APPLICATIONS NOTE

GC Analysis of Non-Purgeable Solvents in Pharmaceutical Discharges

Dual-Column Approach to US EPA Method 1671 Using a Stabilwax®/Stabilwax® DB Column Pair

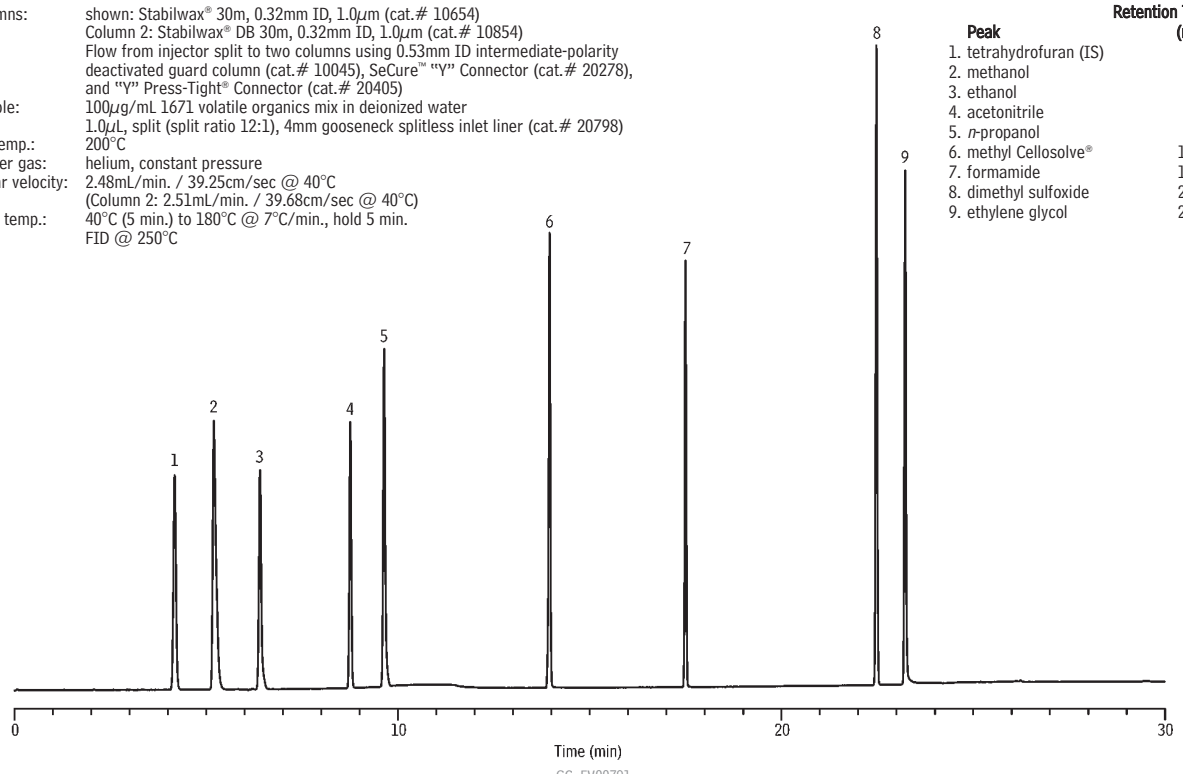
US Environmental Protection Agency Method 1671, *Volatile Organic Compounds Specific to the Pharmaceutical Manufacturing Industry by GC/FID,* is used to monitor concentrations of non-purgeable organic solvents in the aqueous discharge from pharmaceutical manufacturing facilities. The target analytes in this method - water-soluble solvents used in manufacturing pharmaceutical products - include basic organic solvents (e.g., primary amines methyl amine, dimethylamine, diethylamine, and triethylamine), and acidic organic solvents (e.g., simple alcohols methanol, ethanol, and ethylene glycol). Because the solvents listed in the method have varying chemical properties, selection of a suitable column is critical.

Method 1671 is a performance-based method and, therefore, as long as all acceptance criteria are met, the chromatography may be tailored to specific analytes and advantageous techniques. The first step in developing any chromatographic analytical method is the selection of the stationary phase. Currently, no single analytical column has the capacity to effectively elute both basic primary amines and acidic alcohols. Most likely, a column with base-deactivated functionality will be needed to limit the tailing and increase the resolution of the primary amines. Using a base-deactivated column, however, will cause adsorption and peak tailing of the acidic alcohols. Likewise, enhancing the chromatography of the acidic constituents will have negative effects on the basic constituents. Considering that both basic and acidic properties can be present in a single test mixture, we have developed a procedure that employs separate analytical columns for each set of analytes.

Figure 1 Excellent resolution and peak symmetry for non-basic pharmaceutical solvents, using a Stabilwax® column.

Columns:	shown: Stabilwax® 30m, 0.32mm ID, 1.0µm (cat.# 10654) Column 2: Stabilwax® DB 30m, 0.32mm ID, 1.0µm (cat.# 10854) Flow from injector split to two columns using 0.53mm ID intermediate-polarity deactivated guard column (cat.# 10045), SeCure™ "Y" Connector (cat.# 20278), and "Y" Press-Tight® Connector (cat.# 20405)
Sample:	100µg/mL 1671 volatile organics mix in deionized water
Inj.:	1.0µL, split (split ratio 12:1), 4mm gooseneck splitless inlet liner (cat.# 20798)
Inj. temp.:	200°C
Carrier gas:	helium, constant pressure
Linear velocity:	2.48mL/min. / 39.25cm/sec @ 40°C (Column 2: 2.51mL/min. / 39.68cm/sec @ 40°C)
Oven temp.:	40°C (5 min.) to 180°C @ 7°C/min., hold 5 min.
Det.:	FID @ 250°C

Peak	Retention Time (min.)
1. tetrahydrofuran (IS)	4.17
2. methanol	5.20
3. ethanol	6.40
4. acetonitrile	8.75
5. *n*-propanol	9.64
6. methyl Cellosolve®	13.95
7. formamide	17.50
8. dimethyl sulfoxide	22.47
9. ethylene glycol	23.22



Polyethylene glycol (PEG) is an excellent stationary phase choice for analyzing organic solvents in a water matrix. First, the polar PEG phase is capable of resolving all of the applicable analytes. Second, and more important, PEG is capable of retaining polar solvents, like water. In this particular application, retention of water is advantageous, because it enables the analyst to inject larger samples without concern about extinguishing the FID. A dimethyl polysiloxane stationary phase will not retain water, and the water peak will extinguish the flame if the injection volume exceeds 0.5µL. Larger sample volumes mean greater sensitivity and more room for developing advantageous chromatographic techniques.

For the comprehensive analysis of EPA Method 1671 target compounds, we chose a dual column configuration consisting of two PEG-based stationary phases: a Stabilwax® column and a base-deactivated Stabilwax® DB column of the same dimensions (30m x 0.32mm x 1.0µm). Using a short length of guard column and a "Y" Press-Tight® Connector (cat.# 20405), we divided the flow leaving the inlet of the GC system to the two columns. The method suggests making a 0.5µL injection into a splitless injection port. By increasing the injection volume to 1µL (made possible by the PEG stationary phases), we were able to use split injections. We set the split ratio at 12:1, with a total column flow of approximately 2.5mL/min. through each column. These injection conditions enhance analyte peak shapes and increase peak heights, especially for the early-eluting amines. Because the injection volume is doubled, and the split injection provides better peak shape, minimum concentration levels (MLs) for the method are easily met.

The Stabilwax® column provides excellent separations and peak shapes for the non-basic analytes (Figure 1). If primary amines are not among the compounds on the target list, this column alone is a good choice. The amines break down almost totally on the Stabilwax® column, however (Figure 2). Conversely, the Stabilwax® DB column, which is designed for analyses of basic compounds, exhibits reliable resolution and peak shape for the amines and non-acidic analytes (Figure 3), but would produce excessive tailing of the methanol and ethanol peaks, and nearly complete breakdown of ethylene glycol. By selecting the appropriate information from this Stabilwax®/Stabilwax® DB dual-column GC/FID analysis, a comprehensive picture for all Method 1671 analytes, acidic and basic, can be obtained.

With the columns selected and analytical conditions tuned, we analyzed a five-point calibration curve for each target analyte, using tetrahydrofuran at a concentration of 100µg/mL as the internal standard. All calibration curves were linear at or below the minimum concentration levels listed in the method. Table 1 summarizes the results.

In analyses of solvents in pharmaceutical discharges according to EPA Method 1671, a Stabilwax®/Stabilwax® DB column pair provides reliable information for all target analytes, acidic and basic, with a single injection.

Figure 2 A Stabilwax® column is not designed for analyzing basic compounds.

Columns:	shown: Stabilwax® 30m, 0.32mm ID, 1.0µm (cat.# 10654) Column 2: Stabilwax® DB 30m, 0.32mm ID, 1.0µm (cat.# 10854) Flow from injector split to two columns using 0.53mm ID intermediate-polarity deactivated guard column (cat.# 10045), SeCure™ "Y" Connector (cat.# 20278), and "Y" Press-Tight® Connector (cat.# 20405)
Sample:	1671 volatile organics mix in deionized water, concentrations indicated on figure.
Inj.:	1.0µL, split (split ratio 12:1), 4mm gooseneck splitless inlet liner (cat.# 20798)
Inj. temp.:	200°C
Carrier gas:	helium, constant pressure
Linear velocity:	2.48mL/min. / 39.25cm/sec @ 40°C (Column 2: 2.51mL/min. / 39.68cm/sec @ 40°C)
Oven temp.:	40°C (5 min.) to 180°C @ 7°C/min., hold 5 min.
Det.:	FID @ 250°C

Peak	Retention Time (min.)	Concentration (µg/mL)
1. tetrahydrofuran (IS)	4.15	100
2. methanol	5.16	40
3. ethanol	6.37	40
4. acetonitrile	8.73	200
5. *n*-propanol	9.62	200
6. methyl Cellosolve®	14.05	200
7. formamide	17.57	500
8. dimethyl sulfoxide	22.51	100
9. ethylene glycol	23.25	500

*Peaks for diethylamine, methylamine, diethylamine, triethylamine breakdown products, 200 µg/mL each.

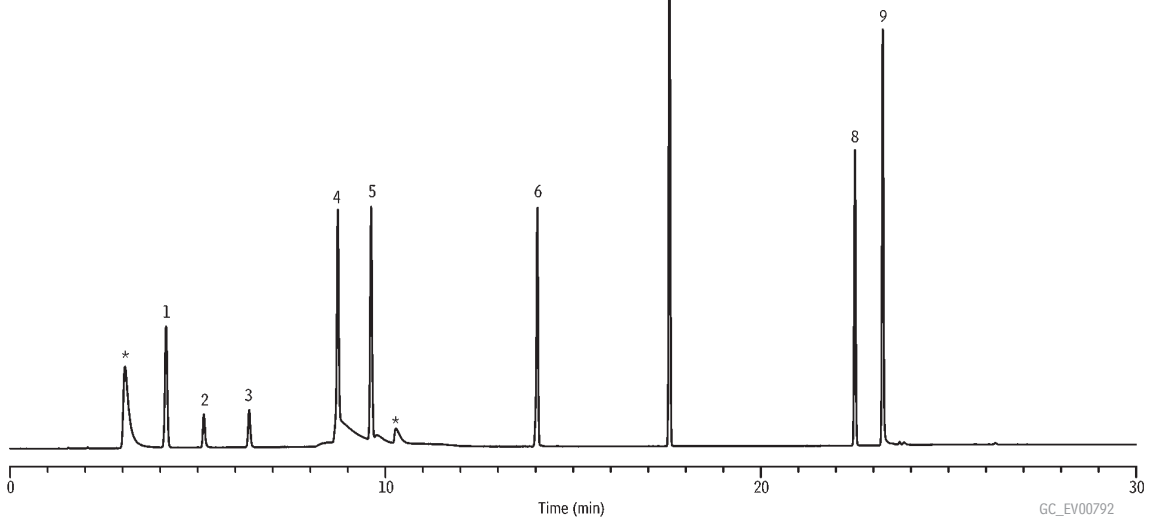


Figure 3 A Stabilwax® DB column provides reliable chromatography for basic and non-acidic pharmaceutical solvents

Columns:	shown: Stabilwax® DB 30m, 0.32mm ID, 1.0µm (cat.# 10854) Column 1: Stabilwax® 30m, 0.32mm ID, 1.0µm (cat.# 10654) Flow from injector split to two columns using 0.53mm ID intermediate-polarity deactivated guard column (cat.# 10045), SeCure™ "Y" Connector (cat.# 20278), and "Y" Press-Tight® Connector (cat.# 20405)
Sample:	1671 volatile organics mix in deionized water, concentrations indicated on figure.
Inj.:	1.0µL, split (split ratio 12:1), 4mm gooseneck splitless inlet liner (cat.# 20798)
Inj. temp.:	200°C
Carrier gas:	helium, constant pressure
Linear velocity:	2.51mL/min. / 39.68cm/sec @ 40°C (Column 1: 2.48mL/min. / 39.25cm/sec @ 40°C)
Oven temp.:	40°C (5 min.) to 180°C @ 7°C/min., hold 5 min.
Det.:	FID @ 250°C

Peak	Retention Time (min.)	Concentration (µg/mL)
1. dimethylamine	1.71	200
2. methylamine	1.76	200
3. diethylamine	2.46	200
4. triethylamine	2.64	200
5. tetrahydrofuran (IS)	4.88	100
6. methanol	7.12	40
7. ethanol	8.01	40
8. acetonitrile	9.82	200
9. *n*-propanol	11.03	200
10. methyl Cellosolve®	15.56	200
11. formamide	18.68	500
12. dimethyl sulfoxide	23.75	100

Ethylene glycol included in sample, but does not elute due to breakdown caused by base deactivation in the DB phase.

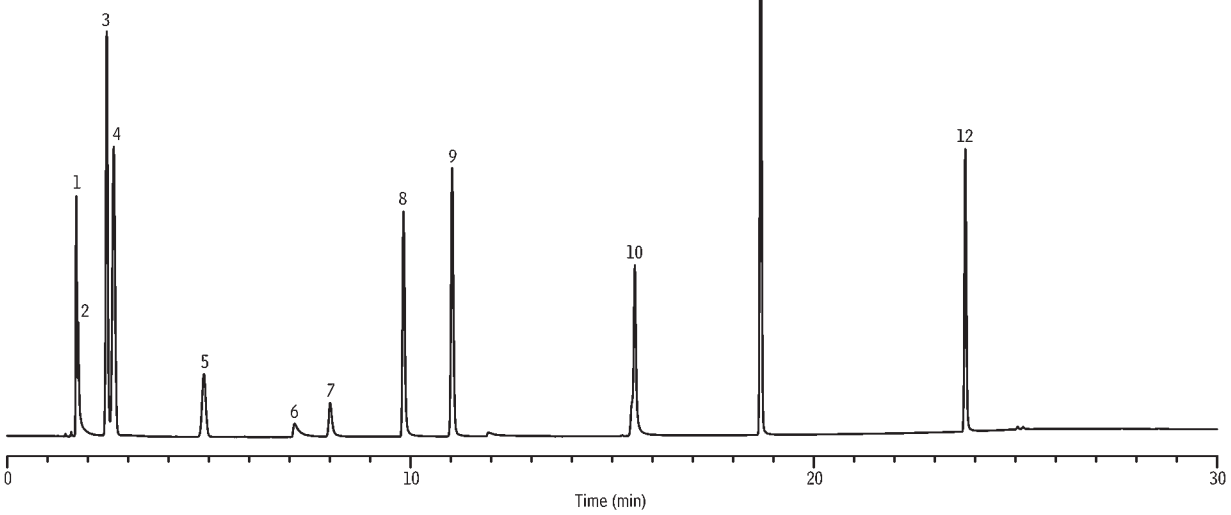


Table 1 A Stabilwax®/Stabilwax® DB column pair assures linear calibration curves at or below the minimum concentration levels listed in Method 1671.

			Calibration Point Concentration (µg/mL)					
Analyte	Column	Minimum Level	Point 1	Point 2	Point 3	Point 4	Point 5	Correlation Coefficient
methylamine	Stabilwax® DB	50	10	25	50	100	200	0.996
methanol	Stabilwax®	2	2	5	10	20	40	0.999
dimethylamine	Stabilwax® DB	50	10	25	50	100	200	0.999
ethanol	Stabilwax®	2	2	5	10	20	40	0.999
acetonitrile	Stabilwax® DB	50	10	25	50	100	200	0.999
n-propanol	Stabilwax® DB	50	10	25	50	100	200	0.999
diethylamine	Stabilwax® DB	50	10	25	50	100	200	0.999
methyl Cellosolve®	Stabilwax®	20	10	25	50	100	200	0.999
formamide	Stabilwax®	100	25	62.5	125	250	500	0.998
ethylene glycol	Stabilwax®	100	25	62.5	125	250	500	0.995
triethylamine	Stabilwax® DB	50	10	25	50	100	200	0.999
dimethyl sulfoxide	Stabilwax®	20	10	25	50	100	200	0.999

free literature

Request the Stabilwax®/MXT®-WAX Fast Facts Flyer (lit. cat.# 59316) for more information. Contact Restek or your Restek distributor.

Stabilwax® Columns (polar phase; Crossbond® Carbowax® polyethylene glycol)

- General purpose columns for FAMEs, flavor compounds, essential oils, amines, solvents, xylene isomers, US EPA Method 603 (acrolein/acrylonitrile).
- Resistant to oxidative damage.
- Temperature range: 40°C to 250°C.
- Equivalent to USP G14, G15, G16, G20, G39 phases.

Our polar-deactivated surface tightly binds the Carbowax® polymer and increases thermal stability, relative to competitive columns. The bonding mechanisms produce a column that can be rejuvenated by solvent washing. Compared to silicone stationary phases, PEG phases are more resistant to damage from strongly acidic or basic volatile compounds, including inorganic acids and volatile inorganic bases.

Stabilwax® Column (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.00	40 to 240/250°C	30-Meter	10654

ordering note

Stabilwax® columns are available with Integra-Guard™ built-in guard columns. Get the protection without the connection!

Stabilwax®-DB Columns (polar phase; Crossbond® base-deactivated Carbowax® polyethylene glycol)

- Application-specific columns for underivatized amines and other basic compounds, including alkylamines, diamines, triamines, nitrogen-containing heterocyclics. No need for column priming.
- Temperature range: 40°C to 220°C.

Stabilwax®-DB columns reduce adsorption and improve responses for many basic compounds, without analyte derivatization or column priming. Use an Rtx®-5 Amine or Rtx®-35 Amine column to analyze oxygenated basic compounds—at low ppm levels, some of these analytes exhibit adsorption on a Stabilwax®-DB column. Stabilwax®-DB is a bonded stationary phase, but avoid rinsing these columns with water or alcohols.

Stabilwax®-DB Column (fused silica)

(Crossbond® Carbowax® polyethylene glycol for amines and basic compounds)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.00	40 to 210/220°C	30-Meter	10854

Universal "Y" Press-Tight® Connectors

- Split sample flow onto two columns.
- Split a single column flow to two detectors—perform confirmation analysis with a single injection.
- Deactivated Press-Tight® connectors assure better recovery of polar and non-polar compounds.
- Siltek® treated connectors are ideal for organochlorine pesticides analysis.
- Fit column ODs from 0.33–0.74mm (Restek 0.1mm–0.53mm ID).

An alternative method of performing dual-column confirmational analyses!

Description	ea.	3-pk.
Universal "Y" Press-Tight® Connector	20405	20406
Deactivated Universal "Y" Press-Tight® Connector	20405-261	20406-261
Siltek® Treated Universal "Y" Press-Tight® Connector	20485	20486

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