratories, and scientific journals in analytical chemistry require validation data for methods to be published.

VALIDATION GUIDELINES

Due to its significance, numerous validation guidelines have been developed for laboratories, ranging from general frameworks to sector-specific standards.

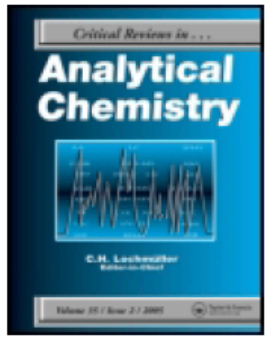


VALIDATION GUIDELINES

Formulation Development and Pharmacokinetic Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani, India

Comparison of various international guidelines for analytical method validation

S. CHANDRAN, R. S. P. SINGH



Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/batc20

Analytical Validation of Quantitative High-Performance Liquid Chromatographic Methods in Pharmaceutical Analysis: A Practical Approach

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[b] Departamento de Farmácia, Universidade Federal de Alfenas, Alfenas, Brazil
Published online: 11 Jan 2012.

Different recommendations and different sets of terminology are found in the different guidelines

The analytical community has yet to reach a consensus on the precise approach to validation, both in general and specifically for LC-MS applications.

DIFFERENT TERMINOLOGY

Guidelines	IUPAC	VIM	ICH	EURACHEM	NordVal	AOAC	EMA	FDA
Reference document	[1]	[2]	[3]	[4a, b, c]	[5]	[6]	[7]	[8a, b]
Selectivity	✓	✓	X	✓	X	✓	✓	✓
Specificity	X	X	✓	X	✓	X	X	X
Ruggedness/Robustness	✓	-	✓	✓	✓	✓	-	-

Table 2
Terms used for trueness in different guidance materials.

Organization	Term	Meaning according to VIM
Eurachem, AOAC, ISO	Accuracy	Accuracy
	Trueness	Trueness
ICH, FDA, EMA	Accuracy	Trueness
IUPAC, NordVal	Trueness	Trueness

Kruve et al. Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II Analytica Chimica Acta 870 (2015) 8–28

1. IUPAC, Harmonized guidelines for single-laboratory validation of method of analyses (IUPAC technical report), Pure Appl. Chem. 74 (5) (2002) 835–855.
2. International Vocabulary of Metrology – _Basic and General Concepts and Associated Terms (VIM), JCGM 200:2012.
3. ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005.
4. (a) Eurachem, Eurachem Guide: The Fitness for Purpose of Analytical Methods, Eurachem, Teddington, 1998; (b) B. Magnusson, U. Örnemark, Eurachem Guide: The Fitness for Purpose of Analytical Methods – _A Laboratory Guide to Method Validation and Related Topics, 2nd ed., Eurachem, Teddington, 2014 Available from www.eurachem. org. ISBN 978-91-87461-59-0. (c) H. Cantwell (ed.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (3rd ed. 2025).
5. NordVal Protocol No. 2, Guide in validation of alternative propietary chemical methods, 2010.
6. AOAC, AOAC Guidelines for Single-laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, Official Methods of Analysis, 19th ed., AOAC, INTERNATIONAL, Gaithersburg, MD, 2012 Appendix K.
7. Guidance on bioanalytical method validation, European Medicines Agency, 2011.
8. (a) U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services Food and Drug Administration, 2001; (b) U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry: Bioanalytical Method Validation, Draft Guidance, U.S. Department of Health and Human Services Food and Drug Administration, 2013. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM368107.pdf.

DIFFERENT RECOMMENDATIONS

Kruve et al. Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II Analytica Chimica Acta 870 (2015) 8–28

Table 1
Recommendations for evaluation of linearity in different validation guidelines and our recommendations.

Guideline	Experiment planning	Evaluation of data
ICH [26]	Min. 5 concentration levels. Dilutions of standard stock solution or weighing different amounts of analyte standard	First evaluated by visual inspection of the plot of signal–concentration relationship. In case of linearity, additional statistical method should be applied, e.g., regression line using least squares method. Correlation coefficient, y-intercept, slope of regression line and residual sum of squares should be used to evaluate linearity In addition: analysis of deviation of actual data points from the regression line (analysis of residuals)
AOAC [27]	6–8 points, approximately equally spaced, measured in duplicates at random. Calibration solutions obtained by dilution of stock solutions	Visual examination is usually sufficient and in addition use of plot of residuals is suggested. An acceptable fit produces random pattern of residuals with a 0 mean
Eurachem [25]	Blank and reference materials or spiked blanks at 6–10 concentrations evenly spaced exceeding ±10% or ±20% of the expected concentration range (2–3 measurements)	Visual inspection of the line and residuals may be sufficient, but statistical test, e.g., goodness-of-fit are recommended
IUPAC [48]	Min. 6 calibration standards at evenly spaced concentrations covering the range. Samples should be analyzed in random order at least in duplicate	Examination of plot of residuals or lack-of-fit test, but preferably both
EMA [14]	Min. 6 calibration standards at evenly spaced concentrations, a blank and a zero sample analyzed at least in duplicate. Calibration standard is defined as matrix spiked with analyte	Matrix-matched calibration. Back-calculated concentrations of the calibration standards should be within 15% of the nominal value (20% for lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ)) for at least 75% of calibration standards
FDA [13a]	A blank sample,[a] a zero sample[b] and 6 min non-zero[c] samples analyzed in duplicate in 6 sequences over several days. Calibration samples should be prepared in the matrix as study samples	Matrix matched calibration. Standard curve is acceptable when 75% of non-zero standards are within 15% of the nominal concentration (20% for LLoQ)
SANCO [4]	Three or more concentrations. Use of two levels is appropriate if the difference of concentrations is below 10 times	Matrix matched calibration. Visual inspection or calculation of residuals. Relative residuals should be within ±20%. Weighted linear regression is preferred
Our recommendations	Solutions with at least 10 different concentrations, approximately equally spaced, with ratio of the highest concentration to the lowest at least 5, containing the matrix components (if possible), measured in random order and at least in duplicate	We recommend a two-stage approach: (1) Linearity is first assessed visually. In clear-cut situations (see Fig. 1 for explanations) visual assessment of linearity is sufficient. (2) In unclear situations (Fig. 1) statistical tests should be used

[a] Blank sample: matrix sample without the analyte and internal standard.
[b] Zero sample: matrix sample with internal standard, without analyte.
[c] Non-zero sample: matrix sample containing both analyte and internal standard [13a].

THE PROJECT

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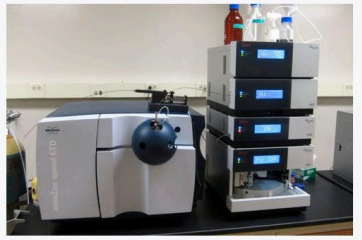
LC-MS quantitative method validation and performance: an exemplified guide

Project No.:	2021-036-1-500
Start Date:	10 Mar 2022
End Date:	
Cite:	https://iupac.org/project/2021-036-1-500
Division:	Analytical Chemistry Division

Objective | Description | Progress | Partners

Objective

Specific guidelines and common experimental protocols for the validation of LC-MS methods are needed. However, existing guidelines are difficult to follow. The application fields involved are numerous, including the pharmaceutical, food, environmental, forensic and clinical ones. Therefore, the aim of this proposal is to review the present status of validation methods critically and provide a broader and easy to follow guide for the validation steps in all the aforementioned areas, with cross-references to definitions and further details. In particular, methods for calculating the detection and quantification limits, calibration, standards, matrix effect, recovery, reproducibility will be provided in an exemplified way. Moreover, we aim to study the best quality control (QC) strategies and External quality assurance (EQA) programs.



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Koit Herodes
Asko Laaniste
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Zoltán Mester
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Progress

Jan 2023 update - The task group including members from North America, United States, Japan, Asia and Europe, will meet for the first time in Europe, in occasion of IUPAC 2023 General Assembly, in August. During on-line meetings the group decided the table of contents for the future report.

Jan 2024 update - During the in-presence meeting in occasion of the GA in The Hague, the task group decided to change the scope of the project including not just an exemplified guide but harmonising the existing guidelines that refer to analytical methodologies and including the specific regulations for LC-MS in one document.

Apr 2024 update - The task group is seeking participation in a survey that will assist in achieving the above stated objective. If you are willing to participate in the survey you will be giving consent for the data to be used in developing the LC-MS validation guidelines. Participation is completely voluntary and all the information collected will be kept private and confidential, to the extent permitted by law. All survey responses will be held anonymous and will not be traceable to you. If you have any questions about this research or about the survey, please contact the Project Leader, Dr. Fabiana Piscitelli of the National Research Council-Institute of Biomolecular Chemistry (email: fpiscitelli@icb.cnr.it).

To participate in the survey, please follow the link here. The survey will remain open through to May 15, 2024.

Any feedback is important to us and we hope that you may forward this survey to your colleagues.

Jan 2025 update - A survey aimed at collecting feedback from the potential stakeholders has been sent out last year and the results have been shown at the last EuChemS conference, held in Dublin last July. TR is in preparation and a workshop will be organized at the end of 2025.

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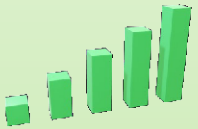
Literature review

we will compare the existing recommendations and guidelines on method validation for small molecules to fill the gap in knowledge and to help improve standardisation and harmonisation of validation procedures between the actual regulatory guidelines and the common strategies used by the LC-MS users in research.

Ask → **Review** → **Report**



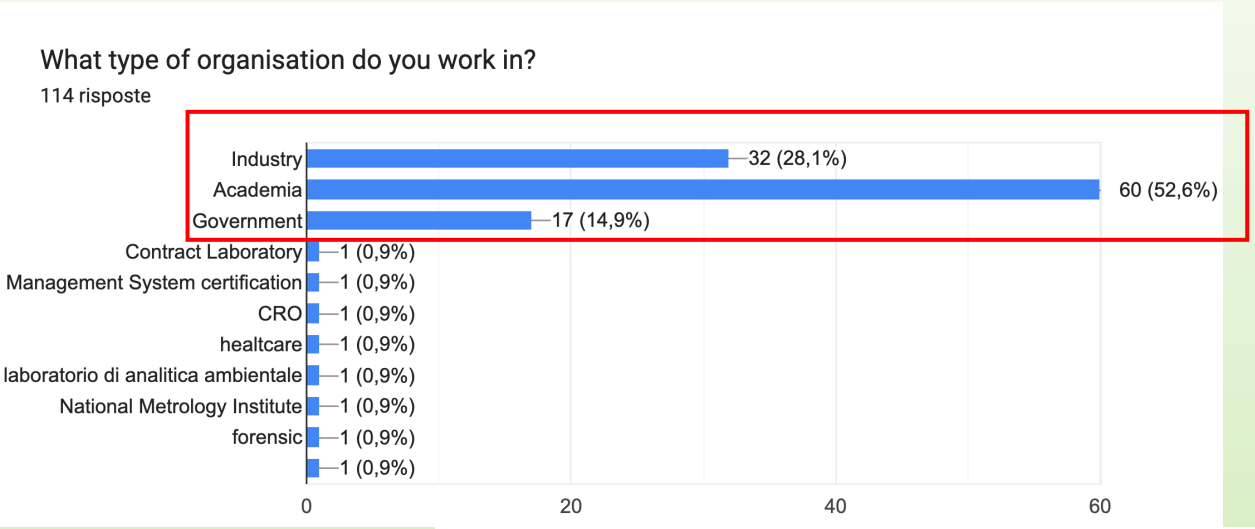
Survey

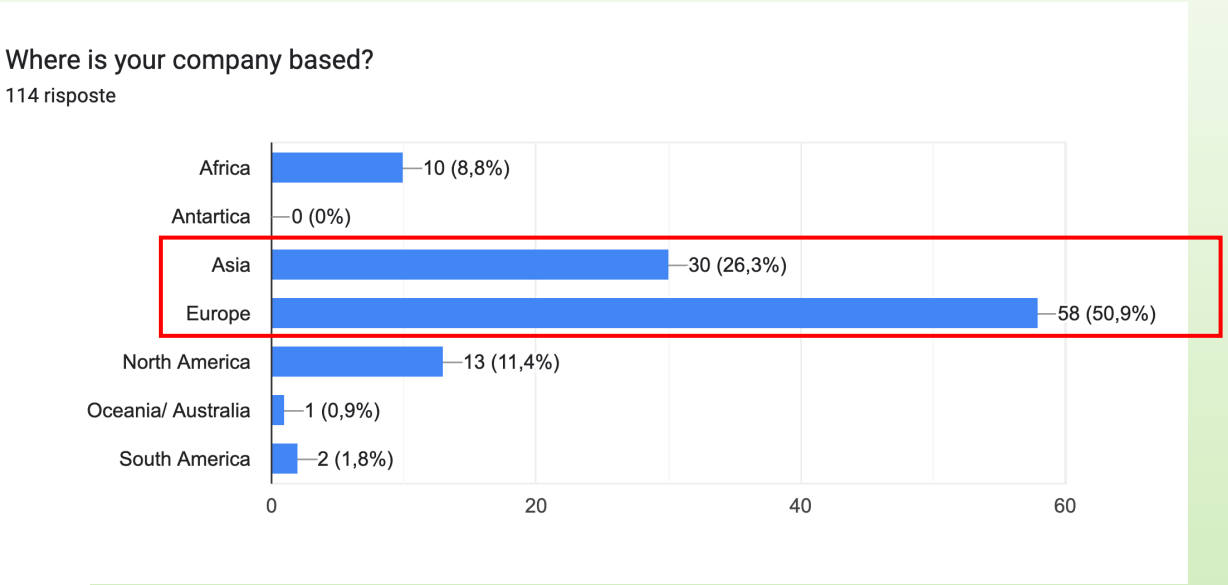
The survey included 89 answers from scientific analytical community with a widespread geographical localization and field of application.

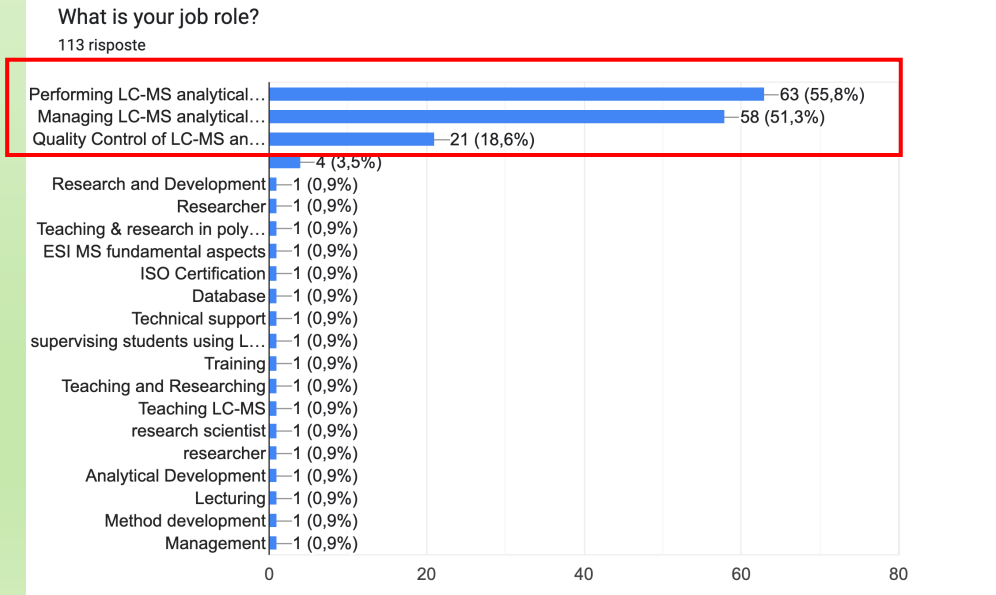
Technical report

The output of the Project will be a publication that builds on well-established standard validation terms and procedures to harmonise validation procedures, create specific guidance for LC-MS and will provide valuable support to practitioners.

SURVEY ON LC-MS VALIDATION METHOD AND BEST PRACTICE

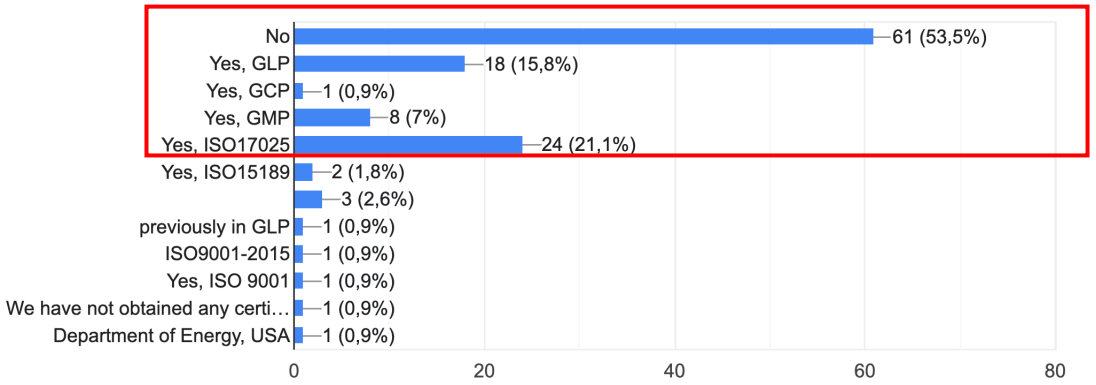






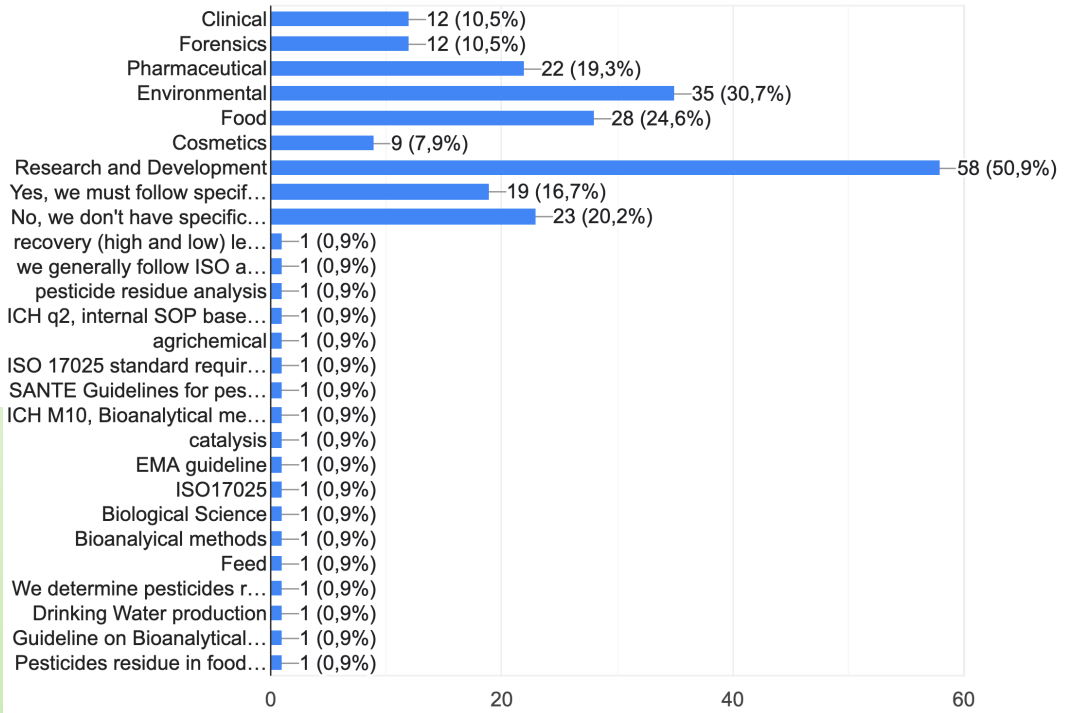
Do you work in a regulated environment?

114 risposte



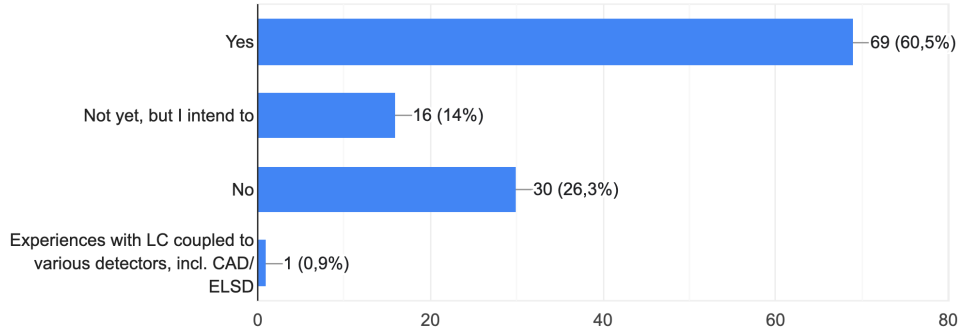
What field/sector are you in? Are there any field specific validation requirements you must follow? If yes, please add details of these required validation documents in 'other' (Can select multiple)

114 risposte



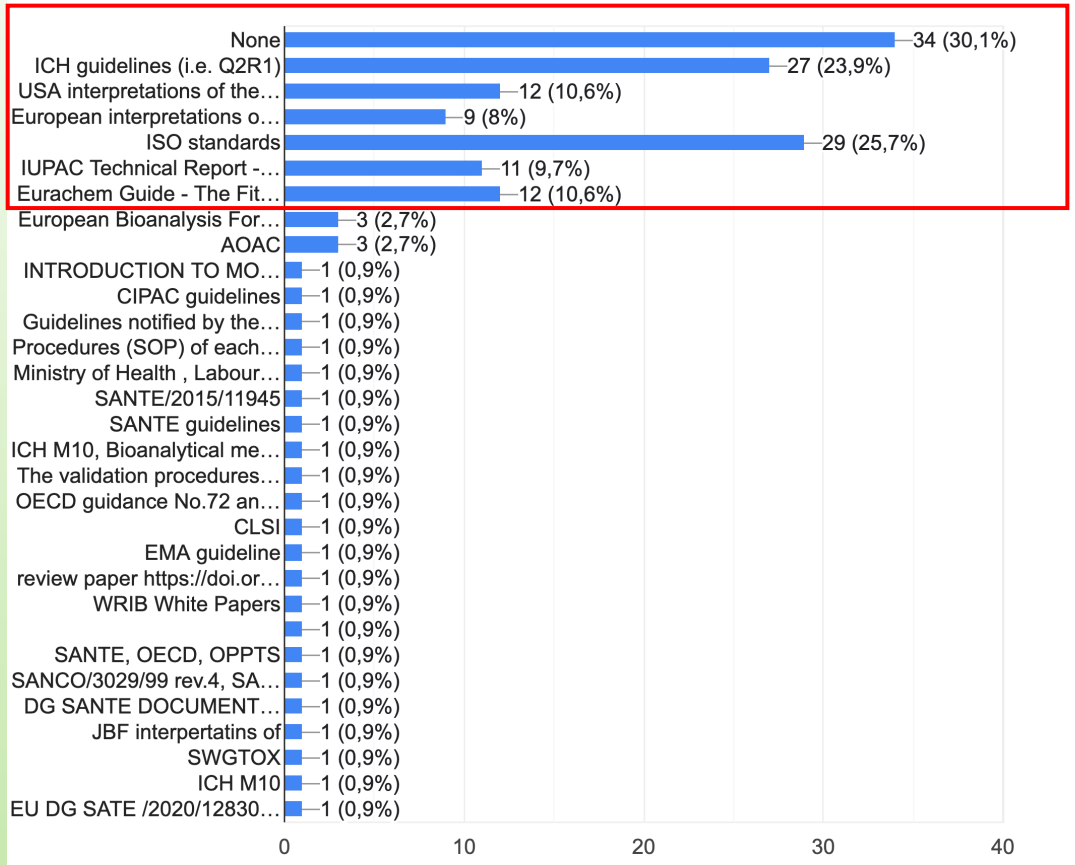
Do you currently carry out method validation for LC-MS?

114 risposte



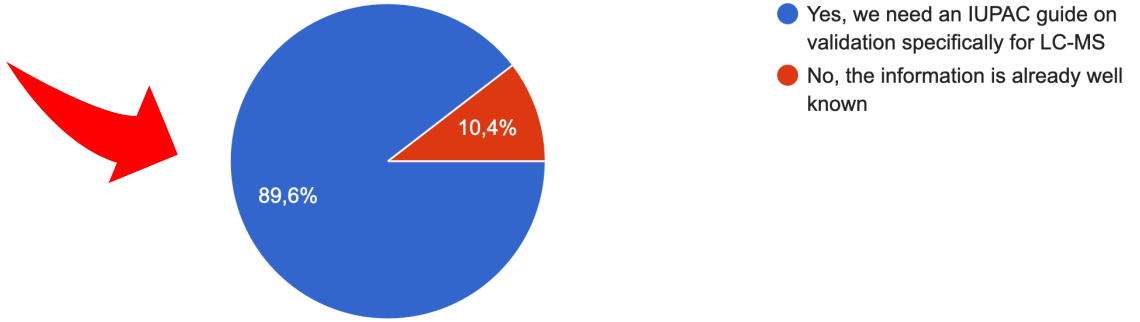
What documents do you currently follow or reference when doing LC-MS validation? Please additionally state specific standard/reference in 'other' if applicable.

113 risposte



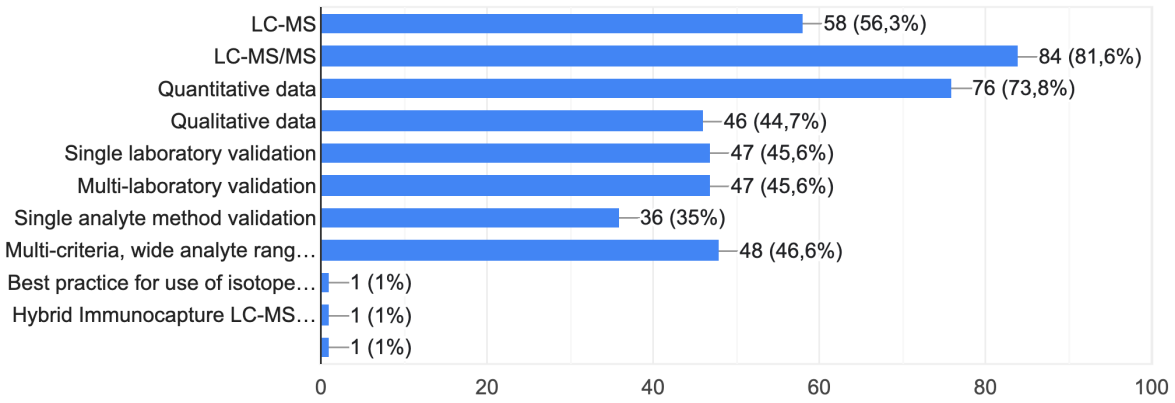
Do you think a IUPAC specific LC-MS validation best practice guide that everyone can use and reference will be useful to you and would you use it?

115 risposte



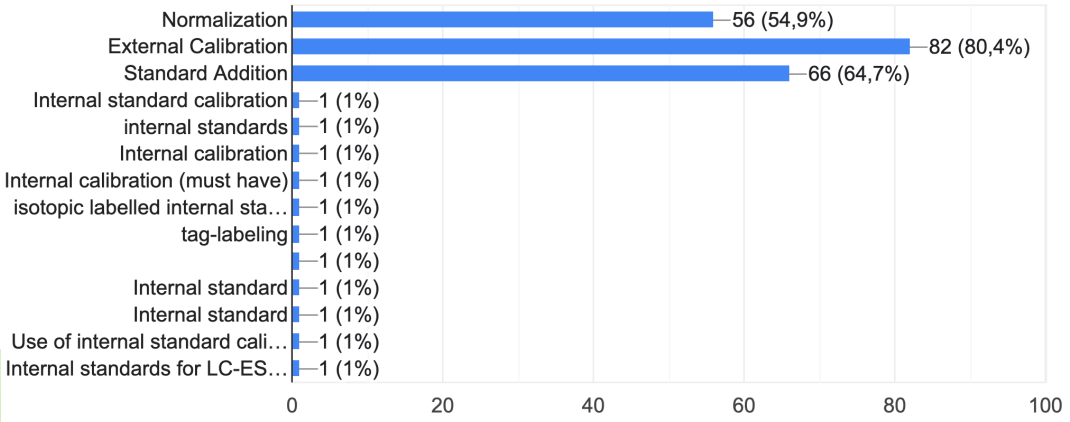
What should the guide focus on? (Can select multiple)

103 risposte



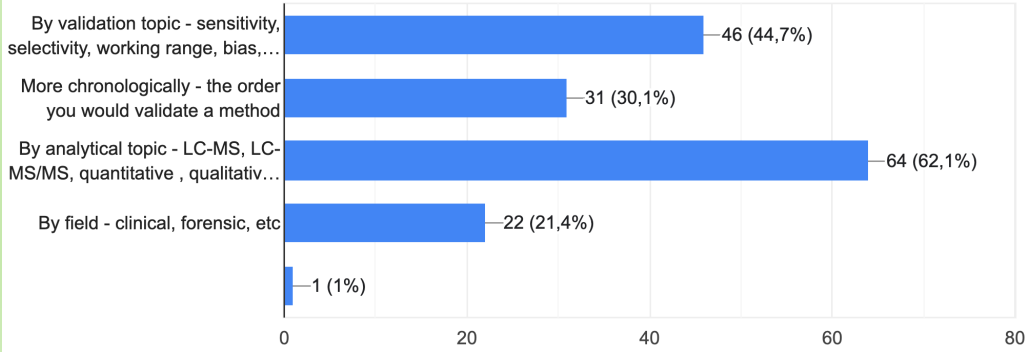
For quantitative methods what kind of quantitation method would you like to see mentioned in the guide? (multiple answers possible)

102 risposte



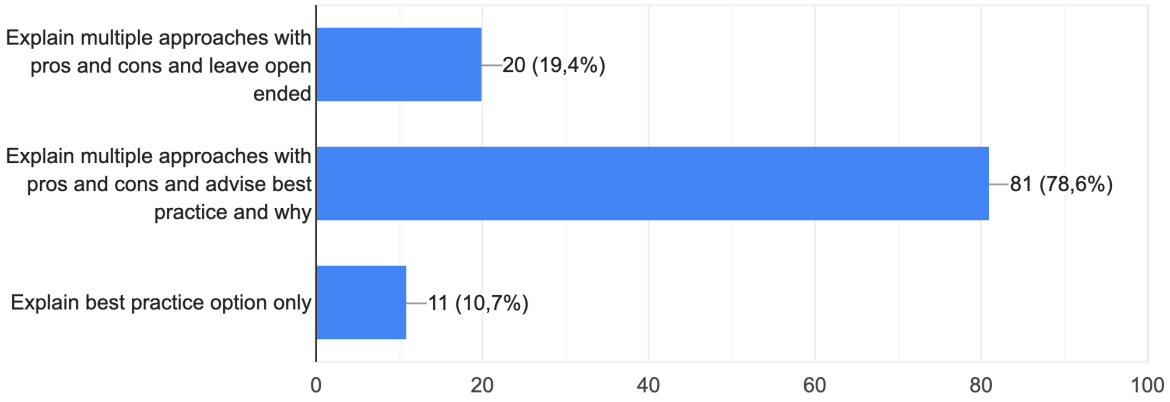
How should the guide be broken down?

103 risposte



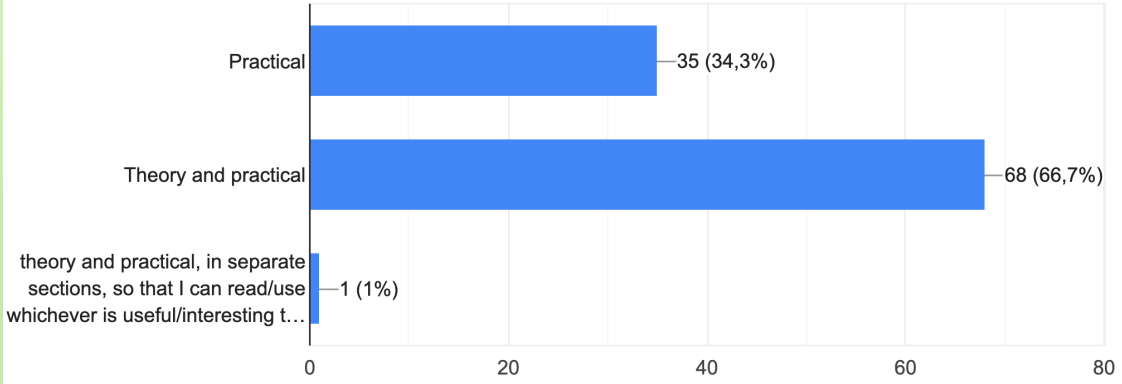
Should the guide discuss all the approaches and then advise on best practice?

103 risposte



Should the guide include a lot of theory, or keep it simple and practical

102 risposte

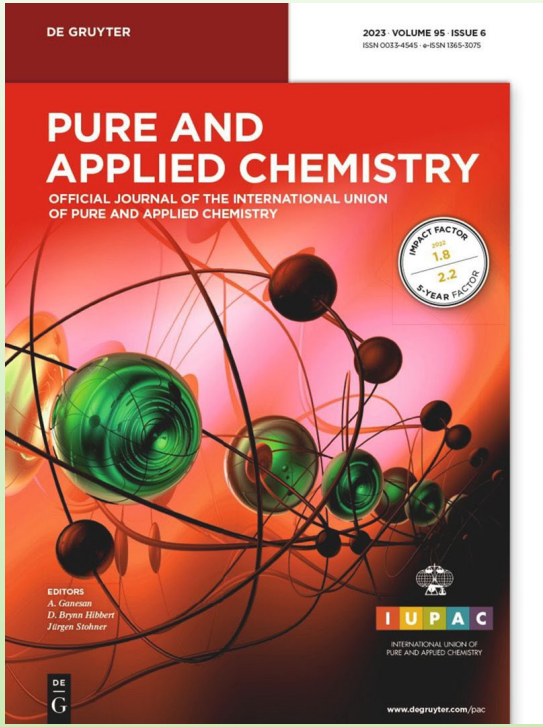


FEEDBACK AND SUGGESTIONS

- ✓ High-throughput requirements: Metabolomics and similar fields often require high-throughput analysis to handle large sample volumes. Traditional validation guidelines may not adequately cover the strategies needed to maintain consistency and reliability at such scales.
- ✓ Data Processing and Analysis: The complexity of data generated by LC-MS, especially in non-targeted studies, requires sophisticated data processing algorithms. Validation guidelines typically focus on the analytical process and do not include the equally important data analysis phase.
- ✓ Regulatory Considerations: In metabolomics, following strict regulatory guidelines often means extensive analysis and significant effort, which does not necessarily coincide with the frequency of analytical method updates. If validation guidelines evolve to provide a more streamlined approach, this would be especially beneficial for exploratory and research-specific applications that can reduce regulatory burden without compromising scientific integrity.
- ✓ Adaptability: Validation protocols should be adaptable so that new technological advances and analytical approaches can be quickly incorporated.
- ✓ Simplification and Standardization: There is a need for simplified and standardized validation protocols that can be easily applied across different laboratories and studies without sacrificing the rigor necessary to obtain reliable and reproducible results.

DISSEMINATION: TECHNICAL REPORT (IN PREPARATION)

	Chapter or paragraph	Title
Section 1	1	Introduction
		1.1 Scope and field of application
		1.2 Terms and definitions
		1.3 Symbols
		1.4 Abbreviations
Section 2	2	LC-MS method development
		2.1 Sample preparation (link to Annex 1)
		2.2 Reference materials (both certified and in-house)
		2.3 Key elements of liquid chromatography
		2.4 Key elements of mass spectrometry
		2.4.1 Ionization
		2.4.3 Quantification
Section 3	3	Method validation
		3.1 Linearity and sensitivity
		3.2 Selectivity and identity confirmation
		3.3 Limit of detection and limit of quantification
		3.4 Trueness, precision and accuracy
		3.6 Ruggedness
		3.7 Matrix effect
		3.8 Measurement uncertainty
		3.9 Expression of results
Section 4	4	Practical tools and softwares
		4.1 Overview
		4.2 Examples and explanations
Annex 1	1	Sample preparation
		1.1 Definition of sample
		1.2 Sample collection
		1.3 Sample containers and handling conditions
		1.4 Concentration of target analyte
		1.5 Extraction methods
		1.6 Protein precipitation



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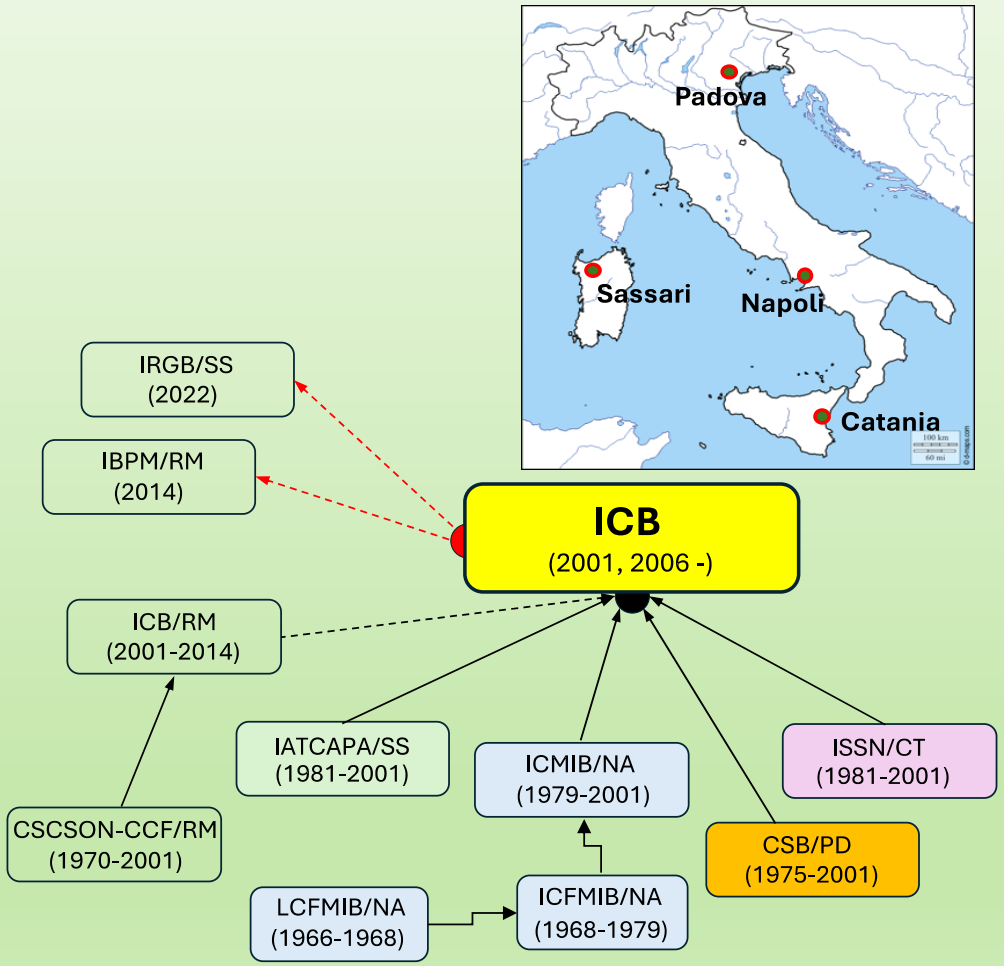

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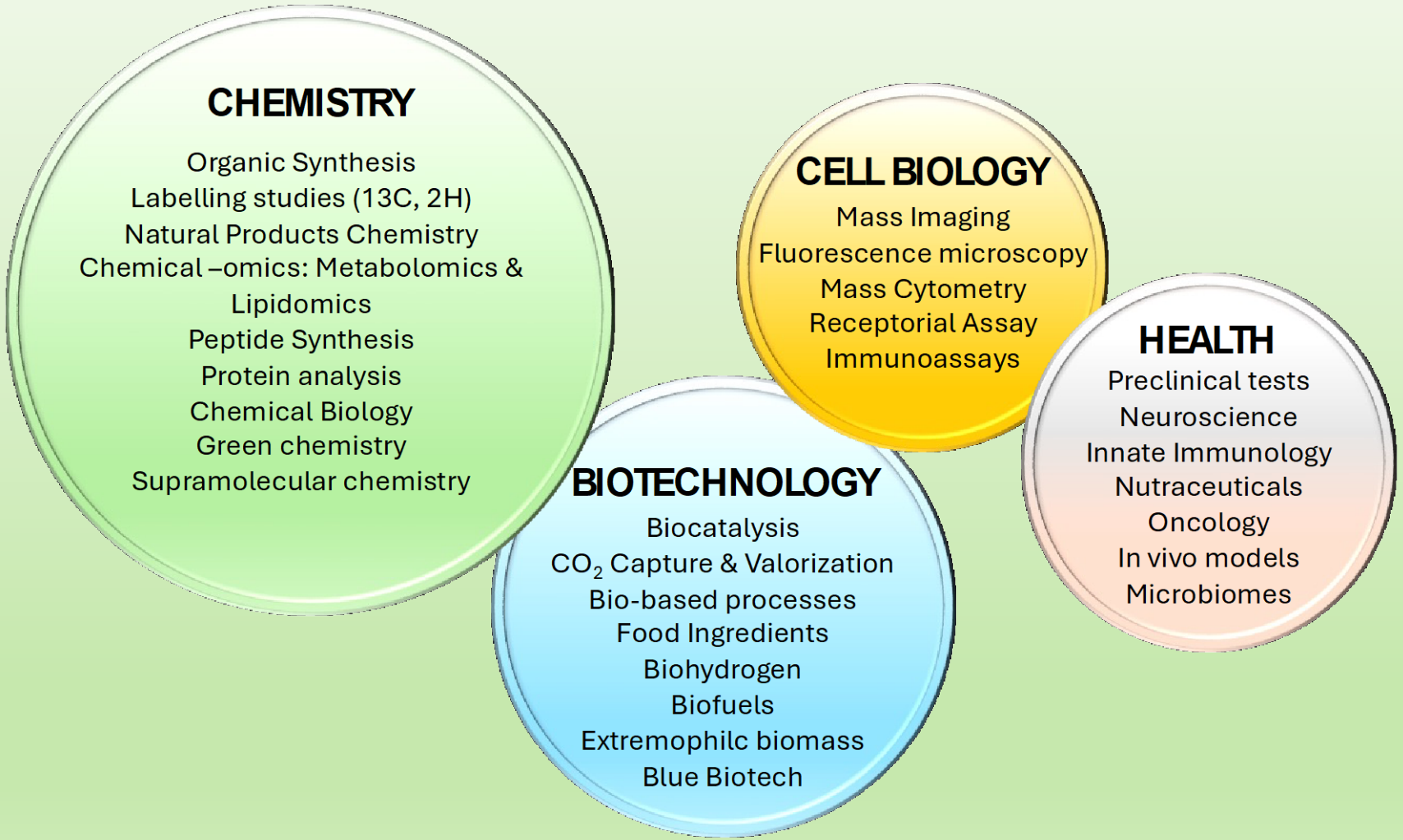
TECHNOLOGY PLATFORMS



Thanks!

Any questions?

your

feedback

truly

matters!