The Determination of Residual Solvents in Pharmaceuticals Using the Agilent G1888 Network Headspace Sampler

Application

Pharmaceuticals

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Abstract

The G1888 Network Headspace Sampler interfaced to 6890N gas chromatographs configured with either an FID or 5973 inert MSD were used for the determination of regulated residual solvents. Standard mixtures in water were used at various concentrations including levels below published acceptance guidelines to demonstrate system performance. Included were Class 1 and Class 2 solvents, according to the International Conference on Harmonization, and those listed in USP 467. Repeatability, inertness, and carryover reduction are improved compared to previous generation samplers, using the automated 70-sample G1888 with Siltek flow path. Integrated control and sequencing of the sampler is incorporated into the Agilent GC ChemStation through an add-on software module.

Introduction

Organic volatile impurities (OVIs) can result from the manufacture of active pharmaceuticals or other drug products. Many are used to enhance yields, improve crystallization, or increase solubility [1]. Other factors such as packaging, transportation, and storage can also impact the level of residual solvents. Gas chromatography (GC) coupled with static headspace sampling, acknowledged as an easy-to-use high-throughput analytical tool for the determination of low-level solvent impurities in drugs, can be found in nearly all Quality Control (QC) laboratories in pharmaceutical manufacturing facilities. Sample prep is relatively simple and the methodology is easily validated as per specific monographs.

General guidelines established by the International Conference on Harmonization (ICH) divide solvents into three classes [2]. The Class 1 solvents should not be used in pharmaceutical manufacture because of toxicity or environmental impact, while use of Class 2 solvents should be limited due to potential toxicity. Class 3 solvents are regarded as posing a lower risk to human health. Solvents listed in USP 467 include a subset of specific Class 1 and Class 2 solvents.

This application note will demonstrate the analysis and quantitation of Class 1 and Class 2 solvents. See Table 1 for a listing of residual solvents.

Table 1A. Class 1 Solvents in Pharmaceutical Products - To Be Avoided [2]

Solvent	Concentration limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 1B. Class 2 Solvents in Pharmaceutical Products [2]

Solvent	Permissible daily exposure (ppm) (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	48.4	4840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

* Usually 60% m-xylene, 14% p-xylene, 9% o-xylene with 17% ethyl benzene.

Table 1C. Solvents in Pharmaceutical Products, According to USP 467

Solvent	Concentration limit (ppm)
Methylene chloride	600
Chloroform	60
Benzene	2
Trichloroethylene	80
1,4-dioxane	380

Residual solvent and other contaminant levels, designated as safe, have trended downward in recent years as information about potential harmful effects of long-term and/or low-level exposures accumulate and as the detection sensitivity of analytical instrumentation improves. For example, based on new toxicity data, a 2003 change in the regulations for residual solvents stipulate a 10-fold reduction of the 1997 PDE (permitted daily exposure) and residual concentration limits for the solvent N-methylpyrrolidone [3]. It also reclassifies tetrahydrofuran from a Class 3 to a Class 2 category solvent with PDE and concentration limitations more restrictive than toluene [3]. Table 1B also lists PDE and concentration limits for Class 1 and Class 2 residual solvents in pharmaceutical products [4].

Experimental

Two systems are described in this work. The first, based on flame ionization detection is considered the system of choice for routine QC work, while the second system with mass selective detection provides unknown determination, possible quantitation of near-coeluting peaks, and solvent confirmation. Ten-milliliter headspace vials were used for all experiments containing 5 mL water as the matrix, with 1-g sodium sulfate added to assist with analyte extraction. The headspace sampler was equipped with a 1-mL sample loop. Since a sufficient flow must be maintained through the system to avoid excessive peak broadening, a split injection is used. A 2:1 split ratio is the lowest recommended with typical 0.53-mm id column flows.

Table 2. Instrument Conditions

FID system	
6890N GC	
Injection port	Volatiles interface
Temperature	160 °C
Split ratio	2:1 to 5:1
Carrier gas	Helium
Carrier flow	9 mL/min
Detector	FID, 250 °C
GC oven program	
Initial temperature	35 °C
Initial time	20 min
Rate	25 °C/min
Final temperature	250 °C
Final time	15 min
Columns	30 m × 0.53 mm × 3 µm DB-624
	30 m × 0.45 mm × 2.55 µm DB-624
G1888A Headspace Sampler	
Loop size	1 mL
Vial pressure	14.0 psig
Headspace oven	85 °C
Loop temp	100 °C
Transfer line temp	120 °C
Equilibration time	30 min, low shake
GC Cycle time	50 min
Pressurization	0.15 min
Vent (loop fill)	0.15 min
Inject	0.5 min

5973 inert system	
6890N GC	
Injection port	Volatiles interface
Temperature	160 °C
Split ratio	2:1 to 5:1
Carrier gas	Helium
Inlet pressure	2.7 psi
Column flow	1.7 mL/min
GC oven program	
Initial temperature	35 °C
Initial time	20 min
Rate	20 °C/min
Final temperature	250 °C
Final time	15 min
Column	30 m × 0.32 mm × 1.8 µm DB-624
G1888A Headspace Sampler	
Same settings as FID system	
5973 inert	
Scan	30 to 200 amu, samples 2
SIM	100 ms dwell
Source temperature	230 °C
Quad temperature	150 °C
Tune	BFB.u
Standards	
USP 467	Restek #36228
	AccuStandard NF-467-R
ICH Class 1 and 2	Restek #36228 (Class 1)
	#36229 (Class 2A)
	#36230 (Class 2B)

Discussion

GC System

Most quality control labs in pharmaceutical manufacturing employ gas chromatography (GC) for the determination of residual solvents that are included in either USP 467 or in the more extensive list covered in ICH guidelines. Capillary GC based on the 624 phase (USP G43) is widely used for solvent separation. A different stationary phase such as DB-1701, DB-5, or DB-WAX (USP G16) can be used in specific methods when coelution is identified. Headspace sampling has many advantages over direct liquid injection including the avoidance of large water injections that can result in column degradation.

Table 3 lists concentrations and identifications of Class 1 and Class 2 solvents used to produce the chromatogram shown in Figure 1.

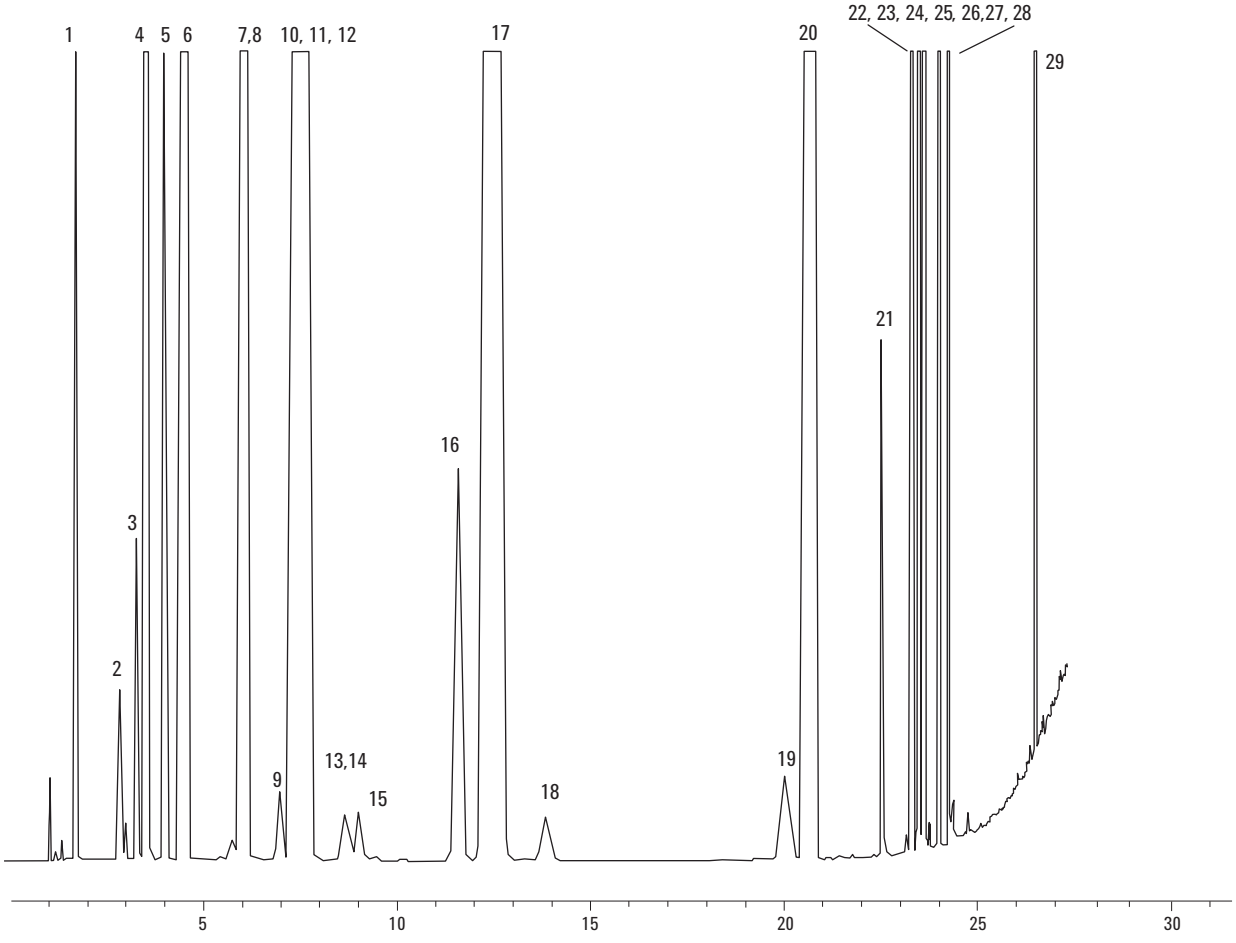

Figure 1. Class 1 and Class 2 residual solvents. G1888, 6890N with FID and volatiles interface.

These concentrations equal the guideline limits based on a 100-mg sample of the pharmaceutical dissolved in 5 mL. USP 467 solvents are shown in Figure 2 at concentrations below the required levels (10 µL Restek std. #36228). Excellent signal-to-noise ratio is still achieved. Concentrations used throughout this work are defined as the analyte concentration present in the headspace vial prior to sampling.

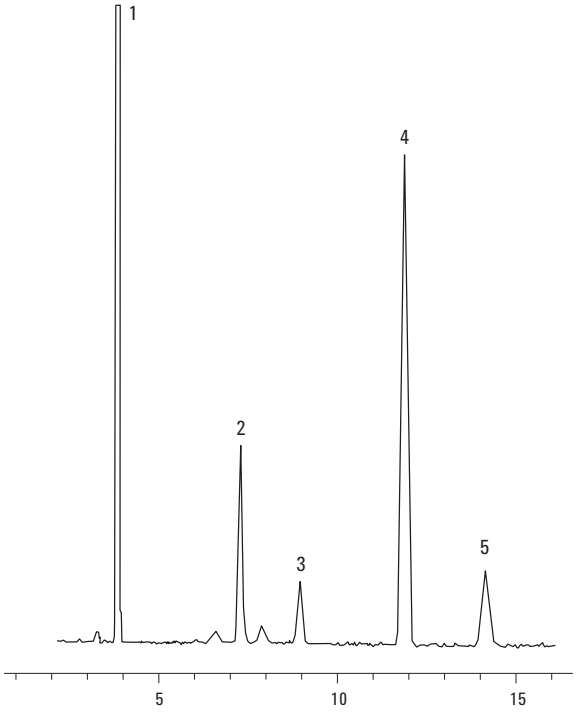

Figure 2. Identifications and concentrations: 1. Methylene chloride 1.2 µg/mL, 2. Chloroform 0.12 µg/mL, 3. Benzene 0.004 µg/mL, 4. Trichloroethylene 0.16 µg/mL, 5. 1,4-dioxane 0.76 µg/mL.

Coelutions that occur on the DB-624 column under the chromatographic conditions and concentrations employed are listed in Table 4. Using the 30 m × 0.45 mm × 2.55 µm DB-624 column, benzene and 1,2-dichloro-ethane can be resolved at a 35 °C oven temperature, as seen in Figure 3.

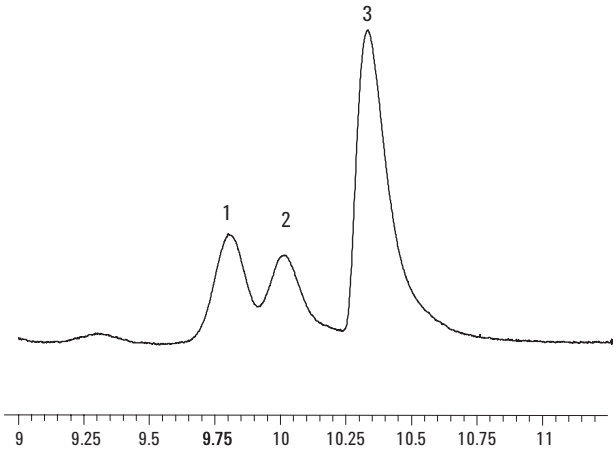

Figure 3. Resolution of benzene and 1,2 dichloroethane. Peak identifications: 1. benzene, 2. 1,2-dichloroethane, 3. 1,2-dimethoxyethane.

Calibration curves for selected solvents included in USP 467 are shown in Figure 4. Linear results are seen over a concentration range that extends well below recommended maximum concentrations. The concentration range is well within the linear dynamic range of the thick film 0.53 mm and 0.45-mm id columns.

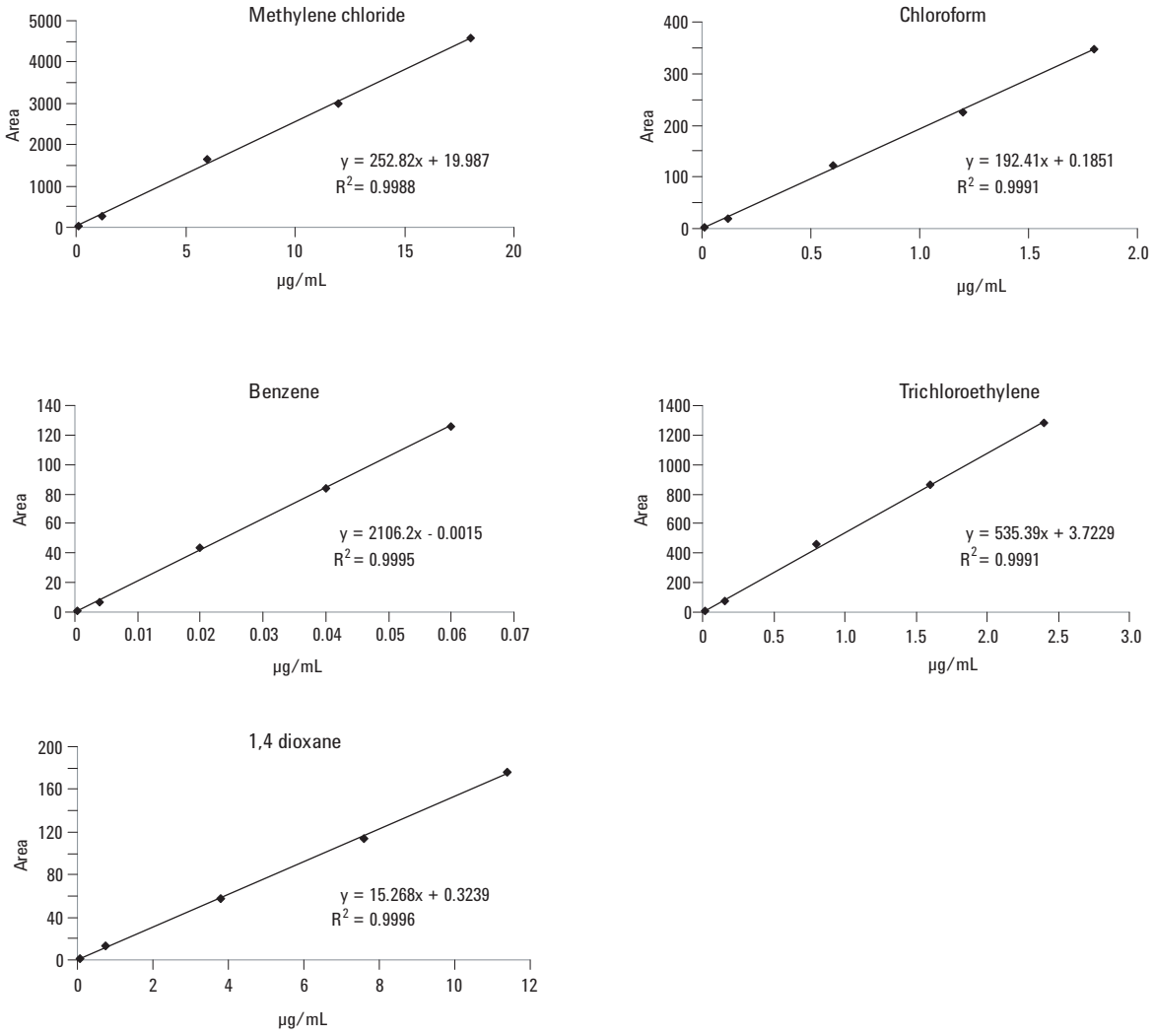

Figure 4. Calibration plots for selected solvents.

Headspace equilibration time is normally set at 60 min; however, in most cases 30 min is sufficient. Figure 5 illustrates an overlay of a 30- and 60-min headspace equilibration for a selected portion of the chromatogram (Class 1 and 2 solvents). Little overall difference is seen in the peak areas; although for some compounds 30-min equilibration produces marginally larger areas.

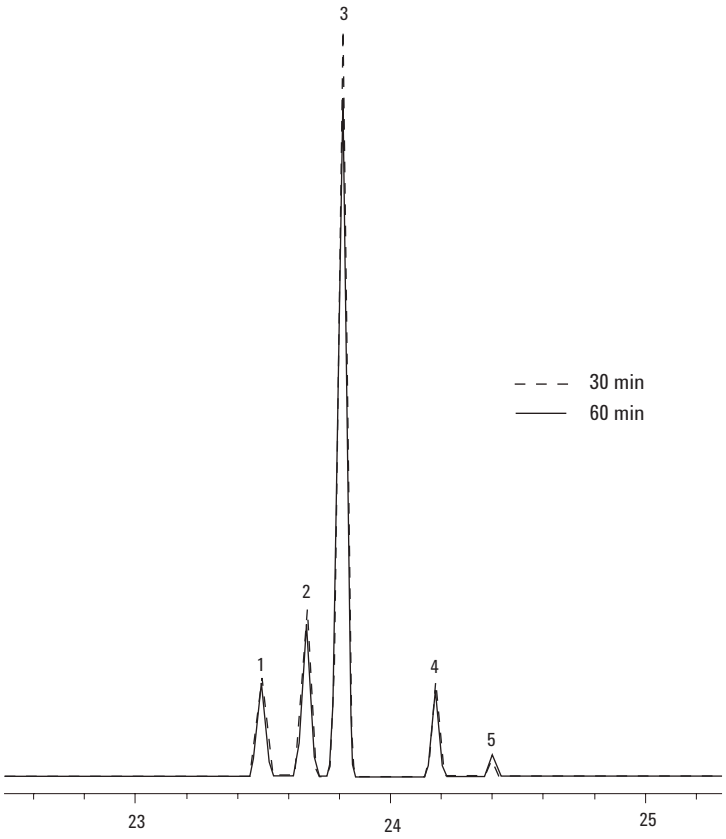

Figure 5. Overlay of selected compounds after 30- and 60-min headspace equilibration times. 1. Chlorobenzene, 2. Ethylbenzene and DMF, 3. m-xylene, p-xylene, 4. o-xylene, 5. N,N-dimethylacetamide.

Table 3. Class 1 and Class 2 Residual Solvent Concentrations*. Table ID Column Corresponds to Chromatogram Numbering

Solvent	ID	Conc. (µg/mL)	Solvent	ID	Conc. (µg/mL)
Methanol	1	60	Trichloroethylene	16	1.6
1,1-Dichloroethane	2	0.16	Methyl cyclohexane	17	236
Acetonitrile	3	8.2	1,4-Dioxane	18	7.6
Methylene chloride	4	12	Pyridine	19	4
Hexane	6	5.1	Toluene	20	17.8
cis-1,2-dichloroethane	7	37.4	2-Hexanone	21	1
Nitrobenzene	8	1	Chlorobenzene	22	7.6
Chloroform	9	1.2	Ethylbenzene	23	7.38
Carbon tetrachloride	10	0.08	N,N-dimethylformamide	24	17.6
Cyclohexane	11	77.6	m-xylene	25	26.04
1,1,1-Trichloroethane	12	30	p-xylene	26	6.08
Benzene	13	0.04	o-xylene	27	3.9
1,2-Dichloroethane	14	0.1	N,N-dimethylacetamide	28	21.8
1,2-Dimethoxyethane	15	2	Tetraline	29	2

*Concentrations shown are headspace vial solution concentrations prior to sampling. Peak 5 (*trans* 1,2 dichloroethane) is not listed in the table as a Class 1 or Class 2 solvent.

Table 4. Coelutions on 0.53-mm id DB-624

Coelution group	Solvents
1 (partial)	Benzene, 1,2 dichloroethane
2	Nitrobenzene, *cis*-1,2 dichloroethene
3	Carbon tetrachloride, Cyclohexane, 1,1,1- trichloroethane*+
4	Ethylbenzene, DMF
5	m-xylene, p-xylene

* Trichloroethane will separate from CCl_4 in absence of cyclohexane
+ Figure 6 shows separation on a 30 m x 0.45 mm x 2.55 µm DB-624 Agilent part no.124-1334.

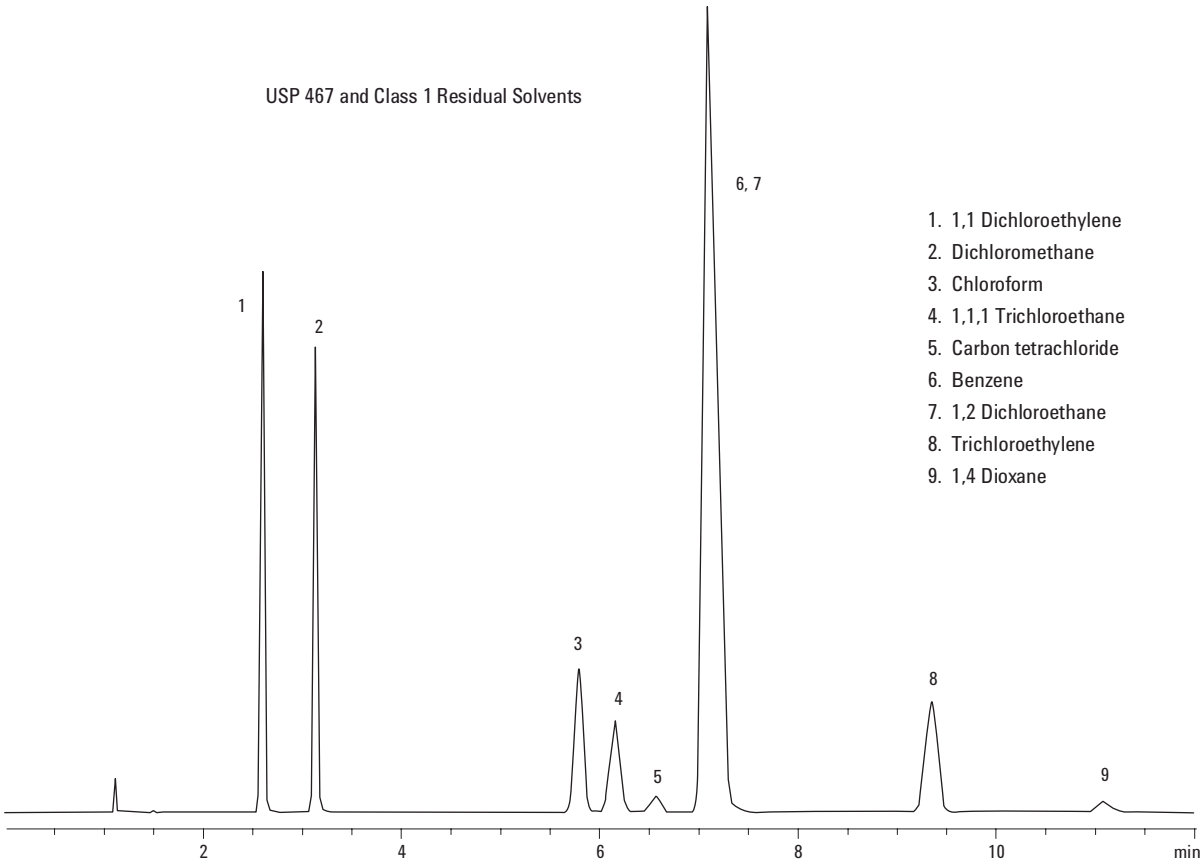

Figure 6. USP 467 and Class 1 solvents at 1 ppm each on the 30 m x 0.45 mm x 2.55 µm DB-624 column. Starting GC oven temperature was 40 °C.

MSD System

A TIC of Class 1 and Class 2 solvents produced with a G1888/6890N/5973 inert system is shown in Figure 7.

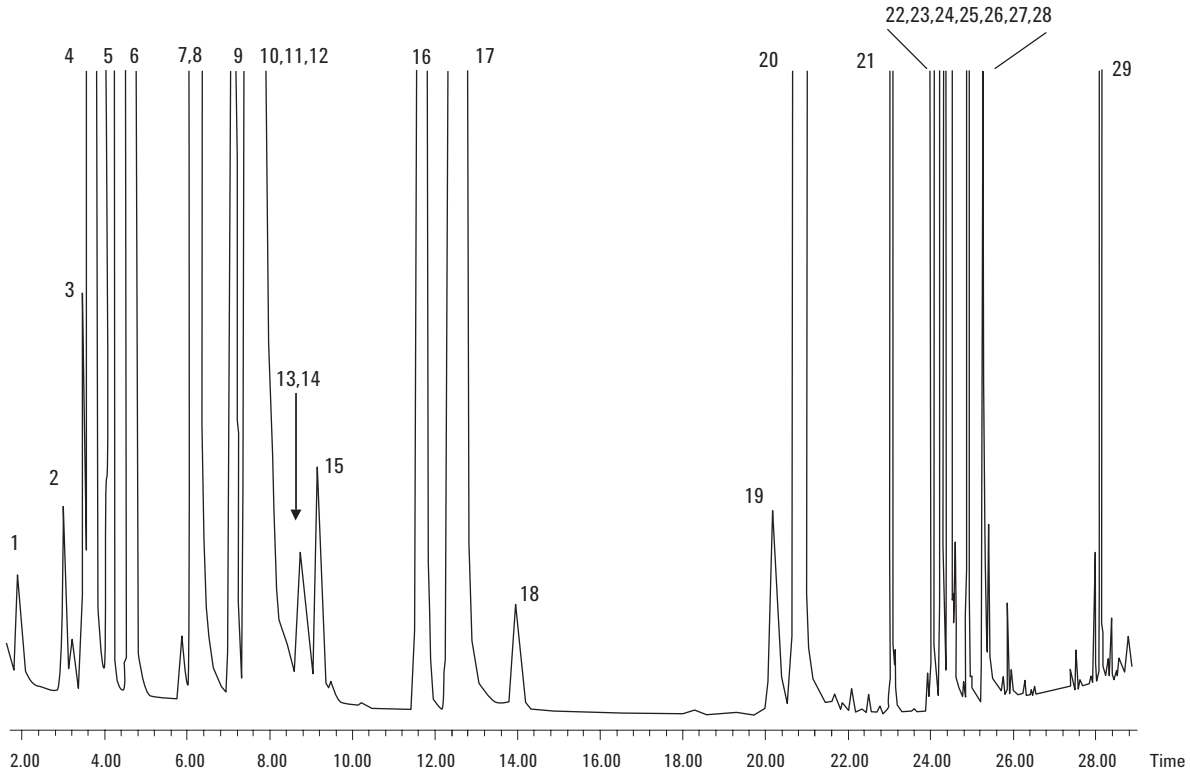

Figure 7. TIC of Class 1 and Class 2 solvents. See Table 3 for compound identifications.

Analyte solution concentrations and peak identifications are as indicated in Table 3. Gas chromatography/mass spectrometry (GC/MS) offers an alternative methodology to flame ionization detection (FID) that can be useful to solve coelution problems or identify unknowns. Also, excellent sensitivity and selectivity can be achieved in Selected Ion Monitoring (SIM) mode, which may be useful for drug manufacturing process development to identify and quantify trace impurities.

Carryover

In practice, nonaqueous solvents are commonly used in testing since extraction of many common solvents used in pharmaceutical manufacturing are not water soluble. Some common solvents include DMA (N.N-dimethylacetamide), DMSO, pyridine, and DMI (1,3-Dimethyl-2-imidazolidinone). Because many of the solvents used are high boiling, the possibility of headspace carryover exists. Improvements in the thermal zone temperature uniformity in the G1888 reduce the condensation of high-boiling materials in various flow path lines and vent valve.

The G1888 incorporates a new feature that allows users to program the vent purge time, labeled "Sequence Vent Purge" in the advanced functions menu. This represents the time interval when the vent line is purged as a post injection event. The default time of 30 seconds can be increased up to an approximate maximum of the cycle time. For the carryover experiments in this work, a vent purge time of 20 min was used to further reduce the possibility of solvent carryover.

One hundred microliters of pure solvent was introduced into 10-mL vials. Larger amounts of solvent will not result in an increase in the amount injected. A 10-vial sequence was set up with alternating solvent and water blank vials using the chromatographic program shown in the experimental section. This test was performed for pyridine, DMSO, and DMA. For all three solvents, carryover ([amount solvent area from blank vial/solvent area from solvent vial] × 100) was under 0.006%. In addition, the solvent areas for all blanks had ±3% RSDs, indicating an absence of trending. As an additional carryover check, 10 consecutive vials of DMA (100 µL per 10-mL vial) were run at a Headspace oven temperature of 100 °C. These were followed by two water blanks. The first blank showed carryover of 0.004% and the second 0.001%.

One of the most effective solvent systems used today by pharmaceutical companies is DMI with a boiling point of 225 °C. Table 5 lists the system set points used in a carryover test with this solvent. Alternating vials of DMI and water blank were run in a headspace sequence. Results are shown in Figure 8.

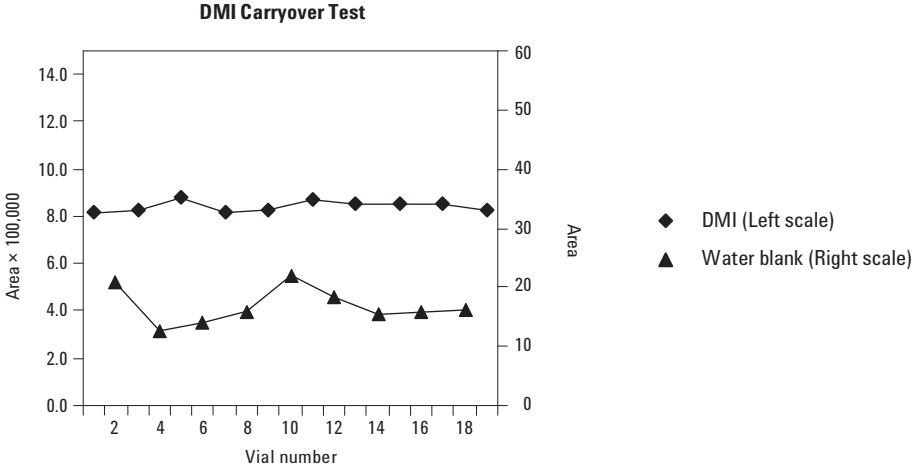

Figure 8. DMI carryover results.

The large concentration of DMI overloads the column and leads to some inconsistency in areas, however, it is not a concern given the purpose of this test. Measured carryover is under 0.003%.

Table 5. Setting Used for DMI Carryover Test

Headspace oven	220 °C
Loop	250 °C
Transfer line	250 °C
Vial eq. time	30 min
Sequence vent purge	20 min
Sample	100-µL DMI in 10-mL vial
Blanks	5-µL water in 10-mL vial
Volatiles interface	250 °C
Split ratio	10:1
Oven program	35 °C (0 min) to 260 °C (15 min) at 25 °C/min

To check for carryover of the actual analytes, a test scheme similar to that used for the pure solvents was chosen. One hundred microliters of the USP 467 standard (Restek # 36007) was placed in 5-mL water/1 g Na_2SO_4. These vials were alternated with pure water blanks in a 10-vial sequence at 85 °C equilibration temperature. No measurable area for the analytes could be integrated in any of the runs.

Conclusions

Manufacturers of pharmaceuticals must ensure that residual solvents, OVIs, and related contaminants are not present in their products, or are present below levels stipulated as safe by regulation. One of the impediments to accurate determination of impurities at very low levels is the tendency for analyte interaction and/or reaction with the internal surfaces of the instrument sample path. To eliminate this problem, a new inert headspace sampler, the G1888 system was developed. This instrument possesses a nonreactive, nonadsorptive sample flow path from the point of injection through detection. This significantly reduces carryover, a common problem with older instrumentation. High temperature heated zones extend the choice of solvent systems that can be used. When coupled to the 5973 inert MSD, which uses a solid inert source, superior results are obtained when the need for unknown identification or confirmation is required. Analytical results obtained for broad classes of solvents, used in medicinal products by the G1888 Headspace Sampler systems, described in this application show reduced carryover, excellent detection sensitivity, and good linear response over the ppm to ppb range.

The methods and procedures outlined in this work illustrate potential approaches to the analysis of residual solvents. Laboratories should perform system suitability studies and validate their methods according to ICH or USP guidelines.

References

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