APPLICATION NOTE

Gas Chromatography

AUTHORS

Rahul Raut, PerkinElmer. Inc.,
Bolton, ON Canada

Miles Snow, PerkinElmer. Inc.,
Bolton, ON Canada

HS-GC-FID: Analysis of Residual Solvents According to USP 467

Introduction

Organic solvents are commonly used in the production of active pharmaceutical ingredients (API) and pharmaceutical products. The solvents are used for varying purposes including as synthesis reaction media, in the separation and purification of API during synthesis, to enhance yield, to impart desired characteristics such as crystal form and solubility, and other uses.The manufacturing process does not always consume or breakdown all of the solvent, thus it is not uncommon for residual amounts of solvent to remain in the final product. Residual solvent levels must meet health-based limits to ensure patient safety. The United States Pharmacopeia (USP) Chapter 467 identifies residual solvents of potential concern for pharmaceuticals, defines acceptable concentration limits, and provides detailed techniques for screening, confirmation, and quantitation of residual solvents, including sample preparation and analytical conditions.[1]

This application note reports the results from the analysis of residual solvents according to USP 467 requirements performed with the PerkinElmer GC 2400™ System with FID Detector and the PerkinElmer HS 2400™ Headspace Sampler, showing improved productivity and lab time optimization. PerkinElmer SimplicityChrom™ Chromatography Data System (CDS) Software manages the analytical workflow, from integrated instrument control to data reporting and reviewing/ approval. It supports compliance with Title 21 of the Code of Federal Regulations (CFR), Part 11, which is necessary in performing USP 467 residual solvent analysis.

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USP 467 identifies three classes of residual solvents, each distinguished by their known or potential health risks for patient safety in pharmaceuticals:

- Class 1 – to be avoided due to carcinogenicity
- Class 2 – to be limited due to toxicity concerns
- Class 3 – low toxicity

As expected, exposure limits for Class 1 and Class 2 solvents are more restrictive than Class 3 solvents.

The Class 1 residual solvents and their concentration limits in pharmaceuticals to meet USP compliance are:

Class 1 Solvent	Concentration Limit (ppm)
Benzene	2
Carbon tetrachloride	4
1,2-Dichloroethane	5
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1,500

The Class 2 residual solvents and their concentration limits in pharmaceuticals to meet USP compliance are:

Class 2 Solvent	Concentration Limit (ppm)
Acetonitrile	410
Chlorobenzene	360
Chloroform	60
Cumene	70
Cyclohexane	3,880
1,2-dichloroethene	1,870
1,2-Dimethoxyethane	100
N,*N*-Dimethylacetamide	1,090
N,*N*-Dimethylformamide	880
1,4-Dioxane	380
2-Ethoxyethanol	160
Ethylene glycol	620
Formamide	220
Hexane	290
Methanol	3,000
2-Methoxyethanol	50
Methylbutylketone	50
Methylcyclohexane	1,180
Methylene chloride	600
Methylisobutylketone	4,500
N-Methylpyrrolidone	530
Nitromethane	50
Pyridine	200
Sulfolane	160
Tetrahydrofuran	720
Tetralin	100
Toluene	890
Trichloroethylene	80
Xylene*	2,170

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

The Class 3 residual solvents each have a concentration limit of 5,000 ppm to meet USP compliance and are:

Class 3 Solvents	
Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Triethylamine

USP 467 identifies gas chromatography with flame ionization detection (GC-FID) as the preferred analytical technique for analysis of residual solvents in pharmaceuticals. It is crucial for the pharmaceutical industry to be able to efficiently and accurately identify and quantify residual solvents in their products and to achieve regulatory compliance as defined by USP 467.

PerkinElmer GC 2400 System with PerkinElmer HS 2400 Headspace Sampler.

Instrumentation

The PerkinElmer GC 2400 System with PerkinElmer HS 2400 Headspace Sampler provides a streamlined solution for the evaluation of residual solvents according to USP 467 Method.

Experimental

Section 7 of USP 467 describes three validated procedures (A, B, C) for Class 1 and 2 residual solvents. This workflow used procedure A to examine Class 1 and Class 2 residual solvents. Those procedures, however, have not been validated for Class 3 solvents. Therefore, a modified alternative method based on procedure A was developed for Class 3 solvents per USP 467 guidelines.

Materials

The standard solutions were prepared as described in Section 8 of USP 467 for water-soluble articles. USP-equivalent residual solvent standard mixtures were obtained from Restek (Maryland, USA). The Class 1, Class 2A, and Class 2B mixtures were diluted to prepare the standard solutions as described in USP 467. The Class 3 mixture was used as-is at 5000 ppm. The consumables used are listed in Table 1.

Table 1: Consumables used for this analysis.

Consumable	PerkinElmer Partnumber
PerkinElmer Elite 624 capillary column, 30 m X 0.32 mm ID X 1.8 µm	N9316203
1 mm ID Straight Liner	N6502037
20 mL Crimp Top Headspace Convenience Kit	N9303992
20 mm Hand Crimper	N9302785
Green Injection Port Septa	N9306218
Ceramic Column Cutter	N9301376
Graphite Vespel Capillary Column Ferrules 0.5 mm ID	09920105
O-ring for Glass Liner	09200714
Triple Filter (Hydrogen & Nitrogen)	N9306110
Moisture/Hydrocarbon Trap (Air)	N9306117
Triple Filter (Helium)	N9306106

Hardware and Software

The PerkinElmer GC 2400 System with the HS 2400 Headspace Sampler and a flame ionization detector (FID) was used for the analysis of residual solvents according to USP 467 methodology. A PerkinElmer Elite 624 column was conditioned according to the recommended protocol in the PerkinElmer Capillary Column Quick Care Guide.

The headspace sampler was configured in injection volume mode. This allows the carrier gas to enter the analytical column at a specified flow rate for a given duration of time. Instrument control and data analysis were completed with the SimplicityChrom CDS Software.

Method

The HS 2400 Headspace Sampler and analysis conditions are described in Table 2 (for Class 1 and Class 2A/2B residual solvents) and Table 3 (for Class 3 residual solvents). The conditions were taken and adapted from procedure A of USP 467 to suit the needs of the analyses.

Table 2: Chromatography conditions for Class 1 and Class 2A/2B residual solvents.

GC Parameters			
Instrument	PerkinElmer GC 2400 System		
Column	PerkinElmer Elite 624, 30 m X 0.32 mm ID X 1.8 µm		
GC Oven Parameters	Initial	Ramp	Final
	40° C (20 min)	10° C/min	240° C (20 min)
Headspace Parameters			
Oven Temperature	80 °C		
Needle Temperature	85 °C		
Transfer Line Temperature	85 °C		
High Pressure Injection	Off		
Column Pressure Program	13 psi hold for 20 minutes, ramp at 0.3 psi/min to 18 psi, hold for 24 min		
GC Cycle Time	70 minutes		
Pressurization Time	1 minute		
Thermostat Time	64 minutes		
Withdrawal Time	0.2 minutes		
Vent Time	5 seconds		
Injection Volume	1.0 mL (0.04 minutes)		
Injection Parameters			
Carrier/mode	Helium/Constant Velocity 35 cm/sec Split Ratio 1:5		
Temperature	Split/Splitless at 200 °C		
Septum Flow	3 mL/min		
Detector Parameters			
Type	FID		
Temperature	250 °C		
Hydrogen	30 mL/min		
Air	400 mL/min		
Make up Gas	Nitrogen 25 mL/min		
Data rate	10 pt/sec, 2 pt/sec Class 1		

Table 3: Chromatography conditions for Class 3 residual solvents.

GC Parameters			
Instrument	PerkinElmer GC 2400 System		
Column	PerkinElmer Elite 624, 30 m X 0.32 mm ID X 1.8 µm		
GC Oven Parameters	Initial	Ramp	Final
	40 °C (5 min)	22 °C/min	60 °C
	60 °C	5 °C/min	80 °C
	80 °C	25 °C/min	240 °C (2 min)
Headspace Parameters			
Oven Temperature	80 °C		
Needle Temperature	180 °C		
Transfer Line Temperature	180 °C		
High Pressure Injection	Off		
Column Pressure Program	13 psi constant		
GC Cycle Time	25 minutes		
Pressurization Time	5 minutes		
Thermostat Time	20 minutes		
Withdrawal Time	0.2 minutes		
Vent Time	5 seconds		
Injection Volume	0.75 mL (0.03 minutes)		
Injection Parameters			
Carrier/mode	Helium/Constant Velocity 25 cm/sec Split Ratio 1:5		
Temperature	Split/Splitless at 200 °C		
Septum Flow	3 mL/min		
Detector Parameters			
Type	FID		
Temperature	300 °C		
Hydrogen	30 mL/min		
Air	400 mL/min		
Make up Gas	Nitrogen 25 mL/min		
Data rate	10 pt/sec		

Results and Discussion

The method results for Class 1, 2, and 3 residual solvents are presented in the following sections.

Class 1 Residual Solvents

Class 1 analytes were prepared following procedure A as described in USP 467. USP System Suitability requirements for Class 1 residual solvents need to have a signal-to-noise ratio (S/N) of not less than (NLT) 5 for 1,1,1-trichloroethane, and NLT 3 for the remaining Class 1 solvents. The SimplicityChrom CDS Software automatically calculates the S/N ratio based on the S/N calculation requirement for USP from a selected region of noise close to the analyte of interest.

Figure 1 shows the chromatogram for Class 1 residual solvents with full separation in less than 12 minutes. Table 4 presents the data for each analyte along with the S/N ratio results. The results demonstrate that the PerkinElmer GC 2400 System with HS 2400 Headspace Sampler is capable of meeting—and more often surpassing—the S/N System Suitability requirements as set forth by USP for Class 1 solvents following procedure A.

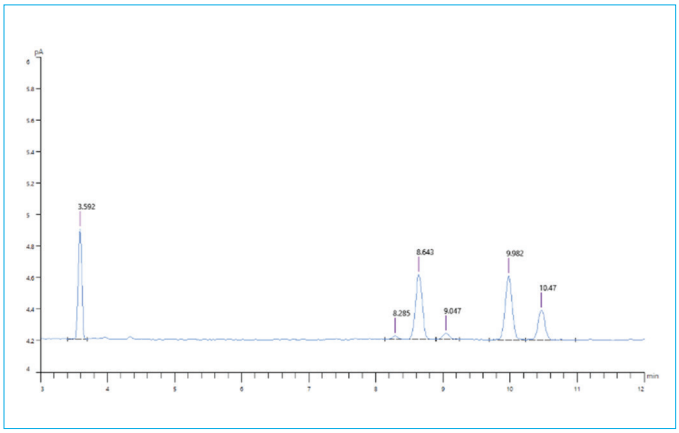

Figure 1: Chromatogram of Class 1 residual solvents mixture critical area.

Table 4: Analyte data for Class 1 residual solvents.

Peak Name	Conc. of Std. (µg/mL)	Retention Time (min)	S/N Ratio	USP Requirement
1,1-Dichloroethene	0.07	3.592	391	NLT 3
1,1,1-Trichloroethane	0.08	8.643	190	NLT 5
Carbon Tetrachloride	0.03	9.047	16	NLT 3
Benzene	0.02	9.982	212	NLT 3
1,2-Dichloroethane	0.04	10.47	101	NLT 3

Class 2 Residual Solvents

Class 2A residual solvents were prepared using procedure A as per USP 467. The USP System Suitability requirements for Class 2A residual solvents require resolution greater than 1.0 between acetonitrile and methylene chloride.

Figure 2 shows the chromatogram for Class 2A residual solvents with full separation in 29 minutes. The resolution achieved between acetonitrile and methylene chloride is 1.4, demonstrating that the PerkinElmer GC 2400 System with HS 2400 Headspace Sampler exceeds the resolution requirement for those analytes as set forth by USP.

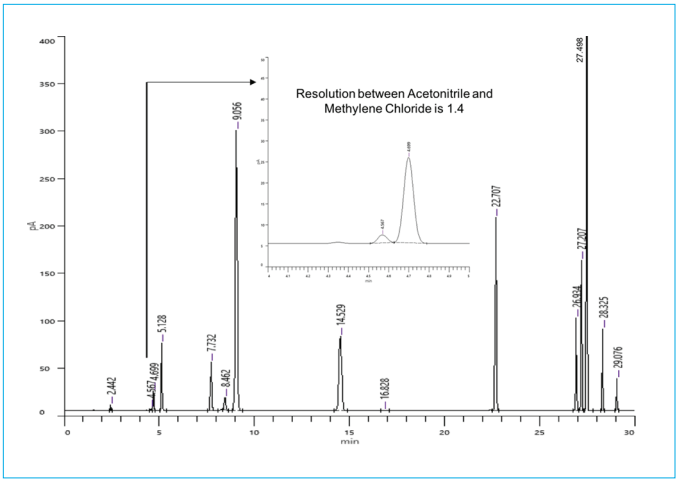

Figure 2: Chromatogram of Class 2A residual solvents mixture.

Table 5 presents the Class 2A residual solvents standard concentration as prepared and their retention time and peak area response for triplicate injections.

Table 5: Analyte data for Class 2A residual solvents.

Peak Name	Retention Time (min)	Concentration (µg/mL)
Methanol	2.442	25.00
Acetonitrile	4.567	3.42
Methylene chloride	4.699	5.00
trans-1,2-Dichloroethene	5.128	7.79
cis-1,2-Dichloroethene	7.732	7.79
Tetrahydrofuran	8.462	6.00
Cyclohexane	9.056	32.33
Methylcyclohexane	14.529	9.83
1,4-Dioxane	16.828	3.17
Toluene	22.707	7.42
Chlorobenzene	26.934	3.00
Ethylbenzene	27.207	3.07
m-Xylene*	27.498	10.85
p-Xylene*	27.498	2.53
o-Xylene	28.325	1.63
Cumene	29.0	0.58

* *m*-Xylene and *p*-Xylene coelute.

Class 2B Residual Solvents

Class 2B residual solvents were prepared using procedure A as per USP 467, but there are no System Suitability criteria explicitly stated for Class 2 solvents in USP 467. Figure 3 shows the chromatogram for Class 2B residual solvents with well-defined peaks at the prepared standard concentration and full separation in less than 35 minutes.

Table 6 presents the data for each Class 2B residual solvent as prepared.

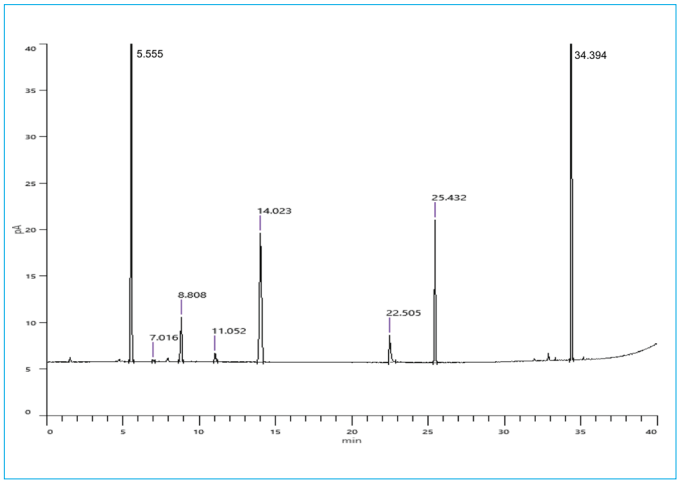

Figure 3: Chromatogram of Class 2B residual solvents mixture.

Table 6: Analyte data for Class 2B residual solvents.

Peak Name	Retention Time (min)	Concentration (µg/mL)
Hexane	5.545	2.17
Nitromethane	7.016	0.42
Chloroform	8.808	0.48
1,2-Dimethoxyethane	11.052	0.82
Trichloroethylene	14.023	0.67
Pyridine	22.505	1.68
Methylbutylketone	25.432	0.42
Tetralin	34.832	0.86

The headspace gas chromatography with flame ionization detection (HS-GC-FID) used in the analysis of residual solvents serves as a simple and highly efficient method. Since the method used an FID detector which has a universal response toward hydrocarbons, retention time stability for peak identification serves a crucial role in the analysis, otherwise the peak responses could be non-distinctive.

Class 3 Residual Solvents

Class 3 residual solvents are the least toxic and therefore have a higher quantitation limit than Class 1 and Class 2 solvents. The concentration limit for each Class 3 solvent is 5,000 ppm. There is no specified method for analyzing class 3 A solvents but usually either procedure A or procedure B with modifications can be adapted and needs to be additionally validated as discussed in USP 467.

Figure 4 shows the chromatogram for Class 3 residual solvents with full separation completed in less than 16 minutes with a modified method. If ethyl formate and 2-propanol are target analytes, they can be resolved by altering the initial oven plateau to run isothermal at 50° C for 5 minutes and other appropriate adjustments, as needed.

Table 7 presents the data for each Class 3 residual solvent as prepared.

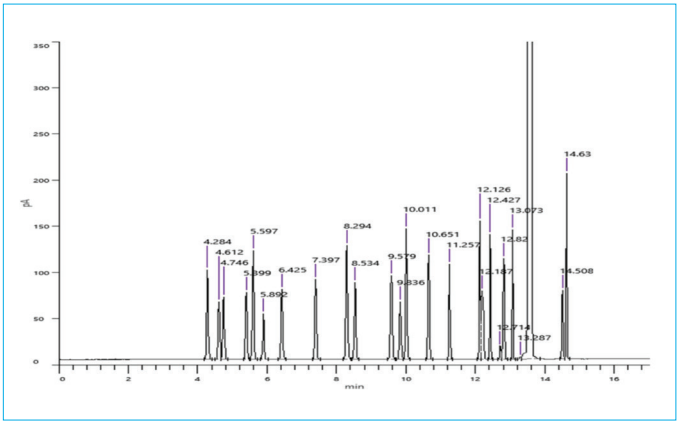

Figure 4: Chromatogram of Class 3 residual solvents mixture.

Table 7: Analyte data for Class 3 residual solvents.

Peak Name	Retention Time (min)	Concentration (µg/mL)
Pentane	4.284	5000
Ethanol	4.612	5000
Ether	4.746	5000
Acetone	5.399	5000
Ethyl formate/2-Propanol*	5.597	5000
Methyl acetate	5.892	5000
tert-Butylmethyl ether	6.425	5000
1-Propanol	7.397	5000
Methylethylketone/Ethyl acetate*	8.294	5000
2-Butanol	8.534	5000
Isopropyl acetate	9.579	5000
2-Methyl-1-propanol	9.836	5000
Heptane	10.011	5000
1-Butanol	10.651	5000
Propyl acetate	11.257	5000
Methylisobutylketone	12.126	5000
3-Methyl-1-butanol	12.187	5000
Isobutyl acetate	12.427	5000
1-Pentanol	12.82	5000
Butyl acetate	13.073	5000
Dimethyl sulfoxide	14.508	5000
Anisole	14.63	5000

** Solvents coelute and thus are integrated and reported as a sum.*

Conclusion

For the analysis of residual solvent analyses of Class 1, 2, and 3 solvents according to USP 467, PerkinElmer GC 2400 System with FID (using a PerkinElmer Elite 624 30 m X 0.32 mm ID X 1.8 µm column) and HS 2400 Headspace Sampler deliver results according to the method requirements with time optimization. The system suitability requirements for Class 1/Class 2 residual solvent were either met or exceeded.

The GC 2400 System with HS 2400 Headspace Sampler provides precise temperature control for demanding separations and coupled with pressure balanced headspace sampling technology, it provides reliable and reproducible runs giving confidence of results, especially for high-throughput laboratories. The SimplicityChrom CDS Software supports compliance with 21 CFR Part 11 requirements, and provides a practical, customizable user experience with multifunctionality and accessibility options.

References

1. USP-NF. 2019. General Chapter USP <467> Residual Solvents, United States Pharmacopeia.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

92537_51794 (708659) PKI