

GC Analysis of Organic Volatile Impurities According to USP <467> Supplement Two of USP 25-NF 20, effective January, 2002

A new test for the gas chromatographic (GC) analysis of Organic Volatile Impurities (OVI) in pharmaceutical products was published in the Third Supplement to the US Pharmacopoeia (USP) XXII-NF XVII, which became effective November 15, 1990. Since its original appearance in the USP, this testing protocol has undergone many revisions and additions.¹⁻⁶ The most recent change was published as USP 25, effective January 1, 2002.¹³ The biggest prior change was to the limit test concentrations, which now match European Pharmacopoeia (EP) concentrations and ICH guidelines for the five USP <467>-regulated solvents (Table I).^{8, 9} The January 2002 revision makes no significant changes.

USP has officially removed the limit test requirements for benzene from any article specified to be tested by <467> for organic volatile impurities, except where a specific limit for benzene is in the individual monograph.¹⁰ The revision was needed because Methods I and V were unable to detect benzene at 2ppm. Method IV, the only method that detects benzene at 2ppm, became official in Supplement Two of USP 24-NF 19.¹¹

Figure 1 shows an analysis using USP <467> Method I on a G27 analytical column with a phenylmethyl guard column. Note that the sample preparation used in this analysis deviates from the method-specified 1:50 dilution in distilled water. A 1:10 dilution in distilled water was used to obtain a detectable amount of benzene by direct injection.

USP also has clarified that a 5m phenylmethyl guard column is not needed for the Method IV headspace analysis.¹⁰ **Figure 2** shows an analysis using Method IV at the revised concentrations, the method-specified sample preparation procedure, a G43 analytical column, and no guard column.

Table I

Limit Test Concentrations for USP <467>

benzene*	2ppm	methylene chloride	600ppm
chloroform	60ppm	trichloroethene	80ppm
1,4-dioxane	380ppm		

* Testing for benzene only required when specified in the individual monograph.

Table II

USP <467> Methods and corresponding chromatographic systems

Method I

G27 with 5m phenylmethyl guard column (5% phenyl/95% methyl polysiloxane) 30m, 0.53mm ID, 5.0µm, (Rtx®-G27 column, cat.# 10279-126)

Sample Introduction: direct aqueous injection

Method IV

G43 (6% cyanopropylphenyl/94% dimethylpolysiloxane) 30m, 0.53mm ID, 3.0µm, (Rtx®-G43 column, cat.# 16085)

Sample Introduction: static headspace

Method V

G43 with 5m phenylmethyl guard column (6% cyanopropylphenyl/94% dimethylpolysiloxane) 30m, 0.53mm ID, 3.0µm (Rtx®-G43 column, cat.# 16085-126)

Sample Introduction: direct aqueous injection

Method VI

Choice of 9 columns, depending on monograph

Sample Introduction: direct aqueous injection

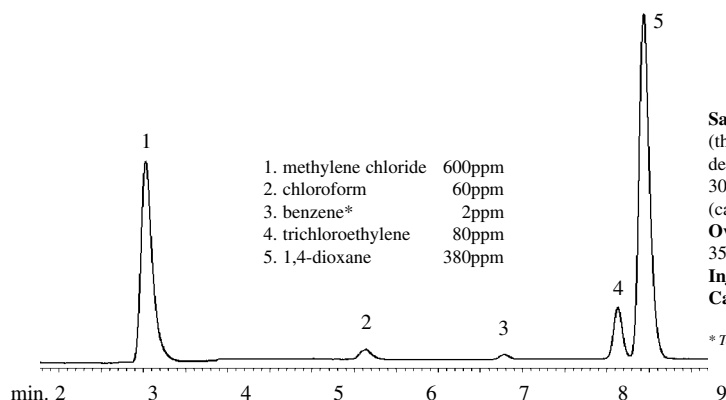
Method for Coated Tablets

0.2% polyethylene glycol, MW 1500 (G39) on graphitized carbon (S7) (0.2% Carbowax® 1500 on 80/100 CarboBlack™ C packed column, cat.# 80122)

Sample Introduction: static headspace

Figure 1

A minor modification of the dilution concentration for Method I allows analysis of 2ppm benzene, using an Rtx®-G27 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the 1:50 dilution in the method was needed to obtain a detectable benzene peak).

30m, 0.53mm ID, 5.0µm Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126).

Oven temp.: 35°C (hold 5 min.) to 175°C @ 8°C/min., to 260°C @ 35°C/min. (hold 16 min.); **Inj. port:** Uniliner® direct injection sleeve 70°C;

Inj. size: 1µL; **Det. temp.:** 260°C; **FID sensitivity:** 1 x 10⁻¹² AFS;

Carrier gas: helium, 4.1psi constant pressure, 35cm/sec. set @ 35°C.

* Testing for benzene only required when specified in the individual monograph.

Figure 3 shows an analysis using USP 24 <467> Method V, a G43 analytical column with a phenylmethyl guard column and, once again, a 1:10 dilution in order to obtain a detectable benzene peak.

USP made changes in 1997 to overcome the difficulties resulting from unregulated solvents coeluting with regulated solvents, and thereby causing over-representation of the latter concentrations using GC/flame ionization detection (FID) methods.¹² GC/mass spectrometry (MS) or a second, validated column having a different stationary phase may be used to confirm the presence of the coeluting unregulated solvent and

report the correct concentration of regulated solvent. **Figures 4, 5, and 6** show the different elution orders for commonly-used pharmaceutical processing solvents on G27, G43, and Stabilwax® columns. The latter are useful secondary columns for confirmational analysis.

We will continue to review changes to pharmaceutical OVI testing. Restek reference materials listed on page 4 of this applications note meet the most recent USP updates. **For more information regarding these applications, please call Restek technical service at 800-356-1688 or 814-353-1300, ext. 4, or your local Restek representative.**

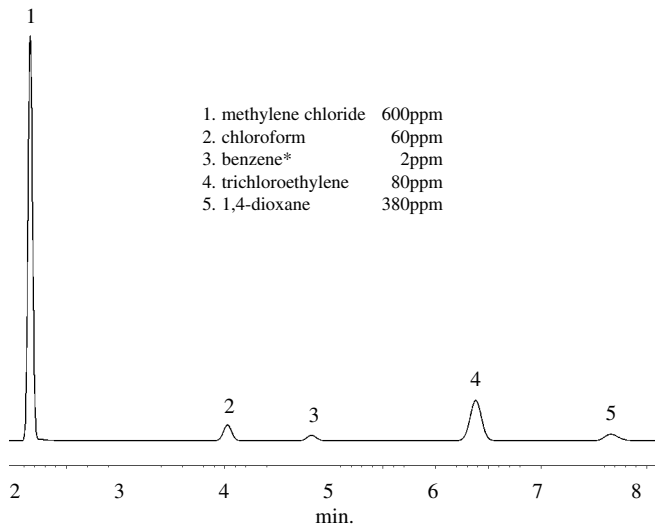
References

1. M.S. Bergren and D.W. Foust, "Comments on USP General Chapter, Organic Volatile Impurities <467>, and Associated Monograph Proposals," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1963-1968.
2. J.A. Krasowski, H. Dinh, T.J. O'Hanlon, R.F. Lindauer, "Comments on Organic Volatile Impurities, Method 1, <467>," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1969-1972.
3. *Pharmacopoeial Forum*, March/April 1991, Vol. 17, No. 2, p. 1653.
4. Fifth Supplement, USP-NF, Organic Volatile Impurities <467>, Nov. 15, 1991, pp. 2706-2708.
5. "Organic Volatile Impurities <467>," *Pharmacopoeial Forum*, May-June 1993, Vol. 19, No. 3, pp. 5335-5337.
6. *Pharmacopoeial Forum*, September/October 1992, Vol. 18, No. 5, p. 4028.
7. USP 24/NF 19, <467> Organic Volatile Impurities, (1877-1878).
8. "ICH Harmonized Tripartite Guideline, Impurities: Guideline for Residual Solvents," *The Fourth International Conference on Harmonization*, July 17, 1997.
9. European Pharmacopoeia, Supplement 1999, pp. 14-15, 208.
10. *Pharmacopoeial Forum*, November - December 1999, Vol. 25, Number 6, (9223 - 9224).
11. Supplement Two, USP 24/NF 19, August 1, 2000.
12. Sixth Supplement, USP-NF, Organic Volatile Impurities <467>, May 15, 1997, pp. 3766-3768.
13. USP 25/NF 20, <467> Organic Volatile Impurities, January 1, 2002.

These references are not available from Restek.

Figure 2

The Rtx®-G43 column provides the resolution and detection limits needed for <467> revised limit test concentrations in USP Method IV. (no guard column)

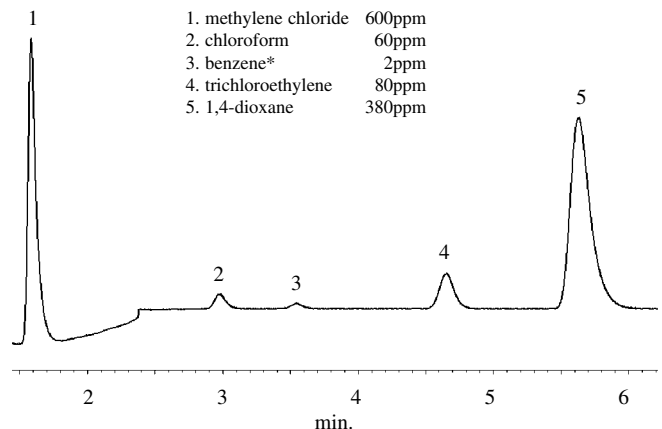


Sample Preparation: 100µL of cat.# 36007 in 5mL distilled water, 1g sodium sulfate in a 20mL headspace vial.
30m, 0.53mm ID, 3.0µm Rtx®-G43 (cat.# 16085)
Oven temp.: 40°C (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.);
Inj. temp.: 140°C, 1mm split sleeve (cat.# 20916);
Det. temp.: 260°C;
FID sensitivity: 1.25 x 10⁻¹¹ AFS;
Carrier gas: helium, 3.5psi constant pressure, 35cm/sec. set @ 40°C;
Split ratio: 2:1; ThermoQuest HS 2000 Headspace Autosampler Vial 80°C, 60 min. shaker on.

* Testing for benzene only required when specified in the individual monograph.

Figure 3

Achieve analysis of 2ppm benzene for Method V, using 1:10 dilution and an Rtx®-G43 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the 1:50 dilution in the method was needed to obtain a detectable benzene peak).
30m, 0.53mm ID, 3.0µm Rtx®-G43 with 5m phenylmethyl Integra-Guard™ (cat.# 16085-126). ThermoQuest Trace 2000 Series. Uniliner® direct injection sleeve.
Oven temp.: 40°C (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.);
Inj. temp.: 140°C;
FID sensitivity: 260°C, 1 x 10⁻¹¹ AFS;
Carrier gas: 4.1psi helium @ 35°C/sec.,
Det. temp.: 260°C

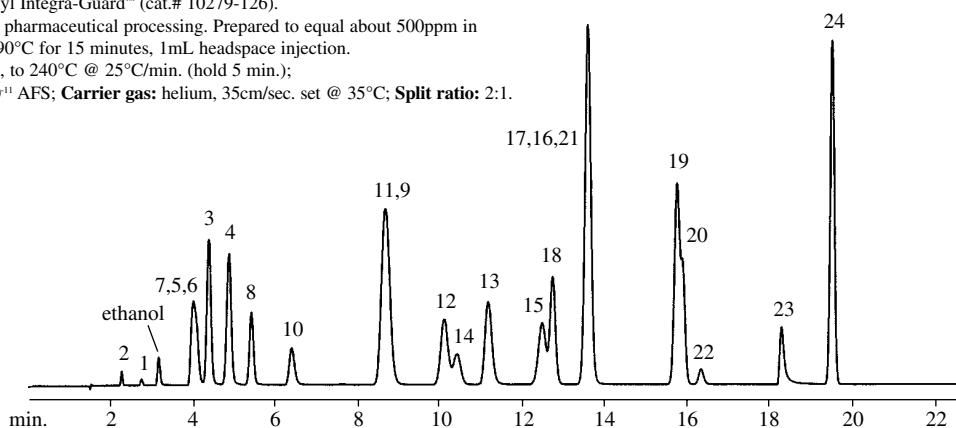
* Testing for benzene only required when specified in the individual monograph.

Figure 4

An Rtx®-G27 column resolves many solvents commonly used in pharmaceutical processing.

30m, 0.53mm ID, 5.0µm Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126).
Headspace injection of 24 common residual solvents for pharmaceutical processing. Prepared to equal about 500ppm in the bulk pharmaceutical. Samples shaken and heated at 90°C for 15 minutes, 1mL headspace injection.
Oven temp.: 35°C (hold 10 min.) to 100°C @ 5°C/min., to 240°C @ 25°C/min. (hold 5 min.);
Inj./det. temp.: 220°C/240°C; **FID sensitivity:** 1.05 x 10⁻¹¹ AFS; **Carrier gas:** helium, 35cm/sec. set @ 35°C; **Split ratio:** 2:1.

- | | |
|-------------------------|---------------------------|
| 1. ethylene oxide | 13. tetrahydrofuran |
| 2. methanol | 14. chloroform |
| 3. diethyl ether | 15. 1,1,1-trichloroethane |
| 4. 1,1-dichloroethene | 16. carbon tetrachloride |
| 5. acetone | 17. benzene* |
| 6. isopropanol | 18. 1,2-dichloroethane |
| 7. acetonitrile | 19. heptane |
| 8. methylene chloride | 20. trichloroethylene |
| 9. n-hexane | 21. n-butanol |
| 10. n-propanol | 22. 1,4-dioxane |
| 11. methyl ethyl ketone | 23. pyridine |
| 12. ethyl acetate | 24. toluene |



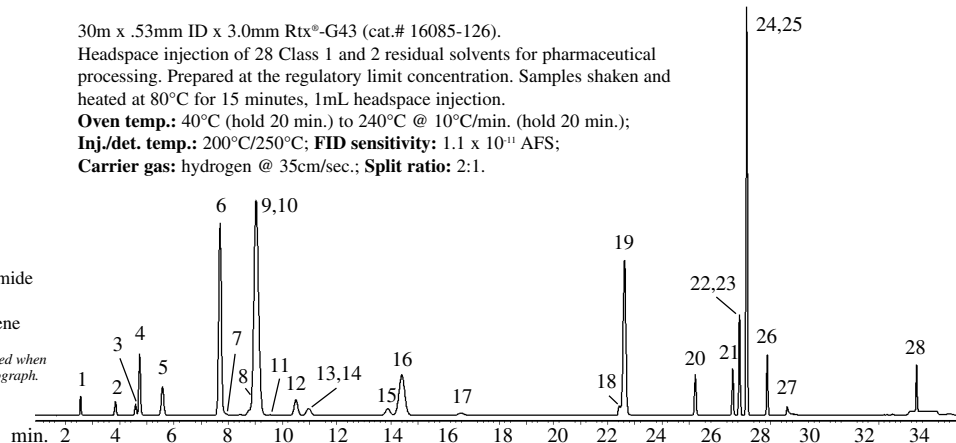
* Testing for benzene only required when specified in the individual monograph.

Figure 5

An Rtx®-G43 column shows excellent resolution of commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

- | | |
|---|-----------------------------------|
| 1. methanol | 16. methylcyclohexane |
| 2. 1,1-dichloroethene | 17. 1,4-dioxane |
| 3. acetonitrile | 18. pyridine |
| 4. methylene chloride (dichloromethane) | 19. toluene |
| 5. hexane (C6) | 20. 2-hexanone |
| 6. cis-1,2-dichloroethene | 21. chlorobenzene |
| 7. nitromethane | 22. DMF |
| 8. chloroform | 23. ethylbenzene |
| 9. cyclohexane | 24. m-xylene |
| 10. 1,1,1-trichloroethane | 25. p-xylene |
| 11. carbon tetrachloride | 26. o-xylene |
| 12. benzene* | 27. N,N-dimethylacetamide |
| 13. 1,2-dimethoxyethane | 28. 1,2,3,4-tetrahydronaphthalene |
| 14. 1,2-dichloroethane | |
| 15. trichloroethylene (1,1,2-trichloroethene) | |

30m x .53mm ID x 3.0mm Rtx®-G43 (cat.# 16085-126).
Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.
Oven temp.: 40°C (hold 20 min.) to 240°C @ 10°C/min. (hold 20 min.);
Inj./det. temp.: 200°C/250°C; **FID sensitivity:** 1.1 x 10⁻¹¹ AFS;
Carrier gas: hydrogen @ 35cm/sec.; **Split ratio:** 2:1.



* Testing for benzene only required when specified in the individual monograph.

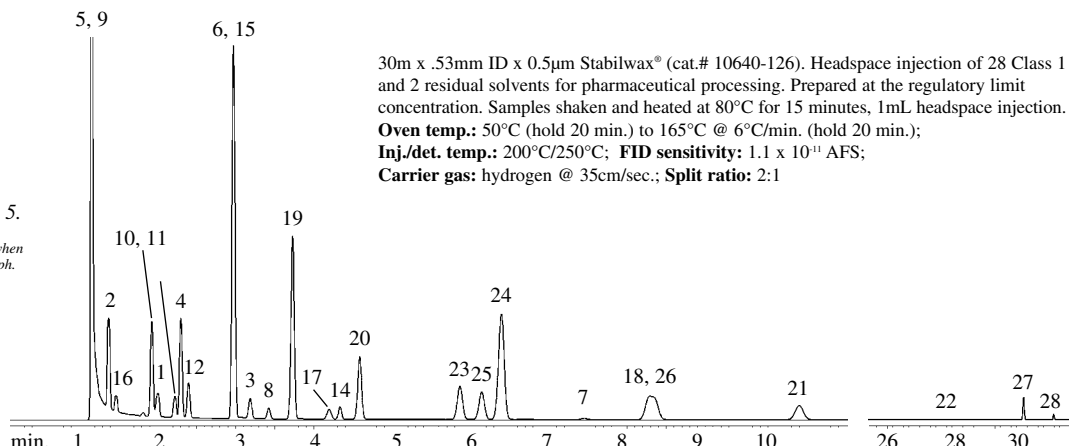
Figure 6

A Stabilwax® column makes an excellent confirmation column for commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

30m x .53mm ID x 0.5µm Stabilwax® (cat.# 10640-126). Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.
Oven temp.: 50°C (hold 20 min.) to 165°C @ 6°C/min. (hold 20 min.);
Inj./det. temp.: 200°C/250°C; **FID sensitivity:** 1.1 x 10⁻¹¹ AFS;
Carrier gas: hydrogen @ 35cm/sec.; **Split ratio:** 2:1

Same peak list as Figure 5.

* Testing for benzene only required when specified in the individual monograph.



Product Listing

These mixes meet requirements of USP 24/NF 19, effective 1/1/2000.

USP <467> Calibration Mix #6

chloroform	60µg/mL	methylene chloride	600
1,4-dioxane	380	trichloroethylene	80

Prepared in methanol, 1mL/ampul

each	10-pk.
36008	36108

USP <467> Calibration Mix #7

chloroform	60µg/mL	methylene chloride	600
1,4-dioxane	380	trichloroethylene	80

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36009	36109

USP <467> Calibration Mix #4

benzene	2µg/mL	methylene chloride	600
chloroform	60	trichloroethene	80
1,4-dioxane	380		

Prepared in methanol, 1mL/ampul

each	10-pk.
36006	36106

USP <467> Calibration Mix #5

benzene	2µg/mL	methylene chloride	600
chloroform	60	trichloroethene	80
1,4-dioxane	380		

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36007	36107

These mixes meet requirements of USP 23/NF 18, effective 1/1/1995–12/31/1999.

USP <467> Calibration Mix #2

benzene	100µg/mL	methylene chloride	500
chloroform	50	trichloroethene	100
1,4-dioxane	100		

Prepared in methanol, 1mL/ampul

each	10-pk.
36002	36102

Restek Trademarks: CarboBlack, Integra-Guard, Rtx, Stabilwax, Restek logo, Uniliner.
Other Trademarks: Carbowax (Union Carbide Corp.)

USP <467> Calibration Mix #3

benzene	100µg/mL	methylene chloride	500
chloroform	50	trichloroethene	100
1,4-dioxane	100		

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36004	36104

USP <467> Method—ethylene oxide standard

500µg/mL in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36005	36105

Rtx®-G27 column with 5m phenylmethyl Integra-Guard™

(5% phenyl/95% methyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	5.00	-60 to 270/290°C	10279-126

Rtx®-G43 column with 5m phenylmethyl Integra-Guard™

(6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085-126

Rtx®-G43 column, USP <467> Method IV

(6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085

Stabilwax® columns

ID	df (µm)	Temp. Limits	30-Meter
0.32mm	0.25	40 to 250/260°C	10624
0.53mm	0.50	40 to 250/260°C	10640

CarboBlack™ packed column

Description	Length	OD	ID	cat.#
0.2% Carbowax® 1500 on 80/100 CarboBlack™ C	2m	1/8"	2mm	80122-*

*Please call for cat.# suffix for your specific GC column configuration.

Custom OVI mixtures are available on request.



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