Application Note

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Analysis of USP <467> Residual Solvents Using the Agilent 8697 Headspace Sampler -XL Tray and Agilent 8890 GC System

Author

Youjuan Zhang
Agilent Technologies,
Shanghai, China

Abstract

This application note demonstrates the analysis of United States Pharmacopeia (USP) <467> class 1, 2A, and 2B residual solvents at their limit concentrations. Using the Agilent 8697 Headspace Sampler -XL Tray coupled with dual-channel GC/FID systems, excellent peak shape, resolution, and repeatability were possible. Both helium and nitrogen carrier gases were tested, with both providing excellent results that exceeded the specifications required in USP <467>.

Introduction

Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The USP Method <467>[1] defines the classification, risk assessment, and analytical procedures for residual solvents. The USP is also aligned with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline for Residual Solvent Q3C (R8) approach.[2]

Methyl isobutyl ketone (MIBK) was added to the class 2 solvent list according to the USP <467> Interim Revision Announcement for 2019 and 2020. The resolution of MIBK and *cis*-dichloroethene should be no less than one in the system suitability test of procedure B of class 2A. Cyclopentyl methyl ether (CPME) and *tert*-butanol (TBA) are recommended to be added to the USP <467> class 2 list according to ICH Q3C (R8) for 2021.

Three types of carrier gas are mentioned in the USP <467> method: helium, nitrogen, and hydrogen. Helium is widely used because of its excellent performance as a carrier gas. However, the ongoing helium shortage has impacted laboratory operation and created cost issues. Nitrogen has also gained in popularity due to its low cost and great performance as a carrier gas. In this application note, method resolution is more than adequate using helium for most analytes.

For this reason, nitrogen carrier gas can be a realistic option to provide good separation without the supply and cost issues of helium or the safety concerns of hydrogen. Both helium and nitrogen were investigated as carrier gases in this application note. By optimizing the acquisition parameters, the results obtained with nitrogen carrier gas are comparable with those obtained with helium carrier gas. All results demonstrated the excellent performance and great reliability of the whole system.

The newly designed 8697 -XL Tray, configured with a dual-column and dual-FID GC system, was used for residual solvents analysis in this study. The 8697 -XL Tray is fully integrated with the GC, which can be detected and configured automatically, as shown in Figure 1. Key HSS intelligent diagnostic tests are built into the smart Agilent 8890 GC System, enabling the GC to interact more closely with the HSS than ever before. All the HSS and GC tests are easy to find on the 8890 touchscreen. These tests prevent leaks throughout the whole system before sample testing, greatly reducing the possibility of unplanned downtime.

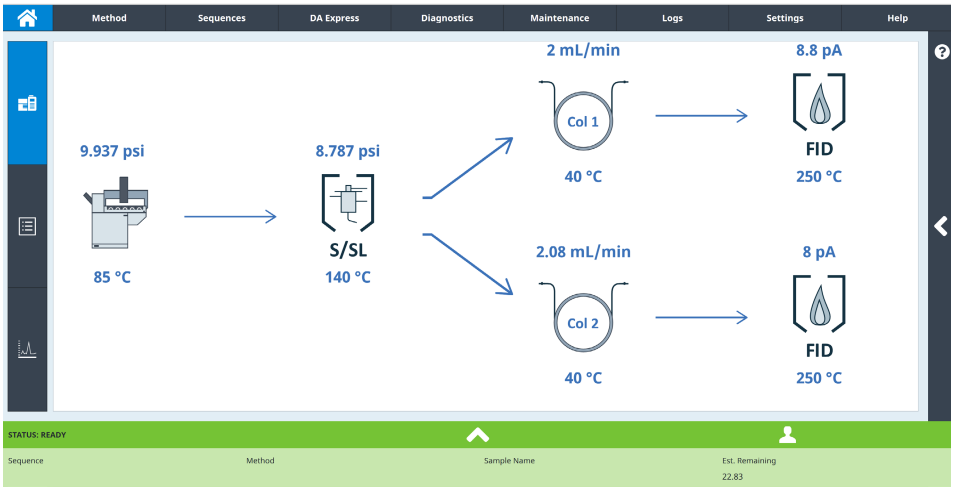


Figure 1. Flowpath of dual-channel configuration on the Agilent 8890 GC System (taken from the browser interface).

Experimental

USP <467> describes three analytical procedures (procedures A, B, and C) for evaluating the levels of all class 1 and 2 residual solvents. Procedure A uses a G43 phase (624-type columns) for initial identification and a limit test. Procedure B uses a G16 phase (wax-type columns) to confirm whether the solvent is above the concentration limit in procedure A. Procedure C is a quantitative test using a G43 or G16 phase if the solvent does not meet the criteria using procedure A and B.

In this application note, both procedure A and B are combined into a single dual-column dual-FID configuration. As shown in Figure 2, samples were introduced through the 8697 -XL Tray, which was directly connected to the split/splitless inlet of an 8890. An unpurged two-way splitter was used to split the sample 1:1 to an Agilent DB-Select 624 UI column and an Agilent DB-WAX UI column, and compounds were detected by associated FIDs.

Both helium and nitrogen were used as carrier gas, and their performance was similar. When using helium carrier gas, method parameters were based on the guidance from previously published work by Agilent[3], as shown in Table 1. When nitrogen was used as carrier gas, resolutions were obtained that were similar to those collected with helium, except for acetonitrile and MIBK. To improve the resolution, a lower initial oven temperature of 38 °C, a lower column flow of 1.5 mL/min, and a lower loop final pressure of 2 psi were used in the nitrogen carrier gas method.

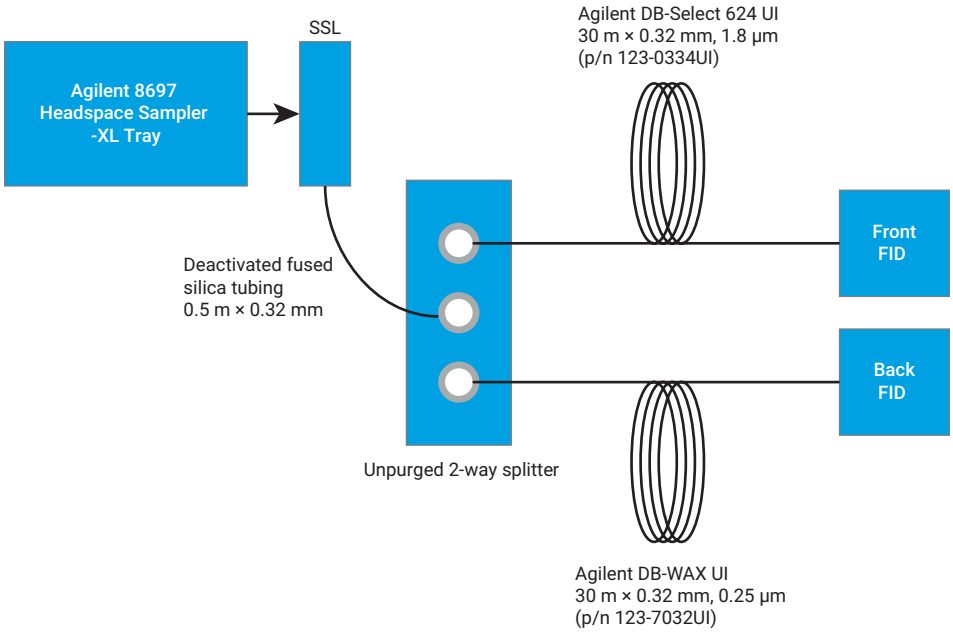


Figure 2. Dual-column, dual-FID GC system for residual solvents analysis.

Table 1. Chromatographic conditions.

Agilent 8697 Headspace Sampler -XL Tray	
Parameter	**Value**
Vial Pressurization Gas	N_2
Loop Size	1 mL
Vial Size	20 mL
Vial Shaking	8,188 shakes/min with acceleration of 770 cm/s^2
Vial Standby Flow	20 mL/min
Vial Equilibration Time	40 min
Inject Time	0.5 min
Oven Temperature	85 °C
Loop Temperature	85 °C
Transfer Line	0.53 mm id, deactivated fused silica (p/n 160-2535-5)
Transfer Line Temperature	100 °C
Vial Fill Pressure	15 psi
Loop Fill Mode	Custom
Loop Ramp Rate	40 psi/min
Loop Final Pressure	Helium carrier gas: 4 psi Nitrogen carrier gas: 2 psi
Loop Equilibration Time	0.05 min
Agilent 8890 GC	
Parameter	**Value**
Septum	9 mm headspace (p/n 5183-4801)
Inlet	SSL, 140 °C, split ratio 10:1
Liner	Straight, deactivated, 2 mm ID (p/n 5181-8818)
CFT Device	Unpurged two-way splitter (p/n G3181B)
Column	Column 1: Agilent DB-Select 624 UI, 30 m × 0.32 mm, 1.8 µm (p/n 123-0334UI) Column 2: Agilent DB-WAX UI 30 m × 0.32 mm, 0.25 µm (p/n 123-7032UI)
Carrier	Helium carrier gas: 2 mL/min, constant flow Nitrogen carrier gas: 1.5 mL/min, constant flow
Tubing	Deactivated fused silica tubing (p/n 160-2625-10)
Oven	Helium carrier gas: 40 °C (5.5 min), then 15 °C/min to 180 °C, hold 2.5 min Nitrogen carrier gas: 38 °C (5.5 min), then 15 °C/min to 180 °C, hold 4 min
FID (Both Channels)	Temperature: 250 °C, hydrogen: 30 mL/min, air: 300 mL/min, make up gas (N_2):25 mL/min
Data Rate	5 Hz

Chemicals and standards

Three standards of residual solvents in dimethyl sulfoxide (DMSO) were from Agilent Technologies:

- **Class 1:** Residual solvent revised method 467 class 1 (p/n 5190-0490)
- **Class 2A:** Residual solvent revised method 467 class 2A (p/n 5190-0492)
- **Class 2B:** Residual solvent revised method 467 class 2B (p/n 5190-0491)

The single standards of methyl isobutyl ketone (MIBK), cyclopentyl methyl ether (CPME), *tert*-butanol (TBA), and cumene were purchased from Alta Scientific Co. Ltd.

MIBK, CPME, TBA, and cumene were spiked into the class 2A solution. Class 1, class 2A, and class 2B residual solvents were prepared at their limit concentrations in deionized water. Six milliliters of each solution was dispensed into 20 mL headspace vials. The final headspace vial concentrations for each solvent are shown in Table 2.

Results and discussion

Optimized instrument conditions are the foundation of excellent test results. It is therefore important to ensure that the system is running well before performing analysis. The new 8697 -XL Tray, with its revolutionary design, is a smart instrument with full GC integration and built-in diagnostics. Headspace parameters and diagnostics can be intuitively displayed on the 8890 touchscreen or browser interface. As demonstrated in Figure 3, automated diagnostics are integrated into the GC for testing and troubleshooting common headspace issues. The leak tests, including a crossport leak test, restriction and pressure decay test, and transfer line leak and restriction test, can ensure that the whole headspace flow path is leak-free before performing sample analysis. The user vial leak test can help detect if the sample vial is correctly capped.

Since all the class 1, 2A, and 2B solvents were prepared at their USP <467> limit concentrations, some solvents showed very low response, such as carbon tetrachloride, 1,4-dioxane, 1,2-dimethoxyethane, and nitromethane. Meanwhile, acetonitrile and pyridine showed not only low response but also peak tailing on the DB-Select 624 UI column. The integration of these peaks is strongly influenced by the baseline. Therefore, special attention should be paid when optimizing the integration parameters.

System suitability requirements

USP <467> details the system suitability requirements for class 1, 2A, and 2B residual solvents. These requirements are mandatory.

For procedure A:

- The signal-to-noise (S/N) ratio for 1,1,1-trichloroethane in the class 1 solution is no less than five
- The S/N ratio of other peaks in class 1 is no less than three
- The resolution between acetonitrile and methylene chloride in class 2A is no less than one

For procedure B:

- The S/N ratio for benzene in class 1 solution is no less than five
- The S/N ratio of other peaks in class 1 is no less than three
- The resolution between MIBK and *cis*-dichloroethene in class 2A solution is no less than one

Helium carrier gas results

Figures 4, 5, and 7 illustrate the analysis of classes 1, 2A, and 2B residual solvent mixes on both DB-Select 624 UI and DB-WAX UI columns using helium carrier gas. The S/N value for 1,1,1-trichlororethane calculated following procedure A, and the S/N value for benzene following procedure B, were both much greater than five. All the other solvents exceeded the S/N value of three. The resolution between acetonitrile and methylene chloride in procedure A was 3.3. The resolution between MIBK and *cis*-dichloroethene was five. The resolution of both critical pairs was greater than the acceptance criteria of one. Acetonitrile actually eluted between *cis*-dichloroethene and MIBK, and the resolution between acetonitrile and MIBK is 1.68.

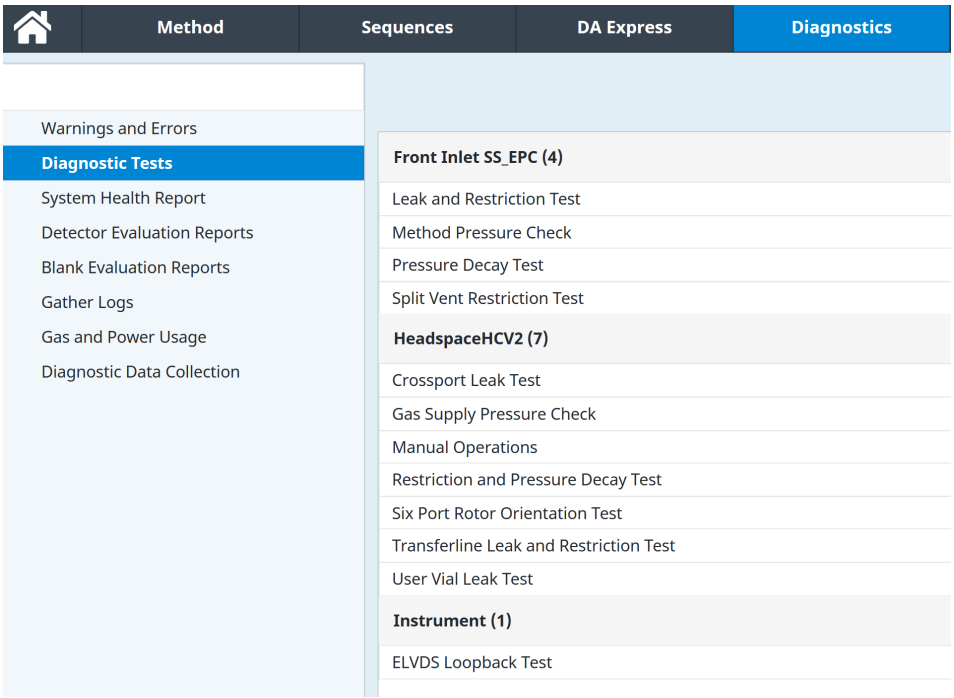


Figure 3. HSS diagnostics tests on the Agilent 8890 GC System (taken from the browser interface).

In general, analytes that coelute in procedure A were resolved well in procedure B, including the four additional solvents added to class 2A. USP <467> does not detail any performance requirements for class 2B solvents. There are no coelutions for class 2B solvents on either column. However, it should be noted that some solvents, such as nitromethane and 1,2-dimethoxyethane, have very low response, and special attention should be paid to the integration of these peaks.

Table 2 lists RSDs for all class 1, 2A, and 2B solvents prepared at their USP <467> limit concentrations. The number of runs for each class was 10. One sample blank was run ahead of each solvent class. Most RSDs are well below 2%, with a maximum of 3.45%. The retention time RSDs are below 0.03%, as shown in Table 2 and Figure 8. Those solvents with higher RSD values generally have a low partition coefficient K (apolar solvents with poor affinity for water) or very low detector response at the limit concentrations, such as carbon tetrachloride, 1,2-dimethoxyethane, or nitromethane. The generally excellent RSD results indicated the high stability and robustness of the whole system.

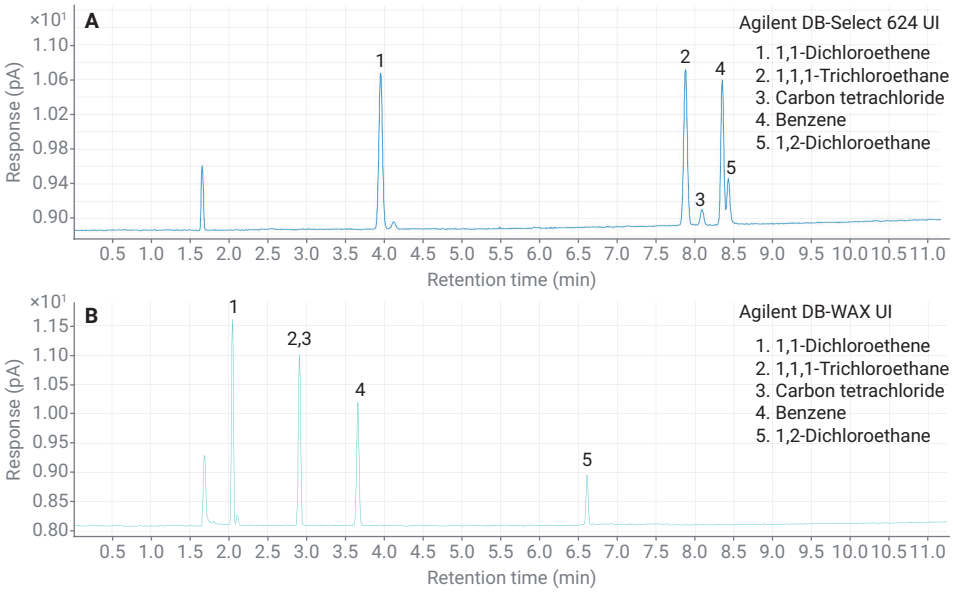


Figure 4. GC/FID chromatograms of class 1 standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using helium carrier gas.

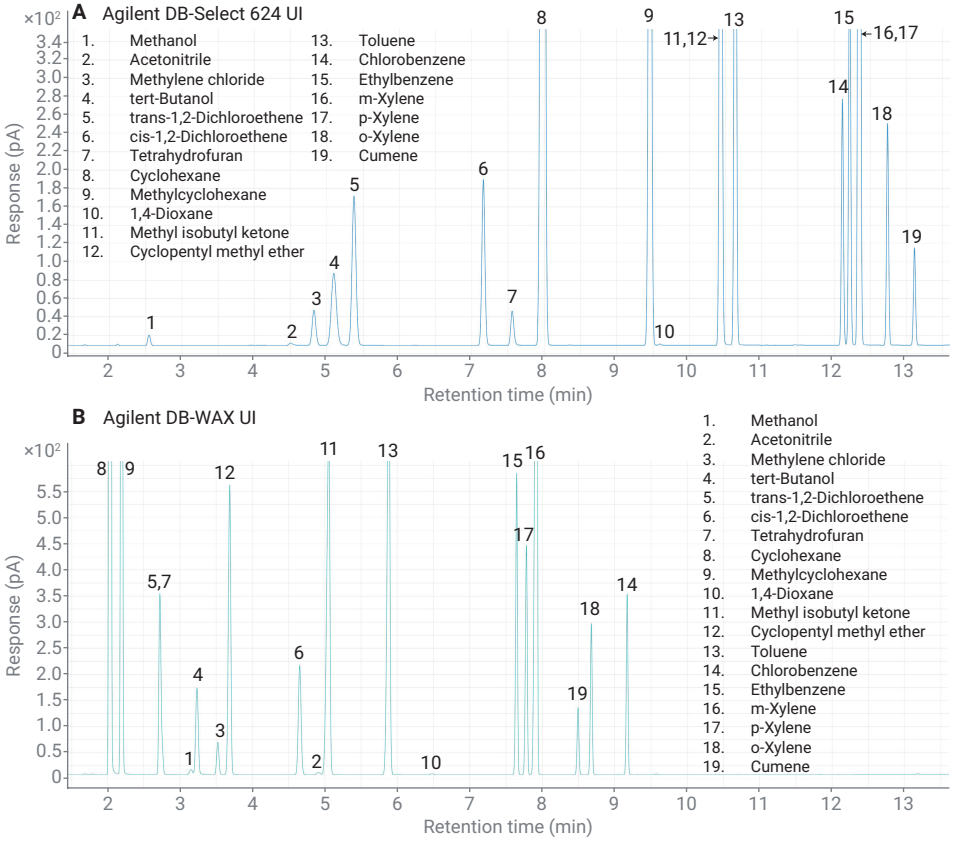


Figure 5. GC/FID chromatograms of class 2A standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using helium carrier gas.

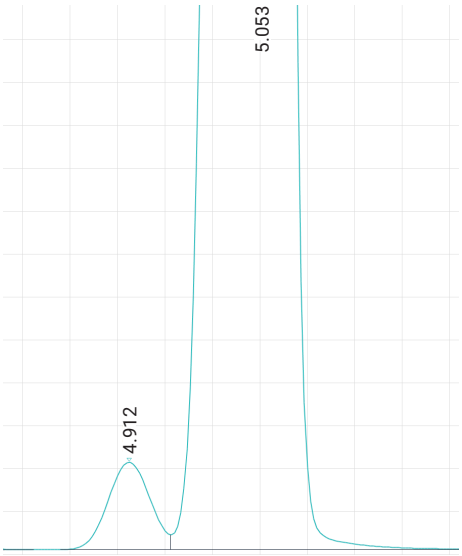


Figure 6. USP resolution of 1.68 between acetonitrile and MIBK on an Agilent DB-WAX UI column using helium carrier gas.

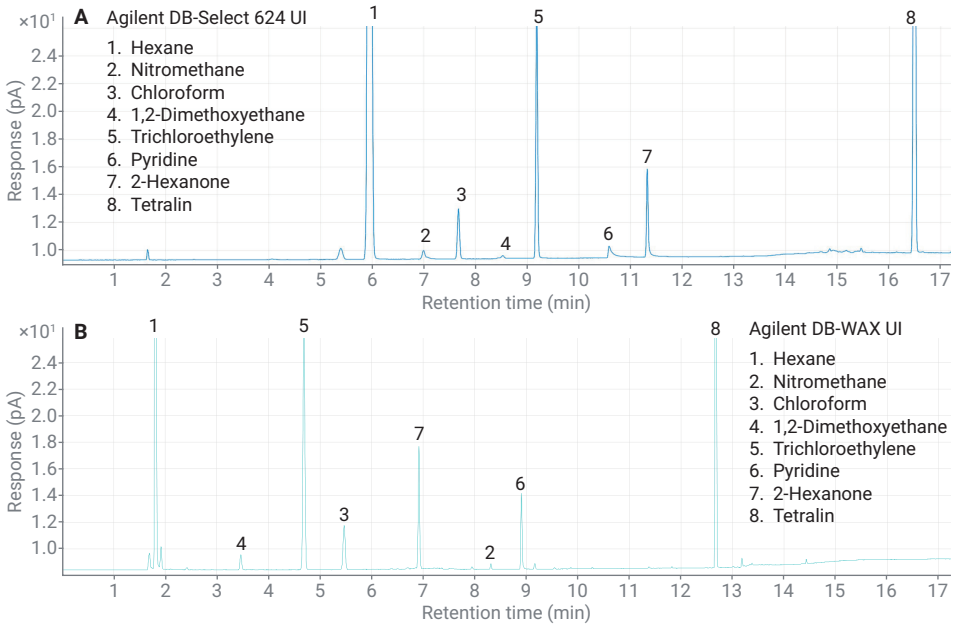


Figure 7. GC/FID chromatograms of class 2B standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using helium carrier gas.

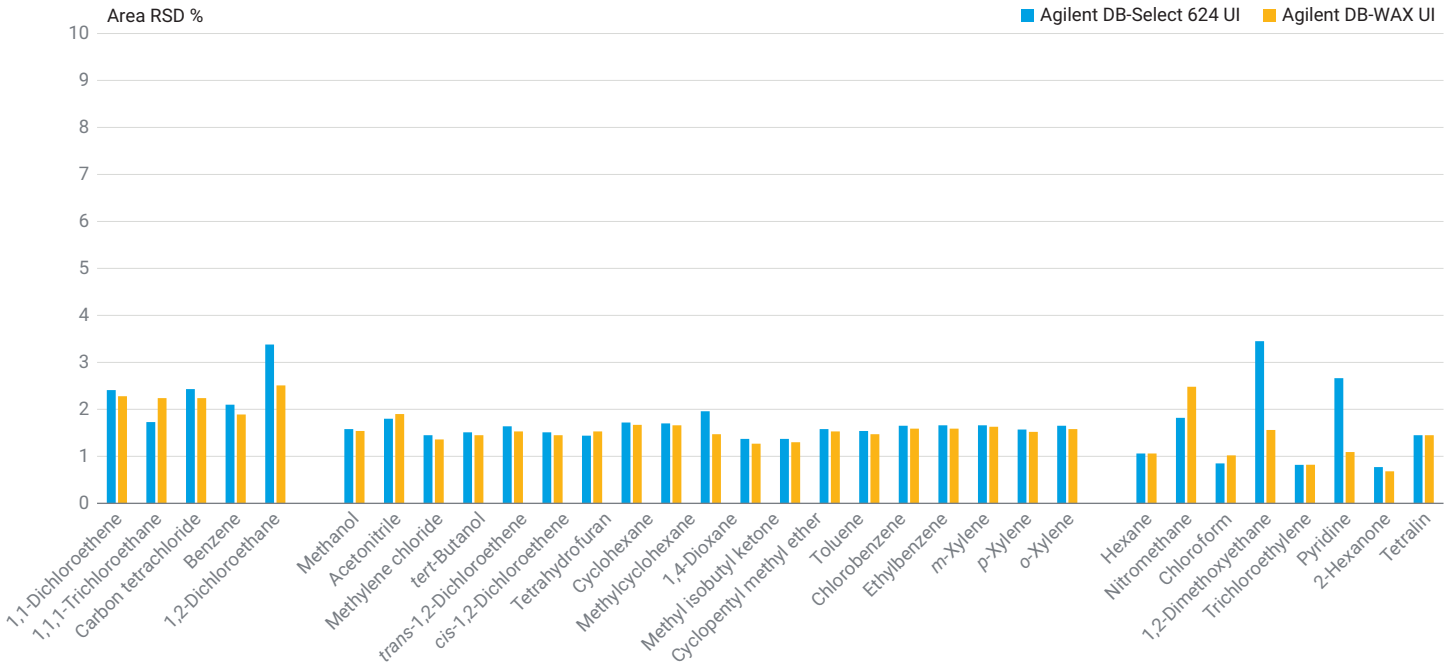


Figure 8. Area RSDs for all solvents on both Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using helium carrier gas.

Table 2. Residual solvents, actual headspace vial concentrations, and repeatability (n = 10) obtained on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using helium carrier gas.

Compound Name	Concentration (µg/mL)	Procedure A (DB-Select 624 UI Column)		Procedure B (DB-WAX UI Column)	
		RT %RSD	Area %RSD	RT %RSD	Area %RSD
Class 1					
1,1-Dichloroethene	0.07	0.011	2.41	0.012	2.28
1,1,1-Trichloroethane	0.08	0.009	1.73	0.012	2.24
Carbon tetrachloride	0.03	0.015	2.43	Coelutes with 1,1,1-trichloroethane	Coelutes with 1,1,1-trichloroethane
Benzene	0.02	0.009	2.1	0.012	1.89
1,2-Dichloroethane	0.04	0.007	3.38	0.007	2.51
Class 2A					
Methanol	25	0.014	1.58	0.016	1.54
Acetonitrile	3.42	0.013	1.8	0.013	1.9
Methylene chloride	5.02	0.009	1.45	0.014	1.36
tert-Butanol	29.17	0.01	1.51	0.014	1.45
trans-1,2-Dichloroethene	7.87	0.008	1.64	0.013	1.53
cis-1,2-Dichloroethene	7.87	0.006	1.51	0.014	1.45
Tetrahydrofuran	6.02	0.004	1.44	Coelutes with *trans*-1,2-dichloroethene	Coelutes with *trans*-1,2-dichloroethene
Cyclohexane	32.33	0.006	1.72	0.016	1.67
Methylcyclohexane	9.88	0.005	1.7	0.015	1.66
1,4-Dioxane	3.18	0.005	1.96	0.009	1.47
Methyl isobutyl ketone	37.5	0.004	1.37	0.01	1.27
Cyclopentyl methyl ether	12.5	Coelutes with MIBK	Coelutes with MIBK	0.012	1.3
Toluene	7.45	0.004	1.58	0.009	1.53
Chlorobenzene	3	0.004	1.54	0.008	1.47
Ethylbenzene	3.08	0.003	1.65	0.007	1.59
m-Xylene	10.88	0.003	1.66	0.014	1.59
p-Xylene	2.55	Coelutes with *m*-xylene	Coelutes with *m*-xylene	0.011	1.63
o-Xylene	1.64	0.003	1.57	0.013	1.52
Cumene	0.58	0.002	1.65	0.016	1.58
Class 2B					
Hexane	2.43	0.022	1.06	0.028	1.06
Nitromethane	0.42	0.02	1.82	0.01	2.48
Chloroform	0.5	0.013	0.85	0.028	1.02
1,2-Dimethoxyethane	0.83	0.023	3.45	0.029	1.56
Trichloroethylene	0.67	0.011	0.819	0.028	0.82
Pyridine	1.67	0.007	2.664	0.01	1.092
2-Hexanone	0.42	0.006	0.77	0.015	0.68
Tetralin	0.84	0.004	1.45	0.005	1.45

Nitrogen carrier gas results

When switching from helium to nitrogen carrier gas, the initial method parameters were aligned with the helium method parameters. All the solvents met the requirements of the USP <467> method, except for the resolution between acetonitrile and MIBK. With nitrogen as carrier gas, MIBK and CPME coeluted on the DB-Select 624 UI column, while acetonitrile and MIBK were poorly resolved on the DB-WAX UI column. According to USP <467> requirements, any coelutions that occur in procedure A should be resolved in procedure B, so that accurate quantitative results can be obtained. A lower initial oven temperature, a lower column flow, and a lower loop final pressure helped to achieve improved resolution of acetonitrile and MIBK on the DB-WAX UI column. Their improved resolution of 1.17 met the requirements for class 2A solvents, as shown in Figure 11. The optimized nitrogen method shown in Table 1 was also used for class 1 and 2B solvents acquisition. The results for all the analytes met the specifications of USP <467>. Figure 9, 10, and 12 illustrate the analysis of classes 1, 2A, and 2B residual solvent mixes on both DB-Select 624 UI and DB-WAX UI columns using nitrogen carrier gas.

Table 3 lists RSDs for all class 1, 2A, and 2B solvents with 10 replicates for each class using the optimized nitrogen method. The typical RSD of the peak area ranged from 0.92% to 4.44%, while the retention time RSDs are well below 0.03%, as shown in Table 3 and Figure 13.

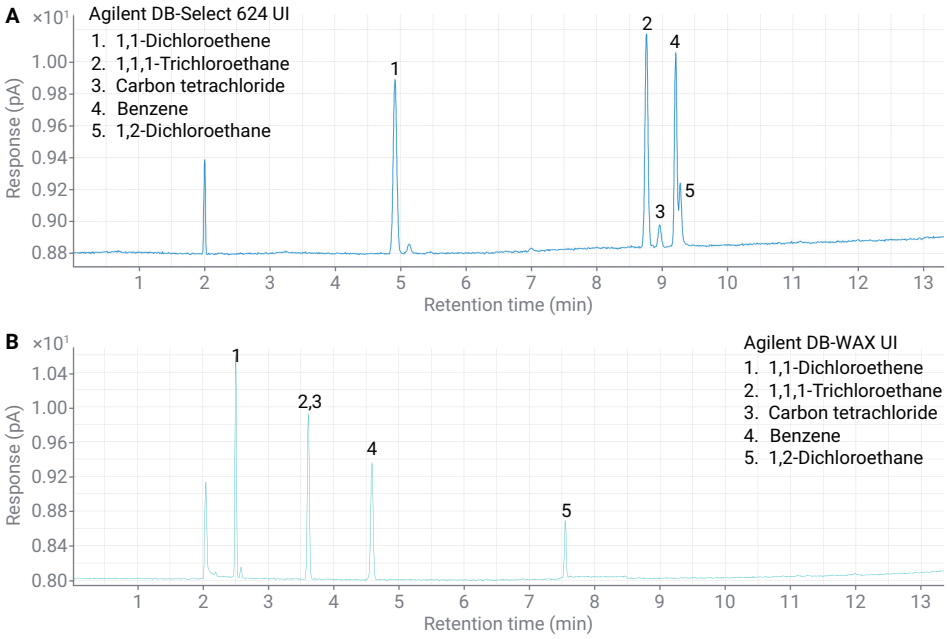


Figure 9. GC/FID chromatograms of class 1 standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using nitrogen carrier gas.

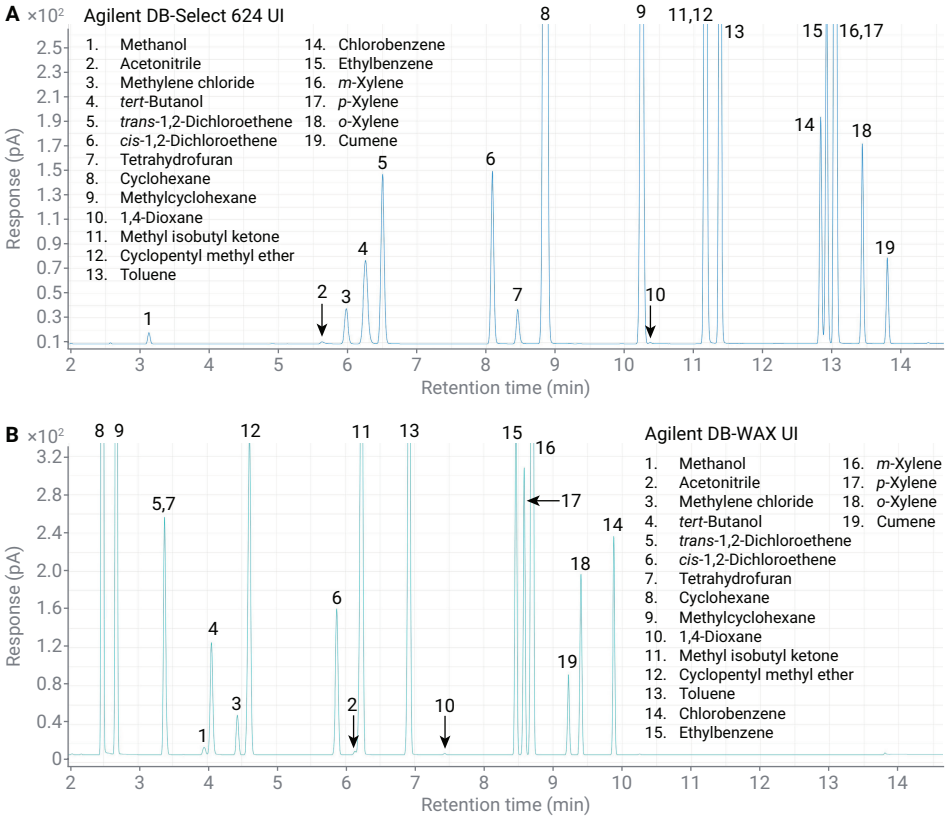
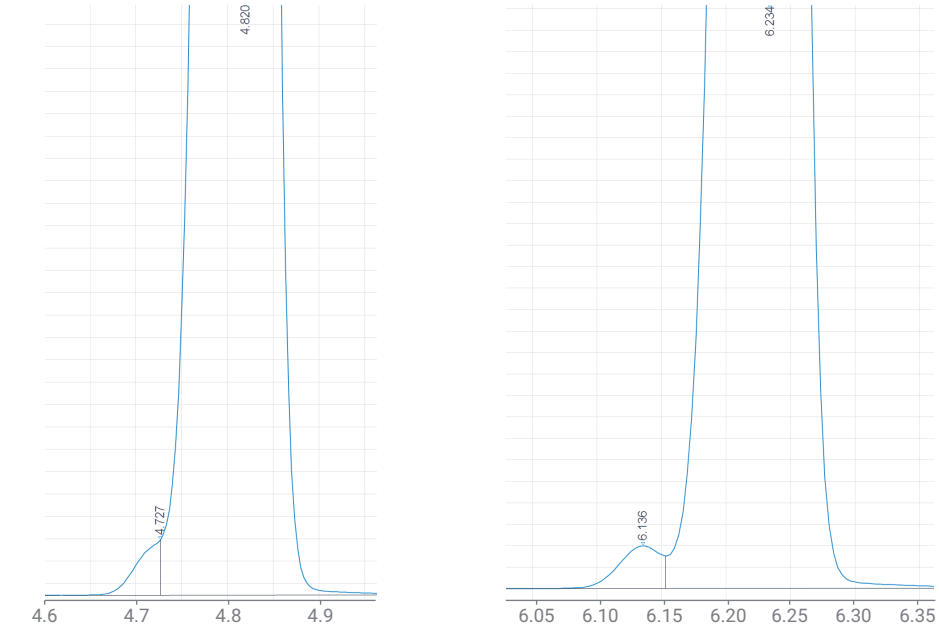


Figure 10. GC/FID chromatograms of class 2A standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using nitrogen carrier gas.



No resolution value for acetonitrile and MIBK

Resolution of acetonitrile and MIBK: 1.17

Figure 11. USP resolution between acetonitrile and MIBK on an Agilent DB-WAX UI column using nitrogen carrier gas. Original helium carrier gas method parameters using nitrogen carrier gas are compared with an optimized method based on nitrogen carrier gas.

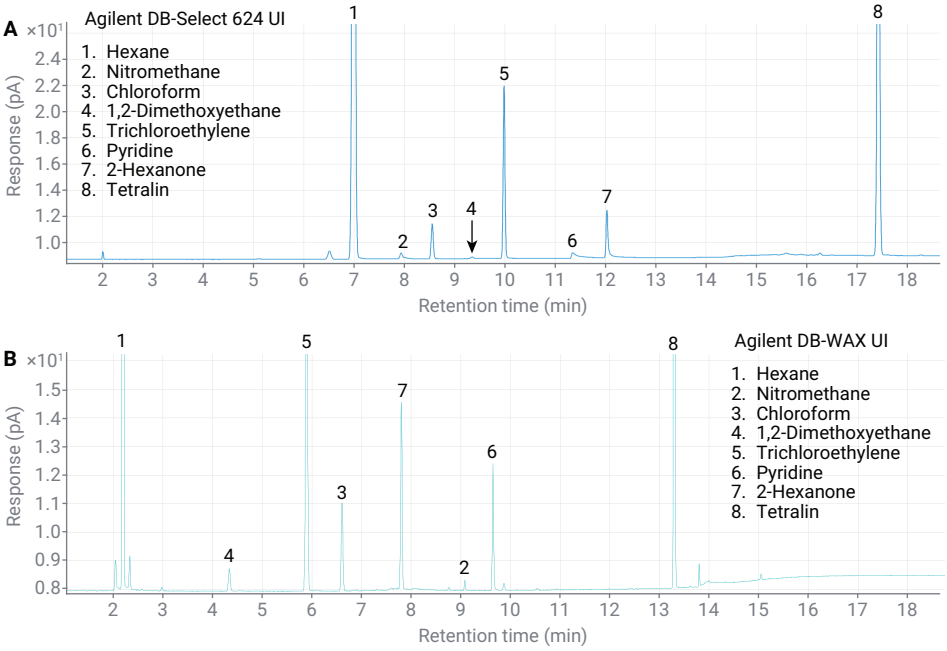


Figure 12. GC/FID chromatograms of class 2B standard solution on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using nitrogen carrier gas.

Table 3. Residual solvents, actual headspace vial concentrations, and repeatability (n = 10) obtained on Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using nitrogen carrier gas.

Compound Name	Concentration (µg/mL)	Procedure A (DB-Select 624 UI Column)		Procedure B (DB-WAX UI Column)	
		RT %RSD	Area %RSD	RT %RSD	Area %RSD
Class 1					
1,1-Dichloroethene	0.07	0.012	1.16	0.01	1.21
1,1,1-Trichloroethane	0.08	0.007	1.26	0.009	4.13
Carbon tetrachloride	0.03	0.015	3.33	Coelutes with 1,1,1-trichloroethane	Coelutes with 1,1,1-trichloroethane
Benzene	0.02	0.006	1.21	0.011	1.12
1,2-Dichloroethane	0.04	0.003	2.19	0.006	1.53
Class 2A					
Methanol	25	0.012	1.76	0.007	1.67
Acetonitrile	3.42	0.008	2.05	0.006	2.12
Methylene chloride	5.02	0.004	1.12	0.007	1.11
tert-Butanol	29.17	0.008	1.22	0.006	1.22
trans-1,2-Dichloroethene	7.87	0.005	1.19	Coelutes with tetrahydrofuran	Coelutes with tetrahydrofuran
cis-1,2-Dichloroethene	7.87	0.003	1.13	0.008	1.12
Tetrahydrofuran	6.02	0.003	1.06	0.007	1.12
Cyclohexane	32.33	0.004	1.23	0.01	1.23
Methylcyclohexane	9.88	0.004	1.24	0.008	1.25
1,4-Dioxane	3.18	0.004	2.15	0.005	1.43
Methyl isobutyl ketone	37.5	0.003	0.97	0.008	1.22
Cyclopentyl methyl ether	12.5	Coelutes with MIBK	Coelutes with MIBK	0.006	1.01
Toluene	7.45	0.003	1.16	0.007	1.16
Chlorobenzene	3	0.001	1.08	0.007	1.11
Ethylbenzene	3.08	0.002	1.12	0.003	1.17
m-Xylene	10.88	0.003	1.127	0.013	1.16
p-Xylene	2.55	Coelutes with *m*-xylene	Coelutes with *m*-xylene	0.01	1.15
o-Xylene	1.64	0.002	1.13	0.015	1.14
Cumene	0.58	0.003	1.2	0.015	1.22
Class 2B					
Hexane	2.43	0.004	1.38	0.007	1.65
Nitromethane	0.42	0.008	3.06	0.001	3.38
Chloroform	0.5	0.004	0.99	0.004	1.16
1,2-Dimethoxyethane	0.83	0.016	4.44	0.009	2.8
Trichloroethylene	0.67	0.002	1.2	0.006	1.49
Pyridine	1.67	0.027	4.29	0.013	2.75
2-Hexanone	0.42	0.002	2.16	0.004	1.01
Tetralin	0.84	0.003	0.92	0.003	1.12

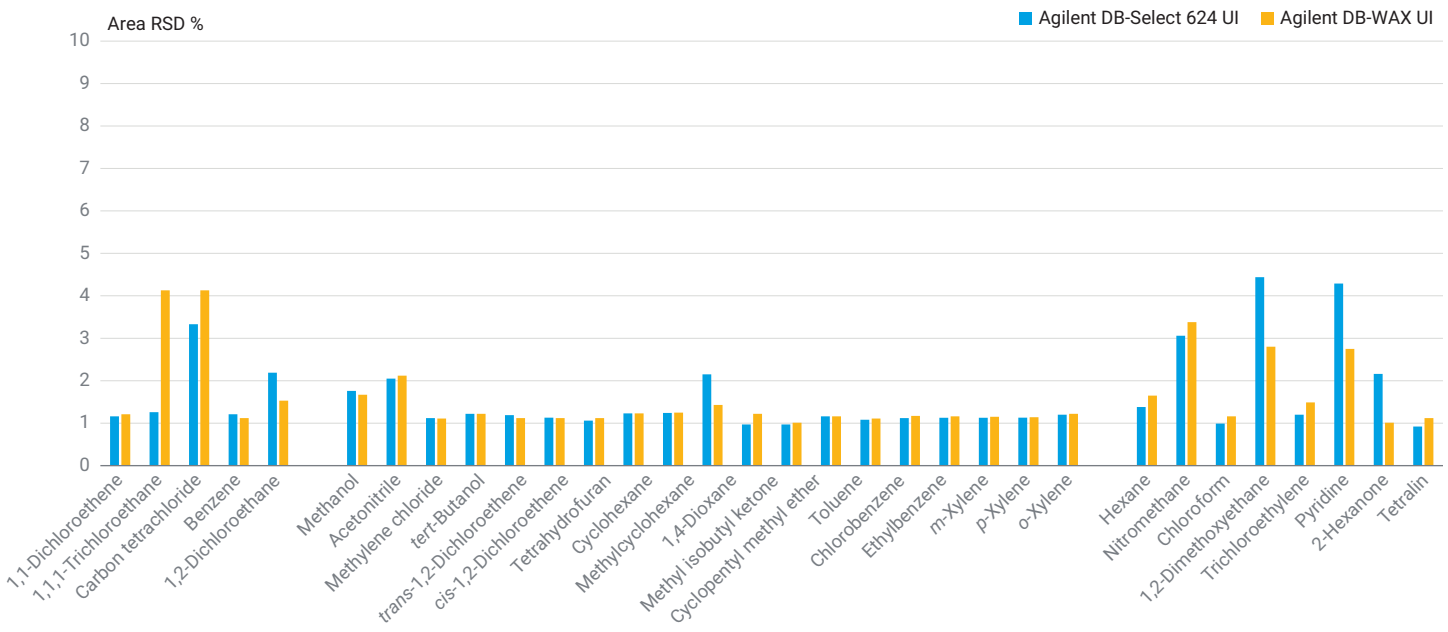


Figure 13. Area RSDs for all solvents on both Agilent DB-Select 624 UI and Agilent DB-WAX UI columns using nitrogen carrier gas.

Conclusion

USP <467> specifies residual solvents analysis using a single column with confirmation on a second column. With a conventional GC system, this analysis requires two separate analytical runs. The 8890, configured with dual columns and FIDs on a single inlet, greatly shortens the total analysis time. The 8697 -XL Tray, integrated with the GC, allows users to carry out specific tests to check that the HSS is operating correctly before sample testing.

The whole system delivers outstanding repeatability for the analysis of residual solvents using either helium and nitrogen carrier gas. Area precision for 10 replicates was less than 3.45% for helium carrier gas and less than 4.44% for nitrogen carrier gas. The typical retention time precision was well below 0.03% for all the analytes.

References

1. USP <467> Residual Solvents. 467 RESIDUAL SOLVENTS (uspnf.com)
2. Impurities: Guidance for Residual Solvents Q3C (R8). Q3C(R8) Impurities: Guidance for Residual Solvents Guidance for Industry | FDA
3. Eisele, I. Residual Solvents Analysis Using an Agilent Intuvo 9000 GC with 8697 Headspace Sampler. *Agilent Technologies application note*, publication number 5994-3075EN, **2021**.

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