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Standard for Mass Spectral Data Acceptance in Forensic Toxicology

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AAFS Standards Board

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Foreword

During the last several decades, mass spectrometry has replaced traditional, less specific techniques such as flame ionization, nitrogen-phosphorus, electron-capture, ultraviolet and fluorescence detection as the preferred technology for the confirmation of drugs, drug metabolites, relevant xenobiotics, and endogenous analytes in forensic toxicology. Although criteria for the acceptance of mass spectrometry data have been promulgated in regulated areas of forensic toxicology, none have been universally applied by practicing forensic toxicologists.

This document addresses this gap by providing minimum standards of practice for the acceptance of mass spectral data used in all forensic toxicology laboratories. Specifically, this standard focuses on minimum criteria for mass spectral data acquired using a low- or high-resolution mass spectrometer that utilizes ionization processes such as electron ionization, chemical ionization, electrospray ionization, or atmospheric pressure chemical ionization.

This document was revised, prepared, and finalized as a standard by the Toxicology Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science. It was originally conceived by the Scientific Working Group for Toxicology (SWGTOX).

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All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

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Standard for Mass Spectral Data Acceptance in Forensic Toxicology

1 Scope

This document provides criteria for the acceptance of mass spectral analyses of small molecules (compounds with an atomic weight of less than 800 daltons) in laboratories conducting any of the following forensic toxicology subdisciplines: postmortem forensic toxicology, human performance toxicology (e.g., drug-facilitated crimes and driving-under-the-influence of alcohol or drugs), non-regulated employment drug testing, court-ordered toxicology (e.g., probation and parole, drug courts, child services), and general forensic toxicology (non-lethal poisonings or intoxications).

The document provides minimum requirements for acquiring data on single- or multiple-stage mass spectrometers using low or high-resolution mass spectrometers. It also provides instruction on the evaluation of mass spectral data when conducting acquisitions in full-scan mode, selected ion monitoring, multiple-stage analyses, or when using high-resolution mass analyzers.

Criteria, requirements and instructions in this document are not intended for the area of breath alcohol toxicology. Further, it is not intended to address the use of matrix assisted laser desorption, inductively coupled plasma, or ion mobility mass spectrometry. It is also not intended to provide criteria for analyte identification in forensic toxicology laboratories.

2 Normative References

The following references are indispensable documents for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*, First Edition

ASB Standard 054: *Standard for Quality Control Program in Forensic Toxicology,* First Edition
[document not yet published, will not be included if it is not published]

ASB Standard 113, *Standard for Identification Criteria in Forensic Toxicology*. First Edition
[document not yet published, will not be included if it is not published]

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
base peak
Most abundant ion in the mass spectrum. In plotting the mass spectrum, all other ions are normalized to the base peak [13].

3.2
concurrently analyzed
Analyzed at or close to the same time under the same analytical conditions (e.g., same instrument and instrumental parameters).

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3.3
diagnostic ion
A MS or MS/MS molecular ion or fragment ion that has structural relevance to the targeted analyte.

3.4
full-scan acquisition
Operation of a mass spectrometer in which abundances of ions for entire mass spectrum are recorded over a defined mass range.

3.5
high resolution mass spectrometry
HRMS
In this document, it refers to a MS instrument that can give at least 10,000 nominal mass resolving power at full width of the peak at half its maximum height (FWHM) for the compound of interest. [1].

3.6
ion ratio
In MS, the ratio of the instrument responses between two previously identified diagnostic ions.

3.7
ionization
The physicochemical process of producing a gas-phase ion. In the mass spectrometer this typically occurs within the ion source. Several mechanisms of ionization exist such as chemical and electron ionization[13].

3.8
isotopomer
Isomers having the same number of each isotopic atom but differing in their positions [13].

3.9
low resolution mass spectrometry
LRMS
A mass spectrometer limited to nominal mass resolution measurements. (see nominal mass)

3.10
mass spectrometry
MS
Study of matter through the formation of gas-phase ions that are characterized using mass spectrometers by their mass, charge, structure, and/or physico-chemical properties [14].

3.11
mass-to-charge ration
The mass of an ion divided by its charge [13].

3.12
monoisotopic mass
m/z
Exact mass of an ion or molecule calculated using the mass of the most abundant isotope of each element [14].

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3.13
MS^n
Multiple-stage mass spectrometry experiments designed to record product ion spectra where n is the number of product ion stages (nth-generation product ions) [14].

3.14
multiple reaction monitoring
MRM
Application of selected reaction monitoring to multiple product ions from one or more precursor ions [14].

3.15 nominal mass
Mass of a molecular ion or molecule calculated using the isotope mass of the most abundant constituent element isotope of each element rounded to the nearest integer value and multiplied by the number of atoms of each element [14].

3.16
precursor ion
Ion that reacts to form particular product ions or undergoes specified neutral losses [14].

3.17
product ion
Ion formed as the product of a reaction involving a precursor ion [14].

3.18
reference material
Material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement [13].

3.19
relative abundance
The abundance of an ion produced in relation to the abundance of the base peak [21].

3.20
resolution
In a mass spectrum, the observed m/z value divided by the smallest difference Δ(m/z) for two ions that can be separated: (m/z)/Δ(m/z) [14].

3.21
selected ion monitoring
SIM
Operation of a mass spectrometer in which the abundances of ions of one or more specific m/z values are recorded rather than the entire mass spectrum [14].

3.22
selected reaction monitoring
SRM
Data acquired from one or more specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry [14].

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3.23
tandem mass spectrometry
MS/MS
Acquisition and study of the spectra of the product ions or precursor ions of m/z selected ions, or of precursor ions of a selected neutral mass loss [14].

4 MS Analysis Criteria Requirements

4.1 General Rules

MS analysis may be performed by several approaches including single-stage or multiple-stage instruments at low- or high-resolution. Data may be acquired using full-scan mode or by monitoring selected ions and may be used to establish evidence of analyte identification. Analyte identifications shall meet the requirements of ASB Std 113: *Standard for Identification Criteria in Forensic Toxicology [document not yet published].*

Selected ions shall be diagnostic ions. Ions that represent a loss of derivatizing agent shall not be considered as structurally relevant. Ions generated through the loss of water or isotopic ions are permissible when suitable alternatives are not available and method validation demonstrates selectivity. Intact isotopomers do not allow for structural elucidation by MS and shall not be used as diagnostic ions. Adducts should not be considered as diagnostic ions in single stage mass analyzer and may be considered diagnostic ions in multiple stage analyzers only after validation proves the uniqueness and utility of the adduct. Method parameters (e.g., scan range, monitored ions, dwell time, gas settings, tune criteria) for MS analysis shall be the same as used during method validation studies and follow the requirements of ANSI/ASB Std 036, *Practices for Method Validation in Forensic Toxicology*.

Method performance shall be continuously monitored with a rigorous quality assurance and quality control (QA/QC) program according to ASB Std 054: *Standard for Quality Control Program in Forensic Toxicology [document not yet published]*. Further, documented regular instrument calibration and maintenance shall follow recommendations of the manufacturer and shall be described in the appropriate laboratory procedures.

4.2 Single-Stage Mass Analysis Using a Low-Resolution Mass Analyzer

4.2.1 Full-Scan Acquisition using a Single-Stage Low-Resolution Mass Analyzer

The following criteria shall be met when using a single-stage low-resolution mass analyzer in full-scan mode:

a) The scan range shall be the same as that established during method validation and include all diagnostic ions.

b) The scan range should be set to at least 50 m/z above the highest anticipated ion to account for possible adducts.

c) No ions shall be present at a relative abundance equal to or greater than 50% of the base peak that are not also present in the reference material spectrum. Background subtractions may be performed to achieve this requirement but shall be documented when used.

d) One of the following two requirements shall be met:

1. ratios of diagnostic ions shall agree with those calculated from a reference material given the tolerances shown in Table 1; OR
2. the spectrum shall be compared to a library by a minimum of a forward match (which can be complemented by a reverse match) and be above a pre-defined comparison threshold or match factor.

4.2.2 SIM Acquisition using Single-Stage Low-Resolution Mass Analyzer

A minimum of a single diagnostic ion may be monitored using a single-stage low-resolution mass analyzer in SIM mode. When two or more diagnostic ions are measured, ion ratios shall agree with those calculated from a concurrently analyzed reference material given the tolerances shown in Table 1.

4.3 Multiple-Stage Mass Analysis (MS/MS or MS^n) Using Low-Resolution Mass Analyzer

4.3.1 Product Ion Scan Acquisition Using a Low-Resolution Mass Analyzer

The following criteria shall be met when using a multiple-stage low-resolution mass analyzer in product ion scan mode:

a) The precursor mass shall have unit resolution or better.

b) The scan range for product ion spectra shall be the same as that established during method validation and should be set to at least 50 m/z above the highest anticipated ion to account for possible adducts.

c) A minimum of a single transition of a diagnostic ion shall be monitored.

d) No ions shall be present at a relative abundance equal to or greater than 50% of the base peak that are not also present in the reference material spectrum. Background subtractions may be performed to achieve this requirement but shall be documented when used.

e) One of the following two requirements shall be met:

   1. ratios of diagnostic ions shall agree with those calculated from a reference material given the tolerances shown in Table 1; OR

   2. the spectrum shall be compared to a library by a minimum of a forward match (which can be complemented by a reverse match) and be above a pre-defined comparison threshold or match factor.

4.3.2 MRM Acquisition Using Low-Resolution Mass Analyzer

The following criteria shall be met when using a multiple-stage low-resolution mass analyzer in MRM mode:

a) The precursor mass shall have unit resolution or better.

b) A minimum of a single product ion transition from a single diagnostic precursor ion shall be monitored.

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c) When two or more product ion transitions are measured (from either the same or different precursor ions), ion ratios shall agree with those calculated from a concurrently analyzed reference material given the tolerances shown in Table 1.

4.4 Single-Stage Mass Analysis Using High-Resolution Mass Analyzer

The following criteria shall be met when using a single-stage high-resolution mass analyzer:

a) A minimum of a single diagnostic ion shall be monitored.

b) The scan range shall be the same as that established during method validation and include all diagnostic ions. The scan range should be set to at least 50 m/z above the highest anticipated ion to account for possible adducts.

c) No ions shall be present at a relative abundance equal to or greater than 50% of the base peak that are not also present in the reference material spectrum. Background subtractions may be performed to achieve this requirement but shall be documented when used.

d) The spectrum shall contain the molecular species and any monitored diagnostic ions within 5 ppm of either the theoretical or the measured m/z value of a concurrently analyzed reference material.

e) One of the following two requirements shall be met:

   1. ratios of diagnostic ions shall agree with those calculated from a concurrently analyzed reference material given the tolerances shown in Table 1; OR

   2. The spectrum shall be compared to a library by a minimum of a forward match (which can be complemented by a reverse match) and be above a pre-defined comparison threshold or match factor.

4.5 Multiple-Stage Mass Analysis (MS/MS or MS^n) Using A High-Resolution Mass Analyzer

4.5.1 Product Ion Scan Acquisition Using a High-Resolution Mass Analyzer

The following criteria shall be met when using a multiple-stage high-resolution mass analyzer in product ion scan mode:

a) The precursor mass shall have better than unit resolution.

b) The scan range for product ion spectra shall be the same as that established during method validation and should be set to at least 50 m/z above the highest anticipated ion to account for possible adducts.

c) A minimum of a single transition from a diagnostic precursor ion shall be monitored.

d) No ions shall be present at a relative abundance equal to or greater than 50% of the base peak that are not also present in the reference material spectrum. Background subtractions may be performed to achieve this requirement but shall be documented when used.

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f) The spectrum shall contain the molecular species and any monitored diagnostic ions within 5 ppm of either the theoretical or the measured m/z value of a concurrently analyzed reference material.

g) One of the following two requirements shall be met:

1. ratios of diagnostic ions shall agree with those calculated from a concurrently analyzed reference material given the tolerances shown in Table 1; OR

2. the spectrum shall be compared to a library by a minimum of a forward match (which can be complemented by a reverse match) and be above a pre-defined comparison threshold or match factor.

4.5.2 MRM Acquisition Using High-Resolution Mass Analyzer

The following criteria shall be met when using a multiple-stage high-resolution mass analyzer in MRM mode:

a) The precursor mass shall have better than unit resolution.

b) A minimum of a single product ion transition from a single diagnostic precursor ion shall be monitored.

c) When two or more product ion transitions are measured (from either the same or different precursor ions), ion ratios shall agree with those calculated from a concurrently analyzed reference material given the tolerances shown in Table 1.

Table 1—Maximum Acceptable Ion Ratio Limits[1]

Relative Intensity (% of base peak)	Electron Impact Ionization (relative)	All Other Ionization Techniques (relative)
Greater than 50%	20%	20%
20 to 50%		25%
10 to 20%		30%
Less than 10%	50%	50%

[1] Adapted from Commission of the European Communities (2002). Official Journal of the European Communities; Guidance Document for Laboratories and Inspectors, National Laboratory Certification Program, Research Triangle Park, NC, 2002; and U.S. Department of Health and Human Services Food and Drug Administration, Center for Veterinary Medicine (2001).

Annex A
(informative)

Bibliography

1] Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data for the FDA Foods and Veterinary Medicine Program US Food & Drug Administration Office of Foods and Veterinary Medicine September 2015

2] Berendsen, B.J., L.A. Stolker, and M.W. Nielen, . "The (Un) Certainty of Selectivity in Liquid Chromatography Tandem Mass Spectrometry." *Journal of the American Society for Mass Spectrometry*, 2012. 24, 154-163.

3] Clinical and Laboratory Standards Institute, & International Federation of Clinical Chemistry and Laboratory Medicine (2007). Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline (CLSI document C50-A). Wayne, Pennsylvania: Clinical and Laboratory Standards Institute.

4] Commission of the European Communities (2002). Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C3044) (Text with EEA relevance) (657). Official Journal of the European Communities.

5] Cooper, G. A., Paterson, S., & Osselton, M. D. (2010). The United Kingdom and Ireland Association of Forensic Toxicologists Forensic toxicology laboratory guidelines. Science and Justice, 50, 166-176.

6] Couchman, L., & Morgan, P. (2011). LC-MS in analytical toxicology: some practical considerations. Biomedical Chromatography. 25, 100-123.

7] Guidance Document for Laboratories and Inspectors, National Laboratory Certification Program, Research Triangle Park, NC, 2002.

8] Habib Jiwan, J. L., Wallemacq, P., & Herent, M. F. (2010). HPLC-high resolution mass spectrometry in clinical laboratory? Clinical Biochemistry, 44, 136-147.

9] Hoyt, D. W., Finnigan, R. E., Nee, T., Shults, T. D., & Butler, T. J. (1987). Drug Testing in the Workplace-Are Methods Legally Defensible? A Survey of Experts, Arbitrators, and Testing Laboratories. Journal of the American Medical Association, 258(4), 504-509.

10] Lehotay, S. J., Mastovska, K., Amirav, A., Fialkov, A. B., Alon, T., Martos, P. A., Kok, A. D., & Fernandez-Alba, A. R. (2008). Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques. Trends in Analytical Chemistry, 27(11), 1070-1090.

11] Maurer, H. H. (2005). Advances in analytical toxicology: the current role of liquid chromatography–mass spectrometry in drug quantification in blood and oral fluid. Analytical and Bioanalytical Chemistry 381, 110-118.

DRAFT

12] Peters, F. T., & Remane, D. (2012). Aspects of matrix effects in applications of liquid chromatography–mass spectrometry to forensic and clinical toxicology—a review. Analytical and Bioanalytical Chemistry 403, 2155-2172.

13] OSAC Preferred Terms. Retrieved from http://lexicon.forensicosac.org/Term/Home/Index. Accessed March 14, 2018

14] Pure Appl. Chem., Vol. 85, No. 7, pp. 1515–1609, 2013. http://dx.doi.org/10.1351/PAC-REC-06-04-06

15] RTI International (2010). The National Laboratory Certification Program Manual for Urine Laboratories, October (rev. July 2011).

16] Sauvage, F. L., Gulier, J. M., Lachatre, G., & Marquet, P. (2008). Pitfalls and Prevention Strategies for Liquid Chromatography–Tandem Mass Spectrometry in the Selected Reaction Monitoring Mode for Drug Analysis. Clinical Chemistry. 54, 1519-1527.

17] Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (2011). Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations. Retrieved from http://www.swgdrug.org/

18] Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology, Journal of Analytical Toxicology, Volume 37, Issue 7, 1 September 2013, Pages 452–474, https://doi.org/10.1093/jat/bkt054

19] U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research, & Center for Veterinary Medicine (2001). Guidance for Industry Bioanalytical Method Validation. Retrieved from http://www.fda.gov/cder/guidance/index.htm

20] U.S. Department of Health and Human Services Food and Drug Administration, Center for Veterinary Medicine (2001). Guidance for Industry Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues US FDA/CVM. May 1, 2003 Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052658.pdf

21] WADA (2003). Identification Criteria for Qualitative Assays Incorporating Chromatography and Mass Spectrometry (TD2003IDCR).

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