Quantitative Analysis of Specially Denatured Alcohol and Articles Using HS-GC-MSD

Scope and Application

The objective of this method is to qualitatively identify and simultaneously quantify ten (10) common volatile denaturants found in specially denatured alcohol (SDA) and articles made with SDA, and to verify compliance with applicable regulations.

This method utilizes gas chromatography with a mass spectrometer detector and headspace extraction unit to identify and quantify methanol, acetone, isopropyl alcohol, tert-butanol, methyl ethyl ketone, ethyl acetate, heptane, n-propyl acetate, methyl isobutyl ketone, and toluene. Acetone–d_6 and toluene–d_8 are used as internal standards.

Regulatory Tolerances

27 CFR Parts 20 and 21 prescribe the composition of SDA formulas and the requirements for articles made with SDA.

Levels and Limitations

1. Due to tert-butanol's extremely high melting point (24-26°C), there may be a need to gently warm the tert-butanol before it is used.

2. Toluene is present in SDA 2-B and SDA 2-C at a level of 0.50% by volume. A quantitation level lower than 0.60% by volume may be achieved by using the GC-MS in single ion monitoring (SIM) mode.

3. Tert-butanol is present in SDA 39, 39-A, 39-B, 40, 40-A, and 40-B at or slightly below the detection limit. However, tert-butanol is not the only denaturant present in these SDA formulas. Although the level of tert-butanol is below the quantitation limit, GC or LC analysis of the other denaturants can verify compliance with regulations
For all 10 denaturants:

Detection Limit (% by volume)	Quantitation Limit (% by volume)	Linear Range (% by volume)	Interferences
0.120	0.60	0.60 - 10.0	None

Equipment

Instrumentation:

GC:	Agilent 6890 and Chemstation Software, or equivalent
Column:	Agilent DB-624, 30 m x 25mm I.d, 0.25 µm film thickness
Carrier Gas:	Helium, constant flow, 1 mL/min
Temperature:	45°C initial, hold 5 min; ramp at 2°C/min to 50°C, hold 1 min; next ramp at 10°C/min to 70°C; then at 40°C/min to a final temp. of 200°C Total run time = 14.75 min.
Injector:	200:1 split with 50 µL sample loop
MSD Scan Parameters:	Group 1: Start time 1.60 min, 28.0 - 200 amu Group 2: Start time 2.70 min, 45.5 - 200 amu Group 3: Start time 3.10 min, 28.0 - 200 amu

Headspace Sampler:	*Agilent 7697A, or equivalent*
Temperature:	***Oven, 90 ºC*** ***Loop, 100 ºC*** ***Transfer Line, 110 ºC***
Time:	***GC-cycle, 20 min*** ***Vial equilibration, 10 min*** ***Pressurization, 0.20 min*** ***Loop fill, 0.20 min*** ***Loop equilibration, 0.20 min*** ***Injection, 0.20 min***
Extractions:	***2 per vial***
Shake Speed:	***High***

Glassware and Supplies:

Class A volumetric pipets, volumetric flasks, and other assorted laboratory glassware
Amber bottles and tops
50 µL micropipettor
100 µL Eppendorf pipettor
Eppendorf plastic tips
20 mL headspace vials
20 mm headspace crimp caps
Vial crimper

Reagents, Sample Preparation, and Handling

Reagents:

Methanol, ***≥ 99.0% purity***
Acetone, ≥ 99.***0***% purity
Isopropyl Alcohol, ***≥ 99.0% purity***
tert-Butanol, ***≥ 99.0% purity***
Methyl Ethyl Ketone, ***≥ 99.0% purity***
Ethyl Acetate, ***≥ 99.0% purity***
Heptane, ***≥ 99.0% purity***
n-Propyl Acetate, ***≥ 99.0% purity***
Methyl Isobutyl Ketone, ***≥ 98.5% purity***
Toluene, ***≥ 99.0% purity***
Acetone – d_6, 99.9 atom % D
Toluene – d_8, 99.6 atom % D
Ethyl Alcohol, 200 Proof

Reagent Preparation:

Prepare 10% denaturant standard by:

Transferring 10 mL of each of the 10 denaturants into a 100 mL volumetric flask.

Prepare other denaturant standards by:

1. Transferring 20 mL of the 10% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 8% by volume.

2. Transferring 20 mL of the 8% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 6.4% by volume.
3. Transferring 15 mL of the 6.4% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 3.84% by volume.
4. Transferring 10 mL of the 3.84% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 1.54% by volume.
5. Transferring 10 mL of the 1.54% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 0.62% by volume.
6. Transferring 5 mL of the 0.62% standard into a separate 25 mL volumetric flask and dilute to volume with 200 proof ethanol. This will correspond to 0.124% by volume.

Prepare laboratory control sample (LCS) by:

Transferring 5 mL of the 10% standard into a separate 10 mL volumetric flask and dilute to volume with 200 proof ethanol**.** This will correspond to 5% by volume.

Prepare internal standard solution by:

Separately pipetting 2 mL of each acetone-d_6 and toluene-d_8 into a 100 mL volumetric flask and dilute to volume with 200 proof ethanol. This will correspond to 2% by volume.

Store standards, laboratory control sample, and internal standard solution in amber bottles with parafilm at room temperature.
All solutions are stable for 6 months or until r^2 declines to less than 0.99.

Sample Preparation

Samples that are expected to contain more than 10% of a denaturant should be diluted accordingly. Otherwise, no dilution is needed.

Transfer 50 µL of each standard, LCS, and sample into a separate 20 mL headspace vial and add 50 µL of the internal standard solution to each vial.

Note: Care should be taken to ensure accurate pipetting when working with viscous samples.

Procedure

1. Run an ethanol blank at the beginning of the sample set and a minimum of 3 standards on the GC. The choice of standards will depend on the level of denaturant expected in the sample.

2. Generate a calibration curve based on area using the system software. The correlation coefficient (r^2) should be ≥ 0.99. If the correlation coefficient is not ≥ 0.99, follow the quality control procedure outlined below.

3. Run samples and the LCS, followed by an ethanol blank at the end of the run.

4. Run the LCS after every 10 injections.

5. Any samples above the range of the standard curve need to be diluted and re-tested. Conversely, if the sample is too dilute, re-inject with a less diluted sample.

Quality Control

1. An ethanol blank should be run at the beginning and end of each sample set to check for carry over.

2. Standards and the LCS should be run at the beginning of each sample set.

3. After every 10th sample, run the LCS and check for drift. If $r^2 < 0.99$ or the LCS differs by >10% of the expected value, prepare new vials and run standards and samples again.

4. If the new results for the standards or LCS fall outside of the expected ranges, follow the QPD for Nonconforming Work (SSD:QPD:201).

Sources of Uncertainty

Sample dilution
Standard preparation
Instrument performance

Calculations

Quantitative analysis of denaturants is achieved by calibration with internal standards. Acetone, ethyl acetate, heptane, isopropanol, methanol, methyl ethyl ketone, methyl isobutyl ketone, n-propyl acetate, and tert-butanol are calibrated using acetone- d_6. Toluene is calibrated using toluene d-8.

Confirmation and positive quantitation of denaturants are accomplished by using the retention times, as well as the target-to-qualifier ion ratios. Denaturants are calculated as percent (%) by volume.

Denaturant	Retention Time (min)	Target Ion (m/z)	Qualifier Ions (m/z)
Acetone- d_6 (Internal Standard)	3.316	46	64,28
Acetone	3.386	43	39,58
Ethyl Acetate	6.414	43	61,70
Heptane	9.145	71	43,57
Isopropanol	3.540	45	43,59
Methanol	2.221	31	29,32
Methyl Ethyl Ketone	6.252	43	57,72
Methyl Isobutyl Ketone	11.855	43	58,85
n-Propyl Acetate	10.993	43	61,73
tert-Butanol	4.105	59	41,57
Toluene d-8 (Internal Standard)	11.940	98	99,100
Toluene	12.006	91	39,65

Reporting Results

Report results as follows:

Component	Precision	Format
Methanol	1 decimal	x.X % by volume
Acetone	2 decimals	x.XX % by volume
Isopropyl Alcohol	2 decimals	x.XX % by volume
tert-Butanol	2 decimals	x.XX % by volume
Methyl Ethyl Ketone	1 decimal	x.X % by volume
Ethyl Acetate	1 decimal	x.X % by volume
Heptane	1 decimal	x.X % by volume
n-Propyl Acetate	No decimal	Xx % by volume
Methyl Isobutyl Ketone	2 decimals	x.XX % by volume
Toluene	2 decimals	x.XX % by volume

Safety Notes

Normal laboratory safety protocol should be followed.

References

Title 27 Code of Federal Regulations Parts 20 and 21

Location of Validation Package

Quality System Files

Required Training, Certification, and Re-certification

1. In-house or vendor provided training on GC and Chemstation operation.
2. Initial certification is achieved by running 3 blind samples in triplicate with results of precision and reproducibility in agreement with the results in the validation package.
3. Annual proficiency testing.

Revision History

Revision 0 – Initial Revision

Revision 1 – Added headspace sampler and parameters in the equipment section; clarified the purities of the reagents; updated location of validation package