# Revised USP 467 Residual Solvent Method

## Satisfy New Method Requirements with Restek Columns and Standards

By Rick Lake, Pharmaceutical Innovations Chemist

- Overview of the new USP 30/NF 25 procedure.
- New reference standards - stock mixes, custom preparations.
- Optimize your testing within the constraints of the method.

Organic volatile impurities (OVIs), commonly referred to as residual solvents, are trace level chemical residues in drug substances and drug products that are byproducts of manufacturing, or that form during packaging and storage. The United States Pharmacopeia recently revised the general chapter on residual solvent analysis, USP 467, to mirror the International Conference on Harmonization (ICH) guidelines. This revision, effective July 1, 2007, replaces previous methods that were not consistent with the ICH guidelines.

The revised procedure consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection (GC/FID), and is divided into two sections based on sample solubility – water soluble and water insoluble articles. Altogether, the test method consists of three separate procedures – A, B and C–that are designed to identify, confirm and quantify residual solvents in pharmaceuticals.

Procedure A is the first step in the identification process and is performed to screen samples for residual solvents. A series of residual solvent mixes, consisting of Class 1 and Class 2 mixes A and B, are analyzed along with the system suitability and test solutions on an Rtx®-624 column – equivalent to an Rtx®-1301 (G43) column (Figures 1-3). If a peak in the sample matches a retention time, and exceeds the response of the corresponding standard, the analyst proceeds to Procedure B for verification of the analyte.

Once a residual solvent is identified, Procedure B is performed to confirm analyte identity. We recommend a Stabilwax® (G16) capillary column as a confirmation column because it yields an alternate selectivity compared to an Rtx®-624 column or an Rtx®-1301 (G43) column. (See our OVI retention time index at www.restek.com/ovi). The same reference mixes are analyzed with an acetonitrile/trichloroethylene system suitability solution. If a residual solvent is verified, Procedure C is used to quantify the analyte by comparison to a specific, individual standard for the analyte identified. For water-insoluble articles, the procedure is the same, except dimethylformamide and 1,3-dimethyl-2-imidazolidinone are used as the diluent and Class 2 Mix C (higher boiling point solvents mix) is analyzed as a reference solution.

**Figure 1** USP Residual Solvent Class 1 standard solution on an Rtx®-624 (G43) column.

| | |
|---|---|
| **Column:** | **Rtx®-624 30m, 0.32 ID, 1.8µm (cat. # 10970)** |
| Inj.: | Headspace injection (split ratio 1:5),1mm Split liner Siltek® deactivated (cat. # 20972-214.1) |
| Inj. temp.: | 140°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 2.16 mL/min., 35.3 cm/sec. |
| Oven temp.: | 40°C for 20 min., to 240°C @ 10°C/min., hold for 20 min. |
| Det.: | FID @ 240°C |

| **Headspace Conditions** | |
|---|---|
| Instrument: | Tekmar HT3 |
| Transfer Line Temp.: | 105°C |
| Valve Oven Temp.: | 105°C |
| Sample Temp.: | 80°C |
| Sample Equil. Time: | 45 min. |
| Vial Pressure: | 10psi |
| Pressurize Time: | 0.5 min. |
| Loop Fill Pressure: | 5psi |
| Loop Fill Time: | 2.00 min. |
| Inject Time: | 1.00 min. |

**Sample:**
USP 467 Class 1 Standard Solution (cat.# 36279) in 20mL headspace vial

1. 1,1-dichloroethene
2. 1,1,1-trichloroethane
3. carbon tetrachloride
4. benzene
5. 1,2-dichloroethane

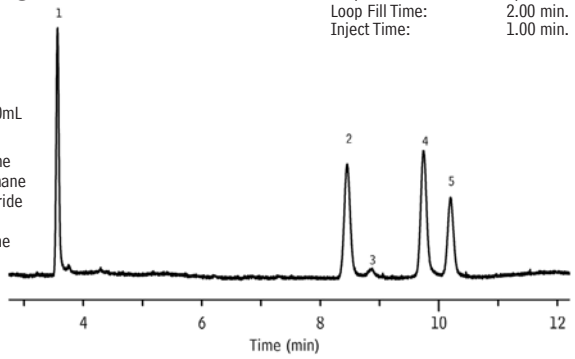
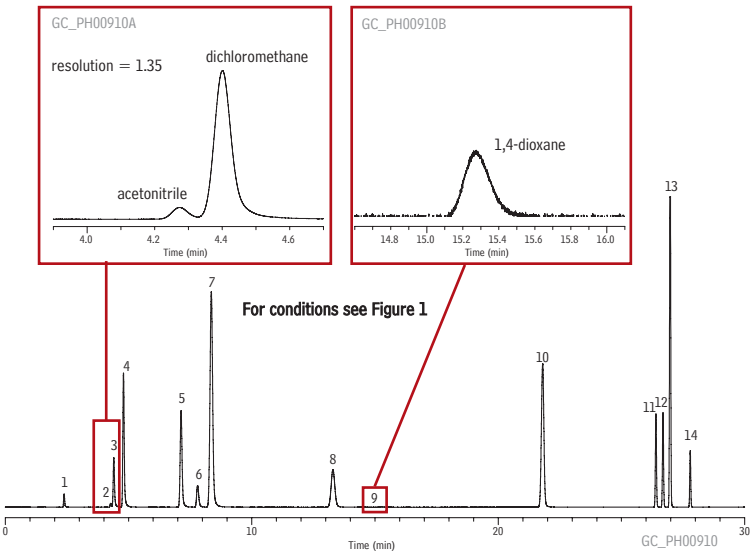


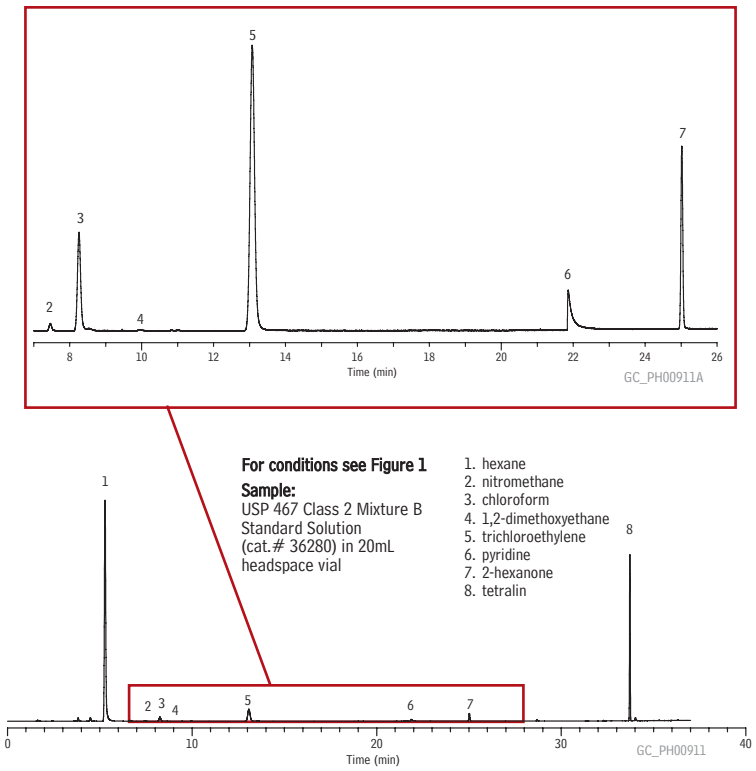

**Figure 2** USP Residual Solvent Class 2 Mixture A standard solution on an Rtx®-624 (G43) column.




**Sample:** USP 467 Class 2 Mixture A Standard Solution (cat.# 36271) in 20mL headspace vial

1. methanol
2. acetonitrile
3. dichloromethane
4. *trans*-1,2-dichloroethene
5. *cis*-1,2-dichloroethene
6. tetrahydrofuran
7. cyclohexane
8. methylcyclohexane
9. 1,4-dioxane
10. toluene
11. chlorobenzene
12. ethyl benzene
13. *m*-xylene / *p*-xylene
14. *o*-xylene

**Figure 3** USP Residual Solvent Class 2 Mixture B standard solution on an Rtx®-624 (G43) column.




Restek can supply all your USP 467 materials and can help you optimize your testing within the constraints of the method. Visit us on the web at www.restek.com or contact our Technical Support team at 800-356-1688, ext.4, for solutions to your residual solvent testing needs and tips on optimizing your analysis.

### Residual Solvents - Class 1

| | | | |
|---|---|---|---|
| benzene | 10mg/mL | 1,1-dichloroethene | 40 |
| carbon tetrachloride | 20 | 1,1,1-trichloroethylene | 50 |
| 1,2-dichloroethane | 25 | | |

In dimethyl sulfoxide, 1mL/ampul
cat. # 36279 (ea.)

### Residual Solvents Class 2 - Mix A (15 components)

| | | | |
|---|---|---|---|
| acetonitrile | 2.05mg/mL | methylcyclohexane | 5.90 |
| chlorobenzene | 1.80 | methylene chloride | 3.00 |
| cyclohexane | 19.40 | tetrahydrofuran | 3.45 |
| *cis*-1,2-dichloroethylene | 4.70 | toluene | 4.45 |
| *trans*-1,2-dichloroethylene | 4.70 | *m*-xylene | 6.51 |
| 1,4-dioxane | 1.90 | *o*-xylene | 0.98 |
| ethylbenzene | 1.84 | *p*-xylene | 1.52 |
| methanol | 15.00 | | |

In dimethyl sulfoxide, 1mL/ampul
cat. # 36271 (ea.)

### Residual Solvents Class 2 - Mix B (8 components)

| | | | |
|---|---|---|---|
| chloroform | 60µg/mL | nitromethane | 50 |
| 1,2-dimethoxyethane | 100 | pyridine | 200 |
| *n*-hexane (C6) | 290 | tetralin | 100 |
| 2-hexanone | 50 | trichloroethylene | 80 |

In dimethyl sulfoxide, 1mL/ampul
cat. # 36280 (ea.)

## did you **know**?

Restek offers a full day seminar on headspace analysis. Join us September 26, in Edison, NJ for a day of learning focused exclusively on headspace principles, techniques, and applications (cat.# 65563). To register, visit us online at **www.restek.com/seminar**

## for **more** info

- Technical poster: *Comprehensive Dual-Column Analysis of Residual Solvents in Water-soluble Articles Using Dynamic Headspace and Modular Accelerated Column Heating.* **www.restek.com/usp467**
- *A Technical Guide for Static Headspace Analysis Using GC*, cat.# 59895A.
- OVI retention time index **www.restek.com/ovi**

### Rtx®-624 (G43) Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

| ID | df (µm) | temp. limits | length | cat. # |
|---|---|---|---|---|
| 0.32mm | 1.80 | -20 to 240°C | 30-Meter | 10970 |
| 0.53mm | 3.00 | -20 to 240°C | 30-Meter | 10971 |

### Stabilwax® (G16) Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

| ID | df (µm) | temp. limits | length | cat. # |
|---|---|---|---|---|
| 0.32mm | 0.25 | 40 to 250°C | 30-Meter | 10624 |
| 0.53mm | 0.25 | 40 to 250°C | 30-Meter | 10625 |

### Siltek® 1mm Split Liners for Agilent GCs

Use this liner for increased sensitivity. Exclusive Siltek® deactivation makes liner inert to active sample components.

| Benefits/Uses: | ID*/OD & Length (mm) | cat.# ea. | cat.# 5-pk. |
|---|---|---|---|
| for purge & trap inlet splitting or sample <1µL | 1.0 ID 6.3 OD x78.5 | 20972-214.1 | 20973-214.5 |

*Nominal ID at syringe needle expulsion point.

# GC Inlet Liner Deactivations for Basic Drug Analysis

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Base-deactivated inlet liners are inert to basic drugs, for greater responses.
- Inertness of Rtx®-5 Amine column is enhanced for basic compounds.
- Use this liner / column combination for the lowest %RSDs for basic drugs.

Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence by GC/MS. Typical limits of detection are 1-15ng/mL, depending on the sample matrix. For basic drugs (e.g., Figure 1), selecting the proper surface treatment for the GC inlet liner is important, because this parameter can affect responses. The surface of a glass inlet liner contains active silanol groups (Si-OH) that can act as electron pair acceptors, and react with nitrogen or oxygen electron pair donors in basic drug molecules (Figure 2).[1] These reactions usually are rapid and reversible, but they are expressed chromatographically as broad, tailing peaks and/or reduced responses. To eliminate these acid-base reactions, make chromatographic peaks sharp, Gaussian, and easy to integrate, and thereby help ensure reproducible and accurate responses, the -OH groups on the glass surface must be deactivated.

We evaluated several alternatives for deactivating inlet liners to determine the best deactivation chemistry for the analysis of basic drugs. Standards composed of the free base forms of the drugs shown in Figure 1 were prepared at concentrations of 5, 10, 25, 50, and 100 ng/mL for analysis on a 15m, 0.25mm ID, 0.25µm Rtx®-5 Amine column (5% diphenyl/95% dimethyl polysiloxane stationary phase). The analysis of these drug standards was repeated on a series of 4mm ID single gooseneck liners that had been treated with different deactivation techniques, as well as an untreated liner. Three replicate analyses were performed on each liner to determine which deactivation treatment offered the highest and most consistent response for these basic drugs.

We used these results to generate box plots that display the range of data distribution, or variation – an indication of the reproducibility of the performance. We chose phencyclidine (PCP) and cocaine plots to represent the nitrogen-containing and nitrogen/oxygen-containing drugs, respectively (Figure 2). The line in each box indicates the mean response.

The data show that undeactivated liners and liners that received intermediate polarity treatment provided poorer responses or reproducibility, com-

**Figure 1** Basic compounds can react with silanol groups on glass liner surfaces, causing poor chromatography.

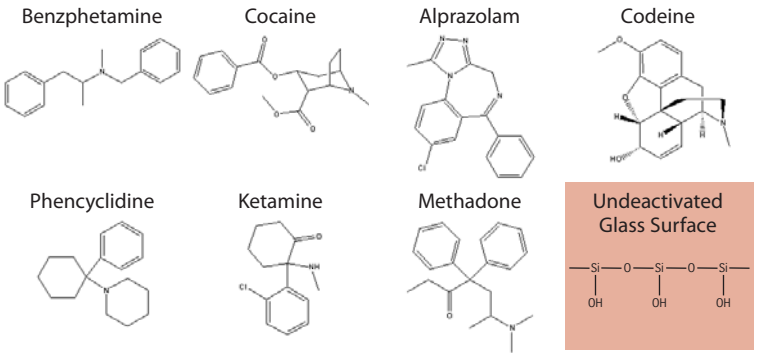



**Figure 2** A base-deactivated inlet liner provides highest mean responses for PCP.

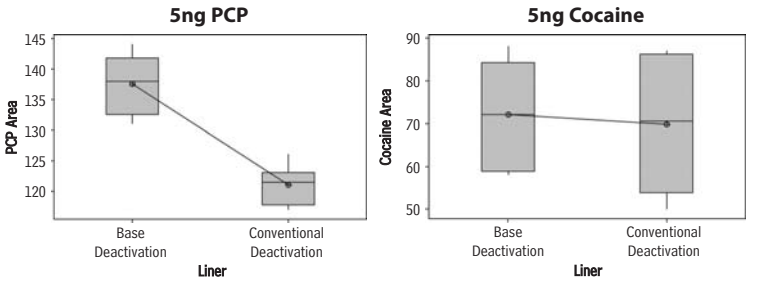



**Figure 3** Linearity plots for all drugs, analyzed using a base-deactivated inlet liner and an Rtx®-5 Amine column.