

Analysis of USP <467> Residual Solvents with Improved Repeatability Using the Agilent 7697A Headspace Sampler

Application Note

Pharmaceuticals

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Abstract

The 7697A Headspace Sampler coupled to a 7890 GC was used for the analysis of USP <467> Class 1 and Class 2 residual solvents at their limit concentrations in aqueous solution. Repeatability was generally better than 2.5% RSD for Class 1, Class 2A, and Class 2B solvents. Configurations were tested including the split/splitless inlet and volatiles interface. Both 10 mL and 20 mL vials were used with each inlet. Headspace control software fully integrated into the multitechnique Chemstation was used for method development and final analysis. Additional configurations for dual column confirmation using FID-FID and FID-MSD are described.



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Introduction

Analysis of residual solvents in pharmaceuticals is extremely important to protect patient safety. Quality assurance (QA) labs routinely use United States Pharmacopeia (USP) Method <467> for this purpose. [1] The basic gas chromatographic/static headspace method is widely used worldwide for quality control. It closely follows ICH Q3C guidelines.

Residual solvents in pharmaceuticals may remain from the manufacturing process of the active pharmaceutical ingredients (APIs) or final product. One needs to monitor and control the levels of residual solvents for a number of reasons including safety, effect on crystalline form, solubility, bioavailability, and stability. All drug substances, excipients, and products must be monitored.

The analysis followed the guidelines of USP <467> for all parameters, except the vial equilibration time and GC oven program rates, for a significant reduction in analysis time and cycle time. Analysis methodologies that deviate from the USP monograph can be used. However, validation and equivalence to the original USP procedures may be required. Each class of solvents was run separately. Therefore, a shorter GC oven program could be used.

USP <467> specifies three procedures as follows for Class 1 and Class 2 residual solvents:

1. Procedure A: Identification and limit test
2. Procedure B: Confirmatory test (if solvent is above limit)
3. Procedure C: Quantitative test

Procedure A uses a G43 phase (Agilent 624 columns, VF-624ms or DB-624, p/n 123-1334) in this work) and Procedure B uses a G16 phase (HP-INNOWax, p/n 19091N-113). In general, analytes that coelute on one of these phases do not coelute on the other. Since the primary objective of this work centers on evaluating repeatability, only the 624 column was used for calculation of RSD's. Other configurations using dual FID's (624 and INNOWax columns) or FID/MSD are also illustrated.

The static headspace based method has historically suffered from poor repeatability when analyzing solvents at or below their USP 467 limit concentrations. Use of advanced pneumatics, excellent thermal zone control, and precise timing yield improved repeatability and precision for residual solvent analysis with the 7697A compared to previous generation samplers.

Experimental

USP <467> Procedure A was used in this work to investigate the performance of the 7697A. The 7890 GC was configured

with both a split/splitless inlet and a volatiles interface. A 2 mm deactivated straight liner (p/n 5181-8818) was used in the split/splitless inlet. Good results can also be obtained with a 4 mm straight liner (p/n 210-3003) as well.

Class 1, class 2A, and class 2B residual solvents were prepared at their limit concentrations in purified water. Clean organic free water is extremely important for good standard preparation. 250 mL solutions of each class were prepared at their final concentrations and then 6 mL was transferred into either 10 or 20 mL vials using a Brand Dispensette connected to a 500 mL glass bottle. Only PTFE-faced headspace vial septa were used. Salt was not added to the vials. Agilent part numbers for the residual solvent standards are:

Class 1: 5190-0490

Class 2A: 5190-0492

Class 2B: 5190-0513

The 7697A was interfaced to the inlets using 0.45 mm id deactivated fused silica tubing (Restek Siltek p/n 10018). Interface to the split/splitless inlet was through the septum into a 2 mm straight deactivated liner. With the volatiles interface, a siltite ferrule and special internal nut (engineered for the VI) was used to attach the fused silica transfer line to the interface body. Thirty meter 0.32 mm id columns were used for this work as they represent a good compromise between resolution, speed, capacity and ease of use. Narrow bore columns could also be used to increase resolution if needed.

Vial pressure is controlled from a Pneumatic Control Module (PCM) located in the 7697A, while carrier flow is routed from the inlet EPC to the headspace sampler and back to the 7890 inlet. The vent flow path is designed to minimize solvent condensation via user setable purging between runs. The headspace sample loop is 1.0 mL. Helium is used for carrier and vial pressurization. Vial Pressures set by the PCM are at "Normal Temperature and Pressure" (NTP) not gauge. This absolute pressure mode allows for consistent results regardless of geographic location where temperature and pressure vary.

A system diagram is shown in Figure 1. Application specific parameters are given in Table 1.

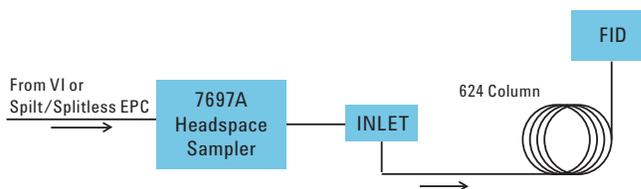


Figure 1. System diagram used for repeatability study.

Table 1. System Parameters for the Analysis of Residual Solvents

| Inlet | Inlet temperature | Split ratio |
|-------------------------------|--|---------------|
| Split/splitless | 140 °C | 5:1 |
| Volatiles interface | 145 °C | 5:1 |
| Carrier control | Inlet EPC | Inlet EPC |
| Headspace transfer line | Deactivated 0.45 mm fused silica | |
| Column p/n CP9104 | 30 m × 0.32 mm × 1.80 μm VF-624 ms | |
| Oven program | 40 °C (5 min) to 240 °C (2 min) at 18 °C/min | |
| Headspace zone temperatures | | |
| HS oven | 85 °C | |
| Valve/loop | 85 °C | |
| Transferline | 100 °C | |
| Vial sampling parameters | | |
| | 10 mL Vials | 20 mL Vials |
| Loop size | 1 mL | 1 mL |
| Vial equilibration time | 40 min | 40 min |
| Inject time | 0.5 min | 0.5 min |
| Vial pressurize value | 15 psia (NTP) | 15 psia (NTP) |
| Vial final sampling pressure | 0 ambient | 10 psia (NTP) |
| Pressure equilibration time | 0.10 min | 0.10 min |
| Vial final pressure hold time | 0.05 min | 0.05 min |
| Vial fill flow | 50 mL/min | 50 mL/min |
| Vial pressure ramp rate | 20 psi/min | 20 psi/min |
| Vent vial after extraction | Yes | Yes |
| Vial shaking setting | 2 | 2 |

The 7697A vial sampling event sequence consists of 13 steps as stated below.

1. Standby
2. Pre pressurize vial
3. Flow sensor auto zero
4. Vial pressurization constant flow
5. Vial pressurization constant pressure
6. Vial pressure equilibration
7. Loop fill (Vent vial)
8. Inject with full Vial vent
9. Inject without full Vial vent
10. Sample vial return
11. Remove vial from needle
12. Recovery (purge needle and Vent line)
13. Recovery (purge needle)

These EPC controlled steps give the user control over all aspects of sampling the vial headspace in a concise and reproducible method with minimal carryover. Barometric pressure compensation is also implemented in the EPC modules. Parameters can be set from the 7697A keyboard or Headspace Control Software. Aside from the unique scheduling aspects of headspace auto sampling, the software integration is very

HS Sampling System Pneumatics, Standby

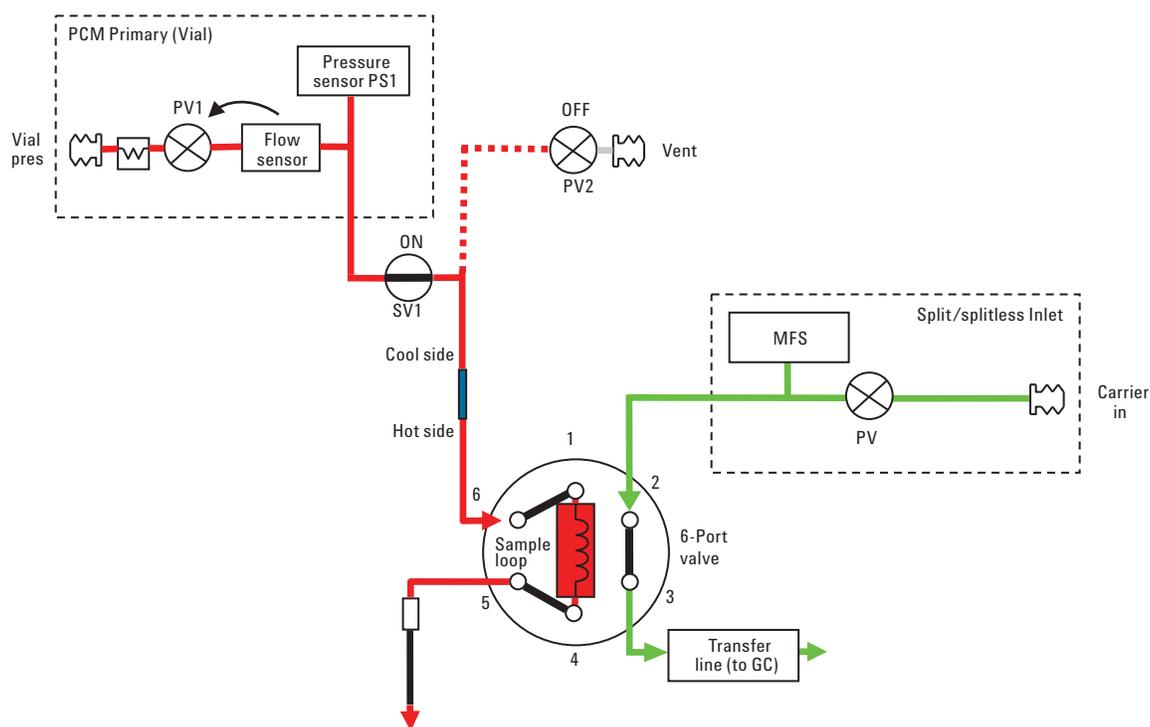


Figure 2. In Standby mode, the PCM EPC is in flow control mode so that the amount of gas used when in Standby can be limited. This flow purges the sample lines, 6-port valve, solenoid valve (SV1), condensation tube, and sample needle. The carrier path is uninterrupted from the GC Inlet EPC and is directed through the 6-port valve and into the transfer line back to the GC inlet. Inlet split line pneumatics not shown for clarity.

Loop Fill (Vial Vent)

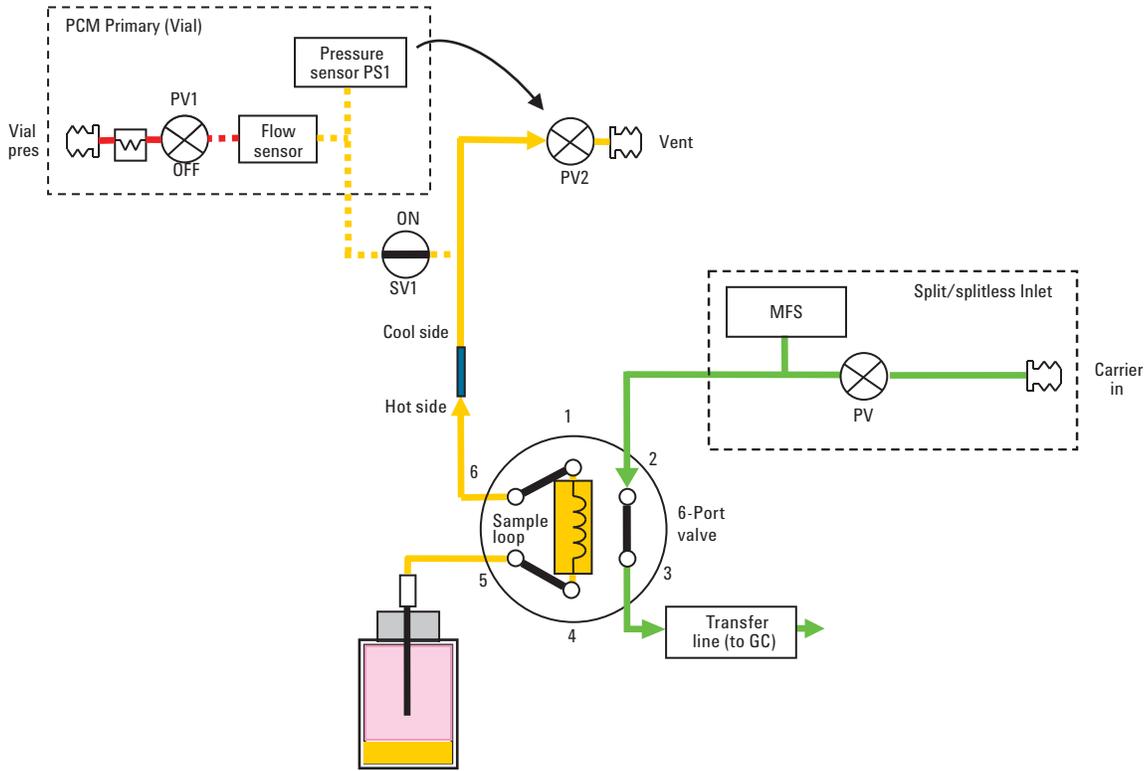


Figure 3. The sample flows from the pressurized vial through the vent. A back pressure regulator formed by PS1 and PV2 controlled by channel 2 of the PCM (Vial) EPC module regulates the absolute exit pressure. This reduces the vent flow and gives a more precise sample amount.

much like that of standard liquid auto samplers. The pneumatic configurations for **Standby** and **Inject** steps are shown in Figures 2 and 3, respectively.

Control of the 7697A is fully integrated in ChemStation. The main parameter input pane is shown in Figure 4.

Figure 4. Parameters are shown for 20 mL vials, where the vial is pressurized to 15 psi (NTP) and vented to 10 psi (NTP) at a rate of 20 psi/min.

Discussion

Table 2 lists RSD's for all Class 1, 2A, and 2B solvents prepared at their USP <467> limit concentrations. The number of runs for each class for all configurations was 20. Two sample blanks were run ahead of each solvent class for all configurations. As can be seen in the table, four configurations were tested: Split/splitless with 10 and 20 mL vials, and volatiles interface with 10 and 20 mL vials.

Most RSDs are well below 2.5% regardless of vial size or inlet type. Repeatability with RSDs in this range cannot be achieved with pressure balanced or conventional valve and loop systems. Those solvents with somewhat higher RSD values generally have low partition coefficients, *k*'s (apolar solvents with poor affinity for water) or very low detector response at USP limit concentrations such as nitromethane. Sample preparation variability will have a larger impact on low *k* solvents. Other solvent systems such as dimethyl sulfoxide (DMSO), dimethyl acetamide (DMAC), 1,3 dimethyl-2-imidazolinone (DMI) or mixed such as DMSO/water will obviously change the response, however, RSD's should be equal if not better than those shown in this work where an aqueous diluent was used.

The use of controlled venting in the Agilent 7697A allows the user flexibility over the final vial pressure when filling the sample loop. As a general rule for method development, the final vial pressure should be set between 2.0 psi (NTP) and 10.0 psi (NTP) in order to achieve the best repeatability. This control leads to better results and depending on the analyte *k* (partition coefficient) value, it can also enhance sensitivity. Three modes of vial pressurization are possible in the 7697A. These are: flow limited to pressure, pressure control at flow of 200 mL/min, and fixed volume. In the systems discussed here, the flow limited mode was used for all experiments.

Representative chromatograms for all three solvent classes are shown in Figure 5. Note that Class 1 solvents benzene and 1, 2 dichloroethane are baseline separated on the Agilent VF-624 ms column.

Table 2. RSDs for Residual Solvent Standards at Limit Concentrations. Standards were Prepared in Aqueous Solution. *n* = 20

| Compound | Split inlet | | VI inlet | |
|------------------------------------|-------------|-------|----------|-------|
| | 10 mL | 20 mL | 10 mL | 20 mL |
| Class 1 | | | | |
| 1,1-dichloroethene | 2.9 | 2.0 | 2.0 | 2.6 |
| 1,1,1-trichloroethane | 2.7 | 2.2 | 1.9 | 3.9 |
| carbon tetrachloride | 3.8 | 4.4 | 3.1 | 3.4 |
| benzene | 3.9 | 2.3 | 1.5 | 3.1 |
| 1,2-dichloroethane | 1.6 | 1.3 | 1.1 | 1.7 |
| Class 2A | | | | |
| methanol | 1.0 | 0.6 | 1.3 | 0.4 |
| acetonitrile | 1.0 | 0.5 | 1.3 | 0.6 |
| dichloromethane | 1.2 | 1.9 | 1.5 | 2.3 |
| <i>Trans</i> -2,3-dichloroethene | 2.4 | 2.5 | 2.9 | 2.8 |
| <i>Cis</i> -1,2-dichloroethene | 1.5 | 2.1 | 1.8 | 2.8 |
| tetrahydrofuran | 0.8 | 0.6 | 1.4 | 0.7 |
| cyclohexane | 3.5 | 2.9 | 4.2 | 2.9 |
| methylcyclohexane | 4.1 | 3.9 | 4.3 | 3.2 |
| 1,4-dioxane | 1.4 | 0.6 | 1.6 | 0.5 |
| toluene | 1.5 | 2.0 | 2.1 | 2.8 |
| chlorobenzene | 1.4 | 2.1 | 1.7 | 3.0 |
| ethylbenzene | 1.6 | 2.2 | 2.1 | 2.7 |
| <i>m</i> -xylene, <i>p</i> -xylene | 1.6 | 2.2 | 2.0 | 2.9 |
| <i>o</i> -xylene | 1.4 | 2.2 | 1.8 | 3.1 |
| Class 2B | | | | |
| hexane | 3.9 | 1.3 | 3.6 | 2.7 |
| nitromethane | 5.4 | 4.8 | 4.4 | 3.3 |
| chloroform | 4.0 | 2.5 | 1.7 | 1.7 |
| 1,2-dimethoxyethane | 1.7 | 1.6 | 4.2 | 4.5 |
| trichloroethene | 5.9 | 2.5 | 2.1 | 1.6 |
| pyridine | 1.7 | 0.8 | 1.5 | 0.9 |
| 2-hexanone | 1.5 | 0.7 | 0.9 | 0.7 |
| tetralin | 2.7 | 2.7 | 1.4 | 1.7 |

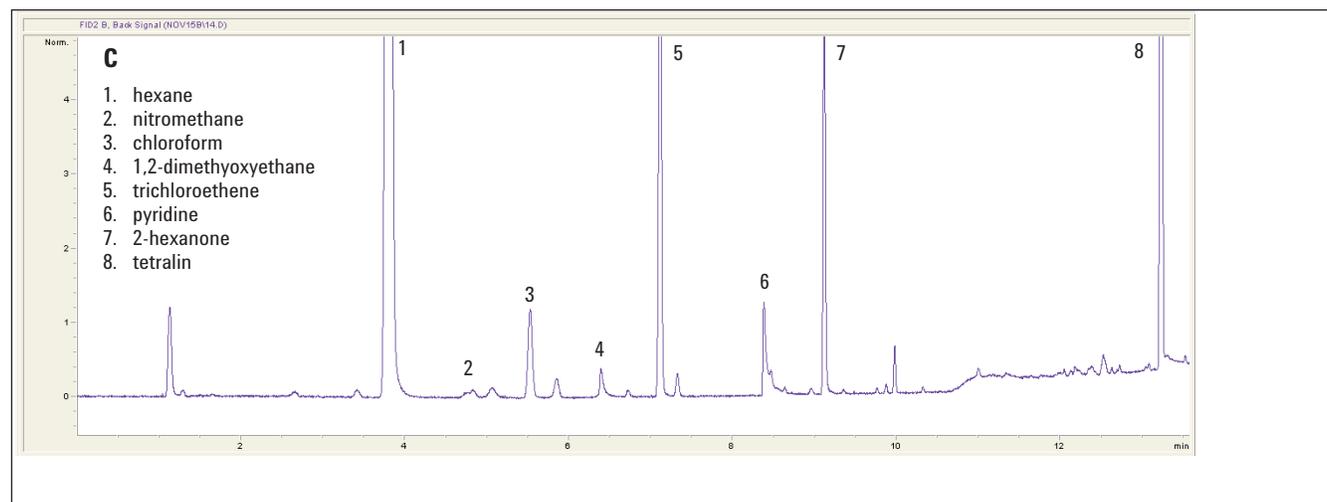
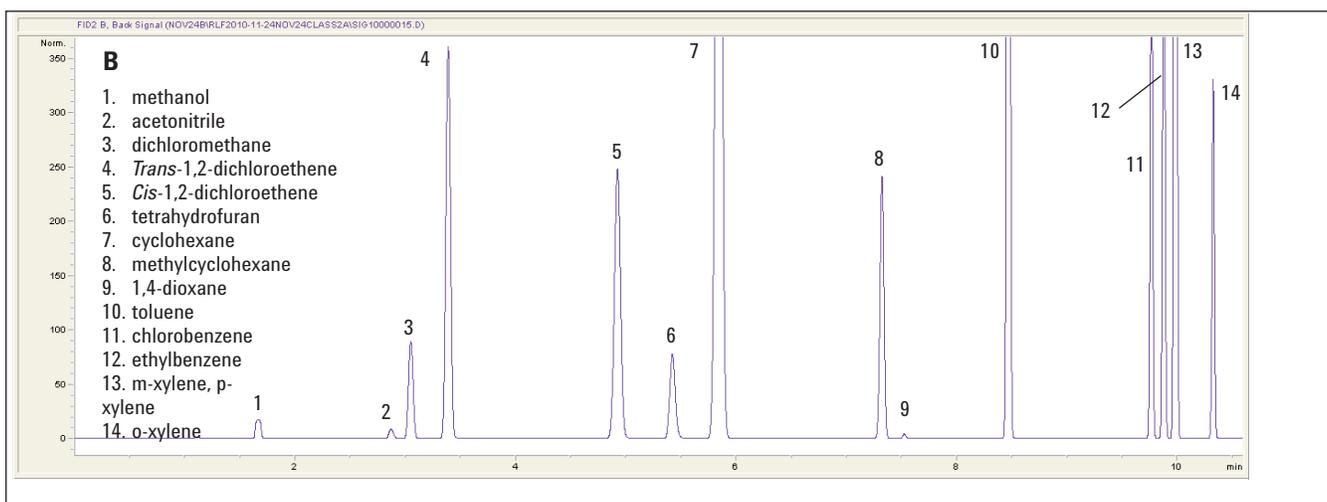
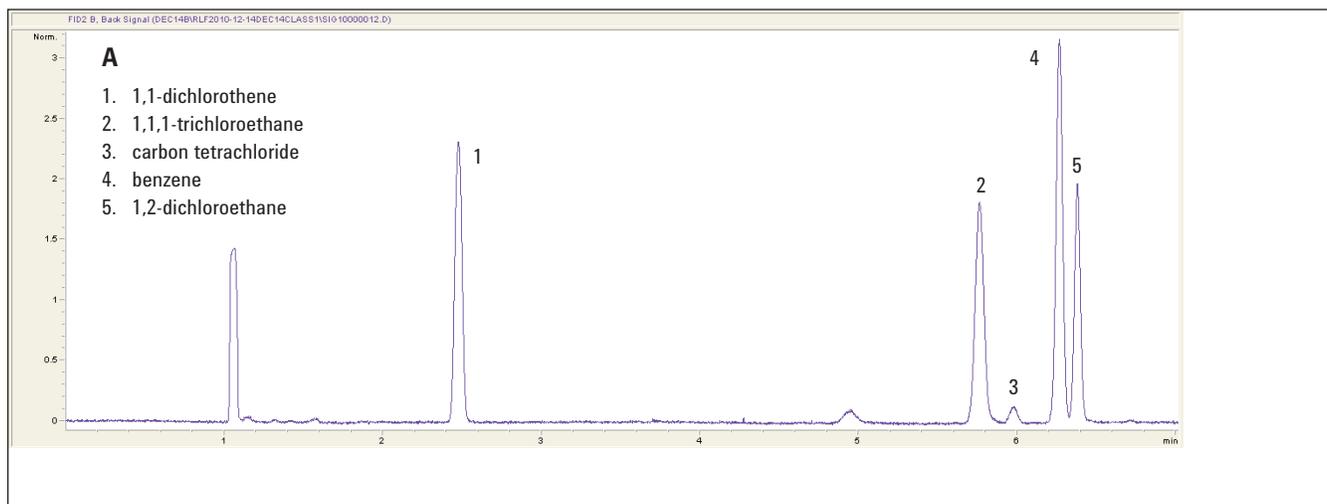


Figure 5. Class 1, class 2A, and class 2B solvents at USP 467 limit concentrations are shown in figures A, B, and C, respectively. All used split/splitless inlet and 20 mL vials.

Additional Configurations

Simultaneous dual channel analysis with Agilent 624 (VF-624ms) and INNOWax columns is possible with the configuration shown in Figure 5. Here a deactivated retention gap connects the inlet to an unpurged splitter. A 1 to 1 split occurs into 30 M × 0.32 mm 624 and INNOWax columns. Now Procedure A and Procedure B of UPS <467> can be accomplished in one run. This configuration can be easily converted to a single column system by simply plugging a port on the CFT splitter using a Siltite/internal nut plug. This is shown in the boxed insert of Figure 6.

Example chromatograms for Class 1, Class 2A, and Class 2B solvents are shown in Figures 7, 8, and 9, respectively. Conditions: volatiles interface at a 7:1 split ratio, 14 psig at 40 °C. Oven: 40 °C (5 min) to 240 °C (2 min) at 18 °C/min. All three classes combined at limit concentrations are shown in Figure 10.

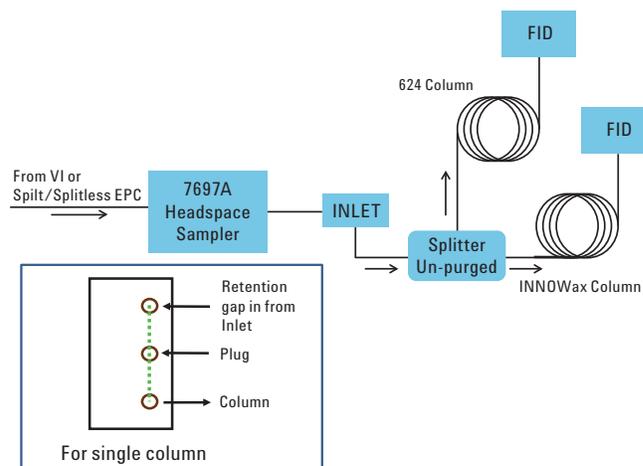


Figure 6. Configuration for dual channel confirmation. Box shows a one column setup with the splitter.

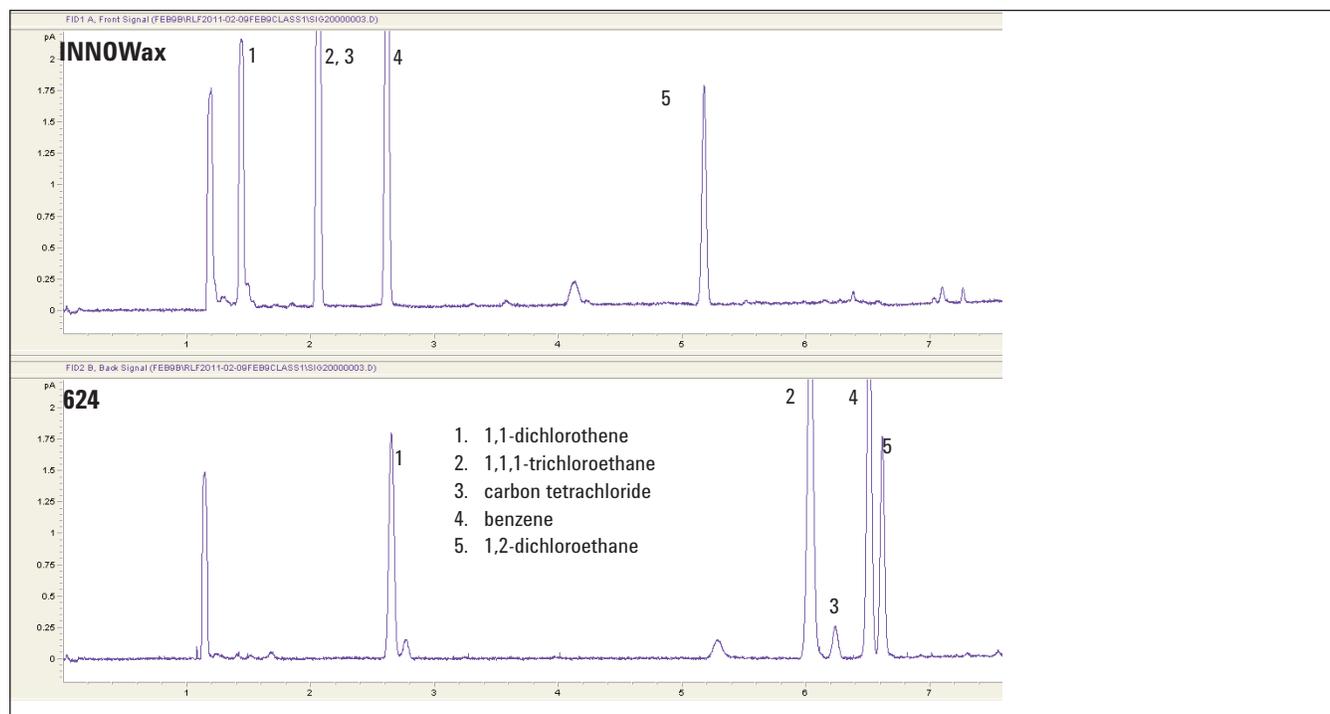


Figure 7. Dual channel analysis of Class 1 solvents at USP 467 limit concentration.

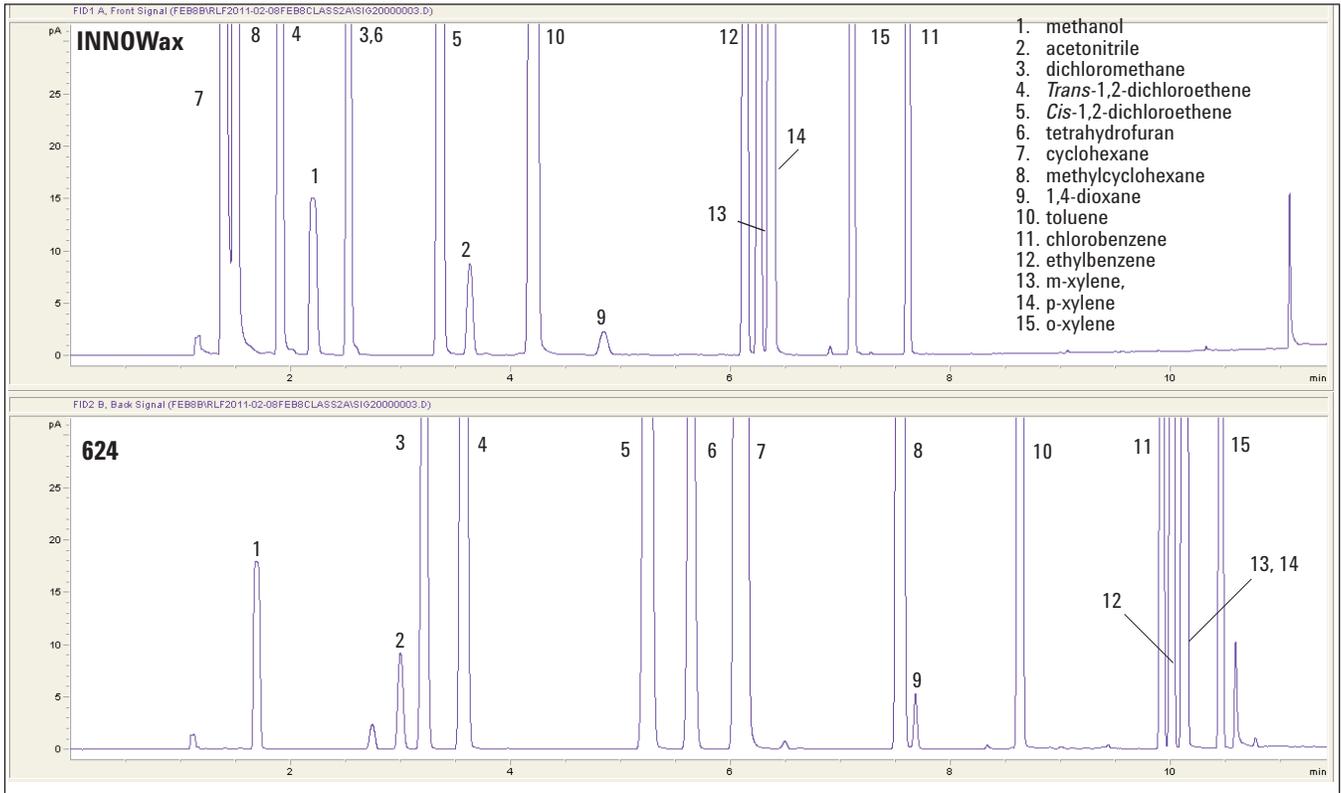


Figure 8. Dual channel analysis of Class 2A solvents at limit concentration.

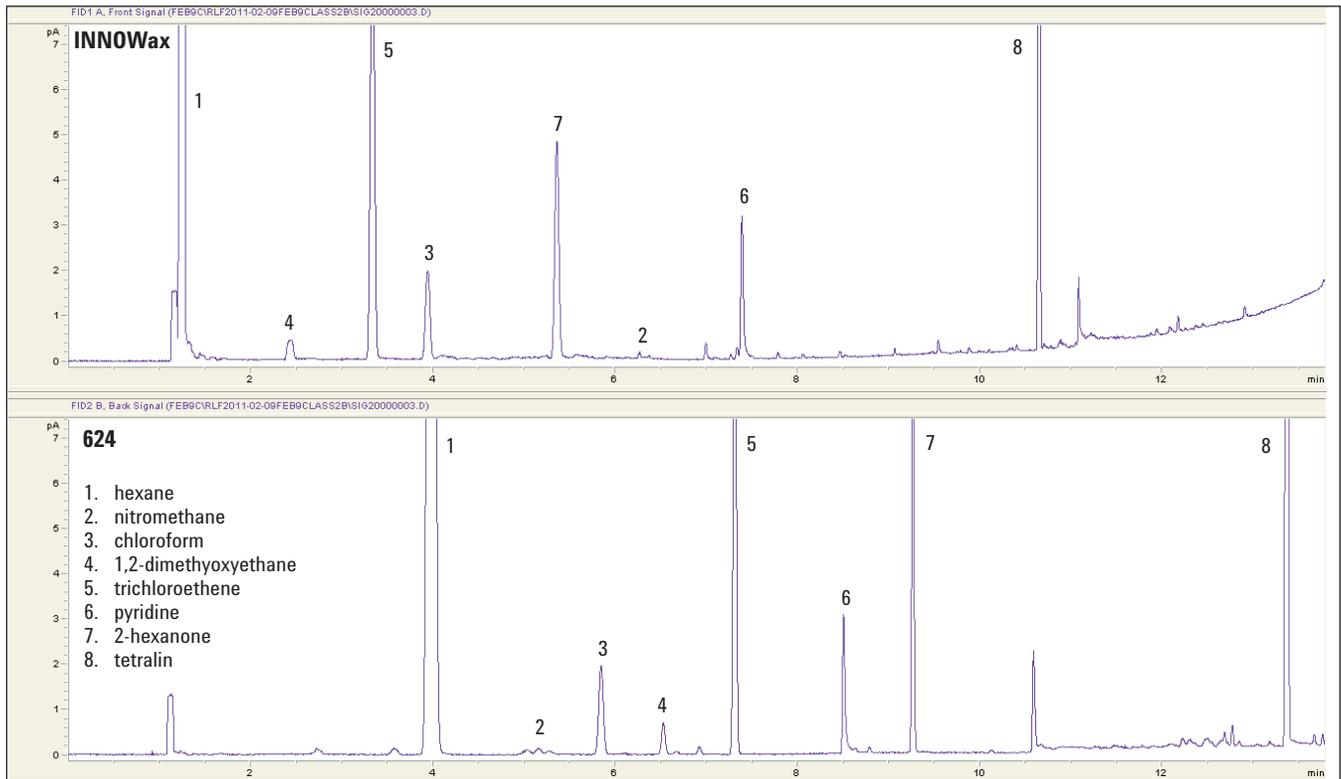


Figure 9. Dual channel analysis of Class 2B solvents at limit concentration.

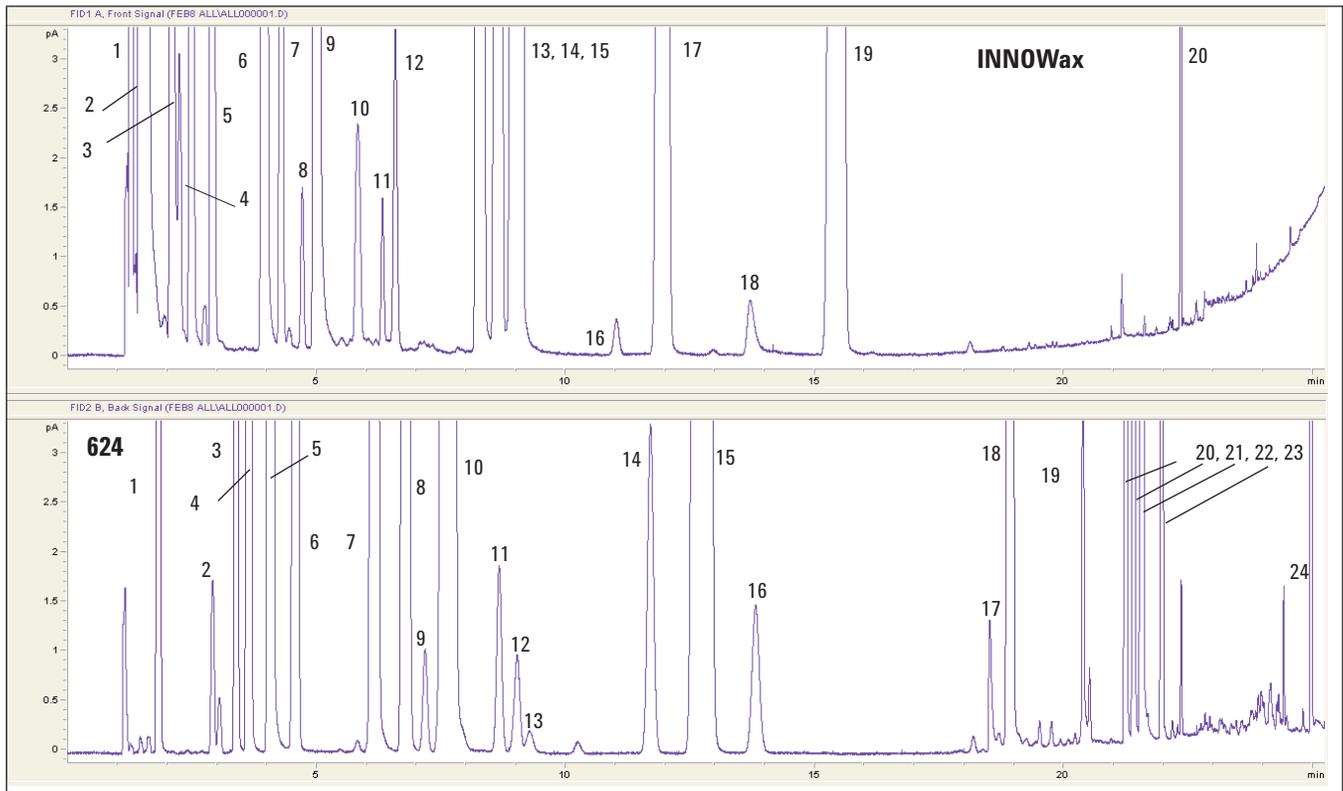


Figure 10. Dual channel analysis of all classes. Oven: 35 °C (17 min) to 240 °C (5 min) @ 20 °C/min. Volatiles interface at a 5:1 split.

Peak IDs for the Agilent 624 column:

1. methanol 2. 1,1 dichloroethene 3. acetonitrile 4. methylene chloride 5. *Trans*-1,2 dichloroethene 6. hexane 7. *Cis*-1,2 dichloroethene, nitromethane 8. tetrahydrofuran 9. chloroform 10. cyclohexane, carbon tetrachloride, 1,1,1-trichloroethane 11. benzene 12. 1,2 dichloroethane 13. 1,2 dimethoxyethane 14. trichloroethene 15. methycyclohexane 16. 1,4 dioxane 17. pyridine 18. toluene 19. 2-hexanone 20. chlorobenzene 21. ethylbenzene 22. *m*, *p*-xylene 23. *o*-xylene 24. tetralin.

Peak IDs for the INNOWax column:

1. hexane 2. cyclohexane, methycyclohexane, 1,1 dichloroethene 3. *Trans* 1,2 dichloroethene, THF, 1,1,1 trichloroethane, carbon tetrachloride 4. methanol, 1,2 dimethoxyethane 5. methylene chloride, benzene 6. *Cis* 1,2 dichloroethene, trichloroethene 7. acetonitrile 8. chloroform 9. toluene 10. 1,4 dioxane 11. 1,2 dichloroethane 12. 2-hexanone 13. ethylbenzene 14. *p*-xylene 15. *m*-xylene, 16. nitromethane (not seen at this scale) 17. *o*-xylene 18. pyridine 19. chlorobenzene 20. tetralin

Coelutions occur on both the 624 and INNOWax columns when all USP Class 1 and Class 2 solvents are combined. Since many of the coelutions are different on the two phases, the dual column configuration can provide clearer identification. Also, in practice, a much smaller group of solvents are typically monitored reducing any ambiguity.

For new drug development and quality control, a dual channel configuration using an FID and Mass Selective Detector can be a powerful tool for residual solvent determinations. The configuration using the Agilent 5975C MSD is shown in Figure 11. This system is particularly suited for the development of generic methods that do not need to follow USP <467> guidelines. MSD analysis helps avoid ambiguity as over 60 solvents are used currently in pharmaceutical manufacturing. When unknown peaks or solvents are present, this system may be the best solution for solvent confirmation and quantitation. Sensitivity will be somewhat reduced in this system due to

detector splitting and the presence of some makeup flow (Table 3).

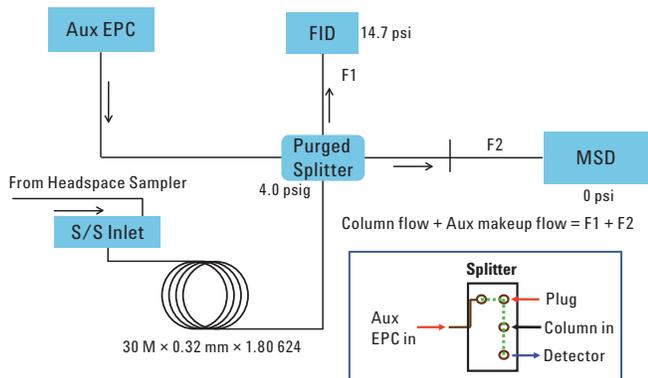
In this configuration the restrictors must be carefully sized to avoid a “not ready” condition on the Aux EPC makeup channel at the maximum programmed oven temperature. This will occur if there is too much restriction causing a flow reversal on the Aux channel. Makeup flow must be positive throughout the run. Table 3. shows flow calculations at several temperatures. Use the flow calculator [2] to calculate desired restrictor dimensions.

Table 3. Flow (mL/min) Calculations for Configuration Shown in Figure 10

| Oven temp | Column flow | MSD** flow | FID flow | Total flow out | Makeup flow |
|-----------|-------------|------------|----------|----------------|-------------|
| 40 °C | 3.5 | 3.8 | 4.3 | 8.1 | 4.6 |
| 150 °C | 3.5 | 2.5 | 2.6 | 5.1 | 1.6 |
| 240 °C | 3.5 | 1.9 | 1.9 | 3.8 | 0.3 * |

* If makeup is negative at max programmed oven temp, then Aux EPC will be in a “not ready” condition. This must be avoided. Check and adjust restrictors as needed.

** Flows take into account 17.1 cm of restrictor at 220 °C (MSD transfer line).



Some solvents will give a smaller response on the FID compared to the MSD. For example, solvents such as 1,4 dioxane and 2-hexanone show higher MSD response. For those solvents with a very low signal/noise or coelution, the use of extracted ion or SIM will greatly improve the chances for a successful determination.

Figure 11. Configuration for dual channel FID-MSD. Restrictor (Splitter to FID) = 80 cm x 0.20 mm, restrictor (Splitter to MSD) = 137 cm x 0.18 mm. Column is DB-624, 30 m x 0.32 mm x 1.80 μ m.

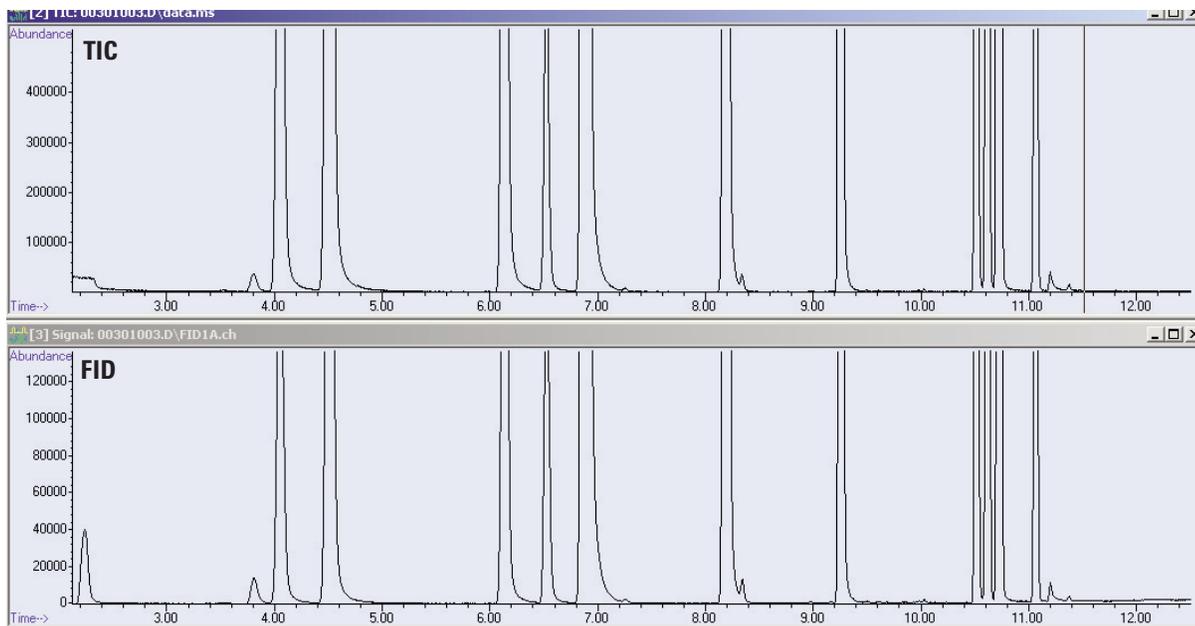


Figure 12. Class 2A solvents at limit concentrations with FID-MSD. 20 mL vials.

Conclusion

The Agilent 7697A Headspace sampler is capable of outstanding repeatability for the analysis of residual solvents. An inert sample path, thermal zones with stability of better than ± 0.1 °C of set point, and flexible EPC controlled vial sampling using absolute pressure all contribute to system performance.

Carryover was essentially nonexistent in all configurations. User programmable flow rate and time events that control needle and vent line purge functions are used to effectively clean the system between runs.

The methods and configurations outlined in this work illustrate a number of possible strategies for the analysis of residual solvents. Laboratories and QA departments should perform system suitability studies and validate their proposed methods according to USP or ICH guidelines. The FID-MSD system is particularly useful when the solvents used in a drug formulation are not known. In addition, this configuration can solve coelution problems by using SIM.

References

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3. Albert E Gudat and Roger L. Firor, "Improved Retention Time, Area Repeatability, and Sensitivity for Analysis of Residual Solvents," Agilent Technologies publication 5989-6079EN.

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