ANSI/ASB Standard 098, First Edition
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Standard for Mass Spectral Analysis in Forensic Toxicology

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Standard for Mass Spectral Analysis in Forensic Toxicology

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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 low- or high-resolution mass spectrometers that utilize ionization processes such as electron ionization, chemical ionization, electrospray ionization, or atmospheric pressure chemical ionization.

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2015 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI), and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practices, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

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 Forensic 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).

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 401 N 21st Street, Colorado Springs, CO 80904.

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

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Keywords: *mass spectrometry, mass spectral data.*

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Standard for Mass Spectral Analysis 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 (e.g., 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. It also provides instructions on the evaluation of mass spectral data when conducting acquisitions in full-scan mode, selected ion monitoring, or multiple-stage analyses.

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 (2019)[a]

ANSI/ASB Standard 054, *Standard for Quality Control Program in Forensic Toxicology,* First Edition (2021)[a]

ANSI/ASB Standard 113, *Standard for Identification Criteria in Forensic Toxicology*, First Edition (2023)[a]

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
base peak
Most abundant ion in the mass spectrum. When plotting the mass spectrum, all other ions are normalized to the base peak.

[a] Available from: https://www.aafs.org/academy-standards-board

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

3.3
deconvoluted mass spectrum
Mass spectrum processed with an algorithm designed to extract a desired signal or signals from raw experimental data in which the desired signals have been complicated (convolved) by some interferences or in some other way.[11]

3.4
diagnostic ion
A MS or MS/MS molecular ion or fragment ion whose presence and relative abundance are characteristic of the targeted analyte.

3.5
full-scan acquisition
Operation of a mass spectrometer in which abundances of ions for entire mass spectrum are recorded over a defined mass range.

3.6
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].

3.7
ion ratio
In MS, the ratio of the instrument responses between two previously identified diagnostic ions.

3.8
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.

3.9
isotopomer
Isomers having the same number of each isotopic atom but differing in their positions.

3.10
low resolution mass spectrometry
LRMS
A mass spectrometer limited to nominal mass resolution measurements. (see nominal mass)

3.11
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 physicochemical properties [11].

3.12
mass-to-charge ratio
The mass of an ion divided by its charge.

3.13
match factor
A mathematical value that indicates the degree of similarity between an unknown spectrum and a reference spectrum.

3.14
molecular ion
Ion formed by the removal of one or more electrons from a molecule to form a positive ion or the addition of one or more electrons to a molecule to form a negative ion.

3.15
monoisotopic mass
m/z
Exact mass of an ion or molecule calculated using the mass of the most abundant isotope of each element [11].

3.16
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) [11].

3.17
multiple reaction monitoring
MRM
Application of selected reaction monitoring to multiple product ions from one or more precursor ions [11].

3.18
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 [11].

3.19
precursor ion
Ion that reacts to form particular product ions or undergoes specified neutral losses [11].

3.20
product ion
Ion formed as the product of a reaction involving a precursor ion [11].

3.21
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.

3.22
relative abundance
The abundance of an ion produced in relation to the abundance of the base peak.

3.23
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) [11].

3.24
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 [11].

3.25
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 [11].

3.26
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 [11].

3.27
target ion
A diagnostic ion used for comparing relative intensities of other monitored ions when calculating ion ratios.

3.28
unit mass resolution
Mass resolution such that it is possible to clearly distinguish a peak corresponding to a singly charged ion from its neighbors 1 dalton away, usually with no more than 5-10% overlap[11].

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 various MS acquisition modes (examples: full-scan, SIM, MRM, SRM) and may be used to establish evidence of analyte

identification. Final analyte identification shall meet the requirements of ANSI/ASB Std 113, *Standard for Identification Criteria in Forensic Toxicology*, First Edition.

4.1.1 Criteria for Monitored Ions

Ions monitored shall be diagnostic. Ions generated through the loss of water, in-source fragmentation, or isotopic ions are permissible when suitable alternatives are not available and method validation demonstrates selectivity. Unless chromatographic separation is achieved, intact isotopomers do not allow for structural elucidation by MS and shall not be used as diagnostic ions. Adducts may be considered as diagnostic ions when validation proves the uniqueness and utility of the adduct. For derivatized analytes, the ion(s) representing the derivatizing agent itself shall not be considered as diagnostic.

4.1.2 MS Parameters

When establishing the upper limit of the scan range in full spectrum acquisitions, both isotopes and adducts should be considered.[b] Selection of precursor ion(s) for multiple-stage MS techniques shall be of appropriate resolution for the mass analyzer used. 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*, First Edition.

4.1.3 Quality Assurance and Quality Control

Method performance shall be continuously monitored with a rigorous quality assurance and quality control (QA/QC) program according to ANSI/ASB Std 054, *Standard for Quality Control Program in Forensic Toxicology*, First Edition. Further, all applicable instrument calibration and maintenance shall follow recommendations of the manufacturer and be properly documented. The laboratory procedures shall describe the instrument calibration and maintenance requirements.

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

In addition to the criteria in 4.1, the following shall be met when using a single-stage low-resolution mass analyzer in full-scan mode.

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

b) When monitoring more than one diagnostic ion:

   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 using an appropriate library search and be above a pre-defined match factor as demonstrated through method validation.

[b] Adduct formation is generally only a concern for some ionization techniques (e.g., electrospray).

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 spectrum. Deconvoluted mass spectra or background subtractions may be used to achieve this requirement but shall be documented when used.

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

In addition to the criteria in 4.1, a minimum of a single diagnostic ion may be monitored using a single-stage low-resolution mass analyzer in SIM mode. When monitoring more than one diagnostic 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

In addition to the criteria in 4.1, the following shall be met when using a multiple-stage low-resolution mass analyzer in product ion scan mode:

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

b) When monitoring more than one diagnostic product ion:

   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 using an appropriate library search and be above a pre-defined match factor as demonstrated through method validation.

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 spectrum. Deconvoluted mass spectra or background subtractions may be used to achieve this requirement but shall be documented when used.

4.3.2 MRM Acquisition Using Low-Resolution Mass Analyzer

In addition to the criteria in 4.1, the following shall be met when using a multiple-stage low-resolution mass analyzer in MRM mode:

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

b) When monitoring more than one diagnostic product ion (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

In addition to the criteria in 4.1, the following shall be met when using a single-stage high-resolution mass analyzer or multi-stage high-resolution mass analyzer for single-stage mass analysis:

a) The spectrum shall contain the molecular ion.

b) All monitored diagnostic ions shall be within a pre-defined mass error and isotopic distribution tolerance as demonstrated through method validation.

c) When monitoring more than one diagnostic ion:

    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 using an appropriate library search and be above a pre-defined match factor as demonstrated through method validation.

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

In addition to the criteria in 4.1, the following shall be met when using a multiple-stage high-resolution mass analyzer in product ion scan mode:

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

b) All monitored diagnostic ions shall be within a pre-defined mass error and isotopic distribution tolerance as demonstrated through method validation.

c) When monitoring more than one diagnostic product ion:

    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 using an appropriate library search and be above a pre-defined match factor as demonstrated through method validation.

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

4.5.2 MRM Acquisition Using High-Resolution Mass Analyzer

In addition to the criteria in 4.1, the following shall be met when using a multiple-stage high-resolution mass analyzer in MRM mode:

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

b) When monitoring more than one diagnostic product ion (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 Permitted Tolerances for Ion Ratios[c]

Relative Intensity (% of base peak or target ion)	Tolerances for Electron Impact Ionization (relative)	Tolerances for all Other Ionization Techniques (relative)
Greater than 50%	± 20%	± 20%
20 to 50%		± 25%
10 to 20%		± 30%
Less than 10%	± 50%	± 50%

[c] 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)

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