Enhanced Productivity for Residual Solvent Analysis in Pharmaceutical Products According to USP 467 by Using a New Valve-and-Loop Static Headspace Sampler

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ABSTRACT

Residual solvents can be present in pharmaceutical products as a result of the manufacturing process or as a contamination during packaging, warehouse storage or transportation. In order to prevent patients from potentially hazardous effects of those chemicals, pharmaceutical products need to be tested to ensure that any solvent residuals have been efficiently removed during manufacturing processes or, if present, their concentration is compatible with the accepted safety requirements. United States Pharmacopeia (USP) Method <467> regulates the testing procedure and establishes recommended acceptable levels and the instrument performance criteria. Gas chromatography coupled with valve-and-loop static headspace sampling (GC-HS) is the technique of choice for identification and quantitation of residual solvents in pharmaceutical products. The USP compliance of the new Thermo Scientific™ TriPlus™ 500 Static Headspace Sampler coupled with the Thermo Scientific™ TRACE™ 1310 GC in a routine testing environment is shown here.

INTRODUCTION

Solvents are widely used in the synthesis of pharmaceutical products, substances and excipients although they cannot always be completely removed during the manufacturing processes. To ensure patients' safety, final products are tested to assess whether the solvents used during the manufacturing processes have been efficiently removed or, if still present, their concentration is within the accepted limits. As organic solvents have relatively low boiling points and are thermally stable the analytical method of choice for Class 1 and Class 2 residual solvent determination is headspace-gas chromatography, with detection using either flame ionization detection (FID) or mass spectrometry (MS). Headspace sampling allows for the extraction of semi-volatile and volatile compounds from complex liquid and solid matrices in a fast and simple way without the need for time-consuming sample preparation. A TriPlus 500 HS autosampler was coupled to a TRACE 1310 GC equipped with a Thermo Scientific™ Instant Connect Split/Splitless SSL Injector and a Thermo Scientific™ Instant Connect FID and used for the determination of residual solvent content in water-soluble and water- insoluble pharmaceuticals according to the United States Pharmacopeia <467> method (USP).[1]

MATERIALS AND METHODS

USP <467> Class 1, Class 2A and Class 2B residual solvent solutions in dimethylsulfoxide (DMSO) were purchased from (Restek®, P/N 36279, 36012, 36280 respectively). Stock and standard solutions for procedures A, B, C were diluted in HPLC-MS grade water or GC headspace grade DMSO as reported in the USP <467> method. Dispersive aspirin (acetylsalicylic acid, 75 mg) and common pain relief tablets (paracetamol, 500 mg and caffeine, 65 mg) were used to prepare sample stock and test solutions as described in the regulation. A second stock of test solutions was prepared at a concentration level five times higher than the limits reported in Table 1, which represent the acceptable amount of residual solvents in the final product.
System compliance, sensitivity, precision, robustness and linearity were assessed for both water-soluble and water–insoluble pharmaceutical products according to USP <467> method, procedures: A, B, C. HS-GC-FID operating parameters, as well as the chromatographic columns, are reported in Table 2.
Data was acquired, processed and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2, a platform compliant with Title 21 of the Code of Federal Regulations, Part 11 (Title 21 CFR Part 11). Simplified e-workflows deliver effective data management, ensuring ease of use, data integrity, and traceability. Moreover several ready-made templates are available for the assessment of ICH method validation procedures.

Table 1. Concentration limits in ppm for Class 1, Class 2A and Class 2B residual solvents

Compound Name	Concentration Limit (ppm)	Compound Name	Concentration Limit (ppm)
Class 1		**Class 2 A**	
1,1-Dichloroethene	8	Methanol	3000
1,1,1-Trichloroethane	1500	Acetonitrile	410
Benzene	2	Dichloromethane	600
Carbon Tetrachloride	4	*trans* 1,2-Dichloroethene	1870
1,2-Dichloroethane	5	*cis* 1,2-Dichloroethene	1870
Class 2B		Tetrahydrofuran	720
Hexane	290	Cyclohexane	3880
Nitromethane	50	Methycyclohexane	1180
Chloroform	60	1,4-Dioxane	380
1,2-Dimethoxyethane	100	Toluene	890
Trichloroethylene	80	Chlorobenzene	360
Pyridine	200	Xylene*	2170
2-Hexanone	50		
Tetralin	100		

* Usually 60% m-xylene, 14% p-xylene, 9% o-xylene, 17% ethylbenzene

Table 2. HS-GC-FID operating parameters according to USP <467> method

TRACE 1310 GC Parameters	Procedure A/C	Procedure B
Inlet Module and Mode:	SSL, split	
Split Ratio:	10:1	20:1
Septum Purge Mode, Flow (mL/min):	Constant, 5	
Carrier Gas, Carrier Mode, Flow:	He, constant flow, 2.2 mL/min	
Oven Temperature Program:	Procedure A/C	Procedure B
Temperature 1 (°C):	40	50
Hold Time (min):	20	20
Temperature 2 (°C):	240	165
Rate (°C/min):	10	6
Hold Time (min):	20	20
FID:		
Temperature (°C):	250	
Air Flow (mL/min):	350	
H_2 Flow (mL/min):	35	
N_2 Flow (mL/min):	40	
Acquisition Rate (Hz):	25	
Chromatographic Column:	Procedure A/C	
TraceGOLD TG-624 (P/N 26085-3390)	30 m × 0.32 mm × 1.8 µm	
	Procedure B	
TraceGOLD TG-WAXMS GC (P/N 26088-1430)	30 m × 0.32 mm × 0.25 µm	

TriPlus 500 HS Autosampler Parameters	
Incubation Temperature (°C):	80
Incubation Time (min):	60
Vial Shaking:	Fast
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	130
Vial Pressure Equilibration Time (min):	1
Loop size (mL):	1
Loop/Sample Path Temperature (°C):	80
Loop Filling Pressure (kPa):	72.4
Loop Equilibration Time (min):	1
Needle Purge Flow Level:	2
Injection Mode:	Standard
Injection Time (min)	1

Figure 1. Class 1 system suitability solution peak-to-peak signal-to-noise (S/N) ratios for water-soluble (a) and water-insoluble (b) products.

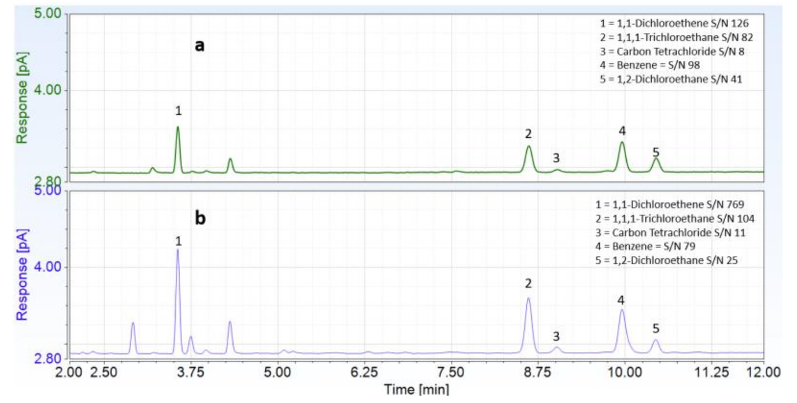
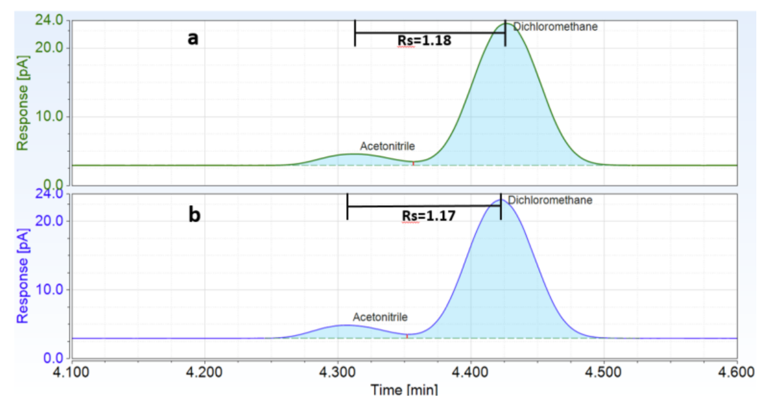


Figure 2. Chromatographic resolution (Rs) between acetonitrile and dichloromethane for water-soluble (a) and water-insoluble products (b).

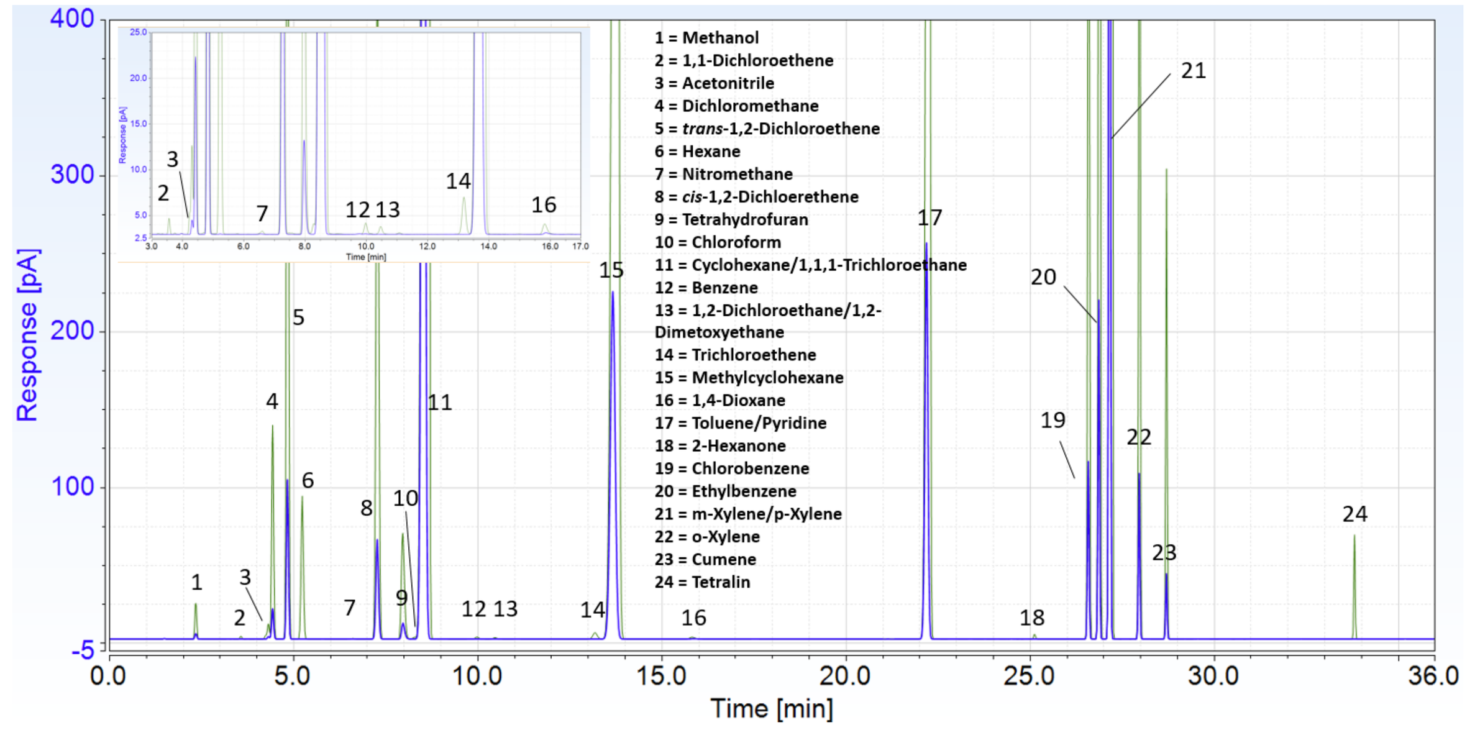


RESULTS

Procedure A: residual solvent screening and identification
Class 1, Class 1 System Suitability, Class 2A standard solutions, and test solutions for water-soluble and water-insoluble pharmaceuticals were prepared as detailed in the USP <467> method and analysed applying the operating conditions reported in Table 2. Peak-to-peak (PtP) signal-to-noise ratio (S/N) for 1,1,1-trichloroethane in Class 1 standard solution was >5:1 and all peaks in Class 1 system suitability showed S/N >3:1 (Figure 1), moreover chromatographic resolution (Rs) between the critical pair, acetonitrile and dichloromethane, was >1 (Figure 2) meeting all the USP <467> method requirements.

Testing a pharmaceutical product failing procedure A
The pharmaceutical products (dispersive aspirin and paracetamol pain relief tablets) spiked with residual solvents were injected into the chromatographic system. The results were compared to the standard solutions. As peaks found in the spiked samples exceeded the limits reported in Table 1, a compound confirmation step was mandatory as described in the procedure B. As an example, the peak profile obtained for dispersive aspirin spiked solution (green) compared to Class 2A standard solution (blue) is reported in Figure 3.

Figure 3. Comparison between peak profiles obtained for water-soluble spiked test solution (green) and Class 2A standard solution (blue).



Procedure B: peak identity confirmation
Class 1, Class 1 System Suitability, Class 2A standard solutions, and test solutions for water-soluble and water-insoluble pharmaceuticals were prepared following the USP <467> method and analysed applying the operating conditions reported in Table 2. PtP S/N for benzene in Class 1 standard solution is >5:1 and all peaks in Class 1 system suitability showed S/N >3:1 satisfying the regulation requirements (Figure 4). The critical pair *cis* 1,2-dichloroethene and acetonitrile is baseline resolved with a chromatographic resolution of 3.8 and 3.9 for water-soluble and water-insoluble Class 2A standard solutions, respectively (Figure 5), meeting the required acceptance criteria (Rs ≥1.0).

Testing a pharmaceutical product matching Procedure B confirmation
The peaks identified (procedure A) were confirmed (procedure B) as their responses were higher than the corresponding standards (Figure 6). Therefore, the levels of these residual solvents must be determined (procedure C).

Figure 4. Peak-to-peak signal-to-noise (S/N) ratios for Class 1 system suitability solutions for water-soluble (a) and water-insoluble (b) products.

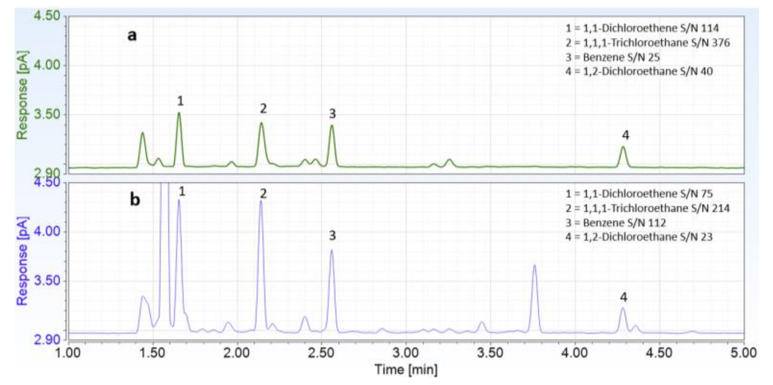
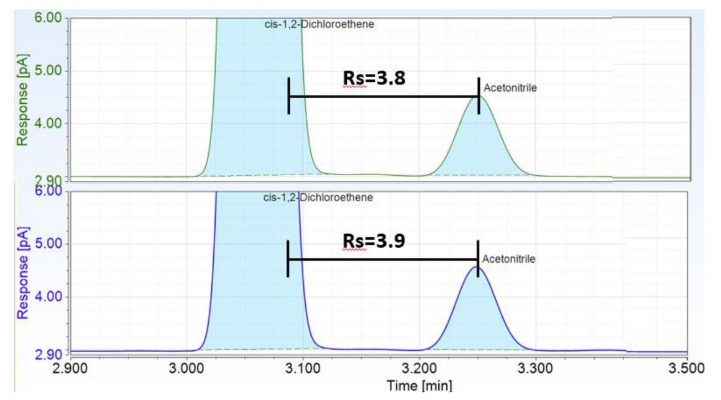


Figure 5. Chromatographic resolution (Rs) between *cis* 1,2-dichloroethene and acetonitrile for water-soluble (a) and water-insoluble products (b).



Procedure C: quantification
Signal-to-noise (S/N) and chromatographic resolution (Rs) requirements for Class 1, Class 1 System suitability solution, and Class 2A standard solution were the same as described and assessed in procedure A.

Quantification of the residual solvents in a pharmaceutical product
Class 1, Class 2A, Class 2B standard and test solutions for quantification have been diluted as described by the USP <467> and injected into the chromatographic system. The calculated amount of each residual solvent (in ppm) identified with procedure A and confirmed in procedure B was derived by applying the formula reported in the USP<467> regulation for water-soluble and water-insoluble pharmaceuticals. Calculated concentrations were consistent with the levels used to fortify the samples. As an example, the peak profile for spiked aspirin compared to spiked standard test solution is reported in Figure 7.

Figure 6. Comparison between peak profiles obtained for water-soluble spiked sample solution (green) and Class 2A standard solution (blue).

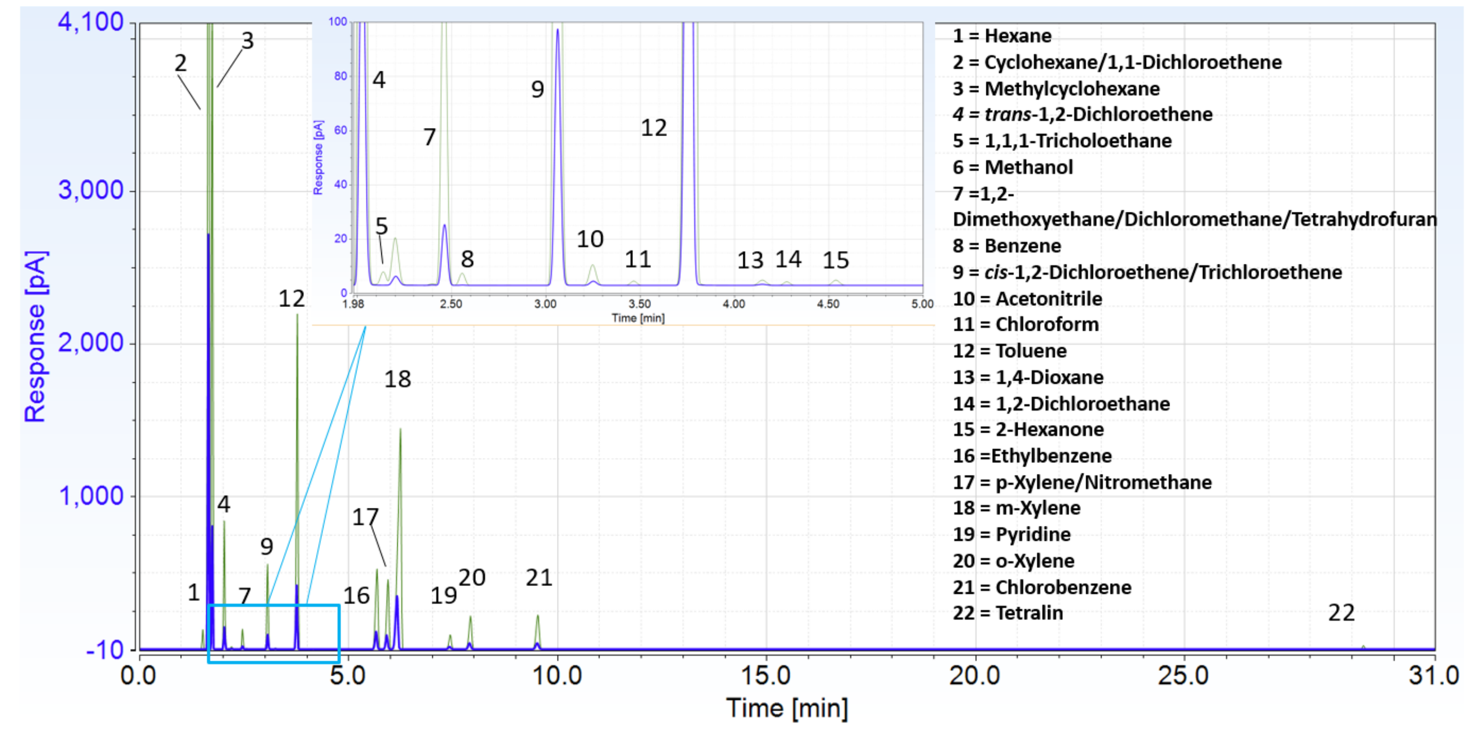
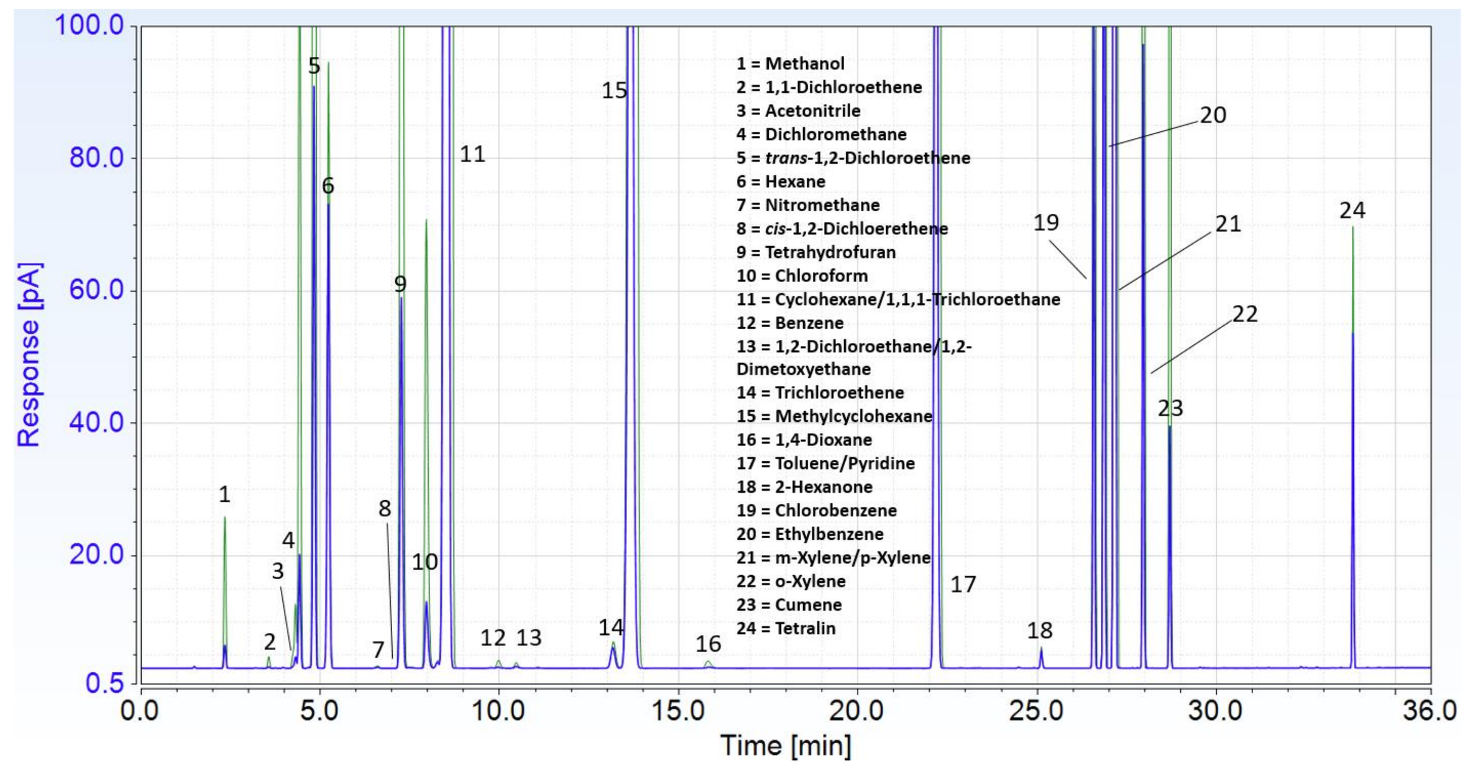
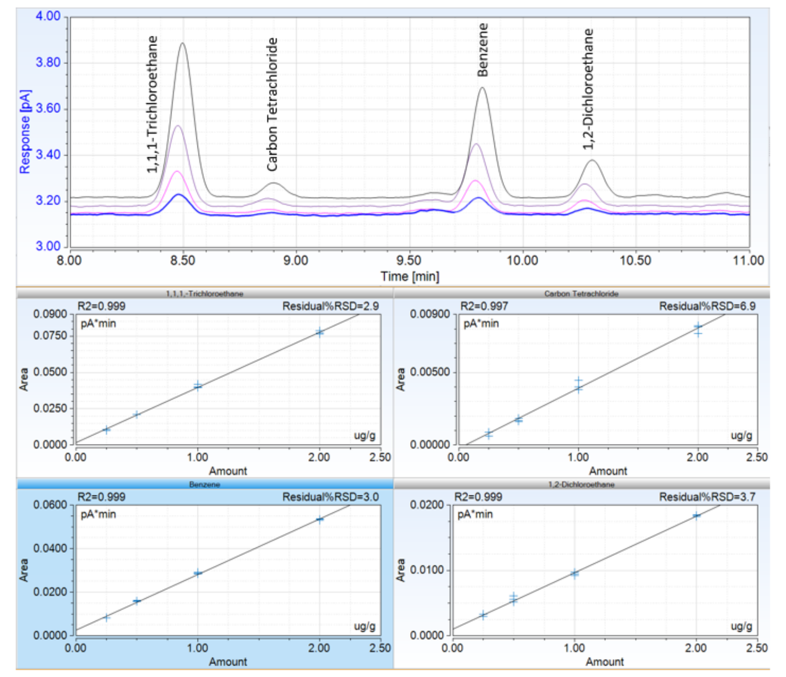

1 = Hexane
2 = Cyclohexane/1,1-Dichloroethene
3 = Methylcyclohexane
4 = trans-1,2-Dichloroethene
5 = 1,1,1-Trichloroethane
6 = Methanol
7 = 1,2-Dimethoxyethane/Dichloromethane/Tetrahydrofuran
8 = Benzene
9 = cis-1,2-Dichloroethene/Trichloroethene
10 = Acetonitrile
11 = Chloroform
12 = Toluene
13 = 1,4-Dioxane
14 = 1,2-Dichloroethane
15 = 2-Hexanone
16 = Ethylbenzene
17 = p-Xylene/Nitromethane
18 = m-Xylene
19 = Pyridine
20 = o-Xylene
21 = Chlorobenzene
22 = Tetralin
Response [pA]
Time [min]

Figure 7. Comparison between peak profiles obtained for spiked aspirin solution (green) and standard test solution (blue).



Linearity
The standard solutions were diluted to prepare four calibration levels at 12.5%, 25%, 50%, and 100% of the concentration limits reported in the USP <467> method. All residual solvents showed excellent linear responses over the calibration range with an average coefficient of determination R^2=0.999. Moreover, the residual values (measured as % RSD of average response factors) were <4%, confirming a good linear response (Table 3). Each calibration level was prepared and analyzed in triplicate (n=3).

Figure 8. Calibration curves (at 12.5%, 25%, 50% and 100% of the concentration limit) for some of the Class 1 residual solvents.



Carry-Over
Carry-over was assessed analyzing a blank vial after n=9 consecutive injections of pure DMSO (200 µL) and resulted to be <0.0015% (Figure 9). The direct column connection to the valve manifold, the highly inert and efficient heated sample path and the effective purging of the loop and the needle contribute to minimize the carryover effect, especially when high boiling residual solvents are injected.

Figure 9. Carryover <0.0015% after 9 consecutive headspace injections of undiluted DMSO.

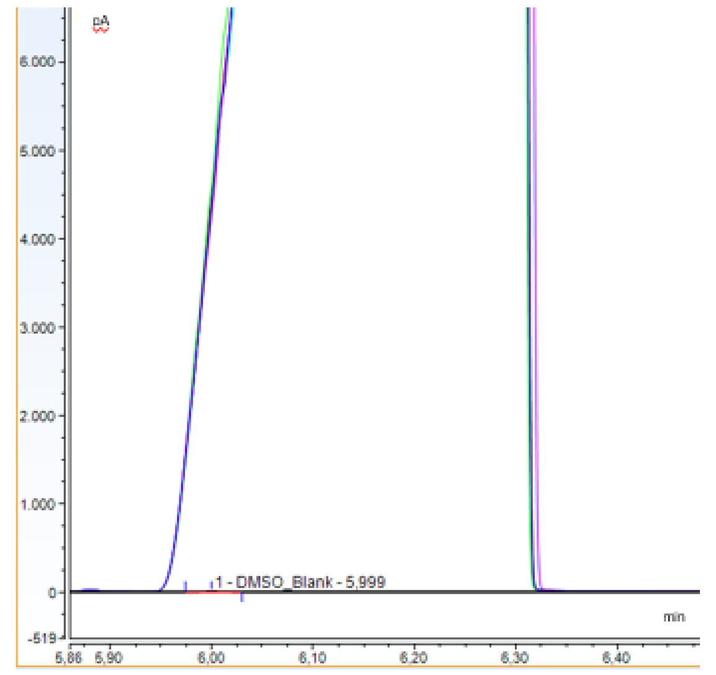


Table 3. Peak area %RSDs obtained from n=18 consecutive injections using water and DMSO as diluents for the concentrated standard solutions, correlation coefficients (R^2) and relative standard deviation of residuals (%RSD) obtained over four calibration levels at 12.5, 25, 50, and 100% of the concentration limits.

Compound name	%RSD (n=18)		Correlation coefficient (R^2)	Residuals standard deviation (%RSD)
	Water	DMSO		
1,1-Dichloroethene	1.5	0.7	1.000	2.0
1,1,1-Trichloroethane	1.0	0.8	0.999	2.9
Carbon Tetrachloride	4.9	2.9	0.997	6.9
Benzene	0.8	0.9	0.999	3.0
1,2-Dichloroethane	1.6	1.0	0.999	3.7
Methanol	0.7	1.4	1.000	1.4
Acetonitrile	0.8	1.6	1.000	1.7
Dichloromethane	3.1	0.7	0.998	4.2
trans 1,2-Dichloroethene	4.0	1.2	0.999	2.9
cis 1,2-Dichloroethene	3.4	0.8	0.998	5.0
Tetrahydrofuran	0.9	1.4	1.000	2.2
Cyclohexane	3.6	2.8	0.999	3.0
Methylcyclohexane	3.0	2.4	1.000	2.5
1,4-Dioxane	1.3	1.9	1.000	1.5
Toluene	3.6	0.8	0.997	5.6
Chlorobenzene	3.3	0.7	0.999	2.8
Ethylbenzene	3.4	0.9	0.997	5.3
m-Xylene	3.3	0.9	0.996	6.0
p-Xylene	3.3	0.9	0.996	6.0
o-Xylene	3.1	0.8	0.997	5.6
Hexane	1.2	0.8	0.998	5.8
Nitromethane	2.9	1.5	0.998	4.8
Chloroform	0.9	1.0	0.999	3.0
1,2-Dimethoxyethane	1.4	0.9	0.997	8.4
Trichloroethene	1.9	0.7	0.999	2.9
2-Hexanone	0.6	0.4	1.000	1.3
Tetralin	0.9	0.6	0.999	3.0

Repeatability
System repeatability was assessed on n=18 consecutive injections for Class 1, Class 2A, and Class 2B standard solutions. The standard solutions were diluted in water or DMSO according to procedure A for water-soluble and water-insoluble products respectively. Sample preparation played a critical role for tested apolar solvents with high partition coefficients. As effect of the low affinity for water, %RSDs were higher when concentrated standard solutions were diluted in water with respect to DMSO. Peak area %RSDs obtained for Class 1, Class 2A, and Class 2B residual solvents are reported in Table 3 with average values <3% for all residual solvent classes when water and DMSO were used as diluent.

Table 5. HS-GC-FID operating parameters

TRACE 1310 GC System Parameters	
Inlet Module and Mode:	SSL, split
Split Ratio:	10:1
Septum Purge Mode, Flow (mL/min):	Constant, 5
Carrier Gas, Carrier Mode, Flow (mL/min):	N_2, constant flow, 2.5
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1
Temperature 2 (°C):	170
Rate (°C/min):	20
FID:	
Temperature (°C):	250
Air Flow (mL/min):	400
H_2 Flow (mL/min):	40
N_2 Flow (mL/min):	40
Acquisition Rate (Hz):	25
Chromatographic Column:	
TG-624 SilMS (P/N 26059-3390)	30 m × 0.32 mm × 1.8 µm

TriPlus 500 HS Autosampler Parameters	
Incubation Temperature (°C):	80
Incubation Time (min):	20
Vial Shaking:	Fast
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	130
Vial Pressure Equilibration Time (min):	1
Loop size (mL):	1
Loop/Sample Path Temperature (°C):	80
Loop Filling Pressure (kPa):	73
Loop Equilibration Time (min):	1
Needle Purge Flow Level:	2
Injection Mode:	Standard
Injection Time (min):	1

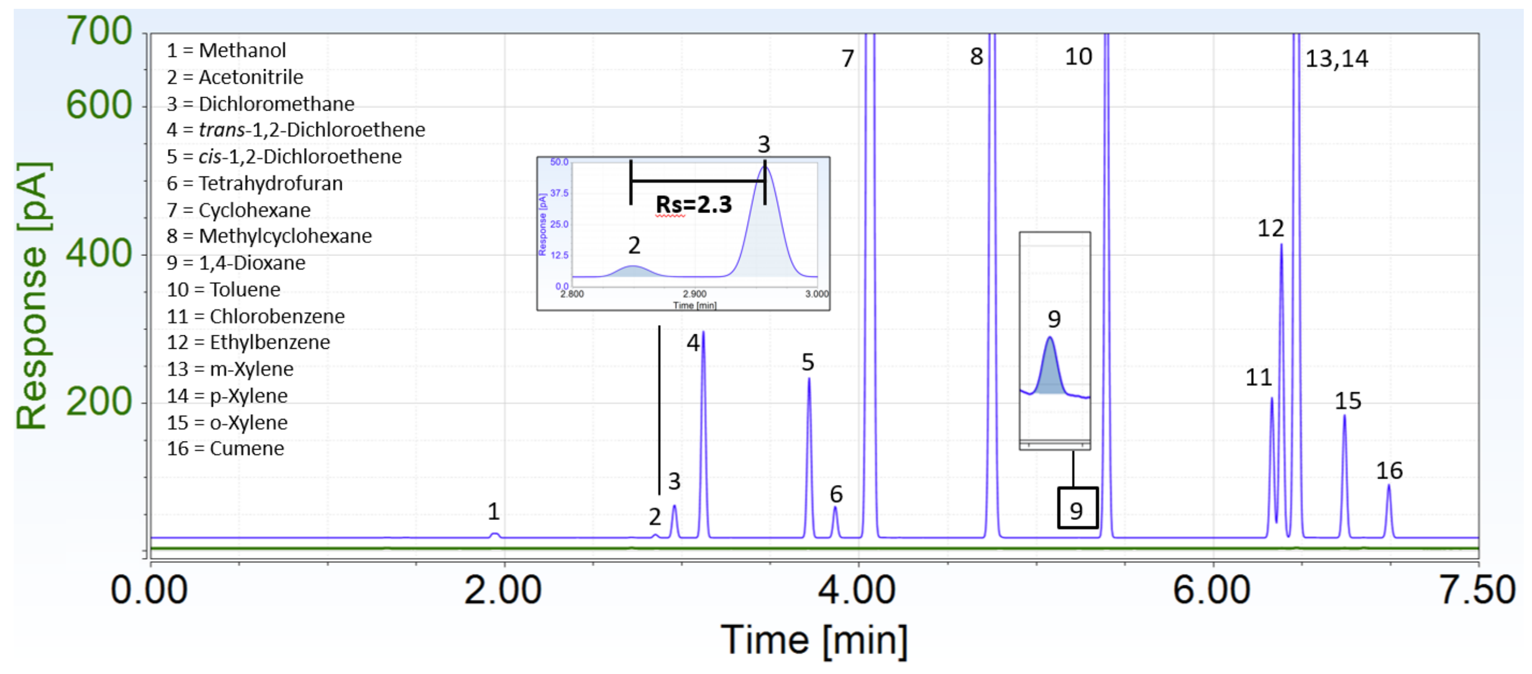
Table 4. Peak area %RSDs obtained from n=12 consecutive injections using DMSO as diluents for the concentrated standard solutions.

Compound name	%RSD (n=12)
Methanol	1.3
Acetonitrile	1.2
Dichloromethane	0.8
trans 1,2-Dichloroethene	1.1
cis 1,2-Dichloroethene	0.9
Tetrahydrofuran	1.0
Cyclohexane	1.8
Methylcyclohexane	1.5
1,4-Dioxane	1.3
Toluene	1.0
Chlorobenzene	0.8
Ethylbenzene	1.0
m-Xylene	1.0
p-Xylene	1.0
o-Xylene	0.9

SIMPLIFIED AND COST-EFFECTIVE ANALYSIS

The USP General Notices and Requirements[2] allows for the use of alternative methods but they shall be validated as described in the general chapter <1225> *Validation of Compendial Procedures.* The USP <467> method for Class 2A solvent was modified as an example of how the residual solvent analysis can be improved with a faster and cost-effective alternative method using the TriPlus 500 Headspace autosampler, a USP G43 equivalent capillary column Thermo Scientific™ TraceGOLD™ TG-624 SilMS column and nitrogen as carrier gas. The TG-624 SilMS capillary column allowed for baseline separation of the critical pair acetonitrile/dichloromethane when a fast heating rate was applied, fulfilling the USP <467> resolution requirement (Rs>1) with a short analysis time. Applying a faster GC oven programming rate, the chromatographic separation of Class 2A residual solvents was achieved in less than 8 minutes (Figure 10) as opposed to 60 minutes (with the UPS <467> default conditions), allowing for more than 7 times improvement in analysis speed without compromising chromatographic resolution and method performance.
The absolute peak area repeatability was assessed on n=12 consecutive injections of a Class 2A standard solutions at the concentration limits (Table 1). Average peak area %RSD was 1.1% (Table4), demonstrating a reliable and precise pneumatic control during sampling and injection. Moreover the effective vial shaking allowed for a three times reduction in incubation time without compromising system reliability. HS-GC-FID operating conditions are reported in Table 5.

Figure 10. Chromatographic separation for Class 2A residual solvent can be achieved in less than 8 minutes using a TG-624 SilMS capillary column and nitrogen as carrier gas.



CONCLUSIONS

- The results presented in this work demonstrate that the new TriPlus 500 HS autosampler in combination with the Trace 1310 GC and FID detector delivers outstanding performance for the analysis of residual solvents in pharmaceutical products meeting or exceeding all USP <467>method requirements.
- The innovative design of the pneumatic control and the flow path inertness ensure excellent repeatability and precision in routine analysis. This was demonstrated by reliable peak area responses obtained (average peak area %RSDs for n=18 consecutive injections was <3%).
- Good linearity (as demonstrated by R^2 and %RSD residual values) was obtained over the calibration range ensuring that the system can be used for routine quantitative assessment of residual solvents in pharmaceutical products.
- The TG-624 SilMS capillary column allowed for fast chromatographic separation of all Class 2A residual solvents in <8 minutes, exceeding the USP <467> chromatographic resolution requirement on the critical pair acetonitrile/dichloromethane (Rs=2.3).
- Sample equilibration was reached in just 20 minutes as a result of the efficient vial spin shaking allowing for a three times reduction in incubation time per sample.

REFERENCES

1. General Chapter USP <467> Organic Volatile impurities, Chemical Tests, United States Pharmacopeia, 2012
2. General Notices and Requirements USP <38>, United States Pharmacopeia, 2015
3. Thermo Fisher Scientific (2018) Residual Solvent Analysis Application Note 10676
4. Thermo Fisher Scientific (2018) Residual Solvent Analysis Technical Note 10679
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TRADEMARKS/LICENSING

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