ION LABS	Standard Operating Procedure **Residual Solvents by Headspace GC**	**SOP Number** **D-754**	**Revision** **2**
		Effective Date 01|24|23	**Page** **Page 1 of 17**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
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1.0 Purpose

This procedure describes a method for the identification and quantification of residual solvents.

2.0 Scope

This procedure applies to the identification and quantification of residual solvents in dietary supplements, dietary ingredients, pharmaceuticals, and cosmetics in the QC laboratory.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development personnel to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **GC** – Gas Chromatography

4.2 **DMSO** – Dimethyl sulfoxide

4.3 **QC** – Quality Control

4.4 **CofA** – Certificate of Analysis

4.5 **USP** – United States Pharmacopeia

4.6 **RS** – Reference Standard

5.0 References

5.1 USP General Chapter <467> Residual Solvents

5.2 USP General Chapter <1467> Residual Solvents

5.3 D-725, SOP, Loss on Drying

5.4 MV-LAB-19-178, Protocol, Validation of a Method for the Limit of Isopropyl Alcohol in Oil

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents: all reagents are GC grade or better.

6.1.1 Millipore deionized water

6.1.2 DMSO

6.1.3 Residual solvent reference standards

6.1.4 Compressed hydrogen, helium, air, and nitrogen

6.2 Supplies and Glassware

6.2.1 Headspace GC vials, with crimp cap enclosures w/ septa

6.2.2 10-mL and 100-mL volumetric flasks

6.2.3 50-mL and 100-mL beakers

6.2.4 200-uL, 1-mL, and 10-mL pipette tips

6.2.5 Weigh boats

6.3 Equipment

6.3.1 Agilent 7697A Headspace Analyzer

6.3.2 Agilent 7890B GC System

6.3.3 Analytical Balance

6.3.4 Vortex

6.3.5 200-uL, 1-mL and 10-mL pipettes

7.0 Procedure Outline

7.1 Procedure A is a limit test used to identify Class 1 and Class 2 residual solvents that may be present.

7.2 Procedure B utilizes chromatographic separation with different selectivity to confirm any residual solvent previously identified by Procedure A. Confirmation by Procedure B is optional; the analyst may choose to proceed directly to Procedure C.

7.3 For residual solvents that have been identified as present by Procedure A/B or identified as likely to be present by the raw material manufacturer, quantitation is necessary. Use Procedure C to quantitate the residual solvents that are present.

7.4 If only specific solvents need to be quantified, Procedure C may be used directly.

7.5 Procedure D (Loss on Drying) may be used to evaluate the limit of Class 3 residual solvents if they are likely to be present. If the LOD is greater than 0.5% (5000 ppm) or the sample is not amenable to LOD (e.g. an aqueous solution) then it is necessary to quantitate the Class 3 solvents that are likely to be present by Procedure C.

7.6 An outline of the procedure for identification and quantitation of Class 1 and 2 residual solvents is shown in Figure 1.

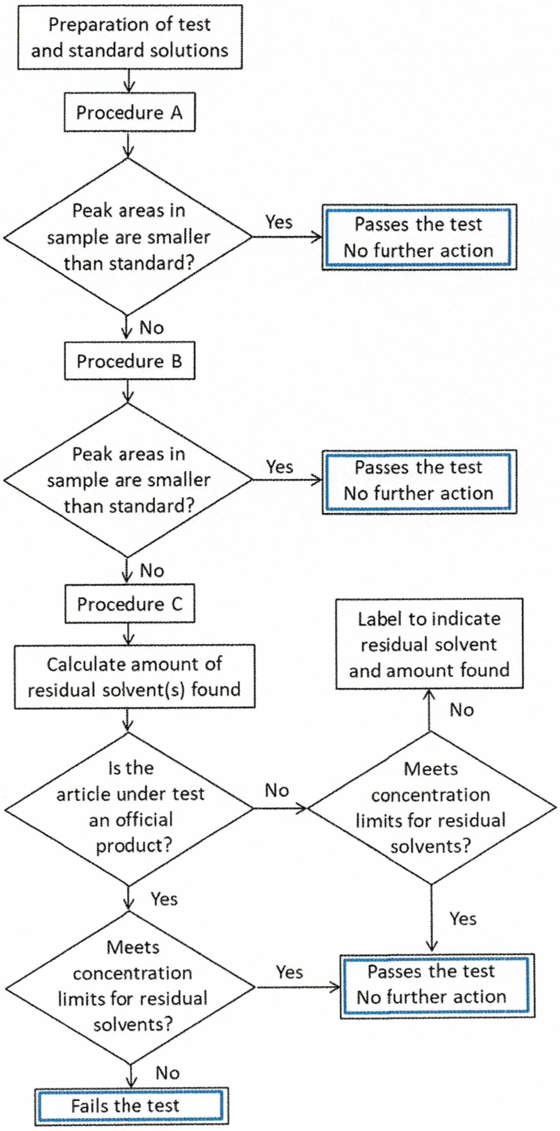

Figure 1: Outline of Procedure for Identification and Quantitation of Residual Solvents

8.0 Limits

8.1 The limits given for each residual solvent in USP <467> are listed in Table 1.

8.2 USP <467> places the each residual solvent in a class based on toxicity:

8.2.1 Class 1 – suspected or known human carcinogens

8.2.2 Class 2 – high toxicity

8.2.3 Class 3 – low toxicity (no health based exposure limit)

Table 1: Limits for Residual Solvents

Solvent	Class	Mixture	PDE (mg/day)	Conc Limit (ppm)
Benzene	1	N/A	N/A	2
Carbon tetrachloride	1	N/A	N/A	4
1,2-Dichloroethane	1	N/A	N/A	5
1,1-Dichloroethene	1	N/A	N/A	8
1,1,1-Trichloroethane	1	N/A	N/A	1500
Acetonitrile	2	A	4.1	410
Chlorobenzene	2	A	3.6	360
Chloroform	2	B	0.6	60
Cumene	2	A	0.7	70
Cyclohexane	2	A	38.8	3880
1,2-Dichloroethene	2	A	18.7	1870
1,2-Dimethoxyethane	2	B	1	100
N,N-Dimethylacetamide	2	C	10.9	1090
N,N-Dimethylformamide	2	C	8.8	880
1,4-Dioxane	2	A	3.8	380
2-Ethoxyethanol	2	C	1.6	160
Ethylene glycol	2	C	6.2	620
Formamide	2	C	2.2	220
Hexane	2	B	2.9	290
Methanol	2	A	30	3000
2-Methoxyethanol	2	C	0.5	50
Methylbutylketone	2	B	0.5	50
Methylcyclohexane	2	A	11.8	1180
Methylene chloride	2	A	6	600
N-Methylpyrrolidone	2	C	5.3	530
Nitromethane	2	B	0.5	50
Pyridine	2	B	2	200
Sulfolane	2	C	1.6	160
Tetrahydrofuran	2	A	7.2	720
Tetralin	2	B	1	100
Toluene	2	A	8.9	890
Trichloroethylene	2	B	0.8	80
Xylene [1]	2	A	21.7	2170
Class 3 Residual Solvents	3	N/A	50	5000

[1] Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene.

9.0 Procedure A (Identification)

9.1 Preparation

9.1.1 Water Soluble Articles

9.1.1.1 Blank Solution - Transfer 6.0 mL of water to a 10-mL headspace vial, apply stopper, crimp cap and mix.

9.1.1.2 Sample Stock Solution – 10 or more dosage units can be pooled and ground by mortar and pestle as necessary. Weigh and transfer about 250 mg of sample to a 25-mL volumetric flask, dissolve and dilute with water to volume and mix.

9.1.1.3 Sample Solution – Transfer 5.0 mL of Sample Stock Solution to a 10-mL headspace vial, add 1.0 mL of water, apply stopper, crimp cap and mix.

9.1.1.4 Class 1 Standard Stock Solution - Add 1.0 mL USP Class 1 residual solvents mixture RS to a 100-mL volumetric flask previously filled with 9 mL DMSO, and dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 100-mL volumetric flask filled about halfway with water. Dilute to volume with water and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask filled about halfway with water. Dilute to volume and mix.

9.1.1.5 Class 1 Standard Solution - Transfer 1.0 mL of Class 1 Standard Stock Solution to a 10-mL headspace vial containing 5 mL water, apply stopper, crimp cap and mix.

9.1.1.6 Class 2 Mixture A Standard Stock Solution - Add 1.0 mL USP Residual Class 2 – Mixture A RS to a 100-mL volumetric flask filled about halfway with water. Dilute to volume with water then mix.

9.1.1.7 Class 2 Mixture A Standard Solution- Transfer 1.0 mL of Class 2 Mixture A Standard Stock Solution to a 10-mL headspace vial containing 5 mL of water, apply stopper, crimp cap and mix.

9.1.1.8 Class 2 Mixture B Standard Stock Solution - Add 1.0 mL USP Residual Class 2 – Mixture B RS to a 100-mL volumetric flask filled about halfway with water. Dilute to volume with water then mix.

9.1.1.9 Class 2 Mixture B Standard Solution - Transfer 1.0 mL of Class 2 Mixture B Standard Stock Solution to an 10-mL headspace vial containing 5 mL of water, apply stopper, crimp cap and mix.

9.1.1.10 System Suitability Solution- Transfer 1.0 mL of Class 1 Standard Stock Solution to a 10-mL headspace vial, add 5.0 mL of Sample Stock Solution, apply stopper, crimp cap and mix.

9.1.2 Water Insoluble Articles

9.1.2.1 Blank Solution - Transfer 1.0 mL of DMSO and 5.0 mL of water to a 10-mL headspace vial, apply stopper, crimp cap and mix.

9.1.2.2 Sample Stock Solution – 10 or more dosage units can be pooled and ground by mortar and pestle as necessary. Weigh and transfer about 500 mg of sample to a 10-mL volumetric flask, dissolve and dilute with water to volume and mix.

9.1.2.3 Sample Solution – Transfer 1.0 mL of Sample Stock Solution to a 10-mL headspace vial, add 5.0 mL of water, apply stopper, crimp cap and mix.

9.1.2.4 Class 1 Standard Stock Solution - Add 1.0 mL USP Class 1 residual solvents mixture RS to a 100-mL volumetric flask previously filled with about 80 mL DMSO, dilute with DMSO to volume and mix. Transfer 1.0 mL of the resulting solution to a 100-mL volumetric flask containing about 80 mL of DMSO, dilute to volume and mix (reserve a portion of this solution for the Class 1 System Suitability Solution).

Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute to volume with DMSO and mix.

9.1.2.5 Class 1 Standard Solution - Transfer 1.0 mL of Class 1 standard stock solution to a 10-mL headspace vial containing 5 mL water, apply stopper, crimp cap and mix.

9.1.2.6 Class 2 Mixture A Standard Stock Solution – Add 1.0 mL USP Residual Class 2 – Mixture A RS to a 100-mL volumetric flask containing about 80 mL of DMSO, dilute to volume with DMSO and mix.

9.1.2.7 Class 2 Mixture A Standard Solution- Transfer 1.0 mL of Class 2 Mixture A Standard Stock Solution to a 10-mL headspace vial containing 5 mL of water, apply stopper, crimp cap and mix.

9.1.2.8 Class 2 Mixture B Standard Stock Solution - Transfer 0.5 mL USP Residual Class 2 – Mixture B RS to a 10-mL volumetric flask containing about 5 mL of DMSO, dilute to volume with DMSO and mix.

9.1.2.9 Class 2 Mixture B Standard Solution - Transfer 1.0 mL of Class 2 Mixture B Standard Stock Solution B to a 10-mL headspace vial containing 5 mL of water, apply stopper, crimp cap and mix.

9.1.2.10 System Suitability Solution- Mix 5.0 mL of Sample Stock Solution with 0.5 mL of the intermediate dilution reserved from Class 1 Standard Stock Solution. Transfer 1.0 mL of the resulting solution to a 10-mL headspace vial containing 5.0 mL of water, apply stopper, crimp cap and mix.

9.2 Analysis

9.2.1 Using the chromatographic conditions outlined in Section 12.1, separately inject equal volumes of headspace of the System Suitability Solution, Class 1 Standard Solution, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard

Solution, Blank Solution, and Sample Solution into the GC system and measure the responses for the peaks-of-interest.

9.3 System Suitability Requirements

9.3.1 The signal-to-noise (S/N) ratio for 1,1,1-trichloroethane in the Class 1 Standard Solution is NLT 5.

9.3.2 The S/N ratio of each peak in the System Suitability Solution is NLT 3.

9.3.3 The resolution between acetonitrile and methylene chloride in the Class 2 Mixture A Standard Solution is NLT 1.0.

9.4 Acceptance Criteria

9.4.1 If the peak response for any peak other than 1,1,1-trichloroethane in the sample solution is ≥ a corresponding peak in any of the standard solutions or 1,1,1-trichloroethane is ≥ 150 times the peak response in the Class 1 standard solution, proceed to Section 10.0 (Procedure B) to confirm the peak.

9.4.2 If no peak response for any peak is ≥ to the peaks in the standard solutions and/or the peak for 1,1,1-trichloroethane is ≤ 150 times the peak from the standard solutions then the sample passes without any need for further tests.

9.5 Method Verification

9.5.1 No additional work is necessary for verification of Procedure A. The System Suitability acceptance criteria satisfy all of the method verification requirements outlined in USP <1467>.

10.0 Procedure B (Confirmation)

10.1 Preparation and Analysis

10.1.1 Use the same Standards and Samples prepared in Procedure A (Section 9.1).

10.2 Analysis

10.2.1 Using the chromatographic conditions outlined in Section 12.2, separately inject equal volumes of headspace of the System Suitability Solution, Class 1 Standard

Solution, Class 2 Mixture A Standard Solution, Class 2 Mixture B Standard Solution, Blank Solution, and Sample Solution into the GC system and measure the responses for the peaks-of-interest.

10.3 System Suitability Requirements

10.3.1 The S/N ratio for benzene in the Class 1 Standard Solution is NLT 5.

10.3.2 The S/N ratio for each peak in the System Suitability Solution is NLT 3.

10.3.3 The resolution between acetonitrile and cis-dichloroethene in the Class 2 Mixture A Standard Solution is NLT 1.0.

10.4 Acceptance Criteria

10.4.1 If the peak response for any peak in the sample solution is ≥ a corresponding peak in any of the standard solutions, proceed to Section 11.0 (Procedure C) to quantify the peak.

10.4.2 If no peak response for any peak is ≥ to the peaks in the standard solutions, then the sample passes without any need for further tests.

10.5 Method Verification

10.5.1 No additional work is necessary for verification of Procedure B. The System Suitability acceptance criteria satisfy all of the method verification requirements outlined in USP <1467>.

11.0 Procedure C (Quantitation)

11.1 Preparation

11.1.1 Water Soluble Articles

11.1.1.1 Blank Solution – Transfer 6.0 mL of water to a 10-mL headspace vial, apply stopper, crimp cap and mix.

11.1.1.2 Standard Stock Solutions – (Note: Only solvents identified as present by Procedure A or identified as likely to be present should be quantified using Procedure C). For each residual solvent transfer an accurately measured volume of the individual RS to a volumetric flask filled about halfway with water, and dilute quantitatively and stepwise, if necessary, with water to obtain a solution having a final concentration of 1/20 the value stated in Table 1. For Class 1 residual solvents, the first dilution should be 1 mL to 100 mL.

11.1.1.3 Standard Solution – Transfer 1.0 mL of each Standard Stock Solution to a 10-mL headspace vial containing 5.0 mL water, apply stopper, crimp cap and mix.

11.1.1.4 Sample Solutions – Same as directed in Section 9.1.1 (Procedure A - Water Soluble Articles).

11.1.1.5 Spiked Sample Solution – For each identified peak, transfer 5.0 mL of Sample Stock Solution to a 10-mL headspace vial and add 1.0 mL of the Standard stock solution, apply stopper, crimp cap and mix.

11.1.2 Water Insoluble Articles

11.1.2.1 Blank Solution – Transfer 1.0 mL of DMSO and 5.0 mL of water to a 10-mL headspace vial, apply stopper, crimp cap and mix.

11.1.2.2 Standard Stock Solutions – (Note: Only solvents identified as present by Procedures A and/or B or identified as likely to be present should be quantified using Procedure C). For each residual solvent transfer an accurately measured volume of the individual RS to a volumetric flask, and dilute quantitatively and stepwise, if necessary, with water to obtain a solution having a final concentration of 1/20 the value stated in Table 1. For Class 1 residual solvents, the first dilution should be 1 mL to 100 mL.

11.1.2.3 Standard Solution – Transfer 1.0 mL of each Standard Stock Solution to a 10-mL headspace vial and add 5.0 mL water, apply stopper, crimp cap and mix.

11.1.2.4 Sample Solutions – Same as directed for Section 11.1.2 (Procedure A - Water Insoluble Articles).

11.1.2.5 Spiked Sample Solution –For each identified peak, transfer 1.0 mL of each Sample Stock Solution to a 10-mL headspace vial, add 1.0 mL of the Standard stock solution and 4.0 mL of water, apply stopper, crimp cap and mix.

11.2 Analysis

11.2.1 Using the chromatographic conditions (Procedure A or Procedure B) that resulted in the best separation for the solvents being quantitated, separately inject equal volumes of headspace for the Blank Solution, Sample Solution, Spiked Sample Solution(s), and Standard Solution(s) into the system and measure the responses for the major peaks.

11.2.2 For each residual solvent, calculate the concentration in ppm of each residual solvent found by the formula:

$$Result = A \times \frac{C}{W} \times \frac{r_u}{r_{st} - r_u}$$

Where A is a constant (A=5 for Water Soluble and A=10 for Water Insoluble articles), C is the concentration of the Standard Stock Solution (µg/mL), W is the weight of sample used to prepare the Sample Stock Solution (g), r_u is the peak response from the Sample Solution, and r_{st} is the peak response from the Spiked Sample Solution.

11.3 System Suitability Requirements

11.3.1 For each peak identified by Procedure A or deemed likely to be present, the S/N ratio is NLT 10 for any solvent except NLT 5 for 1,1,1-trichloroethane or benzene and NLT 3 for any other Class 1 solvent.

11.3.2 The resolution between any peak-of-interest and any adjacent peak is NLT 1.0.

11.4 Acceptance Requirements

11.4.1 If solvent being tested is less than the limit shown in Table 1, the sample passes for that residual solvent.

11.5 Method Verification

11.5.1 If this is the first time that the article has been tested using Procedure C, verification of the method is required. Refer to Section 13.0 for verification requirements.

12.0 Chromatographic Conditions

12.1 Procedure A

12.1.1 Gas Chromatography Conditions

Column:	ZB-624, 30 m x 0.32 mm x 1.8 µm or equivalent
Oven Temp:	40°C
Temp. Gradient:	40°C hold for 20 min, Ramp at 10°C/min to 240°C, and hold for 20 min
Pressure Gradient:	10 psi hold for 20 min, Ramp at 0.21 psi/min to 14.2 psi, and hold for 20 min
Run Time:	60 min
Equil Time:	2 min
Inlet Temp:	140°C
Split Ratio:	5:1
Septum Purge:	2 mL/min
FID Temp:	250°C
Hydrogen:	30 mL/min
Air:	350 mL/min
Helium:	30 mL/min (constant column + makeup)

12.1.2 Headspace Conditions

Oven Temp: 80°C

Loop Temp: 95°C

Transfer Line: 110°C

Equil Time: 50 min

Injection Time: 0.5 min

GC Cycle Time: 67 min

Vial Size: 10 mL

Vial Fill Mode: Default: Flow to Pressure

Fill Pressure: 12 psi

Loop Fill Mode: Default

Extraction Mode: Single Extraction

Post Injection purge: Custom

Purge Flow: 100 mL/min

Purge Time: 2.5 min

12.2 Procedure B

12.2.1 Gas Chromatography Conditions

Column: ZB-WAXplus, 30 m x 0.32 mm x 0.25 µm or equivalent

Oven Temp: 50°C

Temp. Gradient: 50°C hold for 20 min,

Ramp at 6°C/min to 165°C, and hold for 20 min

Pressure Gradient: 10 psi hold for 20 min,

Ramp at 0.12 psi/min to 12.4 psi, and hold for 20 min

Run Time: 60 min

Equil Time:	2 min
Inlet Temp:	240°C
Split Ratio:	5:1
Septum Purge:	2 mL/min
FID Temp:	250°C
Hydrogen:	30 mL/min
Air:	350 mL/min
Helium:	30 mL/min (constant column + makeup)

12.2.2 Headspace Conditions

12.2.2.1 Headspace Conditions are the same as for Procedure A.

13.0 Procedure D (Loss on Drying)

13.1 Record all data and results on Form D-725-F1 Vacuum Oven LOD Test Record.

13.2 Equilibrate a vacuum oven to 105°C.

13.3 Dry a clean weigh bottle just before adding sample.

13.4 Record the weight of the clean and dry weigh bottle (tare weight).

13.5 Add 2.5 ± 0.2 g of the sample to the tared container, and record the sample weight.

13.6 Place the open weigh bottle and lid into the vacuum oven.

13.7 Apply 20 ± 4 inches of vacuum and leave in the oven under vacuum for 2 hours (solids) or 3 hours (oils).

13.8 After the allotted time, remove the sample from the oven, replace the cap, and move the sample to a desiccator to cool for 20 minutes. Use caution when moving samples as they may be hot.

13.9 After cooling, record the weight of the weigh bottle and sample (final weight).

13.10 The % LOD is determined by the following equation:

$$\% LOD = \frac{(Tare\ Wt + Sample\ Wt) - Final\ Wt}{Sample\ Wt} \times 100$$

14.0 Method Verification

14.1 Method verification is only required for Procedure C. Verification involves preparing samples spiked in triplicate at 50%, 100%, and 150% of the limit amount, and measuring recovery of the spiked analyte.

14.2 Preparation of Spiked Sample Solutions

14.2.1 50% spike: Transfer 1.0 mL of Sample Stock Solution to a 10-mL headspace vial, add 0.5 mL of the Standard Stock Solution and 4.5 mL of water, apply stopper, crimp cap and mix.

14.2.2 100% spike: Transfer 1.0 mL of Sample Stock Solution to a 10-mL headspace vial, add 1.0 mL of the Standard Stock Solution and 4.0 mL of water, apply stopper, crimp cap and mix.

14.2.3 150% spike: Transfer 1.0 mL of Sample Stock Solution to a 10-mL headspace vial, add 1.5 mL of the Standard Stock Solution and 3.5 mL of water, apply stopper, crimp cap and mix.

14.3 Analysis

14.3.1 Using the chromatographic conditions (Procedure A or Procedure B) that resulted in the best separation for the solvents being quantitated, separately inject equal volumes of headspace for the Blank Solution, Sample Solution, Spiked Sample Solution(s), and Standard Solution(s) into the system and measure the responses for the major peaks.

14.3.2 For each residual solvent, calculate the recovery in % of each spiked residual solvent.

14.4 Acceptance Criteria

14.4.1 Accuracy – The mean recovery for the nine spiked samples is 80% - 120%.

14.4.2 Precision – The %RSD of the recovery for the nine spiked sample is NMT 20%.

14.5 After verification has been performed, a report should be prepared and stored in the F:\Laboratory\Residual Solvents\ folder.

15.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	06/06/18	New procedure	N/A	J. Maignan
1	11/26/19	Update procedure to comply with the new general chapter USP <1467>. Edit procedure for better flow and brevity. Add Procedure D for determination of Class 3 residual solvents by LOD.	19-0894	S. Sassman
2	01/20/23	Schedule review: updated logo and document format.	CC-23-0036	K. Burris