Validation of Residual Solvent Analysis in Neat Materials by GC/FID Headspace

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Introduction

- In addition to chromatographic purity, determination of residual solvents is critical for complete certification of reference materials.
- Determination of residual solvents in API materials is required to meet the ICH and FDA guidelines.
- USP <467> residual solvents method is available for testing a wide range of solvents. However the testing is lengthy, requires different columns and multiple stages of analyses.
- A simple and rapid GC-Headspace method was developed using a DB ALC column. The method is capable of accurately quantitating eight common residual solvents used in our manufacturing process. DB ALC column is widely used in blood ethanol analysis and has proven highly adaptable to screening and detection of many additional volatile organic solvents.
- Baseline resolution between Methanol, Ethanol, Ether, Dichloromethane, Acetone, Hexane, Chloroform and Ethyl Acetate was achieved within fifteen minutes.
- As in any process, when other solvents are used they must be specifically tested and quantitated

Method Parameters

Instrument Parameters Agilent Technologies, 6890 Gas Chromatograph, G1888 Headspace Autosampler	
Column: DB-ALC1, 30 m length x 0.53 mm ID, 3.0µm film thickness	
Temperature Ramp:	40 C (12 minute hold) to 220 C at 40 C/minute (5.5 minute hold)
Sample Loop:	3.0 mL
Carrier Gas:	Helium
Inlet Temperature:	200°C
Ratio:	10:1
Flow Rate:	Constant Flow @ 2.0 mL/min
Headspace Parameters	
Temp - Oven / Sample / Transfer Line:	60°C / 80°C / 100°C
Shaker Condition:	Low
Vial Equilibration:	10 minutes
Pressurization / Loop Fill / Equilibration:	0.10 minute
Injection Time:	0.5 minute
GC Cycle Time:	28 minutes

- Minimum sample weight: 7 mg
- Diluent: N-methyl pyrrolidinone (NMP)
- A reference standard solution containing all eight residual solvents was certified and used for quantitation.
- 100 µL of reference standard solution was added to headspace vial with 1 mL of NMP.

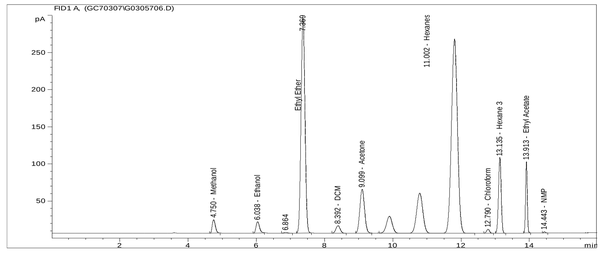
System Suitability

- Six preparations of reference standard were analyzed to establish system suitability and used as an assay standard.
- The reproducibility and resolution between all eight analytes was confirmed.

Analyte	Concentration (mg/mL)	%RSD	Resolution	RRT†
Methanol	1.5	≤3%	N/A	0.65 ± 0.03
Ethanol	1.5	≤3%	≥6	0.82 ± 0.03
Ethyl Ether	1.5	≤3%	≥5	1.00
Dichloromethane	1.5	≤3%	≥3	1.14 ± 0.03
Acetone	1.5	≤3%	≥2	1.24 ± 0.03
Hexanes*	1.5	≤3%	≥6	1.45 ± 0.05
Chloroform	3.0	≤3%	≥8	1.64 ± 0.08
Ethyl Acetate	1.5	≤3%	≥5	1.76 ± 0.1

† RRT is stated for peak identification only
*Hexanes are observed as four main isomers, three elute as a group before chloroform and the fourth elutes after chloroform.

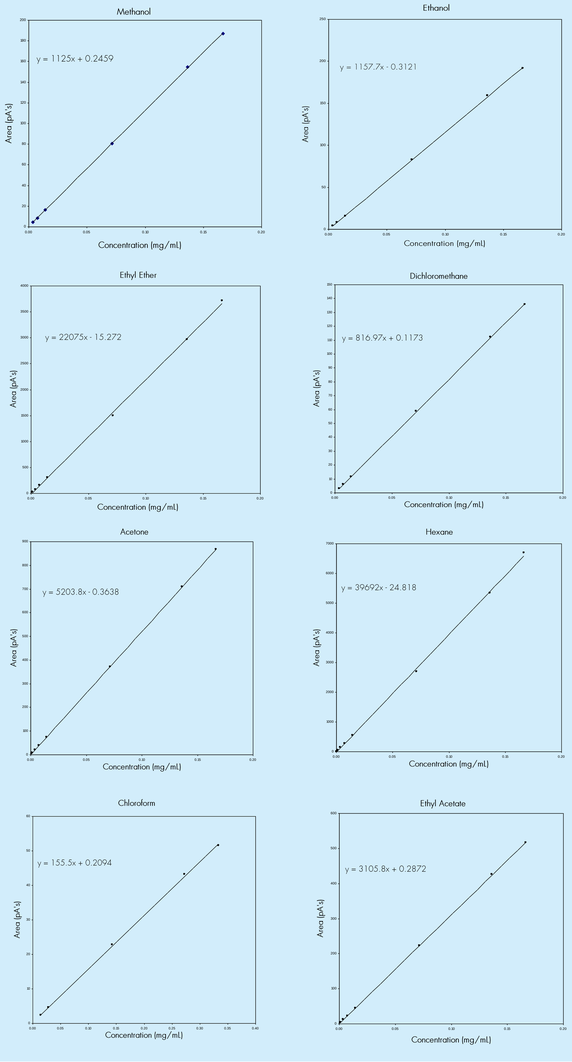
Reference Standard



Linearity and Range

- Linearity was assessed for all eight solvents across the range of 0.000149 mg/mL to 0.167 mg/mL.
- The linear relationship was confirmed by plotting peak area against concentration which had a correlation coefficient ≥ 0.99 and signal/noise ≥ 10.

Analyte	Confirmed Range (mg/mL)	R^2
Methanol	0.0037 to 0.167	0.9999
Ethanol	0.0015 to 0.167	0.9999
Ether	0.00075 to 0.167	0.9995
Dichloromethane	0.0037 to 0.167	1.0000
Acetone	0.00075 to 0.167	1.0000
Hexane	0.000149 to 0.167	0.9995
Chloroform	0.0149 to 0.333	0.9996
Ethyl Acetate	0.00075 to 0.167	1.0000



LOD and LOQ

- Lowest concentration of solvents that can be detected or quantified reliably.
- Based on S/N for peak height
  - Limit of Detection = 3:1 S/N
  - Limit of Quantitation = 10:1 S/N

	LOD		LOQ		
Analyte	mg/mL	ppm	mg/mL	ppm	wt%†
Methanol	0.000678	0.661	0.00229	2.23	0.0327
Ethanol	0.000444	0.432	0.00140	1.37	0.0201
Ethyl Ether	0.000598	0.582	0.00071	0.69	0.0102
Dichloromethane	0.000516	0.503	0.00230	2.24	0.0328
Acetone	0.000181	0.176	0.000424	0.41	0.0061
Hexane	0.000627	0.611	0.00070	0.68	0.0100
Chloroform	0.00247	2.400	0.0124	12.04	0.1766
Ethyl Acetate	0.0000	0.00	0.000188	0.18	0.0027

† wt% determined for a sample with a mass of 7 mg

Accuracy

- Accuracy was assessed at LOQ, 100% and 125% dilution levels of the assay standard in the presence of neat material.
- Five neat materials representative of a broad spectrum of Cerilliant products were used as samples: Acetaminophen, Ephedrine HCl, Morphine, o-p'-DDE, and Oxazepam.
- Accuracy is reported as percent recovery by the assay of known added amount of solvent in the sample.

% Recovery at LOQ and 100%*					
Analyte	Acetaminophen	Ephedrine	Morphine	o,p'-DDE	Oxazepam
Methanol	89.6/100.2	110.5/96.5	100.3/100.2	88.3/102.3	93.2/102.0
Ethanol	113.3/100.6	97.6/97.7	101.1/100.7	103.3/103.4	110.4/102.3
Ether	111.8/98.4	109.5/100.5	112.4/100.0	107.5/100.4	110.2/99.4
DCM	111.9/100.4	107.2/98.8	112.3/100.8	107.2/102.3	112.5/102.2
Acetone	113.3/99.1	118.8/98.5	118.6/99.7	109.0/101.3	111.9/101.2
Hexane	105.3/98.5	102.9/101.1	105.4/100.2	94.4/100.2	101.3/99.0
Chloroform	116.9/101.7	114.5/99.1	118.4/101.9	118.0/104.0	116.6/103.1
Ethyl Acetate	108.6/99.5	102.8/99.0	108.8/99.9	116.0/101.5	112.8/101.4

* LOQ concentration for each solvent is listed in the next Table; 100%: 0.136 mg/mL for all solvents except chlorofom:0.273 mg/mL

LOQ concentration in mg/mL					
Analyte	Acetaminophen	Ephedrine	Morphine	o,p'-DDE	Oxazepam
Methanol	0.0023	0.0023	0.0023	0.0023	0.0023
Ethanol	0.075	0.0014	0.0014	0.0014	0.0014
Ether	0.00071	0.00071	0.00071	0.00071	0.00071
DCM	0.0023	0.0023	0.0023	0.0023	0.0023
Acetone	0.075	0.00042	0.00042	0.075	0.075
Hexane	0.00070	0.00070	0.00070	0.00070	0.00070
Chloroform	0.012	0.012	0.012	0.012	0.15
Ethyl Acetate	0.00019	0.00019	0.00019	0.00019	0.075

- All recoveries at LOQ concentration were within 20%.
- All recoveries at 100% and 125% concentration were within 3%.
- For a given analyte, LOQ concentration varied based on compound class.
- The highest observed LOQ concentration was stated as LOQ for each analyte.

Solvent	LOQ (mg/mL)
Methanol	0.0023
Ethanol	0.075
Ethyl Ether	0.00071
Dichloromethane	0.0023
Acetone	0.075
Hexane	0.00070
Chloroform	0.15
Ethyl Acetate	0.075

Precision

- Expresses the agreement between a series of measurements obtained from multiple analyses of the same homogeneous sample under the prescribed conditions.
- Two Levels of Precision Evaluated
  - Repeatability is demonstrated under the same operating conditions over a short period of time.
  - Intermediate precision, ie- ruggedness, is documented using different analysts, instruments and columns.
- Precision was verified with replicate (five) preparations of Acetaminophen spiked with 100 µL of reference solution. %RSD, relative retention time, resolution and relative response were examined.

Precision Study Results for the Residual Solvents in Acetaminophen

Analyte	%RSD Peak Area		%RSD of Solvent Wt% in Sample	
	Repeatability	Intermediate Precision	Repeatability	Intermediate Precision
Methanol	0.97	8.22	1.19	1.16
Ethanol	1.18	9.65	1.20	1.17
Ethyl Ether	0.83	7.67	1.15	1.15
Dichloromethane	0.84	7.48	1.19	1.16
Acetone	0.63	8.07	1.18	1.15
Hexanes	0.90	7.56	1.14	1.15
Chloroform	1.42	8.60	2.39	2.35
Ethyl Acetate	0.77	7.88	1.19	1.15

- The peak area %RSD from intermediate precision is consistently higher than repeatability. This is a reflection of using two different instruments and columns. On a given day and instrument, the peak area %RSD are under 2%.
- The relative standard deviation of solvent weight % results from intermediate precision are ≤ 2%. The method is precise and accurate despite variations in response from multiple instruments and analysts.

Specificity and Identity

- Specificity is the ability to measure accurately and specifically the analyte in the presence of sample (product and impurities).
- Samples were prepared by adding 100 µL of reference standard to Acetaminophen (7~20 mg) and then spiked with an appropriate level of each analyte.
- Upon spiking, the peak area of each analyte increased relative to the unspiked sample and the relative retention times were verified.

Robustness

- Measurement of the method's capacity to remain unaffected by small but deliberate variation.

Method parameter	Range
Temperature ramp	40°C ± 5°C
Flow rate	2.0 ± 0.5 mL/min
Vial incubation time	10 ± 5
Injection time	0.35 ± 0.5 min
Column	Different lot number

- Results indicated method is tolerant of changes described above except flow rate.
- The flow rate modification resulted in change in relative retention time.

CONCLUSIONS

- Method is reproducible, rapid, accurate and robust.
- Linear from 1 to 166 ppm.
- Accurately quantitates ppm levels of 8 commonly used residual solvents in sample sizes as low as 7 mg.
- The method is applicable to a diverse array of compound classes from pharmaceuticals and illicit drugs to environmental contaminants.

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