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Application News

No. GC-005

Analysis of Residual Solvents in Pharmaceuticals (USP<467>) with Shimadzu GC-2010 Plus and HS-10 Headspace Sampler

■ Introduction

Organic solvents are routinely used in manufacturing pharmaceutical products, yet the presence of these compounds in the final products can be harmful to the consumers. Despite various techniques employed to remove these solvents, small quantities may still remain in the final products, and are commonly known as residual solvents (RS). These solvents are categorized into three classes based on their risks to human health. The United States Pharmacopeia (USP) has published test methods that utilize static headspace sampling and gas chromatography followed by flame ionization detection (GC/FID) to identify and control the level of RS class 1 and 2 (USP<467>). This application note demonstrates the analyses of residual solvents as described in USP<467> carried out with an HS-10 static headspace sampler and a Shimadzu GC-2010 Plus Gas Chromatograph (Figure 1).

■ Instrumentation

A Shimadzu GC-2010 Plus equipped with an advanced flow controller (AFC), a split/splitless injector (SPL) and a Flame Ionization Detector (FID) was used for this study. An HS-10 static headspace sampler with transfer line was used for sample preparation and introduction into the GC through the SPL. A Rxi-624Sil MS column (Restek #13870, G43 equivalent, 30m x 0.32mm x 1.8µm) and a Stabilwax column (Restek #10624, G16 equivalent, 30m x 0.32mm x 0.25µm) were used for the USP<467> Procedure A and Procedure B respectively.

Figure 1: GC-2010 Plus and HS-10 static headspace sampler

■ Standards and Sample Preparation

Class 1, Class 2 Mixture A and Class 2 Mixture B residual solvent standards were purchased from Restek (Cat.# 36279, 36012 and 36280, respectively) and diluted with DMSO (Sigma D4540, GC grade) and deionized water to make the standard stock solutions following USP<467> procedures. For analyses, standard solutions of Class 1, Class 2 Mixture A and Class 2 Mixture B were prepared in 20 mL screw top headspace vials (Shimadzu, 220-94796-01) as described in USP<467>.

■ Analytical Conditions

GC-2010 Plus
- SPL Temp = 140°C
- FID Temp = 250°C, H_2 flow = 40 mL/min, Air flow = 400 mL/min, N_2 Makeup flow = 30.0 mL/min.
- Carrier gas: Helium
- Flow control mode: constant linear velocity @ 35.0cm/s, split ratio = 5.0, purge flow = 3.0 mL/min
- Injection volume: 1mL headspace
- Oven Temp Program
  - o Procedure A: 40°C for 20min, ramp at 10°C/min to 240°C, hold 20min
  - o Procedure B: 50°C for 20min, ramp at 6°C/min to 165°C, hold 20min

HS-10
- Vial equilibration: 60min @ 80°C
- Sample Pathway Temp = 110°C
- Transfer Line Temp = 120°C
- Vial Pressurization: 1.00 min @ 75kPa
- Loop Load Time = 0.50 min
- Injection time = 1.00 min

■ Results

USP RS Class 1

There are five solvents in USP RS Class 1 Mixture. The USP<467> monograph requires that the signal-to-noise ratio (S/N) of 1,1,1-trichloroethane is not less than 5 and that the S/N of each peak is not less than 3 for Procedure A. For Procedure B, which is used to confirm the results from Procedure A, the criteria is that the S/N of benzene is not less than 5 and that the S/N of each peak is not less than 3. As shown in figures 2-3 and table 1, using HS-10 and GC-2010 Plus, all peaks in Procedure A have a S/N above 7. And for Procedure B, the S/N was over 90 for benzene and over 60 for all peaks.
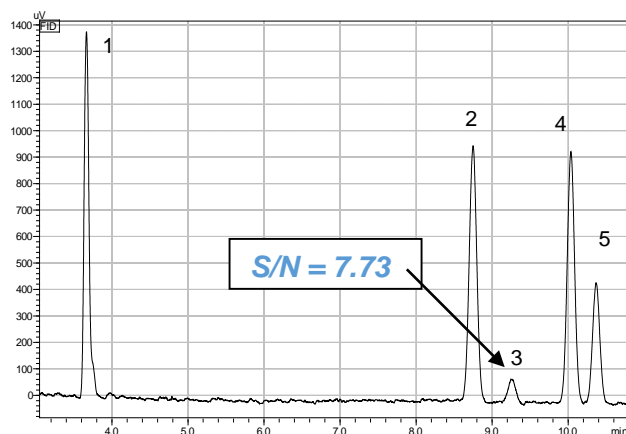

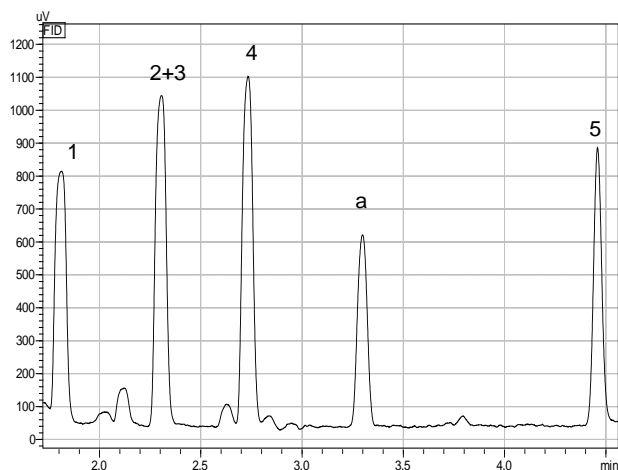**Figure 2**: Chromatogram of Class 1 Standard Solution, Procedure A

Figure 3: Chromatogram of Class 1 Standard Solution, Procedure B (*a: artifact)*

Peak No.	Name	S/N Procedure A	S/N Procedure B
1	1,1-Dichloroethene	116.76	68.15
2	1,1,1-Trichloroehane	81.54	90.11
3	Carbon Tetrachloride	7.73	*co-elute*
4	Benzene	80.19	93.12
5	1,2-Dichloroethane	38.17	72.35

Table 1: Signal-to-noise ratios of peaks in Class 1 Standard Solution, Procedure A and B

USP RS Class 2

Due to the large number of solvents in Class 2, the standards are divided into three mixtures: A, B and C. Solvents in Class 2 Mixture C are not readily detected by HS-GC therefore must be evaluated using other methods. The system suitability requirements for Class 2 Mixture A are that the resolution between acetonitrile and methylene chloride (dichloromethane) is not less than 1.0 for Procedure A, and the resolution between acetonitrile and *cis*-1,2-dichloroethene not be less than 1.0 for Procedure B. As shown in Figure 4 and 5 below, using Shimadzu HS-10 with the GC-2010 Plus, the resolution between acetonitrile and methylene chloride or *cis*-1,2-dichloroethene is > 2.2 for both procedures. No criteria have been stated for Class 2 Mixture B. Separation of the compounds in Class 2 Mixture B are shown in Figures 6 and 7.
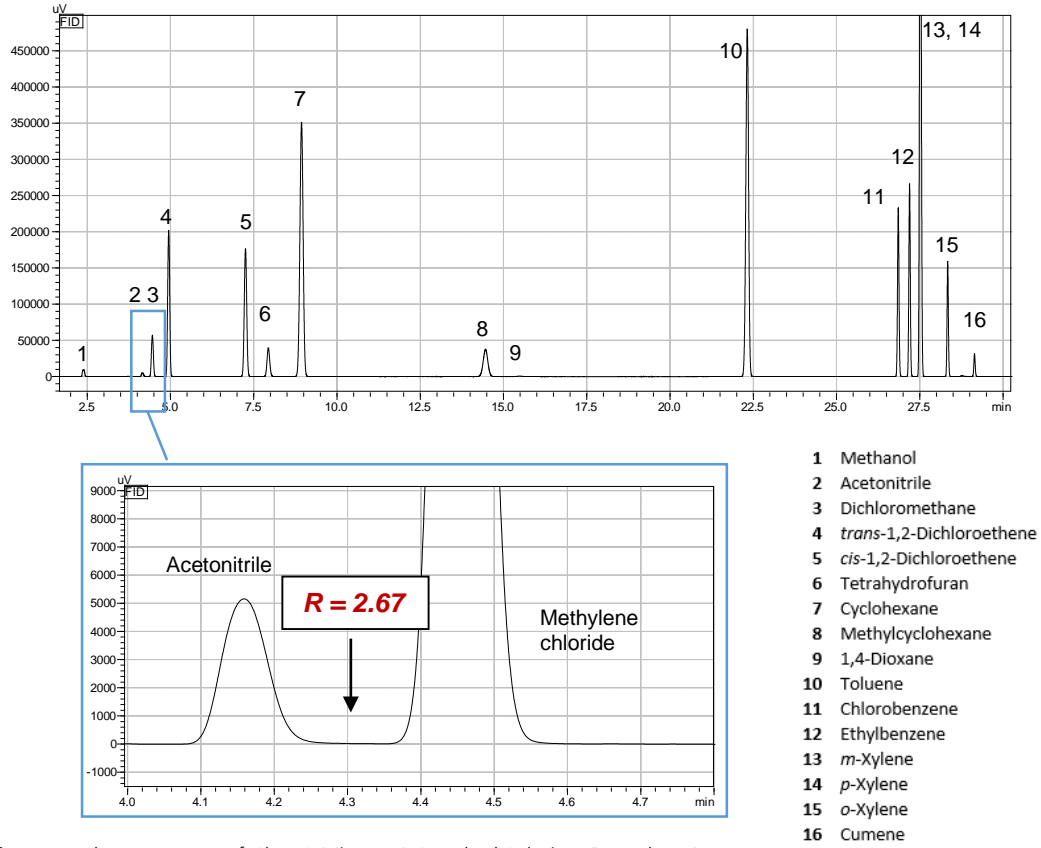
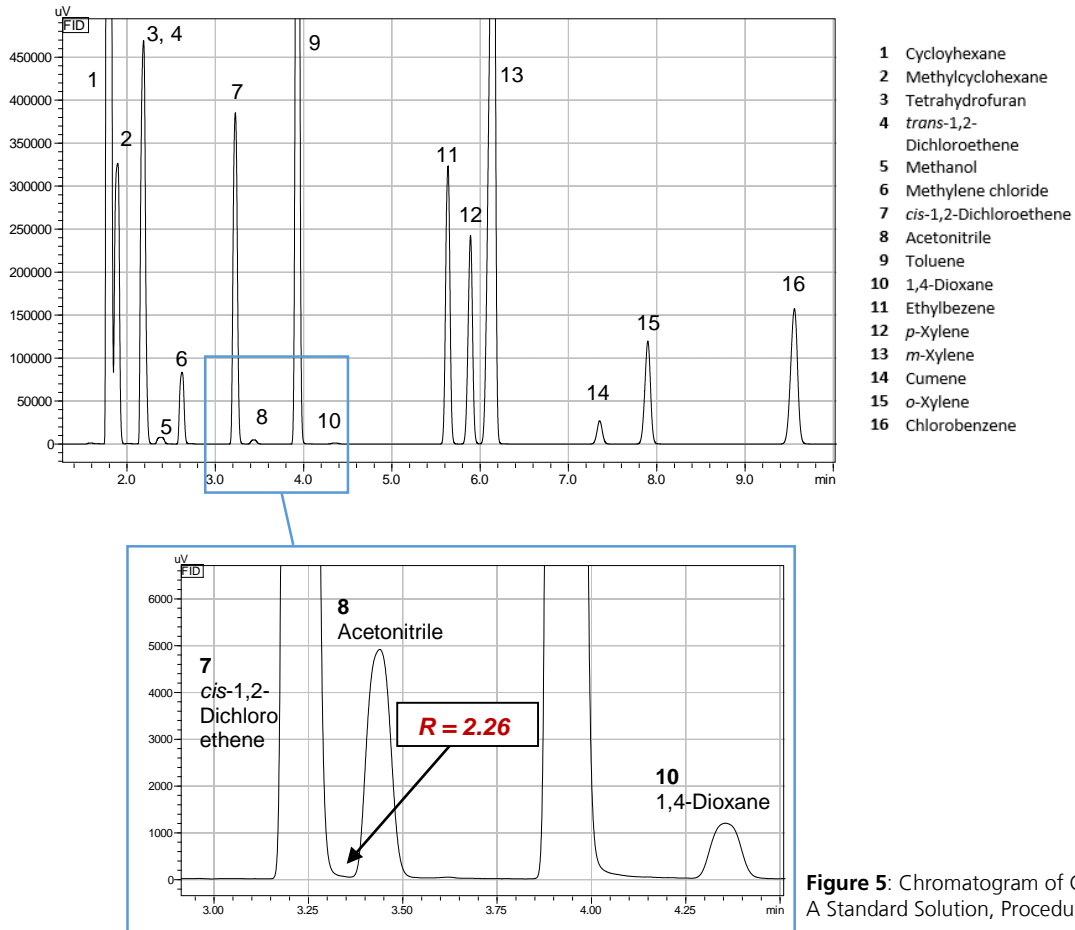**Figure 4**: Chromatogram of Class 2 Mixture A Standard Solution, Procedure A

1 Methanol
2 Acetonitrile
3 Dichloromethane
4 *trans*-1,2-Dichloroethene
5 *cis*-1,2-Dichloroethene
6 Tetrahydrofuran
7 Cyclohexane
8 Methylcyclohexane
9 1,4-Dioxane
10 Toluene
11 Chlorobenzene
12 Ethylbenzene
13 *m*-Xylene
14 *p*-Xylene
15 *o*-Xylene
16 Cumene

1 Cycloyhexane
2 Methylcyclohexane
3 Tetrahydrofuran
4 *trans*-1,2-Dichloroethene
5 Methanol
6 Methylene chloride
7 *cis*-1,2-Dichloroethene
8 Acetonitrile
9 Toluene
10 1,4-Dioxane
11 Ethylbezene
12 *p*-Xylene
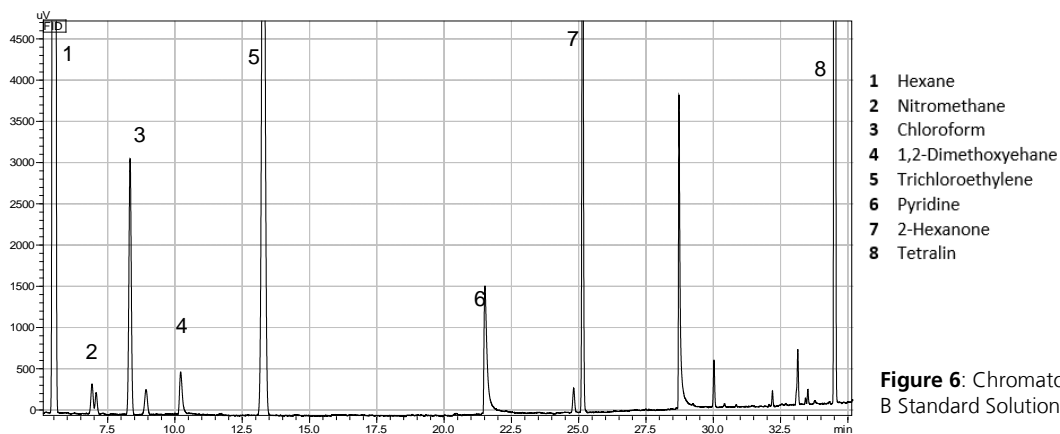13 *m*-Xylene
14 Cumene
15 *o*-Xylene
16 Chlorobenzene

Figure 5: Chromatogram of Class 2 Mixture A Standard Solution, Procedure B

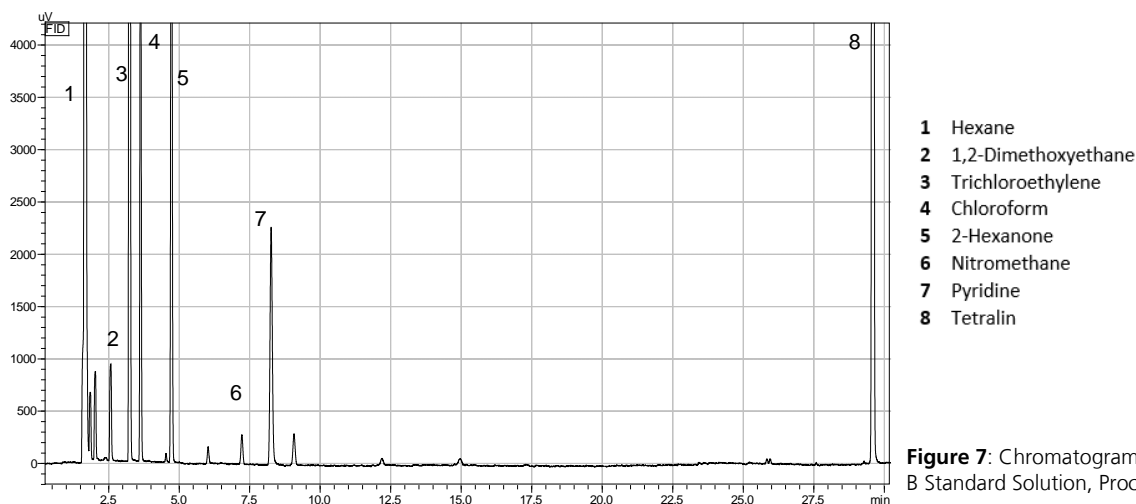**Figure 6**: Chromatogram of Class 2 Mixture B Standard Solution, Procedure A

1 Hexane
2 Nitromethane
3 Chloroform
4 1,2-Dimethoxyehane
5 Trichloroethylene
6 Pyridine
7 2-Hexanone
8 Tetralin

Figure 7: Chromatogram of Class 2 Mixture B Standard Solution, Procedure B

1 Hexane
2 1,2-Dimethoxyethane
3 Trichloroethylene
4 Chloroform
5 2-Hexanone
6 Nitromethane
7 Pyridine
8 Tetralin

Repeatability of Area and Retention Times

Compound	RT (min)	RT % RSD	Area % RSD
1,1-Dichloroethene	3.663	0.012	1.012
1,1,1-Trichloroehane	8.743	0.012	1.707
Benzene	10.030	0.014	1.065
1,2-Dichloroethane	10.361	0.012	1.170
Methanol	2.393	0.094	0.405
Acetonitrile	4.159	0.024	0.473
Dichloromethane	4.456	0.021	1.045
trans-1,2-Dichloroethene	4.946	0.019	1.415
cis-1,2-Dichloroethene	7.252	0.020	1.091
Tetrahydrofuran	7.938	0.024	0.586
1,4-Dioxane	15.484	0.034	0.792
Toluene	22.319	0.006	1.109
Chlorobenzene	26.853	0.003	1.269
Ethylbenzene	27.192	0.004	1.566
o-Xylene	28.337	0.003	1.504
Hexane	5.511	0.003	0.544
Chloroform	8.338	0.009	1.185
1,2-Dimethoxyehane	10.219	0.009	1.756
Trichloroethylene	13.294	0.006	1.523
Pyridine	21.522	0.012	1.442
2-Hexanone	25.149	0.004	0.480
Tetralin	34.509	0.002	1.953

Table 2: Four replicates of different solvents were assayed using Procedure A and the Relative Standard Deviation *(% RSD, n=4)* for retention times and area are reported
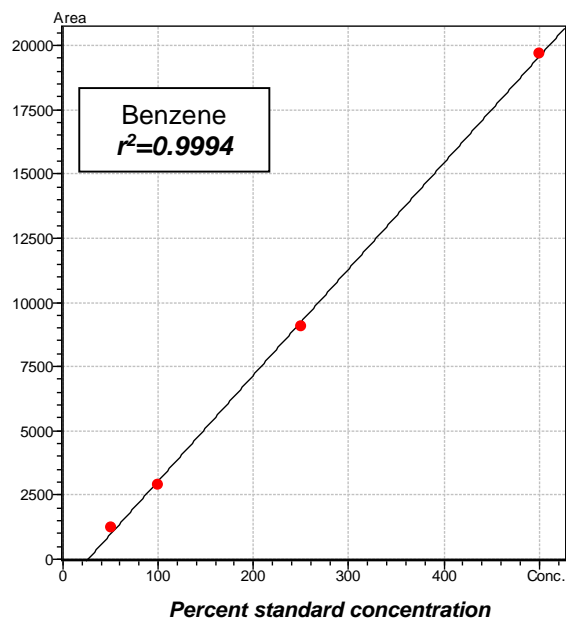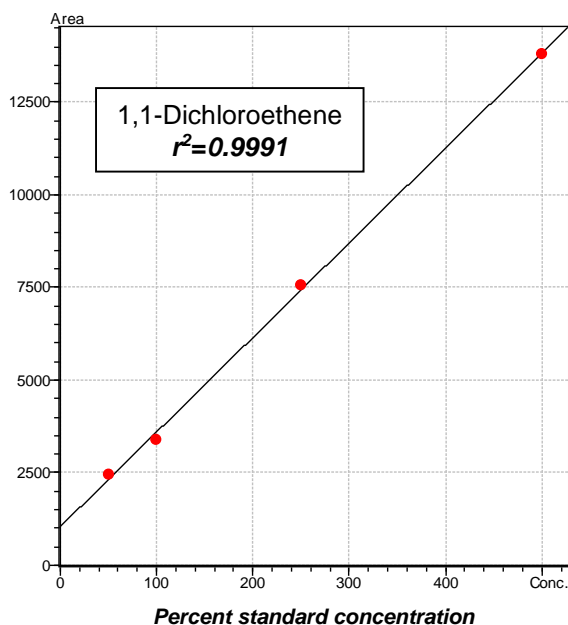
Calibration Curves

Figure 8: Four dilutions of 1,1-Dichloroethene and Benzene corresponding to 50% to 500% of the USP Standard Solution concentration were assayed using procedure A. The calibration curves and the correlation coefficient (r^2) are shown

■ Conclusions

In this study, USP<467> residual solvent analysis was carried out with a Shimadzu HS-10 static headspace sampler and a GC-2010 Plus chromatograph. The results show that all system suitability criteria have been satisfied. In addition, good repeatability and linearity have been achieved.

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