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Editorial

A proposal to standardize the description of LC–MS-based measurement methods in laboratory medicine

The *International Vocabulary of Metrology* defines a measurement method as "*a generic description of a logical organization of operations used in a measurement*" [1]. The current standard for most publications reporting diagnostic mass spectrometry-based measurement methods is to describe the method, as realized, for only *one* laboratory site. Performance characteristics are based on a limited set of experiments that are run within a short period. The same generally applies for kitted solutions as well. Thus, the conventional practice of reporting measurement methods presents, in most cases, a severely limited level of abstraction.

Today's published liquid chromatography–mass spectrometry (LC–MS) methods correspond, more or less, to clinical case reports in the sense that both represent one-off descriptions. However, they differ in that an LC–MS-based test may be used to test thousands of patients, or even millions of patients, whereas a case report is limited in scope. Case reports play an important role as they have utility in paving the path towards a more generalized understanding of a medical condition. This higher-level view is essential for science; we believe that this applies equally to laboratory tests. As such, a crucial question remains unanswered: if one detail of a measurement procedure is modified in relation to the reported protocol – is it still the same method?

This fundamental question raises specific questions about published LC–MS methods, such as: 1) To what extent is it required to re-assess performance data for verification when one (or more) components of the analytical set-up is altered? 2) Is the measurement procedure applicable for use on only one specific instrument type? 3) Less sensitive instruments may require increased injection volume compared to the "index realization", while more sensitive instruments may require reduction of injection volumes or dilution to avoid detector blinding, for example – which compromises the basic principle of isotope dilution internal standardization. Although there are now detailed guidelines for LC–MS method development [2], the reporting framework in the published literature remains variable. Thus, we believe that today's LC–MS methodological standard articles fail to accurately specify the *identity* of a measurement method based on the highly complex technology of LC–MS.

The lack of a reporting standard is a fundamental problem for LC–MS method publications. The number of instrument configurations is nearly infinite and the performance characteristics of any one instrument can fluctuate substantially from hour to hour due to contamination or surface charging phenomena, among other variables. Indeed, uncertainty about the "identity" and sustainability of methods has likely contributed to the limited dissemination of LC–MS following more than two decades of technological availability in diagnostic testing laboratories. To overcome this limitation and introduce a higher level of scientific abstraction in reporting measurement methods for diagnostic application, we, herein, propose a standardized approach to reporting LC–MS-based methods – with a focus on "robustness" of description.

To start, we suggest differentiating between the *fundamental* and *variable* characteristics of a measurement method. In this concept, f*undamental characteristics* are those that can be translated into separate realizations; for example, the mode of ionization (e.g., electrospray in positive polarity) or the *m*/*z* ratio of monitored ions. These characteristics are intended to define the *identity* of a measurement method. Whereas, *variable characteristics* are those that cannot realistically be standardized over time and space; for example, the lot of a chromatographic column or of solvents, or the instrument specific geometry of the ion source that is highly manufacturer dependent, as well as instrument tuning settings. However, these *variable* features, should be thoroughly documented for each realization and, finally, for each analytical series, as changes can have an important impact on the performance of a method over time. Indeed, modifications in products from the side of the supplier of consumables may also substantially compromise a method.

In Table 1 we suggest a preliminary set of 35 fundamental characteristics – defining a measurement procedure generically – and of 15 variable characteristics – which should be documented for each individual implementation and analytical run for traceability. We, furthermore, suggest that well-defined, essential system performance characteristics be included as a fundamental component of any measurement method – e.g., the required mass resolution, a signal readout for direct injection of a pure solution of the measurand, or a signal-to-noise observed for a bottom calibrator sample. Thus, essential pass criteria should be specified both for analytical runs and individual samples. Such "special specifications" could also potentially include tests for carry-over or cross-talk, if critical for a specific diagnostic test.

In order to prove the reproducibility and the upscale- (or "dissemination-") potential of a measurement method itself (developed at *one* site), we suggest a method comparison between two or more method implementations at different sites: these should fully correspond in the *fundamental* characteristics – but differ in as many *variable* characteristics as possible (e.g., manufacturer of columns and solvents). For validation of the measurement method – as a generic description of a logical organization of operations used in a measurement – split aliquots of a significant set of samples should be analyzed comparatively at the different implementation sites. (Use of a shared calibration material may be useful here)

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Table 1
(a) Suggested generic description of fundamental characteristics defining a LC–MS based measurement method addressing small molecule measurands. (b) Suggested list of variable characteristics of a LC–MS based measurement method that cannot be standardized but should be documented for each analytical series.

(a)

#	Fundamental characteristic	Examples and remarks for specification
General & Pre-analytical		
1	Intended use	e.g., supporting the diagnosis of hyperaldosteronism
2	Measurands addressed	e.g., 3 drugs with 6 metabolites (with CAS numbers)
3	Sample matrix, main	e.g., serum
4	Main technology of sample extraction / purification	e.g., protein precipitation, solvent extraction, solid phase extraction (off-line, on-line), immobilized liquid extraction
5	Liquids applied in sample extraction with volumes, temperature and application times	e.g., solvent extraction with dichloromethane (analytical grade), 2 mL, 10 min, horizontal shaker, room temperature, evaporation of the organic phase with N_2 and reconstitution with methanol/water
6	Consumables applied in sample extraction, generic description	e.g., C18 SPE-cartridge, 5 mg
7	Surface specifications	e.g., polypropylene tubes
8	Derivatization, generic description	No/yes. If yes: reagents with concentrations and purity, time and temperature of reactions. Functional group added
LC–MS acquisition conditions		
9	General LC setup	e.g., 2 D-Chromatography; divert valve/splitter
10	Main column geometry	Diameter, length, particle size
11	Main column chemistry	e.g., C18 reversed phase
12	Mobile phase constituents	With stated purity. e.g., acetonitrile, water (HPLC grade)
13	Retention time window of measurands	e.g., 4–5 min
14	Column temperature, range	e.g., 35–37 °C (analytical column), 20–25 °C (extraction column)
15	Total Run time, range	e.g., 5–8 min
16	Flow rate, range	e.g., 0.7–0.9 mL/min (analytical column)
17	Injection volume, range	e.g., 5–8 μL
18	Gradient, generic description	e.g., A: 0.1% formic acid; B: methanol. Starting condition A 70%/B30% – stop condition A 30% / B 70%; 4 min; linear
19	Particular washing procedures	If required in individual cases
20	Main MS ionization mode	e.g., electrospray, positive polarity
21	Mode of ion manipulation	e.g., triple-stage quadrupole with fragmentation; linear ion trap, Orbitrap, time-of-flight, Paul-trap
22	Specific system requirements, generic description	e.g., precursor ion mass accuracy ±0.1 amu; chromatographic separation of defined targets (e.g, baseline separation of methylmalonic acid from succinic acid derivatives)
Run acceptance & quantitation		
23	Internal standard compound(s)	Including labelling patterns with specific locations; range of isotopic purity
24	Recorded ions (precursor ions/product ions) for quantification	Addressing measurands' in source transformation or formation of adducts, etc. Potentially multiple fragment ions per measurand
25	Recorded ions (precursor/product ions) for confirmation with approach of acceptance criteria	Acceptance criterion e.g., determination of branching ratio in calibration samples, branching ratio of unknowns within 3-SD range
26	Mass resolution, ranges	e.g., Precursor ions: 0.8–1.0 amu; product ions 0.4–0.6 amu
27	Number of data points over peaks	Determined by dwell times, interscan delay, number of traces
28	Type of calibration	e.g., external calibration with internal standard or exact matching IS (bracketing calibration, standard addition)
29	Calibration sample number and concentrations (per measurand)	e.g., compound A 3-5-15-30 ng/mL; compound B 5-10-15-20-25 ng/mL
30	Calibration samples matrix	e.g., spiked serum
31	Response/isotopic ratio of lowest/highest calibration samples (per measurand)	e.g., compound A, response 0.1–10.0
32	Specific set of defined pass criteria for runs (per measurand) – specific specifications	e.g., signal-to-noise of the lowest calibration sample (with specification of applied algorithm); maximum deviation of recalculated calibrator concentrations; CV of lowest calibrator recalculated concentration in multiple injections; peak shape criteria (e.g., symmetry, area-to-height, etc. for defined calibration sample) Mean internal standard area of calibration samples vs. unknowns (% deviation) CV of internal standard peak areas CV of retention times of IS Carry-over test, cross-talk test, ion suppression tests etc.
33	Specific set of defined pass criteria for samples (per measurand) – specific specifications	e.g., range of quantifier/qualifier ion ratio (unknown in relation to calibration samples), range of internal standard peak area (unknown in relation to calibration samples) etc.; specific structure of analytical runs (especially for reference measurements)
34	Special considerations	e.g., "does not discriminate between 25 hydroxy vitamin D and epi 25 hydroxyvitamin D"
35	Interpretation of data	Standard of how interpretation is to be performed, also have real patient samples been analyzed and interpreted as part of this method development?

(b)

#	Variable characteristics	Cannot be standardized over longer time in most cases but should to be documented when possible
Pre-analytical & sample preparation		
1	pre-analytical details	e.g., type, brand and lot of sample tubes
2	Tubes, pipette tips	Potential source of contamination or absorption
3	Extraction materials, manufacturer, brand and lot	
4	Solvents, manufacturer, brand and lot	
LC–MS acquisition conditions		
5	MS instrument manufacturer and type	
6	LC instrumentation manufacturer(s) and types	
7	Column manufacturer, brand, lot	
8	Software; manufacturer, version; computer, operating system	
9	Geometry of tubing	e.g., calculated/estimated void volumes, injection loop volume
10	Peak detection and smoothing settings (software dependent)	
11	vacuum conditions	
12	Tuning conditions such as declustering energy, cone voltage, collision energy, source gas flows, spray needle position, heater settings	Highly instrument dependent
Run acceptance & quantitation		
13	Internal standard manufacturers, lots	
14	Standard compounds/calibration samples, manufacturer, lots	
15	Units of reporting	e.g., molar or mass units

Validation pass criteria for a measurement method *and its description*, based on a between-implementation agreement, should be pre-defined.

While the term and concept of metrological traceability (referring to reference and calibration materials) is widely applied in laboratory medicine, the approach described herein aims to achieve conclusive *methodological traceability* for routine MS methods.

Without question, proof-of-concept publications describing the results of a method development at one site may be sound from a scientific perspective – demonstrating the feasibility of a methodological approach in a single observation. However, we propose that the description of a routine diagnostic test should follow distinct principles and should address the potential of implementation at multiple sites as a "laboratory developed test"/"laboratory implemented test". Our work primarily addresses the quantification of small molecule measurands but could potentially be applied with modifications for protein quantification as well.

We envision that a more standardized technical description of measurement methods could help increase trust in LC–MS methods for clinical diagnostic application and thereby foster dissemination of this powerful technology, in the interest of our patients. We are aware of the likely existence of grey area between fundamental and variable characteristics and that the approach suggested herein requires evaluation and optimization in practical application. With this editorial, however, we aim to initiate discussion on the robustness of the technical description of non-research measurement methods for diagnostic application in the community.

Finally, we introduce this proposal from a scientific point of view, with an eye toward clinical applications, not from a regulatory perspective, which may impose additional restrictions on the equivalence of methods. However, we envision that over the long-term this proposal may also be a useful stepping stone toward defining methods that are equivalent from a regulatory perspective, or at least contribute to such discussion. And, of course, we consider this to be a living document subject to change and improvement as the field evolves.

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Michael Vogeser
Carina Schuster
Hospital of the University of Munich (LMU), Munich, Germany
E-mail address: Michael.Vogeser@med.uni-muenchen.de

Alan L. Rockwood
Rockwood Scientific Consulting, Salt Lake City, UT, USA

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