This document has been accepted by the **Academy Standards Board (ASB)** for development as an American National Standard (ANS). For information about ASB and their process please refer to asb.aafs.org. This document is being made available at this stage of the process so that the forensic science community and interested stakeholders can be more fully aware of the efforts and work products of the Organization of Scientific Area Committees for Forensic Science (OSAC). The documents were prepared with input from OSAC Legal Resource Committee, Quality Infrastructure Committee, and Human Factors Committees, as well as the relevant Scientific Area Committee. The content of the documents listed below is subject to change during the standards development process within ASB and may not represent the contents of the final published standard. All stakeholder groups or individuals are strongly encouraged to submit technical comments on this draft document during the ASB's open comment period. Technical comments will not be accepted if submitted to the OSAC Scientific Area Committee or Subcommittees.

Standard for Mass Spectral Data Acceptance in Forensic Toxicology

Draft Document

Standard for Mass Spectral Data Acceptance in Forensic Toxicology

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 nominal 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 originally conceived by the Scientific Working Group for Toxicology (SWGTOX) and further developed by the Toxicology Subcommittee of the Organizational Scientific Area Committee (OSAC). It was prepared and finalized as a standard by the Toxicology Consensus Body of the ASB.

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

Keywords: *Mass spectrometry, mass spectral data*

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 engaged in 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). It is 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.

The focus of the document is on minimum requirements for acquiring data on single- or multiple-stage mass analysis using nominal or high-resolution mass spectrometers. It also provides instructions for 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.

2 Normative References

ASB/ANSI 036 Standard Practices for Method Validation in Forensic Toxicology. It is available at XXX.

ASB/ANSI XX Standard Practices for Analyte Identification in Forensic Toxicology. It is available at XXX.

ASB/ANSI XX Standard Practices for Quality Control in Forensic Toxicology. It is available at XXX.

3 Terms and Definitions

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
diagnostic ion
An ion whose formation reveals structural or compositional information about the target analyte. (14)

3.3
high resolution
HR
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)

3.4
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 ionization and electron ionization.

3.5
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 physio chemical properties. (14)

3.6
monoisotopic mass
Exact mass of an ion or molecule calculated using the mass of the most abundant isotope of each element. (14)

3.7
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)

3.8
multiple reaction monitoring
MRM
Application of selected reaction monitoring to multiple product ions from one or more precursor ions. (14)

3.9
nominal mass analyzer
In this document, it refers to a MS instrument that can achieve resolution of <5000.

3.10
precursor ion
Ion that reacts to form product ions or undergoes specified neutral losses. (14)

3.11
product ion
Ion formed as the product of a reaction involving a precursor ion. (14)

3.12
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)

3.13
relative abundance
The abundance of an ion produced in relation to the abundance of the base peak. (21)

3.14
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)

3.15
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)

3.16
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)

3.17
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

4.1 General Rules

MS analysis may be performed by several approaches including single-stage or multiple-stage instruments at nominal and high resolution. Data may be acquired using full-scan or by monitoring selected ions.

Selected ions shall be diagnostic ions. Ions that represent a loss of derivatizing agent or loss of water shall not be considered as structurally relevant. Intact isotopomers do not allow for structural elucidation by MS and shall not be used as diagnostic ions. Adducts (such as dimers) shall not be considered as diagnostic ions because no meaningful information is obtained from their fragmentation.

Instrument operating conditions (e.g. tune parameters, scan range, monitored ions, ionization energies, dwell time) for MS analysis shall be the same as used during method validation studies.

Method performance shall be continuously monitored with a rigorous quality assurance and quality control (QA/QC) program. Further, documented regular instrument maintenance shall follow recommendations of the manufacturer and described in the appropriate laboratory procedures.

4.2 Single-stage mass analysis using a nominal mass analyzer

4.2.1 Full-scan acquisition for screening:

The following criteria shall be met when using single-stage nominal mass analyzer for screening purposes in full-scan mode:

1) 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 fragment.

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

4.2.2 Full-scan acquisition for confirmation:

The following criteria shall be met when using single-stage nominal mass analyzer for confirmation purposes in full-scan mode:

1) 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 fragment.

2) No ions shall be present at a relative abundance equal to or greater than 50% that are not present in the reference material spectrum.

3) When background subtractions are performed, they shall be documented.

4) Ratios of diagnostic ions:

   a) Shall agree with those calculated from a reference material given the tolerances shown in Table 1; *or*

   b) The spectrum shall be compared to a library by a forward and reverse spectral match algorithm and be above a documented, pre-defined threshold (match factor).

Table 1: Maximum Acceptable Ion Ratio Limits (adapted from references 4,7, and 21)

Relative Abundance	Electron Impact Ionization	All Other Ionization Techniques
Greater than 50%	20%	20%
20 to 50%	20%	25%
Less than 20%	20%	30%

4.2.3 SIM for screening:

When using a single-stage nominal mass analyzer for screening purposes in SIM mode, a minimum of a single diagnostic ion shall be monitored.

4.2.4 SIM for confirmation:

When using a single-stage nominal mass analyzer for confirmation purposes in SIM mode, diagnostic ion ratios shall agree with those calculated from a concurrently analyzed reference material given the tolerance shown in Table 1.

4.3 Multiple-stage mass analysis using a nominal mass analyzer (MS/MS or MS^n)

4.3.1 Product ion scan acquisition for screening:

The following criteria shall be met when using a multiple-stage nominal mass analyzer for screening purposes in product ion scan mode:

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

2) The scan range shall be the same as that established during method validation. The upper end of the scan range for product ion spectra should be set above the precursor ion m/z.

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

4.3.2 Product ion scan acquisition for confirmation:

The following criteria shall be met when using a multiple-stage nominal mass analyzer for confirmation purposes in product ion scan mode:

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

2) The scan range for product ion spectra shall be the same as that established during method validation. The upper end of the scan range for product ion spectra should be set above the precursor ion m/z.

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

4) No ions shall be present at a relative abundance equal to or greater than 50% that are not present in the reference material spectrum.

5) Ratios of diagnostic ions:

    a) Shall agree with those calculated from a reference material given the tolerances shown in Table 1; *or*

    b) The spectrum shall be compared to a library by a forward and reverse spectral match algorithm and be above a pre-defined threshold.

4.3.3 MRM acquisition for screening:

The following criteria shall be met when using a multiple-stage nominal mass analyzer for screening purposes in MRM mode:

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

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

4.3.4 MRM acquisition for confirmation:

The following criteria shall be met when using a multiple-stage nominal mass analyzer for confirmation purposes in MRM mode:

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

2) Ratios of diagnostic product ions 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 a high-resolution analyzer

4.4.1 Single-stage mass analysis with high-resolution acquisition for screening:

When using a single-stage high-resolution mass analyzer for screening purposes, a minimum of a single diagnostic ion shall be monitored.

4.4.2 Single-stage mass analysis with high-resolution acquisition for confirmation:

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

1) 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 fragment.

2) No ions shall be present at a relative abundance equal to or greater than 50% that are not present in the reference material spectrum.

3) The spectrum shall contain the molecular species within 5 ppm of the theoretical monoisotopic mass.

4) Ratios of diagnostic ions:

    a) Shall agree with those calculated from a reference material given the tolerances shown in Table 1; *or*

    b) The spectrum shall be compared to a library by a forward and reverse spectral match algorithm and be above a pre-defined threshold.

4.5 Multiple-stage mass analysis using a high-resolution analyzer (MS/MS or MSn)

4.5.1 Multiple-stage mass analysis with high-resolution acquisition for screening:

When using a multiple-stage high-resolution mass analyzer for screening purposes, a minimum of a single diagnostic ion shall be monitored.

4.5.2 Multiple-stage mass analysis with high-resolution acquisition for confirmation:

The following criteria shall be met when using a multiple-stage high-resolution mass analyzer for confirmation purposes:

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

2) 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 fragment.

3) No ions shall be present at a relative abundance equal to or greater than 50% that are not present in the reference material spectrum.

4) The spectrum must contain the molecular species within 5 ppm of the theoretical monoisotopic mass.

5) Ratios of diagnostic ions:

    a) Shall agree with those calculated from a reference material given the tolerances shown in Table 1; *or*

    b) The spectrum shall be compared to a library by a forward and reverse spectral match algorithm and be above a pre-defined threshold.

Annex A
(informative)

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