OSAC 2025-S-0016
Standard for the Identification and Quantitation of Volatile Compounds in Biological Fluids

Forensic Toxicology Subcommittee
Chemistry: Seized Drugs Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

DRAFT OSAC 2025-S-0016 Standard for the Identification and Quantitation of Volatile Compounds in Biological Fluids

Prepared by
Forensic Toxicology Subcommittee
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The STR consists of an independent and diverse panel, which may include subject matter experts, human factors scientists, quality assurance personnel, and legal experts as applicable. The

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Foreword

Volatile compound analysis is one of the most frequently performed forensic toxicological tests; however, routine volatiles (e.g., acetone, ethanol, isopropanol, and methanol) are excluded from the scope of ANSI/ASB Standard 113, *Standard for Identification Criteria in Forensic Toxicology*. This document addresses the minimum requirements for the identification and quantitation of routine volatiles in biological fluids (e.g., blood, vitreous, urine, tissue homogenates) using headspace gas chromatography-flame ionization detection (GC-FID) or headspace gas chromatography-mass spectrometry (GC-MS).

The draft of this standard was developed by the Forensic Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Keywords: *ethanol, methanol, isopropanol, acetone, forensic toxicology, volatiles, alcohol*

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Standard for the Identification and Quantitation of Volatile Compounds in Biological Fluids

1 Scope

This document establishes the minimum requirements for the identification and quantitation of acetone, ethanol, isopropanol, and methanol in biological fluids (e.g., blood, vitreous, urine, tissue homogenates) by headspace gas chromatography-flame ionization detection (GC-FID) or headspace gas chromatography-mass spectrometry (GC-MS). It does not apply to non-chromatographic techniques (e.g., enzymatic assays, color tests). The analysis of other volatile compounds, including metabolites, is not addressed. The document is intended for 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 ethanol 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 breath alcohol subject testing.

2 Normative References

The following references are documents that are indispensable for the application of the standard. The latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology*

ANSI/ASB Standard 036, *Standard for Test Method Selection, Development, Validation, and Verification in Forensic Toxicology*

ANSI/ASB Standard 037, *Standard for Reporting and Testimony of Forensic Toxicology Results and Opinions*

ANSI/ASB Standard 054, *Standard for a Quality Control Program in Forensic Toxicology Laboratories*

ANSI/ASB Standard 056, *Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology*

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

ANSI/ASB Standard 119, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations*

ANSI/ASB Standard 120, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations*

ANSI/ASB Standard 121, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations*

ANSI/ASB BPR 156, *Best Practices for Specimen Collection and Preservation for Forensic Toxicology*

ANSI/ASB Technical Report 208, *Forensic Toxicology: Terms and Definitions*

3 Terms and Definitions

All applicable terms within this document are defined in ANSI/ASB Technical Report 208, *Forensic Toxicology: Terms and Definitions*.

4 Equipment Requirements

4.1 The following equipment shall be used.

4.1.1 Reagents and other materials, including purified water, internal standard(s) (n-propanol or t-butanol), Certified Reference Materials, and matrix-matched control(s).

4.1.2 Pipette(s) or automated diluter

4.1.3 Headspace vials with nonreactive stoppers and caps

4.1.4 Headspace autosampler system equipped with an incubator, agitator, and gas-tight syringe

4.1.5 Gas chromatograph(s) with capillary column(s)

4.1.6 Flame ionization detector(s) and/or mass spectrometer with electron impact ionization

4.1.7 Instrument controller that includes data acquisition and processing software

4.1.8 Mixer or rotator (optional)

5 Method Development and Validation Requirements

5.1 General Requirements

5.1.1 In addition to the requirements in this document, the laboratory shall adhere to the requirements of ANSI/ASB Standard 036, *Standard for Test Method Selection, Development, Validation, and Verification in Forensic Toxicology*.

5.1.2 The laboratory shall adhere to scope and sensitivity requirements for volatile compounds within the applicable standard(s):

a) ANSI/ASB Standard 119, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations*.

b) ANSI/ASB Standard 120, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations*.

c) ANSI/ASB Standard 121, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations*.

5.1.3 The lower limit of quantitation shall be no greater than 0.020 g/dL (or equivalent) for ethanol and methanol and 0.010 g/dL for acetone and isopropanol.

5.1.4 The upper limit of quantitation shall be no less than 0.300 g/dL (or equivalent) for ethanol and 0.100 g/dL for all other compounds.

5.1.5 Interferent studies shall include 1,1-difluoroethane, acetaldehyde, chloroethane, sevoflurane, common laboratory solvents, and other volatile compounds based on regional trends.

5.1.6 The method's bias and precision shall be within ±10% for ethanol and ±20% for all other compounds.

5.2 Sample Preparation Requirements

5.2.1 The following sample preparation parameters shall be established during method development in accordance with ANSI/ASB Standard 036, *Standard for Test Method Selection, Development, Validation, and Verification in Forensic Toxicology.*

5.2.1.1 Specimens shall be amenable to pipetting prior to sample preparation. If the laboratory homogenizes specimens, there shall be an established procedure for homogenization.

5.2.1.2 Sample preparation shall use a 10-fold or greater dilution with internal standard.

5.2.1.3 Headspace vials shall be agitated prior to headspace sampling.

5.3 Instrument Optimization Requirements

5.3.1 The following instrument specifications shall be established during method development in accordance with ANSI/ASB Standard 036, *Standard for Test Method Selection, Development, Validation, and Verification in Forensic Toxicology*.

5.3.1.1 Analysis shall be performed using headspace gas chromatography-flame ionization detection (GC-FID) or headspace gas chromatography-mass spectrometry (GC-MS).

5.3.1.2 Two different column chemistries to identify volatile compounds shall be employed when solely using GC-FID detection. A single column chemistry may be used with GC-MS.

5.3.1.3 The laboratory shall define which column(s) and detector(s) will be used to generate quantitative results.

5.3.1.4 Incubation parameters (e.g., time, temperature) shall be established to achieve equilibrium in the headspace vial between the sample and the headspace above the sample.

NOTE: Typical incubation times are 5-15 minutes and temperatures between 50-80°C. These parameters depend on the sample volume, headspace vial size, agitation time, and inclusion of other volatile compounds.

5.3.1.5 The injector mode (i.e., sample loop, syringe), volume fill time, and vial pressurization shall be established to ensure consistent sampling.

5.3.1.6 The injection/transfer line temperature shall be at least 10°C above the boiling point of the analytes of interest and the internal standard to maintain the compounds' gaseous phase.

5.3.1.7 The gas chromatograph inlet temperature shall be greater than or equal to the injection/transfer line temperature to maintain the gaseous phase of the compounds.

5.3.1.8 The gas chromatograph column(s) shall be capable of separating the target compounds from their most common interferents with baseline resolution in at least one column.

5.3.1.9 The gas chromatograph carrier gas type and flow rate shall achieve optimal chromatographic performance (e.g., gaussian peak shape, baseline resolution) for the target compounds.

5.3.1.10 The gas chromatograph oven temperature shall be an isothermal or a gradient temperature program and achieve optimal chromatographic performance (e.g., gaussian peak shape, baseline resolution) for the target compounds.

NOTE: An oven temperature is typically held at 40°C (isothermal). Alternatively, a gradient temperature program may start with a 40°C hold until the elution of volatile compounds (within the scope of this document) before an increase to 100°C at 30°C/min.

5.3.1.11 For flame ionization detectors (FIDs), the flow rates of hydrogen, air, and makeup gases shall achieve stable operation of the detector(s), and the detector temperature shall be between 150-200°C to maintain signal stability of the detector(s).

5.3.1.12 For mass spectrometer (MS) detectors, electron impact (EI) ionization shall be used.

5.3.1.13 For MS detectors, data shall be collected in scan mode with a mass range starting at 20 m/z or in selected ion monitoring (SIM) by acquiring the following diagnostic ions:

a) Ethanol: 31, 45, 46 *m/z*

b) Methanol: 31, 32, 29 *m/z*

c) Isopropanol: 45, 43, 27 *m/z*

d) Acetone: 43, 58, 42 *m/z*

e) Internal Standard: n-propanol (31, 42, 59 *m/z*) or t-butanol (59, 57, 41 *m/z*)

NOTE: The diagnostic ions are listed in order of abundance, with the base peak being represented first.

5.4 Observations, Data Processing, and Calculations Requirements

5.4.1 The chromatographic peak area signal-to-noise ratio shall be ≥3.

5.4.2 The chromatographic peaks shall exhibit a Gaussian shape.

5.4.3 The acceptable retention time window shall be established as one of the following:

- The retention time of the compounds (including the internal standard) shall be within ±2% of the retention time of the respective compound in a calibrator or control, or

- The relative retention time of the compound to the internal standard shall be within ±0.5% of the relative retention time of the respective compound in a calibrator or control.

5.4.4 Mass spectral acceptance criteria shall require the ratios of diagnostic ions and spectral library comparisons to adhere to ANSI/ASB Standard 098, Standard for Mass Spectral Analysis in Forensic Toxicology.

5.4.5 Mass spectral scan data shall be evaluated using extracted diagnostic ions (Section 5.3.1.13) and their respective ratios compared to a concurrently analyzed reference material or spectral library.

5.4.6 Mass spectral SIM data shall be evaluated using diagnostic ions and their respective ratios compared to a concurrently analyzed reference material.

6 Procedure Requirements

6.1 Quality Control Requirements

6.1.1 The laboratory shall adhere to calibrator and control requirements within ANSI/ASB Standard 054, Standard for a Quality Control Program in Forensic Toxicology Laboratories, and the following additional requirements.

6.1.2 At least one negative and one positive control for all target compounds shall be matrix-matched and included in each analytical run.

6.1.3 Each analytical run, whether qualitative or quantitative, shall include at least one low positive control for all compounds with a target concentration of no more than 0.030 g/dL.

6.1.4 Each quantitative analytical run shall include at least one high positive control for all compounds with a target concentration of at least 80% of the highest calibrator.

6.1.5 Each quantitative analytical run shall include at least one midlevel positive control for all compounds with a target concentration between the low and high positive controls.

NOTE: A legally defined limit may be considered for the midlevel positive control concentration.

6.1.6 A positive control shall be analyzed at least every 20 injections.

6.2 The laboratory shall define the specimens to be analyzed when multiple specimens (of the same or different matrices) from the same subject are received.

EXAMPLE 1: The analysis of antemortem blood will be performed on the specimen collected at the earliest time in the appropriate specimen container per ANSI/ASB BPR 156, Best Practices for Specimen Collection and Preservation for Forensic Toxicology.

EXAMPLE 2: When a postmortem blood specimen tests positive for ethanol, a vitreous specimen, if submitted, will also be analyzed.

6.3 Sample Preparation and Analysis Requirements

6.3.1 All calibrators, controls, and specimens (collectively called “samples”) shall be handled in the same manner from sample preparation through instrumental and data analysis.

6.3.2 No more than one sample shall be open at a given time.

6.3.3 Samples shall be prepared in an environment (e.g., separate room, fume hood) isolated from the use of common laboratory solvents and other concentrated volatiles.

6.3.4 Tissues and clotted blood shall be homogenized.

6.3.5 Samples shall be mixed prior to aliquoting.

6.3.6 Samples shall be sequentially aliquoted (in the order of the injection sequence), diluted with internal standard, and immediately capped prior to proceeding to the next sample.

6.3.7 Headspace vials shall be loaded onto the headspace autosampler and verified against the instrumental sequence.

6.3.8 Headspace vials shall be agitated prior to headspace sampling.

6.3.9 An independent, secondary check of the headspace autosampler vial position and instrumental method sequence should be performed.

6.3.10 The instrument manufacturer, model, configuration, unique identifier information, and established method parameters shall be documented.

6.4 Analytical Run Data Evaluation Requirements

6.4.1 Chromatographic acceptance shall follow the requirements established during method development and validation.

6.4.2 The internal standard response for case specimens shall be no greater than ±20% of the average internal standard response from the calibrators and/or controls.

6.4.3 Calibration and control acceptance shall adhere to ANSI/ASB Standard 054, *Standard for a Quality Control Program in Forensic Toxicology Laboratories*.

6.5 Metrological traceability shall be maintained by adhering to ANSI/ASB Standard 017, *Standard Practices for Metrological Traceability in Forensic Toxicology.*

6.6 Measurement uncertainty shall be calculated in compliance with ANSI/ASB Standard 056, *Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology*.

6.7 Unless a legal specification defines how the measurement result and expanded uncertainty are to be reported, the laboratory shall report the results as follows:

6.7.1 Reported results shall adhere to ANSI/ASB Standard 037, Standard for Reporting and Testimony of Forensic Toxicology Results and Opinions.

6.7.1.1 Quantitative results shall be obtained from the analysis of at least two separate aliquots for each specimen within the same or different analytical runs.

6.7.1.2 All quantitative results within the calibration range for each specimen shall be averaged using a minimum of three significant figures in the calculation.

6.7.1.3 Each quantitative result shall be within ±10% of the mean of all quantitative results for each specimen.

EXAMPLE: A laboratory may have different acceptance criteria when performing both antemortem and postmortem testing (i.e., antemortem ±5%, postmortem ±10%).

6.7.1.4 The laboratory shall define a mitigation procedure (e.g., repeat analysis or report results qualitatively) to address individual quantitative results outside the acceptance criteria.

6.7.2 The average quantitative result and the expanded uncertainty shall be reported following ANSI/ASB Standard 056, *Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology.*

6.7.3 The laboratory shall have a procedure to address reporting and/or additional testing when a result(s) is outside of the calibration range.