



# Analysis of USP <467> Residual Solvents using Agilent J&W DB-WAX Ultra Inert Capillary GC Columns

Following USP <467> Residual Solvents Procedure B

## Application Note

Pharmaceutical

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### Abstract

This application note highlights the excellent performance of an Agilent J&W DB-WAX Ultra Inert GC column in the analysis of USP <467> classes 1, 2A, and 2B residual solvents.

### Introduction

Residual solvents may persist in pharmaceuticals from the manufacturing process of the active pharmaceutical ingredients (APIs) or final product. The levels of residual solvents should be monitored and controlled for safety, effect on crystalline form, solubility, bioavailability, and stability. All drug substances, excipients, and products must be monitored. Quality assurance (QA) labs routinely use the United States Pharmacopeia (USP) Method <467> for this purpose [1]. The basic method is used worldwide for quality control, and closely follows ICH Q3C guidelines.

Residual solvents have been classified into three main classes based on risk assessment.

- Class 1 solvents are considered hazardous, and should be avoided in the manufacturing process.
- Class 2 solvents are considered less severe toxicity, and should be limited.
- Class 3 solvents are considered less toxic, and pose less risk to human health than either class 1 or class 2 solvents.



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The USP <467> is divided into two separate sections based on sample solubility: water-soluble and water-insoluble articles. Three analytical procedures are used for identification and quantification of the residual solvents (as shown in Figure 1).

- **Procedure A:** Identification and limit test using a G43 phase (624 type columns).
- **Procedure B:** Confirmatory test if solvent is above limit using a G16 phase (wax type columns).
- **Procedure C:** Quantitative test using a G43 phase or G16 phase, depending on which gave fewer co-elutions.

In previous application notes, an Agilent J&W DB-Select 624UI GC column, equivalent to G43 phase, showed excellent performance for USP <467> residual solvent analysis according to USP <467> procedure A [2,3]. Once a residual solvent was identified above the permitted daily exposure (PDE) limit, procedure B was performed to confirm analyte identity. A G16 (WAX) column was used as a confirmation column because it yields an alternate selectivity compared to that of a G43 column. Agilent J&W DB-WAX Ultra Inert columns are engineered for better peak shapes, and are rigorously tested with demanding probes to verify best-in-class inertness. This study investigated the performance of Agilent J&W DB WAX Ultra Inert columns for the analysis of USP <467> residual solvents using procedure B. Example chromatograms for a dual channel configuration using dual columns (DB-Select 624UI and DB-WAX UI) and dual FIDs are also shown in this application note.

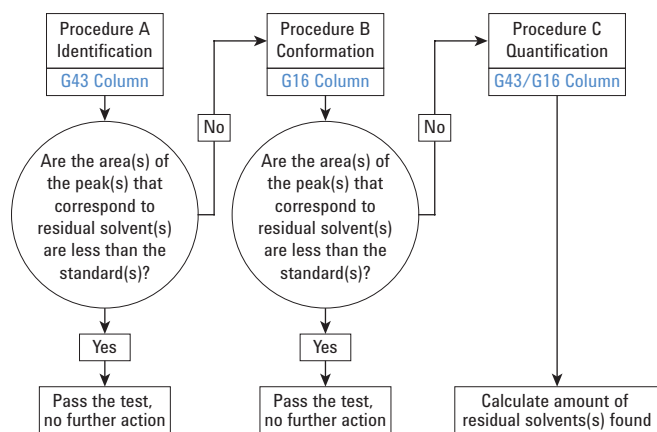


Figure 1. USP <467> Analytical flowchart for residual solvent analysis.

## Experimental

All samples were prepared according to the USP General Chapter <467> methodology.

### Chemicals and reagents

Dimethyl sulfoxide (DMSO) (>99.5%) was purchased from Sigma-Aldrich (Shanghai, China). De-ionized water was from a laboratory water purification system.

### Sample preparation for water-soluble articles

Three stock solutions of residual solvents in DMSO were used:

- Residual Solvent Revised Method 467- Class 1 (p/n 5190–0490)
- Residual Solvent Revised Method 467- Class 2A (p/n 5190–0492)
- Residual Solvent Revised Method 467- Class 2B (p/n 5190–0491)

#### Class 1 solvents

- 1 mL stock solution vial plus 9 mL DMSO diluted to 100 mL with water
- 1 mL from step 1 diluted to 100 mL with water
- 10 mL from step 2 diluted to 100 mL with water
- 1 mL from step 3 + 5 mL water in 20 mL HS vial

#### Class 2A solvents

- 1 mL stock solution vial, diluted to 100 mL with water
- 1 mL from step 1 + 5 mL water in 20 mL HS vial

#### Class 2B solvents

- 1 mL stock solution vial, diluted to 100 mL with water
- 1 mL from step 1 + 5 mL water in 20 mL HS vial

Table 1. Residual solvents, peak number, actual headspace vial concentrations, and repeatability (n = 6) obtained by single column HS-GC-FID system on an Agilent DB-WAX UI column.

No.	Compound	Concentration ( $\mu\text{g/mL}$ )	RSD%
<b>CLASS 1</b>			
1	1,1-Dichloroethene	0.07	2.18
2	1,1,1-Trichloroethylene	0.08	1.37
3	Carbon tetrachloride	0.03	1.37
4	Benzene	0.02	1.86
5	1,2-Dichloroethane	0.04	2.48
<b>CLASS 2A</b>			
1	Cyclohexane	32.50	1.90
2	Methylcyclohexane	9.88	2.33
3	<i>trans</i> -1,2-Dichloroethene	7.87	2.06
4	Tetrahydrofuran	6.03	2.06
5	Methanol	25.17	2.98
6	Dichloromethane	5.02	2.45
7	<i>cis</i> -1,2-Dichloroethene	7.87	0.98
8	Acetonitrile	3.43	1.65
9	Toluene	7.45	1.92
10	1,4-Dioxane	3.18	2.63
11	Ethylbenzene	3.08	2.32
12	<i>p</i> -Xylene	2.55	2.26
13	<i>m</i> -Xylene	10.88	1.67
14	<i>o</i> -Xylene	1.64	2.11
15	Chlorobenzene	3.02	1.17
<b>CLASS 2B</b>			
1	Hexane	2.43	1.82
2	1,2-Dimethoxyethane	0.84	1.11
3	Trichloroethylene	0.67	1.23
4	Chloroform	0.50	1.15
5	2-Hexanone	0.42	1.09
6	Nitromethane	0.42	1.82
7	Pyridine	1.68	2.27
8	Tetralin	0.84	1.98

## Instrumentation

USP <467> Procedure B was used in this work to evaluate the performance of a DB-WAX UI GC column. The analysis followed the guidelines of Procedure B. Table 2 lists the instruments and conditions.

Table 2. Single column GC/FID system conditions.

Parameter	Value
GC system:	Agilent 7890B
Column:	Agilent J&W DB-WAX UI, 30 m $\times$ 0.32 mm, 0.25 $\mu\text{m}$ (p/n 123-7032UI)
Carrier gas:	Helium, 35 cm/s, constant flow mode
Inlet:	Split/splitless, 140 $^{\circ}\text{C}$ , split ratio 5:1
Oven:	50 $^{\circ}\text{C}$ (hold 20 min) to 165 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}/\text{min}$ (hold 20 min)
FID:	250 $^{\circ}\text{C}$
Headspace:	Agilent 7697A Headspace Sampler
Oven temperature:	80 $^{\circ}\text{C}$
Loop temperature:	80 $^{\circ}\text{C}$
Transfer line temperature:	100 $^{\circ}\text{C}$
Equilibration time:	45 min
Sample loop:	1 mL

Figure 2 shows the parallel dual column configuration. The dual channel GC/FID method is described in many Agilent application notes [4,5]. According to these previous described methods, static headspace analysis performed at 85 °C for 40 minutes improved repeatability and reduced analysis time and cycle time. Table 3 summarizes the experimental GC conditions. A DB-WAX UI GC column was tested as a confirmation column in this system. Table 4 lists the flow path consumable supplies.

## Results and Discussion

### Single Column GC/FID System

USP <467> procedure B was used to confirm the peak identification of procedure A. An Agilent J&W DB-WAX UI GC column (G16 column) was tested as a confirmation column using the conditions listed in Table 2. The system suitability requirements for Procedure B were as follows:

- The signal-to-noise ratio (S/N) of benzene in class 1 standard solution is greater than 5.
- The S/N of each peak in class 1 standard solution is not less than 3.
- The resolution of acetonitrile and *cis*-dichloroethene in class 2A standard solution must be greater than 1.

Table 3. Dual column GC/FID system conditions.

Parameter	Value
GC system:	Agilent 7890B
Column 1:	Agilent J&W DB-WAX UI, 30 m × 0.32 mm, 0.25 μm (p/n 123-7032UI)
Column 2:	Agilent J&W DB-select 624 UI, 30 m × 0.32 mm, 1.8 μm (p/n 123-0334UI)
Tubing:	Agilent Ultimate Plus deactivated fused silica tubing, 0.5 m × 0.32 mm (p/n CP803205)
Carrier gas:	Helium, constant flow mode, 15 psi
Inlet:	Split/splitless, 140 °C, split ratio 2.5:1
Oven:	40 °C (hold 5 min) to 240 °C at 18 °C/min (hold 2 min)
FID (both channels):	250 °C
Headspace:	Agilent 7697A Headspace Sampler
Oven temperature:	85 °C
Loop temperature:	85 °C
Transfer line temperature:	100 °C
Equilibration time:	40 min
Sample loop:	1 mL

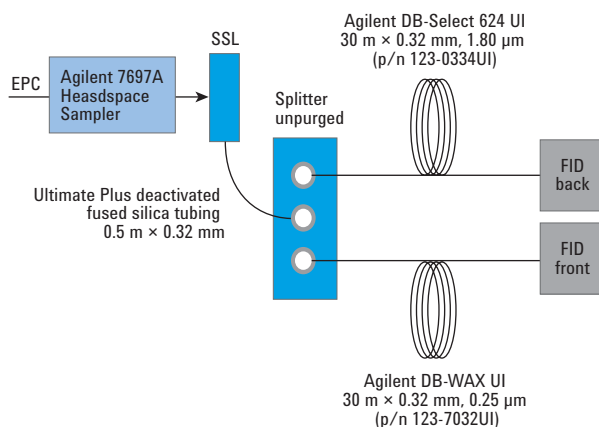


Figure 2. Dual-channel GC/FID System.

Table 4. Flow path supplies.

Parameter	Value
Vials:	Headspace crimp top, flat bottom vials, 20 mL, 100/pk (p/n 5182-0837)
Septa:	Nonstick BTO septa (p/n 5183-4757)
Column nut:	Self-tightening, inlet/detector (p/n 5190-6194)
Internal nut:	CFT capillary fitting (p/n G2855-20530)
Splitter:	Compact splitter, inert (p/n G3181-60500)
Ferrules:	Short graphite: Vespel (15%:85%), 0.32 mm, 10/pk (p/n 5062-3514) UltiMetal Plus Flexible Metal, for 0.32 mm columns, 10/pk (p/n G3188-27502)
Liner:	2 mm, straight, deactivated, liner (p/n 5181-8818)
Inlet seal:	Ultra Inert, gold-plated, with washer (p/n 5190-6144)

Figures 3-5 illustrate the analysis of classes 1, 2A, and 2B residual solvent mixes on a DB-WAX UI GC column. Peaks in the chromatograms can be identified by referring to Table 1. At the concentration limits specified by the monograph, the S/N for benzene in class 1 standard solution was 85.4, and all other compounds exceeded 3. The S/N ratios were 68.3 for 1,1-dichloroethene, 88.5 for 1,1,1-trichloroethane and carbon tetrachloride, and 32.3 for 1,2-dichloroethane, respectively.

Resolution of acetonitrile and *cis*-dichloroethene in class 2A standard solution is specified to be not less than 1.0. Figure 4 shows a resolution of 1.85 for this critical pair on DB-WAX UI. Pyridine peak shape or degree of tailing is an important performance parameter to monitor in the class 2B standard solution. In Figure 5, the USP tailing value for pyridine was 1.02 because of the high inertness performance of the DB-WAX UI GC column.

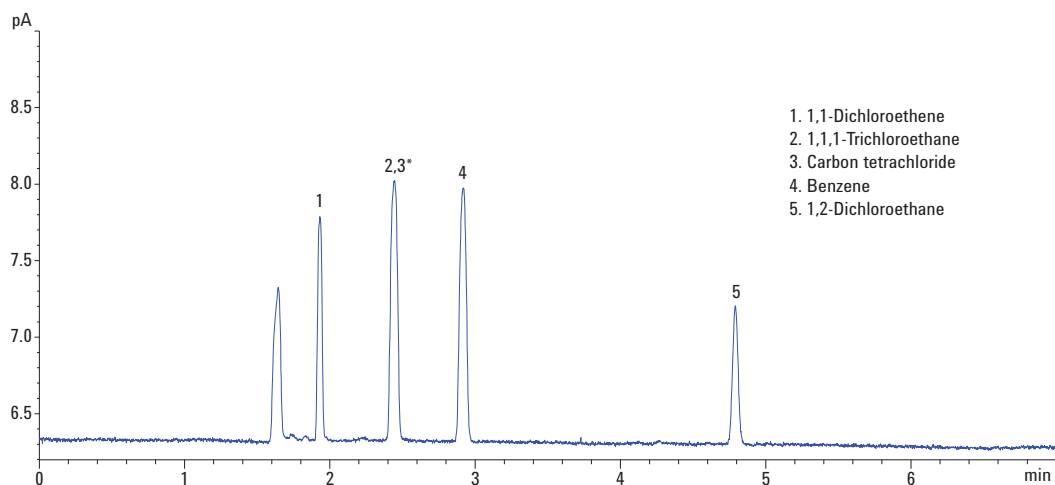


Figure 3. Chromatogram of an USP residual solvent class 1 standard solution resolved on an Agilent J&W DB-WAX Ultra Inert 30 m × 0.32 mm, 0.25 μm GC column.

\* Carbon tetrachloride coelutes with 1,1,1-trichloroethane with the G16 (DB-WAX UI) column, but is separated from all peaks in the class 1 standard with the G43 column.

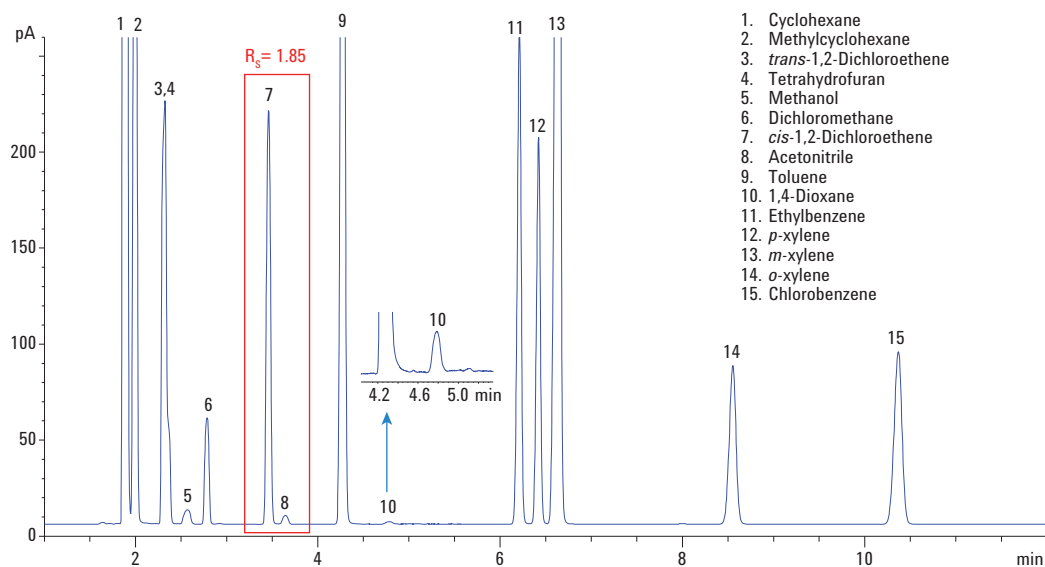


Figure 4. Chromatogram of an USP residual solvent class 2A standard solution resolved on an Agilent J&W DB-WAX Ultra Inert 30 m × 0.32 mm, 0.25 μm GC column.

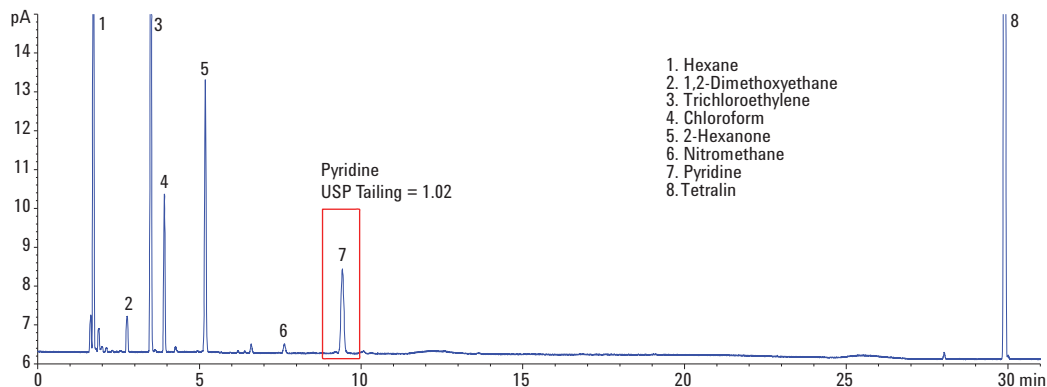


Figure 5. Chromatogram of an USP residual solvent class 2B standard solution resolved on an Agilent J&W DB-WAX Ultra Inert 30 m × 0.32 mm, 0.25 μm GC column.

The area repeatability (RSD%) was evaluated by measuring the class 1, 2A, and 2B residual solvent mixes six consecutive times. Table 1 lists the RSD% obtained on the DB-WAX UI column. The resulting RSD% values were less than 3.0%, which indicated high repeatability and stability of the column, the Agilent 7697A headspace sampler, and the Agilent 7890B GC/FID system.

### Dual Channel GC/FID System

USP <467> procedures A and B can be accomplished in one run with the dual channel configuration shown in Figure 2. Ultimate plus deactivated fused silica tubing connects the inlet to an inert splitter. A 1:1 split occurs into 30 m × 0.32 mm DB-Select 624UI and DB-WAX UI columns. To achieve equivalent results as a single column GC/FID system, the split ratio became 2.5:1. Example chromatograms for classes 1, 2A, and 2B standard solvents are shown in Figures 6, 7, and 8, respectively.

There are known co-elutions on both G43 (DB-Select 624) and G16 (DB-WAX UI) phases for USP residual solvent analysis. Since many of co-elutions are different on the two phases, the dual column configuration can provide clearer identifications, as shown in Figures 6-8.

USP 467 procedure A requires that the S/N for 1,1,1-trichloroethane on a DB-Select 624UI be greater than 5. In addition, the S/N obtained from the other compounds in the class 1 is required to be greater than 3. Resolution between acetonitrile and dichloromethane must be not less than 1 in the class 2A solution. As shown in Figure 6, the S/N for 1,1,1-trichloroethane was 52.8, and for carbon tetrachloride, which had the lowest sensitivity level, the S/N was 6.5. Resolution between acetonitrile and methylene chloride was 3.12 (see Figure 7).

The system suitability requirements for USP 467 procedure B were easily met or exceeded using the DB-WAX UI GC column with a dual channel GC/FID system. The S/N for benzene in class 1 standard solution was 73.5, and all other compounds exceeded 3. The resolution of acetonitrile and *cis*-dichloroethene in class 2A standard solution was 2.78. Furthermore, the high inertness of the DB-WAX UI column provided excellent peak shape for residual solvents; for a challenging compound, pyridine, USP tailing was 1.06.

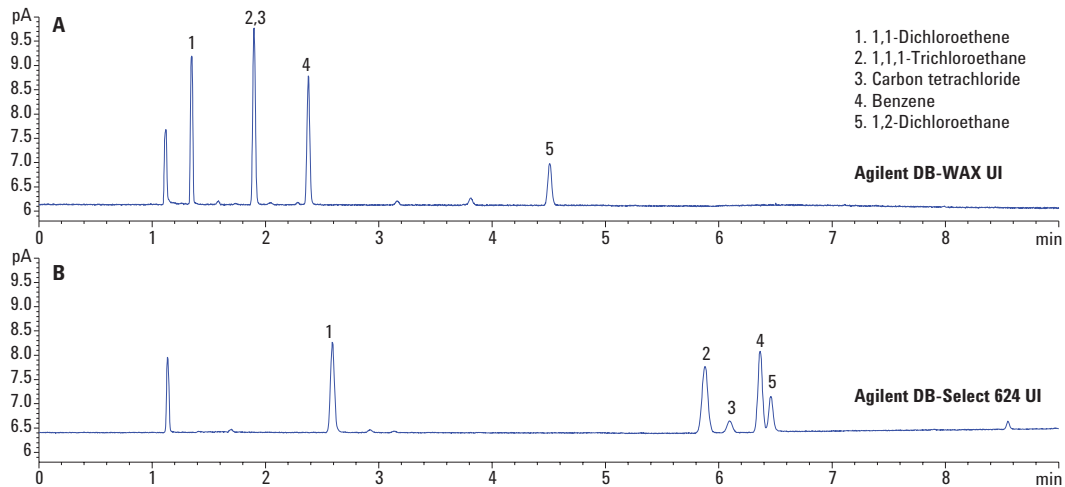


Figure 6. Dual channel GC/FID chromatograms of class 1 standard solution using an Agilent J&W DB-WAX UI and an Agilent DB-Select 624 UI GC column.

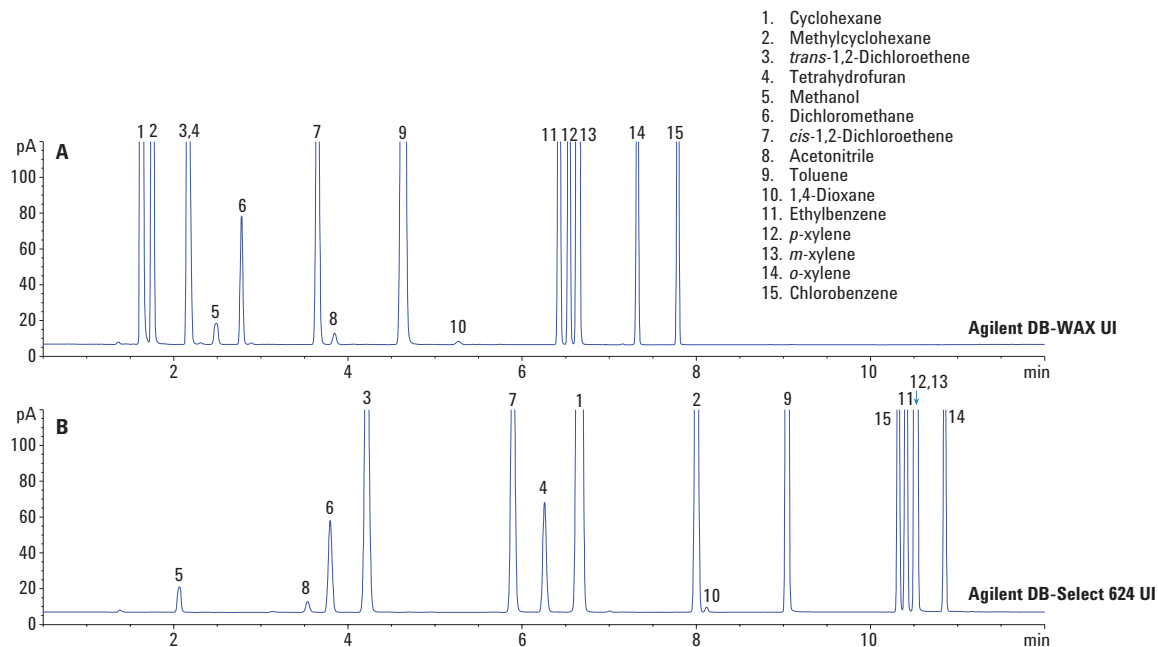


Figure 7. Dual channel GC/FID chromatograms of class 2A standard solution using an Agilent J&W DB-WAX UI and an Agilent DB-Select 624 UI GC column.

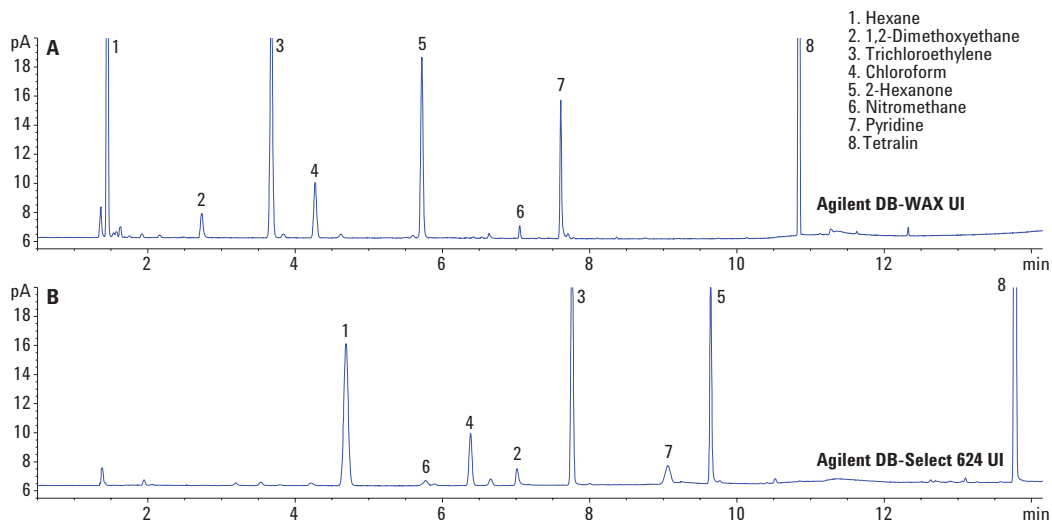


Figure 8. Dual channel GC/FID chromatograms of class 2B standard solution using an Agilent J&W DB-WAX UI and an Agilent DB-Select 624 UI GC column.

## Conclusions

Residual solvents of classes 1, 2A, and 2B were tested using an Agilent J&W DB-WAX UI GC column with single GC/FID system and dual channel GC/FID system. The Agilent J&W DB-WAX UI GC column demonstrated excellent chromatographic performance including good resolution, peak shape, sensitivity, and repeatability for the three classes of residual solvents at USP 467 procedure B method specified limits.

## References

1. USP 32-NF 27, General Chapter USP <467> Organic volatile impurities, United States Pharmacopeia. Pharmacopoeia Convention Inc., Rockville, MD, 8/2009.
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