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Standard for Identification Criteria in Forensic Toxicology

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Foreword

This Standard for Identification Criteria in Forensic Toxicology was developed to provide minimum requirements for the identification of an analyte in forensic toxicology laboratories. The fundamental reason for defining acceptable identification criteria is to ensure confidence and reliability in forensic toxicological test results.

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 Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science and is modeled after The Official Journal of the European Communities Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC).

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.

ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

Keywords: *identification, forensic toxicology*

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Standard for Identification Criteria in Forensic Toxicology

1 Scope

This document sets minimum criteria, based on a point system, for the identification of an analyte during forensic toxicology testing. The document provides a mechanism for laboratories to evaluate each analytical technique to determine if their testing regimen is sufficient to meet or exceed the minimum points required for identification. This document does not address identification of alcohols and routine volatiles, carbon monoxide, cyanide, or metals.

2 Normative References

The following references are documents that are indispensable for the application of the 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 098, *Standard for Mass Spectral Analysis in Forensic Toxicology*. First Edition (2023)[a]

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
analyte
A chemical substance to be identified and/or measured.

3.2
chromatography
A physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.

3.3
concurrently analyzed
Analyzed at or close to the same time under the same analytical conditions (e.g., same instrument and instrumental parameters).

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

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

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.

3.6
interferences
Non-targeted substances (i.e., matrix components, other drugs and metabolites, internal standard, impurities) which may impact the ability to detect, identify, or quantitate a targeted analyte.

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
isomers
Compounds that have the same elemental formula but have different structural configurations and hence different physical and/or chemical properties. [3]

3.10
limit of detection
An estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and identified by the analytical method.

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. [3]

3.12
match factor
A mathematical value that indicates the degree of similarity between an unknown spectrum and a reference spectrum.

3.13
matrix
A specific biological fluid or tissue. Examples include blood, plasma, serum, urine, vitreous fluid, hair, and tissue.

3.14
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). [3]

3.15
minimum identification criteria
Lowest number of points, including a chromatographic separation, that must be achieved within a testing regimen to identify an analyte in a specific matrix.

3.16
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. [3]

3.17
precursor ion
Ion that reacts to form particular product ions or undergoes specified neutral losses. [3]

3.18
product ion
Ion formed as the product of a reaction involving a precursor ion. [3]

3.19
specificity
Ability of a method to distinguish between the targeted analyte and other non-targeted substances.

3.20
specimen
A matrix sample collected from a specific origin for toxicological analysis. Examples include femoral or cardiac blood; left versus right eye vitreous fluid; and liver, brain, or kidney.

4 Requirements

4.1 Background

4.1.1 Toxicological examinations typically begin with screening techniques that rule out the presence of analytes or indicate if further testing is warranted. Screening techniques shall have limits of detection for analytes of interest.

4.1.2 Historically, confirmation of presumptive positive screening results was accomplished using different chemistries or techniques.

4.1.3 The combination of the data obtained from all techniques for a given matrix contributes to the identification of an analyte. For the purposes of identification, data obtained for a given matrix (e.g., blood, vitreous, or kidney) collected from different anatomic sites may be combined.

4.1.4 A wide array of techniques and instrumentation exist within forensic toxicology laboratories for the identification of an analyte. Different techniques offer a range of identification potential. The purpose of this document is to establish a rating system for comparing and contrasting different identification techniques. Each technique is assigned a point value based on its specificity. Techniques can be combined to achieve a total score that meets or exceeds predefined criteria for identification.

4.1.5 Although one hyphenated instrumental technique (e.g., LC-MS/MS) may be sufficient to achieve identification, this alone does not ensure the reliability, reproducibility, quality, and integrity of results. As a matter of good laboratory practice, two aliquots of the same or different matrices from the same subject should be independently analyzed.

4.1.6 While mass spectrometry techniques are commonly used in forensic toxicology for analyte identification, this document does not mandate the use of mass spectrometry. However, in general, mass spectrometry techniques afford more specificity.

4.2 Analytical Methods

4.2.1 Validation

All analytical methods used to generate identification points shall be properly validated to meet the requirements of ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology* (2019) and demonstrate they are fit-for-purpose.

4.2.2 Chromatography

At least one chromatographic or electrophoretic separation technique, including a concurrently analyzed reference standard/positive control of the analyte of interest, shall be performed to achieve identification. Chromatographic or electrophoretic acceptability criteria (retention time, peak shape, resolution, signal to noise) shall be specified in the validated analytical method and shall be met for analyte identification. Identification points for chromatographic and electrophoretic separations are only awarded when a reference standard/positive control is concurrently analyzed.

4.3 Point System Identification

4.3.1 General Requirements

4.3.1.1 To identify an analyte, a minimum of four (4) points shall be achieved by combining no more than three different techniques on a specific matrix.

- Hyphenated techniques count as one technique (e.g., GC-NPD, GC-MS, LC-MS/MS).
- If mass spectrometry is not utilized, at least two different chromatographic separations shall be performed to alter the separation of target analytes and/or interferences.

4.3.1.2 Each identified analyte in each matrix shall independently meet the minimum point criteria. For example, identification points obtained from an immunoassay of blood do not apply to the identification point total for an analyte in a urine sample from the same case.

4.3.1.3 Repetition of the same technique on the same matrix does not earn additional points toward the total needed for identification. For example, repeating the same GC-MS analysis of blood does not earn additional points for an identification.

4.3.1.4 Identification points from an analytical scheme shall only be combined based on the specificity of any given test, as demonstrated by method validation.

— A blood specimen analyzed by ELISA detects the presence of cannabinoids. Points are only allowed for those cannabinoids with sufficient cross-reactivity. During method validation, only carboxy-THC was determined to have sufficient cross-reactivity.[b] A GC-MS analysis confirms the presence of carboxy-THC in the blood specimen, but also detects THC. The points for the ELISA may be used toward the identification of carboxy-THC, but not the THC.

— A blood specimen analyzed by ELISA detects the presence of benzodiazepines. A LC-MS/MS analysis confirms the presence of two different benzodiazepines in the blood specimen: alprazolam and diazepam. During method validation, both drugs demonstrated sufficient cross-reactivity in the benzodiazepine immunoassay. The points for the ELISA may be used toward the identification of both the alprazolam and diazepam.

— A urine specimen analyzed by full scan GC-MS detects only a quetiapine metabolite (norquetiapine) using a spectral library match. A LC-MS/MS analysis confirms the presence of norquetiapine in the urine specimen, but also detects the parent drug, quetiapine, and another metabolite, hydroxyquetiapine. The points for the initial full scan GC-MS may be used toward the identification of norquetiapine, but not toward quetiapine and hydroxyquetiapine.

4.3.1.5 Specific identification of an isomeric compound shall meet the minimum point requirements of this document (e.g., escitalopram, d-amphetamine). Unless differentiation is achieved, it is only acceptable to identify the mixed isomeric compound (e.g., amphetamine or d/l-amphetamine, methorphan or dextro/levomethorphan).

[b] See Section 8.7.2. Estimating LOD for Immunoassays in ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology.*

4.3.2 Assignment of Points to Specific Techniques [c]

Non-Mass Spectrometric Techniques	**Points earned**
Colorimetric Tests (e.g., Fujiwara, Diphenylamine, TLC Visualization Techniques, Trinders)	0.5
Non-instrument Immunoassay (e.g., Dipstick, Lateral flow immunoassay cards, Urine cup)	0.5
Instrument Immunoassay (e.g., ELISA, EMIT, CEDIA, KIMS)	1
Chromatographic or Electrophoretic Separation [d]	1
Each Non-selective Detector (e.g., FID, TCD, UV)	0.5
Each Selective Detector (e.g., NPD, DAD, ECD, Fluorescence)	1
Non-Chromatographic Mass Spectrometric Techniques [e]	
Low Resolution MS (e.g., DART, LDTD, direct infusion)	1
High Resolution MS (e.g., DART, LDTD, direct infusion)	2
MS^n (e.g., DART, LDTD, direct infusion)	2
Chromatographic Techniques with Mass Spectrometric Ion Monitoring	
Chromatographic or Electrophoretic Separation [c]	1
Low Resolution MS	1 per ion
Low Resolution MS^n, precursor product ion transition	2 per ion transition
High Resolution MS	2.5 per ion
High Resolution MS^n, precursor product ion transition	3 per ion transition
Chromatographic Techniques with Mass Spectral Library Matching [f]	
Chromatographic or Electrophoretic Separation [c]	1
Low Resolution Full Scan	2
Low Resolution MS^n, product ion spectrum	3
High Resolution Full Scan	3.5
High Resolution MS^n, product ion spectrum	4

[c] According to 4.2.2, in order to achieve minimum identification criteria, a chromatographic or electrophoretic separation technique and a minimum of four (4) points shall be required.
[d] According to 4.2.2, identification points for chromatographic and electrophoretic separations are only awarded when a reference standard/positive control is concurrently analyzed.
[e] According to 4.5.6, no more than two points shall be awarded for non-chromatographic mass spectrometry techniques.
[f] According to 4.5.3, mass spectrometry library matches shall meet pre-defined library match criteria as specified in the validated analytical method.

4.4 Chromatographic Requirements

4.4.1 Chromatography performed on different stationary phases receives one point for each column chemistry.

4.4.2 Chromatography performed on the same stationary phase employing two different detection techniques is awarded one point for the chromatography and additional points for each detection technique. For example, the same chemistry column with FID and NPD detection would be awarded 2.5 points.

4.5 Mass Spectrometry Requirements

4.5.1 When two or more diagnostic MS ions are measured, ion ratio acceptability criteria shall be met as specified in ANSI/ASB Standard 098, *Standard for Mass Spectral Data Acceptance in Forensic Toxicology* (2023).

4.5.2 When points are awarded for ion transitions, the product ions can derive from the same (e.g., a single precursor ion yields two different product ions) or different precursor ions (e.g., two different precursor ions yield the same or different product ions). All precursor and product ions are required to be diagnostic per ANSI/ASB Standard 098, *Standard for Mass Spectral Data Acceptance in Forensic Toxicology* (2023).

4.5.3 When spectral library searches are conducted, the results shall meet or exceed a predefined match factor that is documented in the laboratory's standard operating procedures and meet the criteria specified in ANSI/ASB Standard 098, *Standard for Mass Spectral Data Acceptance in Forensic Toxicology* (2023).

4.5.4 When ions are acquired in the full scan mode, points may only be awarded for either the library match or extracted ion ratios.

4.5.5 Ionization processes such as electron ionization, electrospray ionization, chemical ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization are considered different techniques. Points awarded for a specific ionization process may only be awarded for either the full scan or the selected ion monitoring mode, but not both. Points for both single- and multi-stage mass spectrometry with the same ionization process are allowed, provided different ions are monitored in each technique.

4.5.6 For non-chromatographic MS and MS^n techniques, a maximum of two points is permitted, regardless of the number of monitored ions.

Annex A
(informative)

Acronyms in Annex B

DAD – Diode array detector
DART – Direct analysis in real time
ECD – Electron capture detector
FID – Flame ionization detector
GC – Gas chromatography
HR – High resolution
IA (I) – Immunoassay (Instrument)
IA (N) – Immunoassay (Non-Instrument)
LC – Liquid chromatography
LDTD – Laser diode thermal desorption
LR – Low resolution
MS – Mass spectrometry
NPD – Nitrogen phosphorous detector
SOP – Standard operating procedure
TCD – Thermal conductivity detector
TLC – Thin layer chromatography
TOF – Time of flight
UV – Ultraviolet

Annex B
(informative)

Examples of Identification Points for Common Forensic Toxicology Laboratory Methods

Technique(s)	Tabulation	Total Points
Combinations Insufficient for Identification		
Colorimetric test + GC-FID	0.5+1+0.5	2
IA (N) + GC-FID	0.5+1+0.5	2
Dual column GC-NPD (must be different column chemistries)	1+1+1	3
IA (I) + HR LDTD-MS^n	1+2	3
LR GC-MS with full scan spectral library match	1+2	3
HR GC-MS with 1 ion	1+2.5	3.5
IA (I) + HR LDTD-MS + full scan spectral library match	1+2+3.5	6.5[g]
Combinations Sufficient for Identification		
LR LC-MS with 3 ions	1+3	4
IA (I) + GC-NPD + LR DART	1+1+1+1	4
LR LC-MS with product ion spectral library match	1+3	4
IA (I) + GC-FID + HR DART	1+1+0.5+2	4.5
IA (I)+ GC-FID + GC-NPD (must be different column chemistries)	1+1+0.5+1+1	4.5
HR LC-MS TOF with full scan spectral library match	1+3.5	4.5
LR GC-MS/MS with 2 precursor product ion transitions	1+2+2	5
IA (I)+ LR GC-MS (3 ions)	1+1+3	5
LR LC-MS/MS with 2 precursor product ion transitions	1+2+2	5
Colorimetric test + LR GC-MS (4 ions)	0.5+1+4	5.5
IA (I)+ HR LC-MS with full scan spectral library match	1+1+3.5	5.5
IA (I) + LR LC-MS full scan spectral library match + GC-NPD	1+1+2+1+1	6
HR LC-MS with 2 ions	1+2.5+2.5	6
LR LDTD-MS + LR LC-MS/MS with 2 precursor product ion transitions	1+1+2+2	6
IA (I)+ HR GC-MS/MS with product ion spectral match	1+1+4	6
Colorimetric test + HR GC-MS (2 ions)	0.5+1+2.5+2.5	6.5
HR GC-MS/MS with 2 precursor product ion transitions	1+3+3	7

[g] NOTE There is no identification, as no chromatographic technique is included.

Annex C
(informative)

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