Analyzing Residual Solvents in Pharmaceutical Products Using GC Headspace with Valve-and-Loop Sampling

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Key Words

USP Method <467>, residual solvents, excipients, headspace analysis, valve and loop sampling

Goal

The purpose of this application note is to describe the analysis of residual solvents in pharmaceutical products in accordance with USP Method <467>.

Introduction

US Pharmacopeia (USP) method <467> delineates the procedure to identify and quantify any organic volatile solvent present in pharmaceuticals and excipients as a result of the production process. The method details the chromatographic system and analytical conditions to be used and addresses the acceptable limits for each solvent. This application note describes the analysis of residual solvents in accordance with USP method <467> using the Thermo Scientific™ TRACE™ 1310 GC, the new Thermo Scientific™ TriPlus™ 300 Headspace autosampler, and the Thermo Scientific™ Dionex™ Chromeleon™ chromatography data system (CDS) software.

The residual solvents tested in this work, are chemical residues present at trace levels in pharmaceutical substances and their products. These solvents may be the result of the manufacturing process or from packaging and storage. Pharmaceutical products should contain low levels of residual solvents as determined by safety data. The USP 467 method sets the concentration limit for each solvent and describes the analysis of 53 solvents grouped according to their health hazards:

- **Class 1** solvents (known to cause unacceptable toxicities or to have environmental effects) will be avoided in the manufacturing process.
- **Class 2** solvents (associated with less severe toxicity) will be limited.
- **Class 3** solvents (less toxic) will be used where practical.

GC headspace (HS) analysis is recommended for identification and quantification of Classes 1 and 2 solvents.

Experimental

Three analytical procedures are used for identification and quantification of the residual solvents:

- **Procedure A** screens for and confirms the solvents' presence using a G43 (volatiles) column.
- **Procedure B** confirms the solvents' identity using a G16 (wax) column.
- **Procedure C**, which also uses a G43 column, is required to quantify the amount of residual solvents present.

The USP method suggests the use of a GC and a valve-and-loop headspace sampling system. Accordingly, a TRACE 1310 GC and the TriPlus 300 Headspace autosampler are used here. The data are collected and processed with Chromeleon 7.1 CDS. A Thermo Scientific™ TraceGOLD™ TG 624 30 m × 0.32 mm × 1.8 µm (P/N 26085-3390) column is used for Procedures A and C. A TraceGOLD TG WaxMS 30 m × 0.32 mm × 0.25 µm (P/N 26088-1430) column is used for Procedure B.

Samples and Standard Preparation

Standard stock solutions for Class 1, Class 2 mix A, and Class 2 mix B residual solvents are prepared as described by the method:

- **Class 1**: transfer 1 mL of USP Class 1 mixture RS (Sigma-Aldrich™ 40131-U) to a 100 mL flask previously filled with 9 mL dimethyl sulfoxide, dilute to volume with water and mix. Transfer 1 mL of this solution to a 100 mL flask previously filled with 50 mL water, dilute to volume with water, and mix. Transfer 10 mL of this solution to a 100 mL flask previously filled with 50 mL water, dilute to volume with water, and mix.

- **Class 2 mix A**: Transfer 1 mL of USP Class 2 mix A RS (Sigma-Aldrich 40132-U) to a 100 mL flask, dilute to volume with water, and mix.

- **Class 2 mix B**: Transfer 1 mL of USP Class 2 mix B RS (Sigma Aldrich 40133-U) to a 100 mL flask, dilute to volume with water, and mix.

Prepare standard solutions for Class 1, Class 2 mix A, and Class 2 mix B residual solvents directly into the headspace (HS) vial as described by the method:

- **Class 1**: Transfer 1 mL of Class 1 standard stock solution to a 20 mL HS vial; add 5 mL of water, cap the vial, and mix.

- **Class 2 mix A**: Transfer 1 mL of Class 2 mix A standard stock solution to a 20 mL HS vial; add 5 mL of water, cap the vial, and mix.

- **Class 2 mix B**: Transfer 5 mL of Class 2 mix B standard stock solution to a 20 mL HS vial; add 1 mL of water, cap the vial, and mix.

Prepare the test stock solution and the test solution as follows:

- **Test stock solution**: Transfer 250 mg of the article under test to a 25mL flask, dissolve, bring to volume with water, and mix.

- **Test solution**: Transfer 5 mL of Test Stock Solution to a 20 mL HS vial; add 1 mL of water, cap the vial, and mix.

Analysis

Procedure A

The chromatographic system is equipped with an instant-connect split/splitless (SSL) injector, an instant-connect FID detector, and a 30m × 0.32 mm × 1.8 µm TG 624 column. Helium is used as carrier gas with a constant flow of 2.2 mL/min and a split ratio of 10:1. The instant-connect injector and instant-connect detector are kept at temperatures of 140 °C and 250 °C respectively.

TriPlus 300 HS parameters are configured as follows:

- Equilibration time of the sample is 45 min with vial shaking set to high
- Oven temperature set to 80 °C
- Manifold temperature set to 90 °C
- Transfer line temperature set to 105 °C

- Pressurization mode is set to **Pressure**, and the pressure is set to 1 bar and the pressure equilibration time to 0.2 min
- Loop filling mode is set to **Pressure**, and the pressure is set to 0.5 bar with an equilibration time of 0.2 min
- Loop size is 1 mL
- Injection mode is **Standard**, and injection time is set to 0.5 min
- Sample line is purged after injection for 1 min at 80 mL/min
- Vial venting is set to **On**

The GC oven parameters are configured as follows:

- Hold at 40 °C for 20 minutes
- Raise at 10 °C/min until oven temperature reaches 240 °C
- Maintain oven temperature at 240 °C for 20 min

According to USP <467>, the signal-to-noise ratio of 1,1,1 trichloroethane in Class 1 standard solution must be no less than 5, and the resolution between acetonitrile and methylene chloride in Class 2 mix A standard solution must be no less than 1. Figure 1 shows the signal-to-noise ratio of 1,1,1-trichloroethane in Class 1 standard solution obtained in this analysis, and figure 2 shows the resolution between acetonitrile and methylene chloride in Class 2 mix A standard solution.
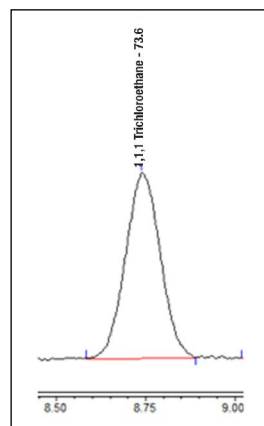

Figure 1. Signal-to-noise ratio (S/N) of 1,1,1-trichloroethane in Class 1 standard solution analyzed according to Procedure A. S/N calculated at 73.6
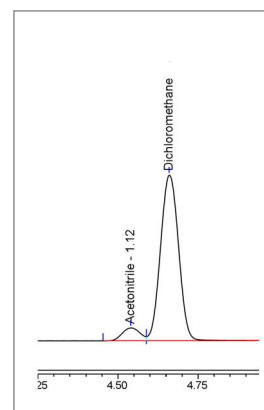

Figure 2. Resolution (R) between acetonitrile and methylene chloride in Class 2 mix A standard solution analyzed according to Procedure A. R = 1.12

If the peak response of any peak of the article under test other than 1,1,1-trichloroethane in the test solution is greater than or equal to a corresponding peak in the standard solution or if the peak corresponding to 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in the Class 1 standard solution, the analyst must proceed to Procedure B to verify the identity of the peak on a different stationary phase, otherwise the article meets the method's requirements.

Procedure B

In Procedure B of USP <467>, the chromatographic system is equipped with an instant-connect split/splitless (SSL) injector, an instant-connect FID detector, and a TraceGOLD TG WaxMS 30 m × 0.32 mm × 0.25 μm column.

The carrier gas used is helium with a constant flow of 2.2 mL/min and a split ratio of 10:1. The instant-connect injector and detectors are kept at temperatures of 140 °C and 250 °C respectively.

The oven ramp is configured as follows:

- Hold at 50 °C for 20 minutes
- Raise at 6 °C per minute until temperatures reaches 165 °C
- Maintain oven at 165 °C for 20 minutes.

This procedure is applied to confirm the peak identification of Procedure A, so a different stationary phase is used to analyze those solvents. The signal-to-noise ratio of benzene (in Class 1 standard solution) must be no less than 5, and the resolution between acetonitrile and *cis*-dichloroethene (in Class 2 mix A standard solution) must be no less than 1. Figures 3 and 4 show the signal-to-noise ratio of benzene in Class 1 standard solution and the resolution between acetonitrile and *cis*-dichloroethene in Class 2 mix A standard solution, respectively.

If the peak response of any peak found in the test solution is greater than or equal to a corresponding peak in the standard solution, the analyst must proceed to Procedure C to quantify the peak; otherwise the article meets the method's requirements.

Procedure C

For each peak identified and verified with Procedures A and B, prepare a standard stock solution according to the following instructions:

- Transfer an accurate volume of an individual standard corresponding to each residual solvent identified to a suitable container.
- Dilute with water to obtain a solution having a final concentration of 1/20 of the concentration limit value as stated by the USP method.

Prepare a standard solution by transferring 1 mL of Class 1 standard stock solution to a 20 mL headspace vial, adding 5 mL of water, capping the vial, and mixing.

Prepare a spiked test solution by transferring 5 mL of test stock solution to an appropriate headspace vial, adding 1 mL of the standard stock solution, capping the vial, and mixing.
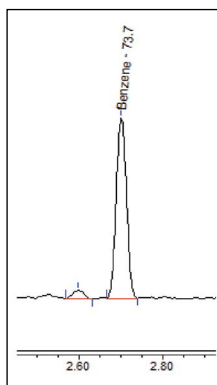

Figure 3. The signal-to-noise ratio (S/N) of benzene in Class 1 standard solution analyzed according to Procedure B. S/N calculated at 73.7
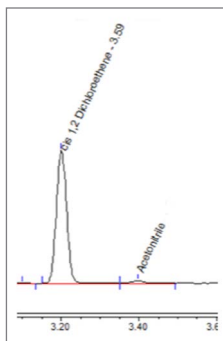

Figure 4. Resolution (R) between acetonitrile and *cis*-dichloroethene in Class 2, mix A standard solution, analyzed according to procedure B. R = 3.59

Perform the analysis following the instructions for Procedure A with the same instrument configuration on the standard solution, the test solution, and the spiked test solution.

Calculate the amount in ppm for each residual solvent found using the formula:

$$5(C/W) \: [ru/(rst-ru)]$$

C is the concentration in μg per mL of the reference standard in the standard stock solution; **W** is the weight in grams of the article under test used to prepare the test stock solution; **ru** and **rst** are the peak responses of each residual solvent obtained from the test solution and the spiked test solution, respectively.

Linearity and repeatability tests

To assess system linearity and the overall system performance, calibration curves are run for the solvents analyzed, preparing the calibration points as follows:

- For Class 1 solvents, prepare 6 calibration points: 2, 1, 0.5, 0.2, 0.1, and 0.05 fold the concentration of Standard stock solution, step 2 for each solvent.
- For Class 2 mix A solvents prepare 5 calibration points: 0.2, 0.1, 0.05, 0.02, and 0.01 fold the concentration of Standard stock solution for each solvent.
- For Class 2 mix B solvents prepare 5 calibration points: 0.2, 0.1, 0.05, 0.02, and 0.01 fold the concentration limit of Standard stock solution for each solvent.

Results and Discussion

Figures 5–9 show examples of the calibration curves of the residual solvents benzene, dichloroethane, THF, *o*-xylene, and Tetraline. All the calibration curves obtained for each standard show outstanding linearity.
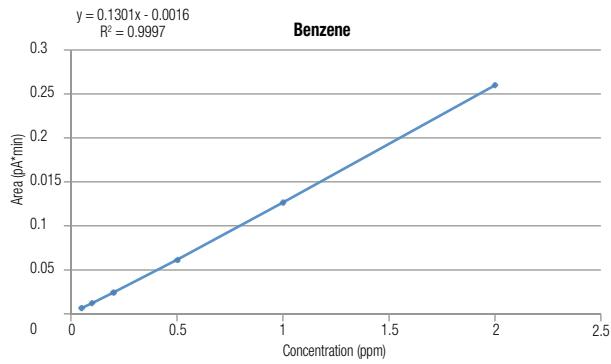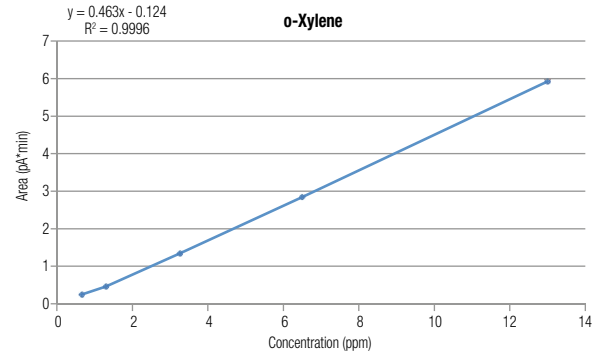

Figure 5. Calibration curve of benzene
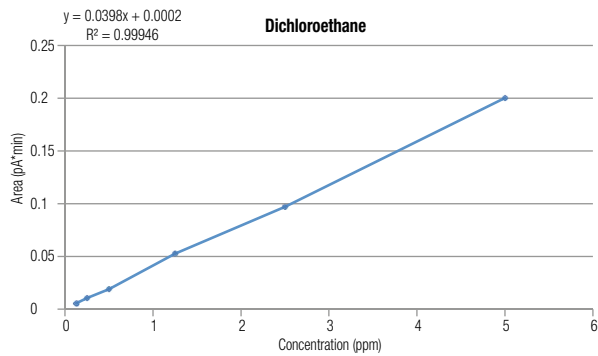

Figure 8. Calibration curve of o-xylene

Figure 6. Calibration curve of dichloroethane

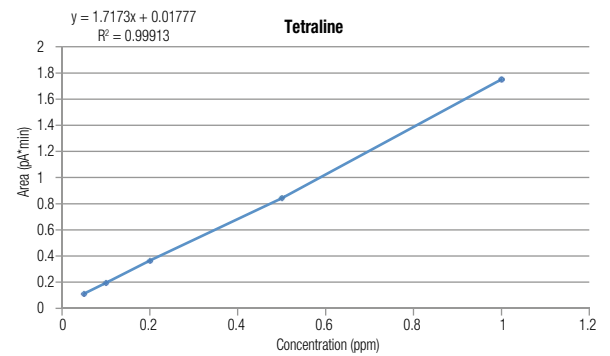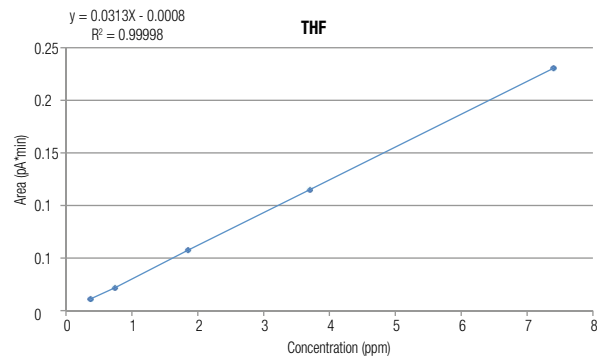Figure 9. Calibration curve of tetraline

Figure 7. Calibration curve of THF

The repeatability was tested for each residual solvent under the conditions of Procedures A and B; results are in Tables 1 and 2. Excellent repeatability for both retention time and peak area were achieved as reported in Tables 1 and 2, even at concentrations much lower than the Safety Concentration limit stated in the USP <467> method.

Table 1. Repeatability for residual solvents using USP <467> Procedure.

Procedure A					
Class 1	RSD % (n = 5)	Class 2 mix A	RSD % (n = 5)	Class 2 mix B	RSD % (n = 5)
1,1-Dichloroethene	0.95	Methanol	0.79	Hexane	1.19
1,1,1-Trichloroethane	1.03	Acetonitrile	0.65	Nitromethane	1.15
Benzene	0.89	Dichloromethane	1.06	Chloroform	0.68
1,2-Dichloroethane	0.94	trans 1,2-Dichloroethene	1.53	1,2-Dimethoxyethane	1.23
		cis 1,2-Dichloroethene	1.38	Trichloroethylene	1.00
		Tetrahydrofuran	0.58	Pyridine	1.43
		Cyclohexane	1.13	2-Hexanone	0.51
		Methylcyclohexane	2.76	Tetraline	0.71
		1,4-Dioxane	1.19		
		Toluene	1.50		
		Chlorobenzene	1.22		
		Ethylbenzene	1.34		
		m-Xylene/p-Xylene	1.14		
		o-Xylene	1.27		

Table 2. Repeatability for residual solvents using USP <467> Procedure B.

Procedure B					
Class 1	RSD % (n = 5)	Class 2 mix A	RSD % (n = 5)	Class 2 mix B	RSD % (n = 5)
1,1-Dichloroethene	0.93	Methanol	1.79	Hexane	1.46
1,1,1-Trichloroethane	1.06	Acetonitrile	1.67	Nitromethane	1.48
Benzene	1.05	Dichloromethane	1.56	Chloroform	0.47
1,2-Dichloroethane	1.04	trans 1,2-Dichloroethene	3.39	1,2-Dimethoxyethane	0.85
		cis 1,2-Dichloroethene	1.03	Trichloroethylene	0.95
		Tetrahydrofuran	1.31	Pyridine	0.82
		Cyclohexane	2.86	2-Hexanone	0.71
		Methylcyclohexane	1.61	Tetraline	0.42
		1,4-Dioxane	2.87		
		Toluene	2.09		
		Chlorobenzene	2.25		
		Ethylbenzene	2.56		
		m-Xylene/p-Xylene	1.89		
		o-Xylene	1.63		

Results and Discussion

The chromatograms for each class mixture are shown in Figures 10 to 17.
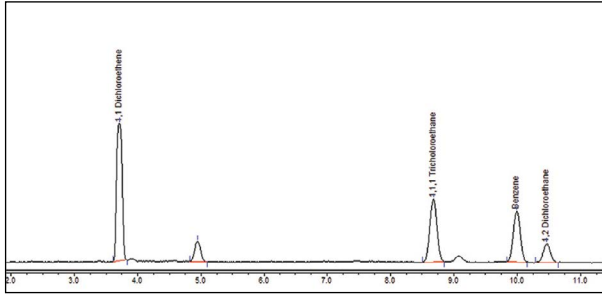

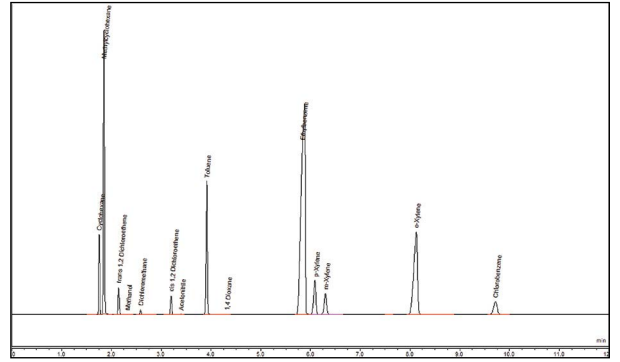Figure 10. Class 1 standard solution analyzed according to Procedure A

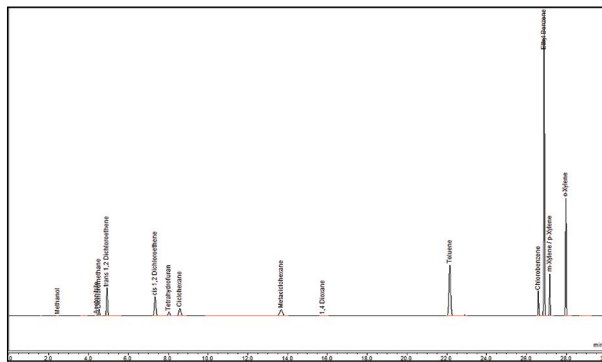Figure 14. Class 2 mix A analyzed according to Procedure B

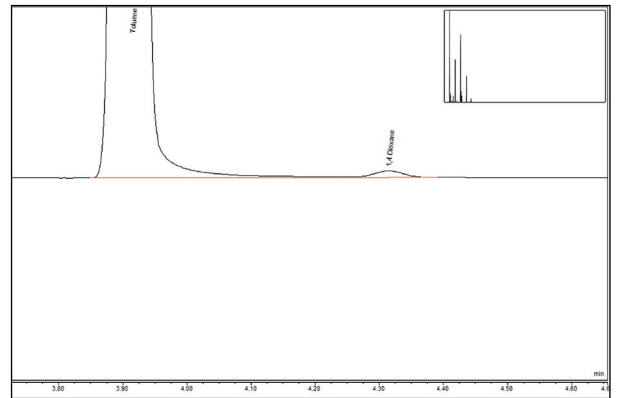Figure 11. Class 2 mix A analyzed according to Procedure A

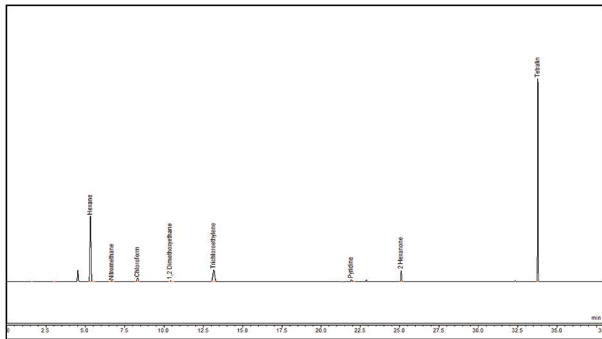Figure 15. Class 2 mix A analyzed according to Procedure B zoomed in on dioxane

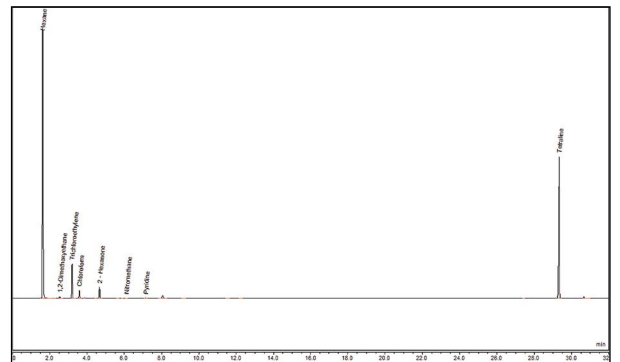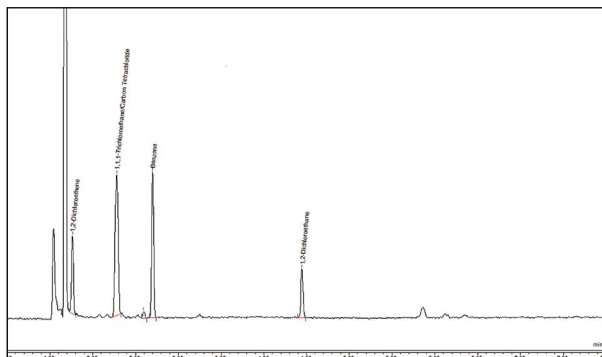Figure 12. Class 2 mix B analyzed according to Procedure A

Figure 16. Class 2 mix B analyzed according to Procedure B

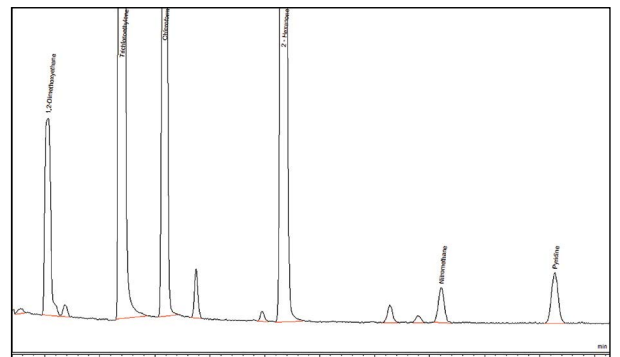Figure 13. Class 1 analyzed according to Procedure B

Figure 17. Class 2 mix B analyzed according to Procedure B

Figure 18 shows the headspace chromatogram obtained when following Procedure A on a sample made by mixing the three solutions (Class 1, Class 2 mix A, and Class 2 mix B).
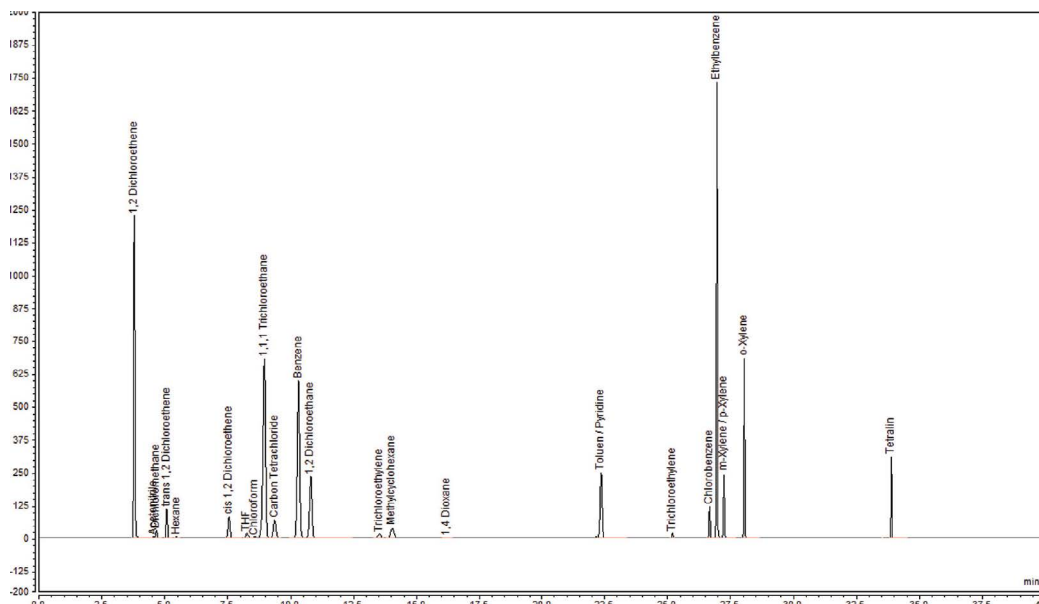

Figure 18. Headspace chromatogram of a sample obtained by mixing the three solutions tested according to procedure A. (Class 1, Class 2 mix A and Class 2 mix B)

Conclusion

All the USP <467> method acceptance criteria were met, and the chromatograms show good peak shapes and resolution. These data demonstrate that headspace-gas chromatography is a quantitative technique that easily meets the requirements of the USP <467> method. The system configuration of the TRACE 1310 GC, TriPlus 300 Headspace, and Chromeleon CDS is a solid, fully integrated, and reliable platform to perform the analysis according to the USP <467> method with full auditing capability. Operating this system configuration and method in a regulated environment is highly possible obtaining accurate, dependable results.

References

United States Pharmacopeia, Chemical Tests, <467> Residual Solvents, official from August 2012.

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