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Standard for Identification Criteria in Forensic Toxicology

Draft Document

Standard for Identification Criteria in Forensic Toxicology

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.

This standard was developed by the Toxicology Subcommittee of the Organizational Scientific Area Committee 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). This standard 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.

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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 low molecular weight analytes (e.g., ethanol, carbon monoxide, cyanide) or metals.

2. Normative References

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

ASB/ANSI 098 Standard for Mass Spectral Data Acceptance in Forensic Toxicology. Draft. It is available at XXX.

3. Terms and Definitions

For purposes of this document, the following definitions and acronyms 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
diagnostic ion
A pre-identified MS or MS/MS fragment ion that has structural relevance to the targeted analyte. It is not appropriate to use fragment ions with little structural relevance such as the loss of derivatizing agent-derived fragments, isotopomers, or certain adducts (including, for example, dimers).

3.4
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.

3.5
interferences
Non-targeted analytes (i.e., matrix components, other drugs and metabolites, internal standard, impurities) which may impact the ability to detect, identify, or quantitate a targeted analyte.

3.6
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.

3.8
isobaric compounds
Compounds that have the same nominal mass but have different exact masses.

3.9
isomers
Compounds that have the same elemental formula but have different structural configurations and hence different physical and/or chemical properties. IUPAC

3.10
isotopomeric isomers
Isotopic isomers that have the same atomic composition, and therefore the same exact mass, but differ in their structural arrangement.

3.11
low resolution mass spectrometry
LRMS
Measurement of the mass-to-charge (m/z) ratio of an ion's aggregate atomic masses to within 1 m/z of the ion's exact mass. A low resolution mass spectrometer is commonly known as a nominal mass analyzer.

3.12
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 physiochemical properties. IUPAC

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).

3.14
match factor
A mathematical value that indicates the degree of similarity between an unknown spectrum and a spectrum from a previously analyzed standard.

3.15
minimum identification criteria
Lowest number of points, including a chromatographic separation, achieved within a testing regimen to identify an analyte.

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

3.17
product ion
Ion formed as the product of a reaction involving a precursor ion. IUPAC

3.18
specificity
Ability of a method to distinguish between the analyte being measured and other substances.

3.19
transition ratio
In MS^n, the ratio of the instrument response between a product ion and its precursor ion.

4. Background

4.1 Toxicological examinations typically begin with screening techniques. The purpose is to rule out the presence of analytes that are detectable by these techniques, or to indicate when further testing may be warranted. Screening techniques have limits of detection for analytes of interest.

4.2 As a general matter of scientific and forensic toxicology practice, confirmation of presumptive positive screening results is accomplished using one or more techniques and based upon a different chemical principle.

4.3 The combination of the data obtained from each screening and confirmatory technique contributes to the identification of a drug, metabolite, or other analyte.

4.4 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.

4.5 Although one analytical technique may be sufficient to achieve identification, this alone does not ensure the reliability, reproducibility, quality, and integrity of results. In addition, two independent aliquots of the same specimen or two matrices from the same case should be analyzed.

4.6 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 and are awarded higher point values.

5. Analytical Methods

5.1 Validation

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

5.2 Chromatography

At least one chromatographic or electrophoretic separation technique shall be performed to achieve identification. Chromatographic acceptability criteria (retention time, peak shape, resolution, signal to noise, etc.) shall be specified in the validated analytical method and shall be met for analyte identification.

6. Point System for Identification

6.1 Assignment of points to specific techniques [a]

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	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 [b]	
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 Mass Spectrometric Techniques	
Chromatographic or Electrophoretic Separation	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
Spectral Library Matching [c]	
Chromatographic or Electrophoretic Separation	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

[a] In order to achieve minimum identification criteria, a chromatographic or electrophoretic separation technique and a minimum of four (4) points shall be required.
[b] No more than two points shall be awarded for non-chromatographic mass spectrometry techniques.
[c] Mass spectrometry library matches shall meet pre-defined library match criteria as specified in the validated analytical method.

6.2 General requirements

6.2.1 A minimum of four (4) points shall be achieved by combining no more than three different techniques. (Hyphenated techniques count as one technique, such as GC-NPD.)

6.2.2 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.

6.2.3 Repetition of the same method does not earn additional points toward the total needed for identification.

6.2.4 When known isobaric and isotopomeric compounds exist, they shall be evaluated during method validation. If chromatographic separation and/or spectrometric differentiation is not attained for isobaric or isotopomeric compounds, identification of the specific analyte is not achieved and reporting shall reflect this limitation (e.g., citalopram/escitalopram or d/l amphetamine).

6.3 Chromatographic requirements

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

6.3.2 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.

6.4 Mass spectrometry requirements

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

6.4.2 Spectral library searches may be conducted and 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 the current *ASB/ANSI 098 Standard for Mass Spectral Data Acceptance in Forensic Toxicology.*

6.4.3 Ionization processes such as electron ionization, electrospray ionization, chemical ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization are considered different techniques.

6.4.4 For non-chromatographic high resolution MS and MS^n techniques, only two points are awarded regardless of the number of ions monitored. Additional points may be gained for a spectral library match; however, a chromatographic or electrophoretic separation is still required.

Annex A
(informative)

Acronyms in Annex B

CEDIA – Cloned enzyme donor immunoassay
DAD – Diode array detector
DART – Direct analysis in real time
ECD – Electron capture detector
EMIT – Enzyme multiplied immunoassay
ELISA – Enzyme linked immunoassay
FID – Flame ionization detector
GC – Gas chromatography
HR – High resolution
KIMS – Kinetic interaction of microparticles in solution
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 Assignment of Identification Points

Technique(s)	Tabulation	Total Points
Combinations Insufficient for Identification		
Colorimetric test+ GC-FID	0.5+1+0.5	2
ELISA + HR LDTD-MS^n	1+2	3
LR LC-MS with full scan spectral library match	1+2	3
HR GC-MS with 1 ion	1+2.5	3.5
ELISA + HR LDTD-MS + full scan spectral library match	1+2+3.5	6.5 NOTE: There is no identification, as no chromatographic technique is included.
Combinations Sufficient for Identification		
LR LC-MS with 3 ions	1+3	4
EMIT + GC-NPD + LR DART	1+1+1+1	4
LR LC-MS with product ion spectral library match	1+3	4
ELISA + GC-FID + HR DART	1+1+0.5+2	4.5
ELISA + 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
ELISA + LR GC-MS (3 ions)	1+1+3	5
Colorimetric test + LR GC-MS (4 ions)	0.5+1+4	5.5
EMIT + HR LC-MS with full scan spectral library match	1+1+3.5	5.5

ELISA + 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+4	6
CEDIA + HR GC-MS with product ion spectral match	1+1+4	6
Colorimetric test + HR GC-MS (2 ions)	0.5+1+5	6.5
HR GC-MS/MS with 2 precursor product ion transitions	1+3+3	7

Annex C
(informative)

Bibliography

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)

Murray, K. K.; Boyd, R. K.; Eberlin, M. N.; Langley, G. J.; Li, L.; Naito, Y., Definitions of terms relating to mass spectrometry (IUPAC Recommendations 2013). *Pure and Applied Chemistry* **2013,** *85* (7), 1515-1609. http://dx.doi.org/10.1351/PAC-REC-06-04-06

ASB/ANSI 054 Standard for a Quality Control Program in Forensic Toxicology Laboratories. Draft. It is available at XXX.