Application News

No. GC-2111

Gas Chromatography

Method Validation for the Detection and Quantitation of Blood Volatiles Using Headspace Gas Chromatography with Dual Flame Ionization Detection and AOC-6000 Plus Autosampler

■ Introduction

Blood alcohol content (BAC) analysis by gas chromatography with dual flame ionization detection (GC-FID) has been the gold standard for volatile detection and quantitation in forensic toxicology laboratories for many years due to its ease of automation, sensitivity, specificity, accuracy, cleanliness, and robustness. Using a multi-injection rail system autosampler (AOC-6000 Plus) with headspace capabilities, a single headspace injection is split between two chromatographic columns, each with different column chemistries to allow for enhanced specificity of volatile analytes, and each leading to a dedicated FID. The peak areas obtained from the two chromatograms are used to calculate the BAC in biological samples.

A method to identify and quantify methanol, ethanol, isopropanol, and acetone using n-propanol as an internal standard was developed and validated by the Miami-Dade Medical Examiner Department (MDME) Toxicology Laboratory utilizing a Shimadzu Nexis GC-2030 with dual FIDs, an AOC-6000 Plus autosampler, and two GC columns with different chemistries. The results of the method validation are detailed in this application note.

■ Experimental

See Shimadzu Application News number GC-2104A for detailed preparation steps for the aqueous calibrator solutions, whole blood positive controls, and internal standard solution, as well as the sample preparation and instrumental parameters used for the method validation.

■ Method Validation

The method was validated following the published ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*. The following validation criteria were determined: calibration model, limit of detection (LOD), limit of quantitation (LOQ), bias, within-run and between-run precision, carryover, dilution integrity, selectivity and specificity, prepared sample stability on the autosampler, and sample re-injection stability.

■ Results and Discussion

Calibration Model and LOQ

A calibration curve with six non-zero calibration standards was prepared, and each was analyzed in single over five runs. After plotting the data and performing a statistical analysis on the individual calibration curves in Excel, the working ranges were determined to be 0.020-0.506 g/dL for methanol, 0.020-0.505 g/dL for ethanol, 0.020-0.502 g/dL for isopropanol, and 0.002-0.051 g/dL for acetone. The data showed that all analytes exhibited a non-linear relationship, so a quadratic curve fit was chosen with a weighing factor of 1/x. All calibration curves had an r^2 ≥0.999. The LOQ was determined to be 0.020 g/dL for methanol, ethanol, and isopropanol and 0.002 g/dL for acetone.

LOD

An instrument shooter prepared at half the concentration of the lowest calibration standard was analyzed in single over five days. All analytes were qualitatively identified in all five samples. The LOD was administratively set at 0.010 g/dL for methanol, ethanol, and isopropanol and at 0.001 g/dL for acetone.

Bias and Precision

Whole blood positive controls prepared at low, mid, and high concentrations were analyzed in triplicate over five days (n=15). The average bias was calculated to be within the pre-determined maximum of ±10% of the target concentrations as prepared for Methanol, ±5% for Ethanol and Isopropanol, ±20% for Acetone at the low concentration, and ±10% for Acetone at the mid and high concentrations. The within-run and between-run precision were calculated to be below the pre-determined maximum of <10% for all analytes.

Carryover

A negative control without ISTD was prepared and analyzed immediately following a sample prepared at twice the concentration of the highest calibration standard. No carryover was observed in the negative control for any of the analytes or ISTD.

Dilution Integrity

A 1:1 dilution sample (50 µL) of the high-concentration whole blood positive control was prepared and analyzed in triplicate over five days along with undiluted (100 µL) aliquots of the high-concentration whole blood positive control also prepared and analyzed in triplicate over five days. It was determined that a 1:1 dilution following the sample volume reduction protocol was acceptable with bias calculating to within ±5% of the target concentrations for all analytes.

Endogenous Interferences

Ten different sources of whole blood, serum, ocular fluid, and bile, as well as six different sources of liver tissue and brain tissue from postmortem cases at the MDME known to be negative for all analytes, were aliquoted without ISTD and analyzed. No endogenous interferences were observed in any of the whole blood, serum, ocular fluid, or bile specimens; however, some liver and brain tissue specimens produced peaks with concentrations below the validated LOQ that were identified as some of the target analytes on one or both of the GC columns. Identification of an analyte on both GC columns could have been due to the actual presence of the target analyte produced during decomposition of the sample in storage.

Exogenous Interferences

A group of commonly used and abused solvents and volatiles were analyzed on the instrument to determine if any peaks interfered with the peak response of any of the target analytes or ISTD. One drop of liquid solvent was added into separate empty 20 mL headspace vials and crimped. Gaseous samples were sprayed into empty 20 mL headspace vials and crimped. After 15 minutes of equilibration at room temperature, 1 mL of headspace was added to individual pre-crimped 20 mL headspace vials with a gastight syringe. Table 1 illustrates the retention time (RT) and relative retention time (RRT) information that was obtained.

Table 1: Retention times and relative retention times of commonly used and abused solvents.

	SH-Rtx-BAC Plus 1		SH-Rtx-BAC Plus 2	
Analyte	**RT (min)**	**RRT**	**RT (min)**	**RRT**
1,1,1,2-Tetrafluoroethane	0.816	0.351	0.737	0.384
1,1-Difluoroethane	0.845	0.364	0.745	0.388
Methanol	1.008	0.434	0.932	0.486
Ethanol	1.307	0.563	1.142	0.595
Chloroform	1.318	0.568	2.197	1.145
Isopropanol	1.631	0.702	1.323	0.690
Acetonitrile	1.711	0.737	1.486	0.775
Acetone	1.786	0.769	1.239	0.646
Dichloromethane	1.918	0.826	1.342	0.700
n-Propanol (ISTD)	2.322	1.000	1.918	1.000
Hexane	3.000	1.292	1.280	0.667
Ethyl Acetate	Not Detected	Not Detected	1.980	1.032
n-Butyl Chloride	Not Detected	Not Detected	2.164	1.128
Toluene	Not Detected	Not Detected	Not Detected	Not Detected
Dimethylformamide	Not Detected	Not Detected	Not Detected	Not Detected

Sample Stability
Six "Set A" (pre-injection) and six "Set B" (no injection) low- and high-concentration whole blood positive controls were prepared. All six Set A low- and high-concentration whole blood positive controls were analyzed at t=0 hours along with a calibration curve. Sets A-1 and B-1 were analyzed at t=6 hours; sets A-2 and B-2 were analyzed at t=12 hours; sets A-3 and B-3 were analyzed at t=24 hours; sets A-4 and B-4 were analyzed at t=36 hours; sets A-5 and B-5 were analyzed at t=48 hours; sets A-6 and B-6 were analyzed at t=72 hours. The analyte area responses and concentrations of all analytes in the Set B low- and high-concentration whole blood positive controls that were left to sit on the autosampler at room temperature without prior injection were stable with <5% change over 72 hours. The analyte area responses and concentrations of all analytes in the Set A low- and high-concentration whole blood positive controls that were left to sit on the autosampler at room temperature with a prior injection were stable with <20% change over 72 hours.

The results of the method validation were within all pre-established acceptability limits. The method has been successfully applied to over 2,500 postmortem cases received by the MDME Toxicology Laboratory since the completion of its validation. Specimens analyzed included postmortem blood, serum, ocular fluid, bile, liver tissue, and brain tissue.

■ Conclusion

BAC analysis is one of the most common types of tests performed in a forensic toxicology laboratory, and a reliable instrument for this type of testing is essential for a laboratory's success. Utilizing a Shimadzu Nexis GC-2030 with dual FIDs, an AOC-6000 Plus autosampler, and two GC columns with different chemistries provides the laboratory with exactly that. The validated method presented provides a sensitive, accurate, and robust procedure for the identification and quantitation of methanol, ethanol, isopropanol, and acetone in postmortem specimens, and also allows for the qualitative identification of other commonly used and abused solvents.

First Edition: November 2021

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com

For Research Use Only. Not for use in diagnostic procedure.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Shimadzu disclaims any proprietary interest in trademarks and trade names used in this publication other than its own. See http://www.shimadzu.com/about/trademarks/index.html for details.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject

© Shimadzu Corporation, 2021