Residual Solvents per USP 467 using Zebron™ ZB-624*PLUS*™ and ZB-WAX*PLUS*™ GC Columns

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Overview

Residual solvents in pharmaceutical products are defined as organic volatile chemicals that are used or produced in the manufacture of a drug substance, excipients, or preparation of a drug product[1]. These trace concentrations of chemical residues can pose toxicity concerns and it is the responsibility of the drug manufacture to ensure that these residues are removed, or only present in levels that are supported by safety data. Because of the variety of toxicity concerns, solvents have been classified based on their potential health risks. The International Conference on Harmonization (ICH) guidelines (Q3C) references a risk assessment based classification in which residual solvents are categorized into 3 classes of compounds.[1,2] This classification system has been harmonized with the USP general chapter <467> Residual Solvents. Testing is only required for those solvents used in the manufacturing or purification process of drug substances, excipients, or products. This allows each company to determine which solvents it uses in production and subsequently develop testing procedures that are fit for purpose.

Identification, Control, and Quantification of Residual Solvents

Class 1 solvents are known, or strongly suspected carcinogenic compounds and are considered to have an unacceptable toxicity or deleterious environmental impact. This class of solvents should not be employed in the manufacture of drug substances, excipients, and drug products unless their use can be strongly justified in a risk-benefit assessment.[3] When a Class 1 compound is used or produced in conjunction with a drug product, it is the responsibility of the drug manufacturer to implement an acceptable validated procedure for the identified and quantified potential residue. Class 2 can be a potential nongenotoxic animal carcinogen, neurotoxic, or teratogenicity compound. These solvents have suspected reversible toxicity levels and concentrations of these compounds should be limited. If Class 2 residual solvents are present at a greater concentration then the Concentration Limit specified by USP <467> or have a daily dosage greater than 10 g, then they should be identified and quantified. Class 3 solvents have depicted low toxicity potential at levels normally and generally associated with the drug manufactured processes. However, if Class 3 solvents are present, then a nonspecific method may be utilized for analytical procedure when the amount of Class 3 solvent Loss from Drying USP <731> exceed 0.5 %.

It is the responsibility of the drug manufacturer to qualify the purity of all the components used in the manufacturing of the drug product. If Class 1 solvents are likely to be present, they should be identified and quantified through USP <467> procedures or equivalent validated chromatographic technique. If Class 2 or 3 solvents are present at greater than their permitted daily exposure (PDE), PDE values outlined in USP <467>, or greater than 0.5 % Loss on Drying <731>, respectively, they should be identified and quantified through <467> procedures or an equivalent validated chromatographic technique.

Table 1.
Classification of Residual Solvents by Risk Assessment

Residual Solvent Class	Risk Assessment	Appropriate Analytical Procedure
Class 1	• Known/strongly suspected human carcinogens • Environmentaly hazardous • Solvents to be avoided	If Class 1 solvents are used or produced and are not removed by a process, then these solvents should be identified (Procedure A & B) and quantified (Procedure C)
Class 2	• Nongenotoxic animal carcinogens • Possible irreversible toxicity • Suspected reversible toxicity • Solvents to be limited	If Class 2 solvents are present at greater than the Concentration Limit specified by <467>, Option 1 or 2 limits, then these solvents should be identified (Procedure A & B) and quantified (Procedure C)
Class 3	• Solvents with low toxic potential • No health-based exposure limit [Class 3 residual solvents have PDEs of 50 mg or more per day]	If Class 3 solvent limit in an individual monograph is greater than 50 mg per day, then that solvents should be identified (Procedure A & B) and quantified (Procedure C)

Experimental Conditions

The analysis of applicable residual solvents under USP <467> comprises a static headspace extraction, G43 or G16 phase column depending on procedure, and a flame ionization detection (FID). The methodology is segmented into two separate sections based on sample solubility. However, procedures for water-soluble and water-insoluble articles are analogous and differ by the diluent used in standard and sample preparation. For water-soluble articles, water is used, whereas dimethylformamide or dimethylsulfoxide is used for water-insoluble articles. The routine analysis residual solvent reference material is separated into 3 test mixtures – Class 1 Mixture, Class 2 Mixture A, and Class 2 Mixture B.

USP <467> test method consists of a three-part procedure denoted as Procedure A, B, and C. The analytical design for Procedure A is identification, Procedure B is confirmation, and Procedure C is quantification of the identified residual solvents found to be present in drug substance or product.[3]

- Procedure A (Identification): G43, 6 %-cyanopropyl – 94 % dimethylpolysiloxane
- Procedure B (Confirmation): G16, Polyethylene Glycol
- Procedure C (Quantification): G43 or G16, depending on selectivity

Procedure A – Identification

The first step in USP <467> is the qualitative identification of "likely to be present" Class 1 solvents, Class 2 solvents if present at greater than PDE, and Class 3 solvents if Loss from Drying USP <731> is greater than 0.5 %. The identification analysis is performed on a G43 (Zebron ZB-624*PLUS*) GC column and for the purpose of this technical note the water-insoluble article methodology was utilized for demonstration and therefore dimethylsulfoxide was used as the diluent. Class 1 standard and system suitability solutions and Class 2 Mixture A standard solutions are assayed under the given operating conditions to determine suitability of the chromatographic system.

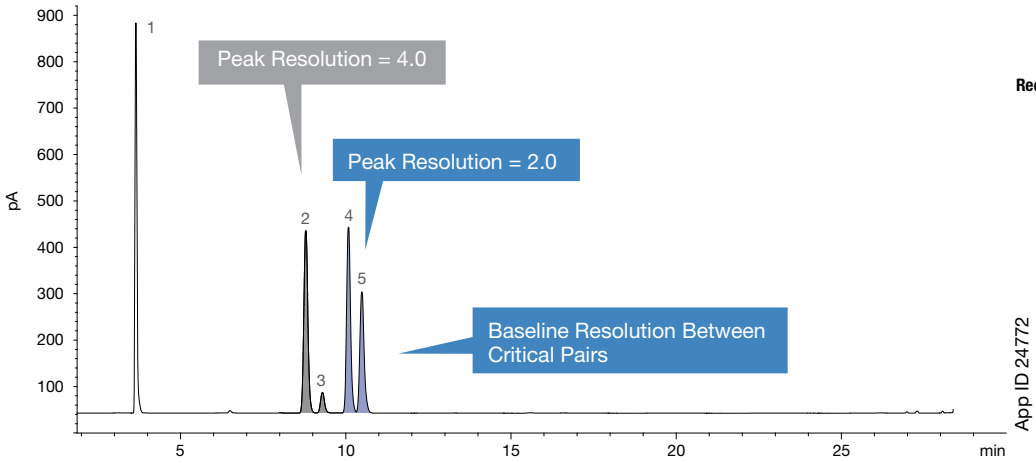
The requirements for Class 1 system suitability solution is a signal to-noise ratio, for all peaks, not less than 3. For the Class 1 standard solution, 1,1,1-trichloroethane signal-to-noise must be greater than 5 to pass suitability. Finally, for Class 2 Mixture A solution, peaks associated with solvents acetonitrile and dichloromethane must have a resolution value not less than 1. Once system suitability has been confirmed, the test solutions are then assayed along with the Class 1 and Class 2 Mixtures A and B standard solutions. If a major peak is determined in the sample that corresponds to a retention time and has a response greater than or equal to that of a corresponding reference material, then proceed to Procedure B for verification of the analyte. However, an exemption is made for 1,1,1-trichloroethane, where a response greater than 150 times the peak response denotes an amount above the percent daily exposure limit.

System Suitability Requirements: Procedure A

- Signal-to-noise ratio of 1,1,1-trichloroethane not less than 5 for Class 1 Standard Solution
- Signal-to-noise ratio for all peaks in Class 1 Standard Solution, not less than 3
- Resolution between acetonitrile and methylene chloride not less than 1.0 for Class 2 Mix B

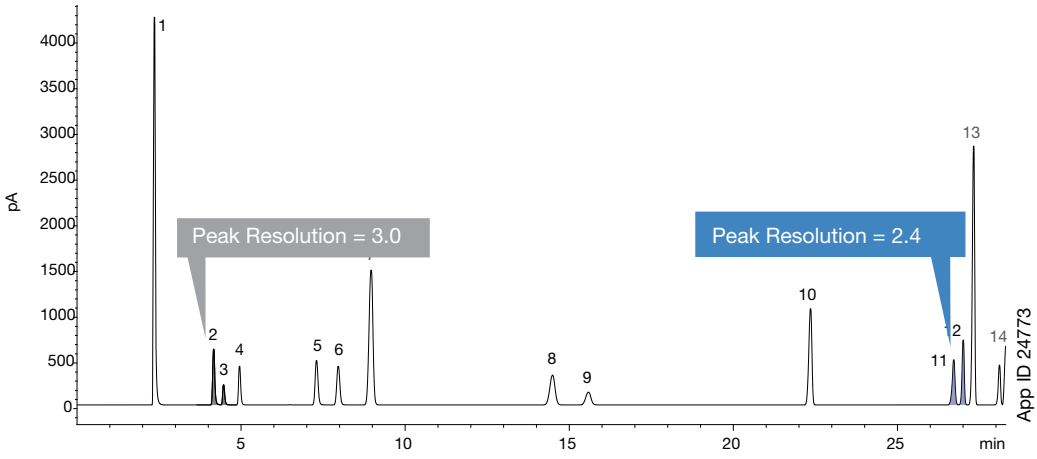
Class 1 Standard Solution was analyzed with a ZB-624*PLUS* to demonstrate signal-to-noise ratio for all peaks were greater than 3; a peak associated with 1,1,1-trichloroethane was confirmed greater than 5 (**Figure 1**). Additionally, Class 2 Mixture B was analyzed with a ZB-624*PLUS* to verify an acetonitrile and methylene chloride resolution value greater than 1.0 (**Figure 2**). **Figures 1** through **3** illustrate the analysis of Class 1, Class 2 Mixture A and Mixture B by Procedure A utilizing the ZB-624*PLUS* column.

Figure 1.
USP Class 1 Standard Solution on Zebron™ ZB-624*PLUS*™ GC Column



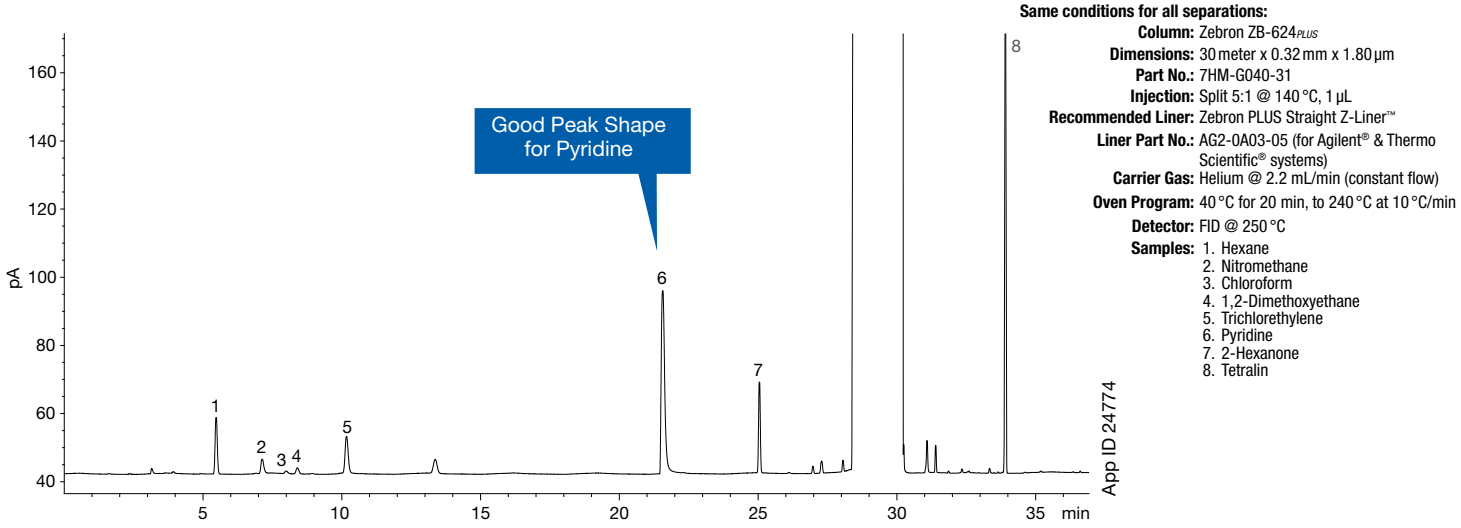
Same conditions for all separations:
Column: Zebron ZB-624*PLUS*
Dimensions: 30 meter x 0.32 mm x 1.80 µm
Part No.: 7HM-G040-31
Injection: Split 5:1 @ 140 °C, 1 µL
Recommended Liner: Zebron PLUS Straight Z-Liner™
Liner Part No.: AG2-0A03-05 (for Agilent® & Thermo Scientific® systems)
Carrier Gas: Helium @ 2.2 mL/min (constant flow)
Oven Program: 40 °C for 20 min, to 240 °C at 10 °C/min
Detector: FID @ 250 °C
Sample: 1. 1,1-Dichloroethene
2. 1,1,1- Trichloroethane
3. Carbon tetrachloride
4. Benzene
5. 1,2-Dichloroethane

Figure 2.
USP Class 2 Mixture A Standard Solution on Zebron ZB-624*PLUS* GC Column



Sample: 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 Class 2 Mixture B Standard Solution on Zebron™ ZB-624*PLUS*™ GC Column



Procedure B – Confirmation

If a peak, from the test solution, corresponds in equal or greater response to reference peaks from either the Class 1 standard solution or either of the two Class 2 mixtures standard solutions, then Procedure B is implemented; to confirm analyte identity. Procedure B methodology utilizes an orthogonal GC column phase, G16 (Zebron ZB-WAX*PLUS*). For confirmation, the reference residual solvent solutions and mixtures are prepared as directed for Procedure A. However, the system suitability requirements differ, the Class 1 standard solution must have a benzene response greater than 5, signal-to-noise ratios no less than 3 for each peak, a minimum resolution value of 1 between acetonitrile and cis-dichlorethene, and in the Class 2 Mixture A solution the resolution of acetonitrile and cis-dichloroethene must not be less than 1. If the analyte identified in Procedure A corresponds with the retention time of the reference peak (utilizing the ZB-WAX*PLUS* phase) and exceeds the peak response of the reference materials, the analyte must be quantified utilizing Procedure C. For peaks identified as 1,1,1-trichloroethane with a response is greater or equal to 150 times the peak response of the Class 1 standard solutions reference peak, then must be quantified utilizing Procedure C.

System Suitability Requirements: Procedure B

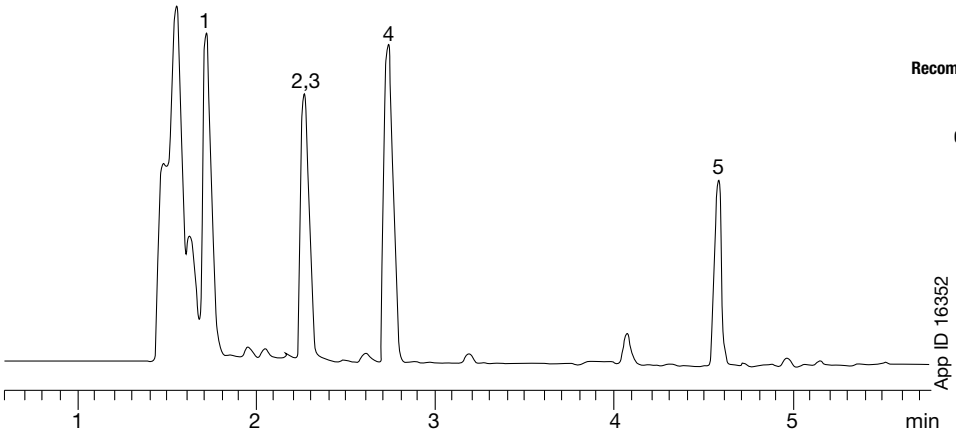
- Signal-to-noise ratio of Benzene (Peak 4) not less than 5 for Class 1 Standard Solution
- Signal-to-noise ratio of each peak of each Class 1 System Suitability Solvent should be >3
- Acetonitrile and trichloroethylene resolution greater than 1.0 for Class 2 Mixture A solution

At the concentration limits specified by the monograph, our signal-to-noise ratio for benzene was 104.2; and all other compounds exceeded 3 (**Figure 4**).[4] Resolution between acetonitrile and trichloroethylene was 1.52 (**Figure 5**). **Figures 4 – 6** illustrate the analysis of Class 1, Class 2 Mixture A, and Mixture B by Procedure B utilizing a ZB-WAX*PLUS* column.

Procedure C - Quantification

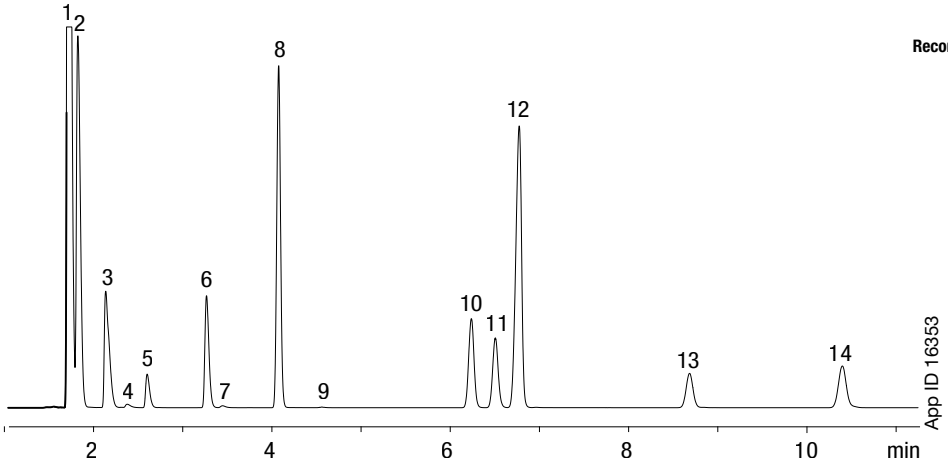
If a peak has been identified (Procedure A) and confirmed (Procedure B) as a Class 1 or Class 2 residual solvent and is equal or greater in response to reference peaks from either the Class 1 standard solution or either of the two Class 2 mixtures standard solutions, then Procedure C is implemented to quantify the analyte. Procedure C methodology dictates analyzing the sample against the specific individual reference material for the analyte identified. Individual standards are prepared by diluting the analyte in solution to a concentration of 1/20 of the concentration limit outlined in the method. The standards are run following the procedure and column selectivity utilized in either Procedure A or B, depending upon which procedure provided the best and most relevant separation. USP <467> Residual Solvent method was developed and implemented to identify (Procedure A), confirm (Procedure B), and quantify (Procedure C) volatile organic chemicals used in the manufacturing or purification process of drug substances, excipients, or products. Within these outlined procedures Gas Chromatography is relied upon as the analytical technique and the prescribed procedures utilize both a G43 phase GC column (Zebron ZB-624*PLUS*) and a G16 phase GC column (Zebron ZB-WAX*PLUS*). In this technical note, both the ZB-624*PLUS* and ZB-WAX*PLUS* demonstrated exceptional selectivity and easily passed dictated system suitability criteria for all procedures.

Figure 4.
USP Class 1 Standard Solution on Zebron™ ZB-WAX*PLUS*™ GC Column



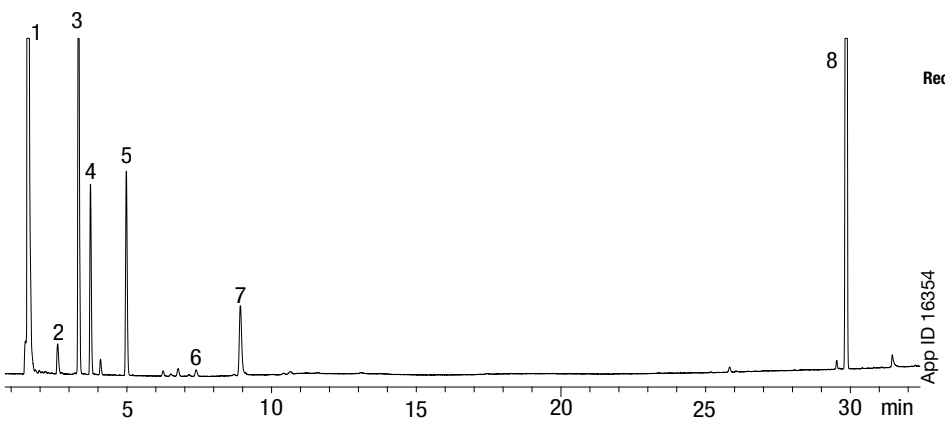
Column: Zebron ZB-WAX*PLUS*
Dimensions: 30 meter x 0.32 mm x 0.25 µm
Part No.: 7HM-G013-11
Injection: Split 5:1 @ 140 °C, 1 mL
Recommended Liner: Zebron PLUS Straight Z-Liner™
Liner Part No.: AG2-0A03-05 (for Agilent® and Thermo Scientific® systems)
Carrier Gas: Helium @ 35 cm/sec (constant flow)
Oven Program: 50 °C hold 20 min to 165 °C @ 6 °C/min hold 20 min
Detection: FID @ 250 °C
Sample:
1. 1,1-Dichloroethene
2. Carbon tetrachloride
3. 1,1,1-Trichloroethane
4. Benzene
5. 1,2-Dichloroethane

Figure 5.
USP Class 2 Mixture A Standard Solution on Zebron™ ZB-WAX*PLUS*™ GC Column



Column: Zebron ZB-WAX*PLUS*
Dimension: 30 meter x 0.32 mm x 0.25 µm
Part No.: 7HM-G013-11
Injection: Split 5:1 @ 140 °C, 1 mL
Recommended Liner: Zebron PLUS Straight Z-Liner™
Liner Part No.: AG2-0A03-05 (for Agilent® and Thermo Scientific® systems)
Carrier Gas: Helium @ 35 cm/sec (constant flow)
Oven Program: 50 °C hold 20 min to 165 °C @ 6 °C/min hold 20 min
Detector: FID @ 250 °C
Sample:
1. Cyclohexane
2. Methylcyclohexane
3. Methanol
4. THF
5. Dichloromethane
6. cis-1,2-Dichloroethene
7. Acetonitrile
8. Toluene
9. 1,4-Dioxane
10. Ethylbenzene
11. p-Xylene
12. m-Xylene
13. o-Xylene
14. Chlorobenzene

Figure 6.
USP Class 2 Mix B Standard Solution on Zebron™ ZB-WAX*PLUS*™ GC Column



Column: Zebron ZB-WAX*PLUS*
Dimension: 30 meter x 0.32 mm x 0.25 µm
Part No.: 7HM-G013-11
Injection: Split 5:1 @ 140 °C, 1 mL
Recommended Liner: Zebron PLUS Straight Z-Liner™
Liner Part No.: AG2-0A03-05 (for Agilent® and Thermo Scientific® systems)
Carrier Gas: Helium @ 35 cm/sec (constant flow)
Oven Program: 50 °C hold 20 min to 165 °C @ 6 °C/min hold 20 min
Detector: FID @ 250 °C
Sample:
1. Hexane
2. 1,2-Dimethoxyethane
3. Trichloroethylene
4. Chloroform
5. Methlbutylketone
6. Nitromethane
7. Pyridine
8. Tetralin

Conclusions

USP <467> Residual Solvent was developed and implemented to identify (Procedure A), confirm (Procedure B), and quantify (Procedure C) volatile organic chemicals used in the manufacturing or purification process of drug substances, excipients, or products. Within these outlined procedures Gas Chromatography is relied upon as the analytical technique and the procedures utilize both a G43 phase GC column (Zebron ZB-624*PLUS*) and a G16 phase GC column (Zebron ZB-WAX*PLUS*). In this technical note, both the Zebron ZB-624*PLUS* and Zebron ZB-WAX*PLUS* demonstrated exceptional selectivity and easily passed dictated system suitability criteria for all procedures.

References

1. United States Pharmacopeia and National Formulary (USP 38-NF 33). Rockville, MD: United States Pharmacopeial Convention; 2018. http://www.uspnf.com/uspnf/pub/index?usp=38&nf=33&s=2&officialOn=December%201,%202015. Accessed March 19, 2018
2. Osterberg, R.E. Impurities: Residual Solvents ICH: Q3C. Presented at the 2007 USP/PDA Joint Conference: Residual Solvents, North Bethesda, Maryland, 2007.
3. <467> Organic Volatile Impurities. General Notices and Requirements: Applying to Standards, Tests, Assays, and Other Specifications of the United States Pharmacopeia. Material Provided at the 2007 USP/PDA Joint Conference: Residual Solvents, North Bethesda, Maryland, 2007.
4. Phenomenex Inc., Understanding the Revisions to USP Monograph <467>: Residual Solvents. Torrance, CA: Accessed March 20, 2018

Ordering Information

Zebron™ ZB-624*PLUS*™ GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
20-Meter			
0.18	1.00	-20 to 300/320	7FD-G040-22
30-Meter			
0.25	1.40	-20 to 300/320	7HG-G040-27
0.32	1.80	-20 to 300/320	7HM-G040-31
0.53	3.00	-20 to 300/320	7HK-G040-36
60-Meter			
0.25	1.40	-20 to 300/320	7KG-G040-27
0.32	1.80	-20 to 300/320	7KM-G040-31
0.53	3.00	-20 to 300/320	7KK-G040-36

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G040-27-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

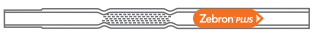
Zebron™ ZB-WAX*PLUS*™ GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
10-Meter			
0.10	0.10	20 to 250/260	7CB-G013-02
15-Meter			
0.25	0.25	20 to 250/260	7EG-G013-11
0.53	1.00	20 to 230/240	7EK-G013-22
20-Meter			
0.18	0.18	20 to 250/260	7FD-G013-08
30-Meter			
0.25	0.25	20 to 250/260	7HG-G013-11
0.25	0.50	20 to 250/260	7HG-G013-17
0.32	0.25	20 to 250/260	7HM-G013-11
0.32	0.50	20 to 250/260	7HM-G013-17
0.32	1.00	20 to 230/240	7HM-G013-22
0.53	1.00	20 to 230/240	7HK-G013-22
60-Meter			
0.25	0.15	20 to 250/260	7KG-G013-05
0.25	0.25	20 to 250/260	7KG-G013-11
0.25	0.50	20 to 250/260	7KG-G013-17
0.32	0.25	20 to 250/260	7KM-G013-11
0.32	0.50	20 to 250/260	7KM-G013-17
0.53	1.00	20 to 230/240	7KK-G013-22

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G013-11-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

Ordering Information

Zebron™ PLUS GC Inlet Liners

Description	Application	Inlet Style	Dimensions ID x L (mm)	Deactivation	Part No.	Unit
For 5890, 6890 and 7890 Models						
Straight Z-Liner™ 	Dirty samples, Volatiles, High initial oven temperatures	S/SL	4 x 78.5	PLUS Inert	AG2-0A03-01 AG2-0A03-05 AG2-0A03-25	ea 5/pk 25/pk

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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