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APPLICATION NOTE 10676

Routine-grade performance of a new static headspace autosampler for the analysis of residual solvents according to USP <467> method

Authors

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Keywords

USP <467>, residual solvents, pharmaceuticals, valve and loop, static headspace, gas chromatography, GC, headspace-gas chromatography, HS-GC, flame ionization detector, FID, TriPlus 500

Goal

The aim of this work was to evaluate the performance of the new Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace (HS) Autosampler for the determination of residual solvent content in water-soluble and water-insoluble pharmaceuticals according to the United States Pharmacopeia <467> method (USP).[1]

Introduction

Organic solvents are widely used in the synthesis of pharmaceutical products and cannot always be completely removed during the manufacturing processes. To ensure 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.

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According to the International Conference on Harmonization (ICH) guidelines,[2] the USP <467> method[1] describes the assay procedure and classifies residual solvents based upon their toxicities, setting the concentration limits according to their health hazard:

- Class 1: solvents with unacceptable toxicities
- Class 2: solvents with less severe toxicity
- Class 3: solvents with low toxicity

These classes do not provide a full list of all the solvents that can be used in the manufacturing processes; therefore, the final products should be screened according to the solvents used during the production/purification processes.

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 static headspace sampling coupled to gas chromatography (HS-GC), with either flame ionization detection (FID) or mass spectrometry (MS) as detectors of choice. Class 3 solvents can also be determined with a nonspecific method such as weight loss on drying.[1] 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.

The new Triplus 500 HS autosampler offers an innovative design of the pneumatic circuit with a direct connection between the heated valve and the GC column. This translates into highly precise sample introduction and excellent peak area repeatability. Additionally, continuous purging of the sample path ensures system robustness and reliability reducing the risk of contamination and carryover (important when high boiling residual solvents are analyzed). In this study, the results from the analysis of residual solvent according to the USP <467> criteria obtained with the TriPlus 500 HS autosampler and FID as detector of choice are reported. USP <467> system compliance, sensitivity, precision, robustness, and linearity were assessed according to the workflow described in the USP <467> method for both water-soluble and water–insoluble pharmaceutical products.

Experimental

In all the experiments, a TriPlus 500 HS autosampler was coupled to a Thermo Scientific TRACE™ 1310 GC equipped with a Thermo Scientific™ Instant Connect Split/Splitless SSL Injector and a Thermo Scientific™ Instant Connect FID.

Chromatographic separation of target chemicals was obtained on a Thermo Scientific™ TraceGOLD™ TG-624 GC column, 30 m × 0.32 mm × 1.8 µm (P/N 26085-3390) for procedure A and C, and on a Thermo Scientific™ TraceGOLD™ TG-WAXMS GC column, 30 m × 0.32 mm × 0.25 µm (P/N 26088-1430) for procedure B.

HS-GC-FID operative conditions, according to the USP <467> method, are given in Tables 1A and 1B.

Table 1A. GC-FID analytical parameters for the TRACE 1310 GC used for residual solvent content determination according to the USP <467> method, procedures: A, B, C

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 (mL/min)	He, constant flow, 2.2	
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	

Table 1B. TriPlus 500 HS autosampler analytical parameters used for residual solvent content determination according to the USP <467> method, procedures: A, B, C

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

Data acquisition, processing, and reporting

Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2, a software platform compliant with Title 21 of the Code of Federal Regulations, Part 11. Integrated instrument control ensures full automation from instrument set-up to raw data processing, reporting, and storage. Simplified e-workflows deliver effective data management ensuring ease of use, data integrity, and traceability. Chromeleon CDS also offers the option to scale up the entire analytical process in the laboratory from a single workstation to an enterprise environment.[3]

Sample preparation

USP <467> Class 1, Class 2A, and Class 2B residual solvents solutions in dimethylsulfoxide (DMSO) were sourced from Restek (P/N 36279, 36012, 36280, respectively). Stock and standard solutions for procedures A, B, C were diluted in water or DMSO as reported in the USP <467> method.[1] HPLC-MS grade water and GC headspace grade dimethylsulfoxide (DMSO, purity ≥ 99.9%) were used as diluents. Over-the-counter purchased aspirin (acetylsalicylic acid, 75 mg) and pain relief tablets (paracetamol, 500 mg, and caffeine, 65 mg) were used to prepare sample stock and test solutions as described in the regulation. To test the complete USP <467> workflow, a second stock of test solutions was prepared at a concentration level five times higher than the limits reported in Table 2, which represent the acceptable amount of residual solvents in the final product.

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

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

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

Results and discussion

Procedure A: residual solvent screening and identification

By using the Chromeleon CDS e-workflow a significant reduction in the number of steps needed to set up the analytical sequence was obtained, speeding up the analysis time and ultimately boosting laboratory productivity. Class 1, Class 1 System Suitability, Class 2A standard solutions, and test solutions for water-soluble and water-insoluble pharmaceuticals were prepared using 20 mL crimped capped vials (P/N 20-CV). The following USP <467> method performance criteria were met for water-soluble and water-insoluble products:

- The 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). PtP S/N calculations were made automatically in Chromeleon CDS according to the method described in the USP <621> system suitability section.[4]
- Chromatographic resolution (Rs) between the critical pair, acetonitrile and dichloromethane, was automatically calculated using the chromatography data system applying the formula for electronic integrator reported in the USP <621> system suitability section,[4] and confirmed to be >1, meeting the acceptance criteria as required by the regulation (Figure 2).

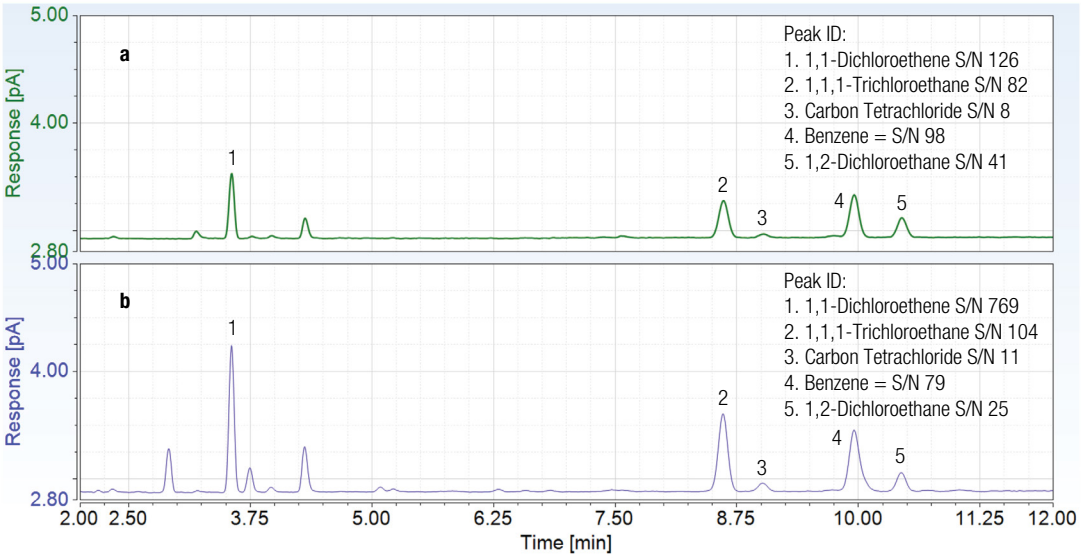

Figure 1. Peak-to-peak signal-to-noise (S/N) ratios for Class 1 system suitability solutions for water-soluble (a) and water-insoluble (b) products. Peaks with no annotation could not be identified from the FID data.

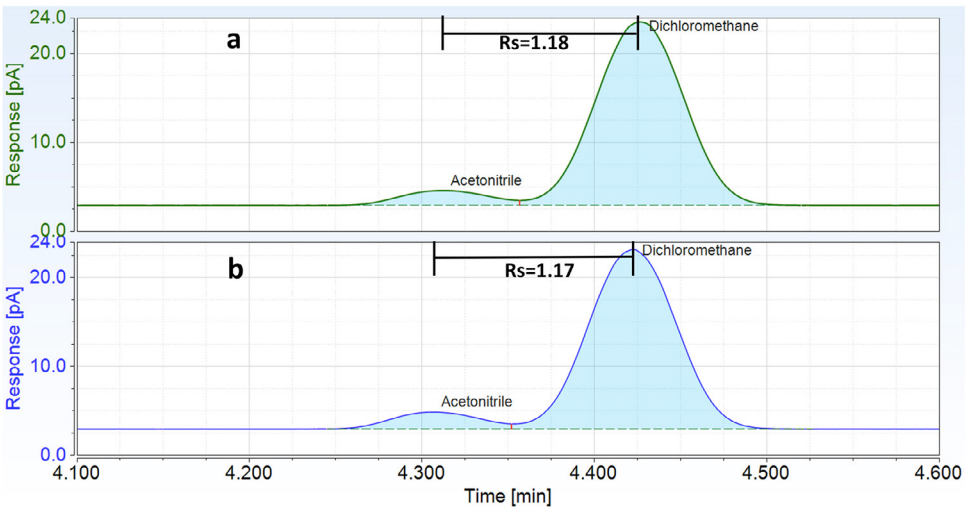

Figure 2. Chromatographic resolution (Rs) between acetonitrile and dichloromethane for water-soluble (a) and water-insoluble products (b). Resolution met the regulation requirements (Rs ≥1.0) with calculated values of 1.18 and 1.17 for water-soluble and water-insoluble pharmaceuticals, respectively.

Testing a pharmaceutical product that passes procedure A requirements

The pharmaceutical products (dispersive aspirin and paracetamol pain relief tablets) were analyzed unspiked for their residual solvent content. The results were compared with the standard solutions and confirmed that all solvents used during manufacturing process were efficiently removed as no residual solvents were detected (Figure 3). As an example, the peak profile obtained for dispersive aspirin unspiked solution (green) compared to Class 2A standard solution (blue) is reported in Figure 3.

Testing a pharmaceutical product failing procedure A

The pharmaceutical products (dispersive aspirin and paracetamol pain relief tablets) were spiked with residual solvents and 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 2, 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 4. Class 2A residual solvent peaks detected in the spiked sample solution showed higher peak areas compared to the ones in the corresponding Class 2A standard solution.

Peaks:

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. Ethylbenzene
13. *m*-Xylene
14. *p*-Xylene
15. *o*-Xylene
16. Cumene

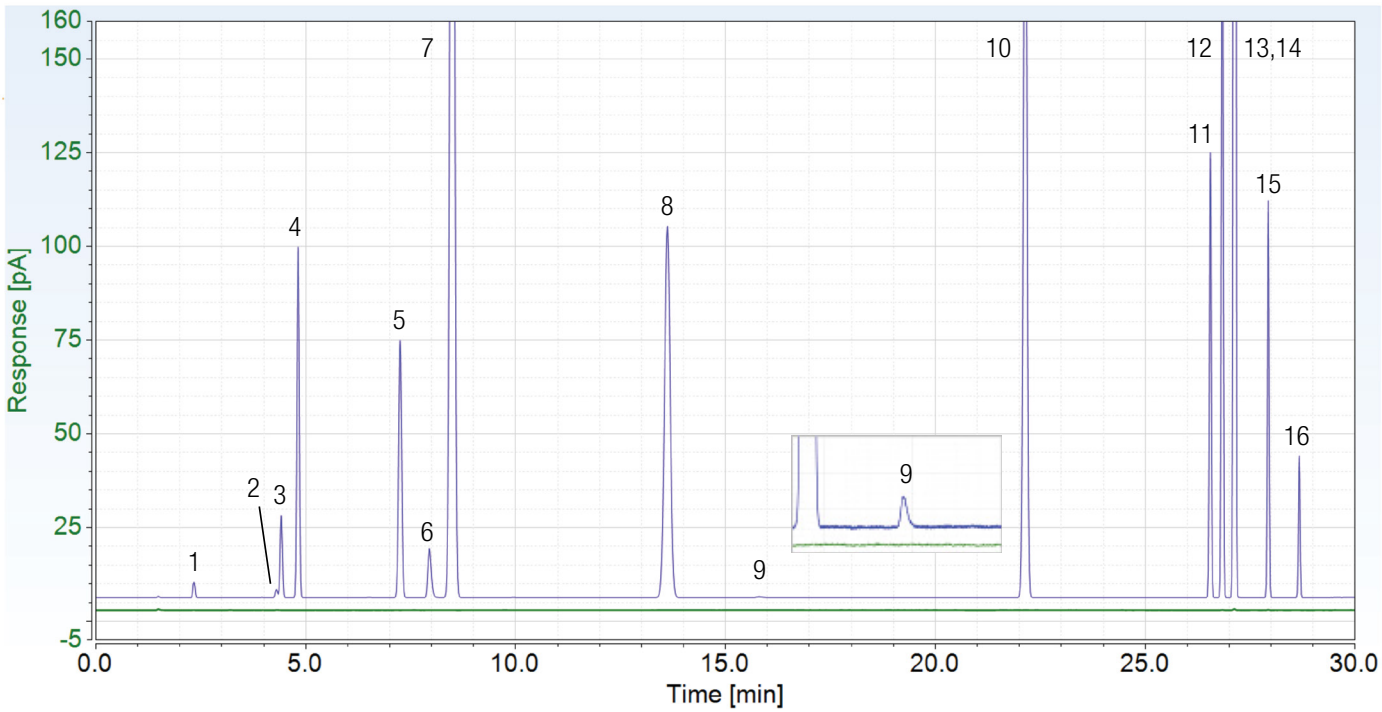

Figure 3. Comparison between Class 2A standard solution (blue) and acetylsalicylic solution (green). Criteria are met as no residual solvent peaks could be detected in the test sample.

Peaks:

1. Methanol
2. 1,1-Dichloroethene
3. Acetonitrile
4. Dichloromethane
5. *trans*-1,2-Dichloroethene
6. Hexane
7. Nitromethane
8. *cis*-1,2-Dichloerethene
9. Tetrahydrofuran
10. Chloroform
11. Cyclohexane/1,1,1-Trichloroethane
12. Benzene
13. 1,2-Dichloroethane/1,2-Dimetoxyethane
14. Trichloroethene
15. Methylcyclohexane
16. 1,4-Dioxane
17. Toluene/Pyridine
18. 2-Hexanone
19. Chlorobenzene
20. Ethylbenzene
21. *m*-Xylene/*p*-Xylene
22. *o*-Xylene
23. Cumene
24. Tetralin

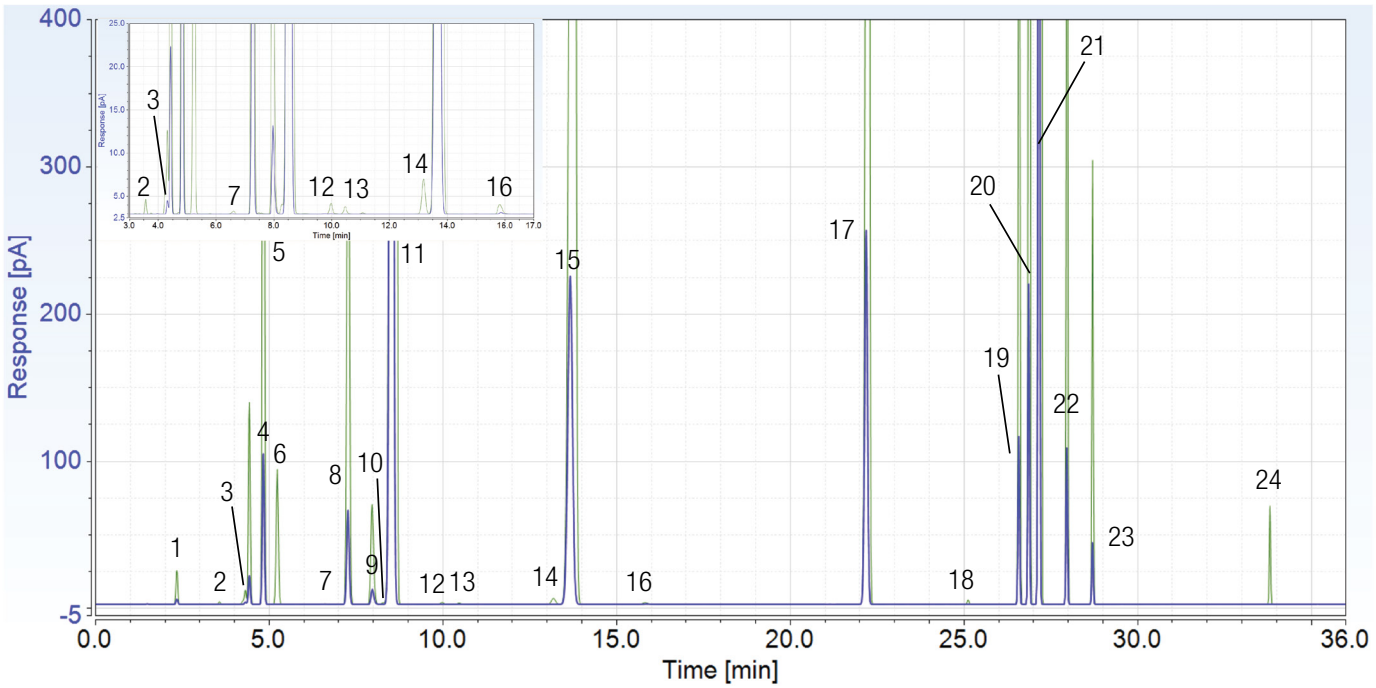

Figure 4. Comparison between peak profiles obtained for water-soluble spiked test solution (green) and Class 2A standard solution (blue). Class 2A peaks in the spiked solution showed higher responses compared to Class 2A standard solution. Unmatched green peaks belonged to either Class 1 or Class 2B residual solvents.

Procedure B: peak identity confirmation

According to the USP <467> for procedure B, the chromatographic TraceGOLD TG-624 GC column was replaced with a WAX column (TraceGOLD TG-WAXMS GC column, 30 m × 0.32 mm × 0.25 µm, P/N 26088-1430). Class 1, Class 1 System Suitability, Class 2A standard solutions and test solutions for water-soluble and insoluble pharmaceuticals were analyzed using the parameters reported in Table 1.

System sensitivity and resolution requirements have been assessed for procedure B:

- 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 5). Calculations for S/N were made automatically by the data system according to the method described in the USP <621> system suitability section.

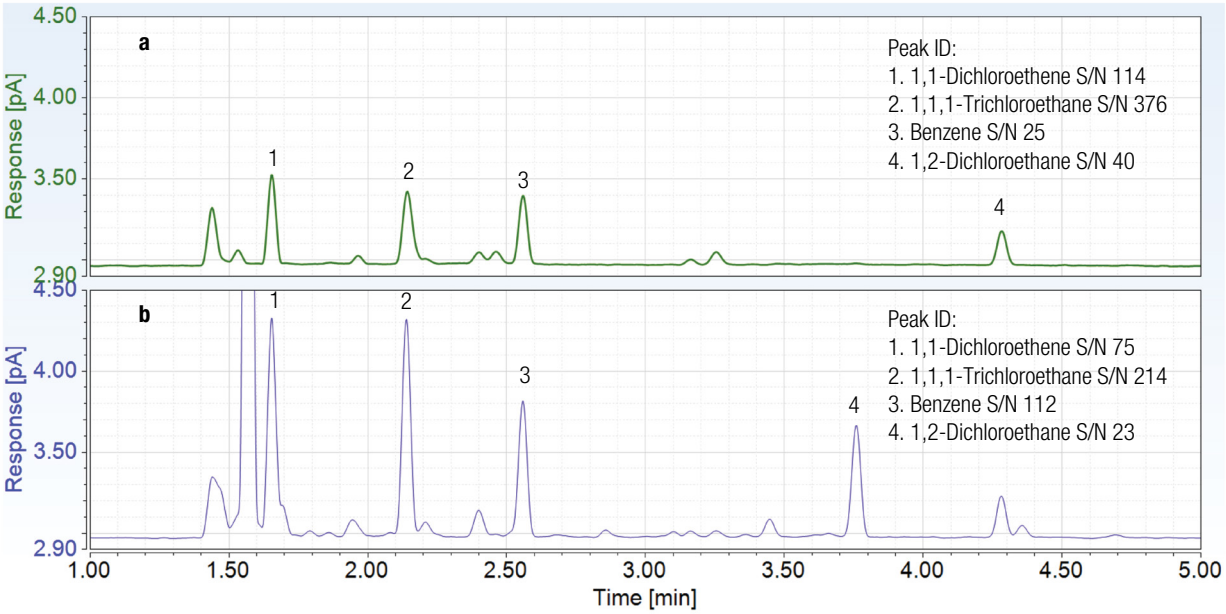

Figure 5. Peak-to-peak ratios for Class 1 system suitability solutions for water soluble (a) and water-insoluble (b) products. Peaks with no annotation could not be confirmed from the FID data.

- 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 6), meeting the acceptance criteria required (Rs ≥1.0). Chromatographic resolution has been automatically determined in Chromeleon CDS by applying the formula for electronic integrator reported in the USP <621> system suitability section.

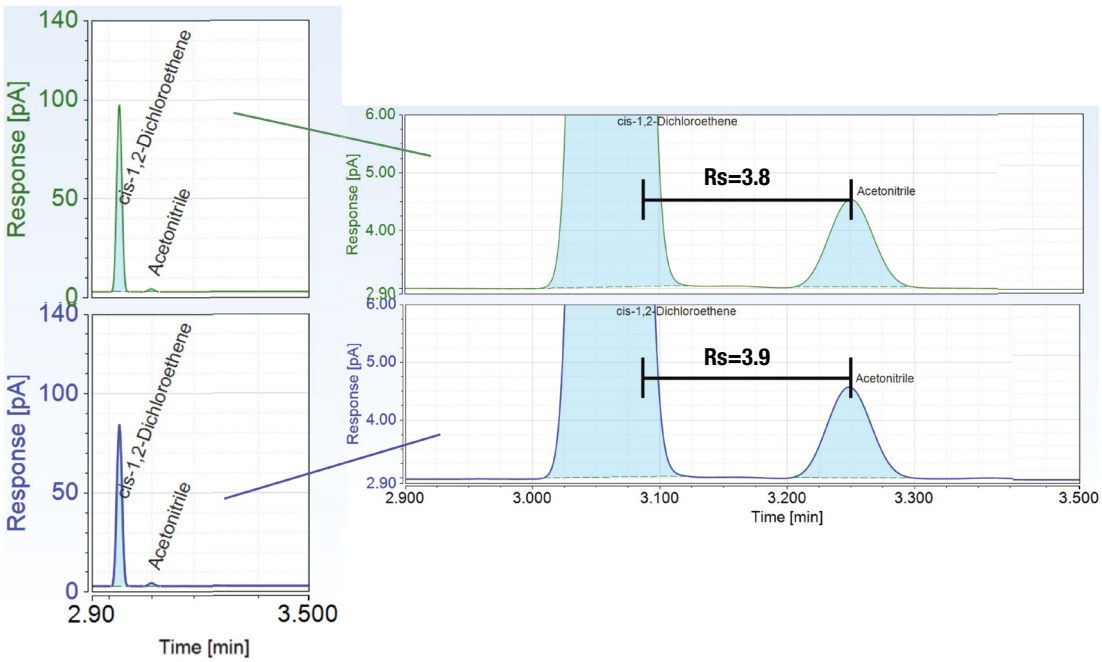

Figure 6. Chromatographic resolution for critical pair *cis*-1,2-dichloroethene/acetonitrile for water-soluble (top) and water-insoluble products (bottom). Peaks are baseline resolved meeting the USP <467> chromatographic resolution requirements (Rs ≥1.0).

Testing a pharmaceutical product matching Procedure B confirmation

Class 1, Class 2A, Class 2B standard solutions and spiked test solutions for water-soluble and water-insoluble pharmaceuticals were injected into the chromatographic system and the peak profiles were compared.

The peaks identified (procedure A) were confirmed (procedure B) as their responses were higher than the corresponding standards. Therefore, the levels of these residual solvents must be determined (procedure C). The chromatographic profile for water-soluble spiked solution and Class 2A standard solution is reported as an example in Figure 7.

Procedure C: quantification

Class 1, Class 1 System Suitability, and Class 2A standard solutions were injected into the chromatographic system. HS-GC parameters applied for procedure C are reported in Table 1. 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.

Peaks:

1. Hexane
2. Cyclohexane/1,1-Dichloroethene
3. Methylcyclohexane
4. *trans*-1,2-Dichloroethene
5. 1,1,1-Tricholoethane
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

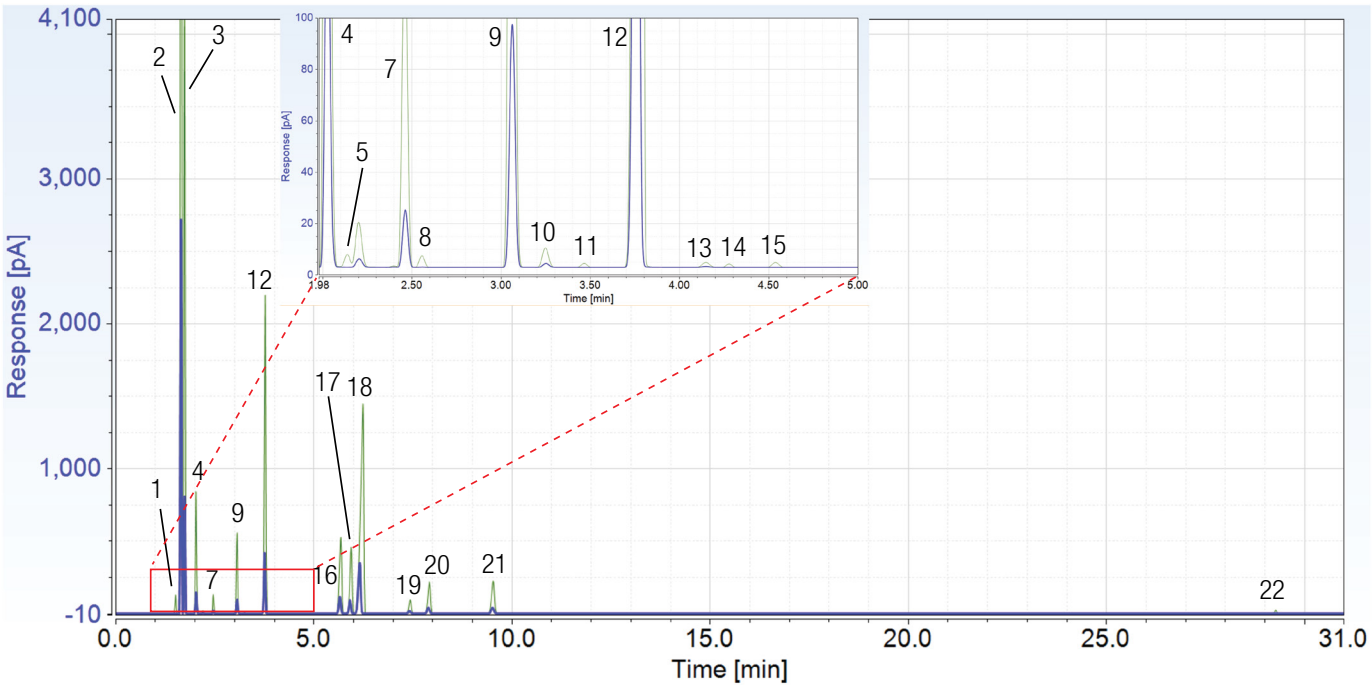

Figure 7. Comparison between peak profiles obtained for water-soluble spiked sample solution (green) and Class 2A standard solution (blue). Class 2A peaks in the spiked solution showed higher responses compared to Class 2A standard solution. Unmatched green peaks belonged to either Class 1 or Class 2B residual solvents.

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. As an example, the peak profile for spiked aspirin compared to spiked standard test solution is reported in Figure 8.

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.

System 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.

Peaks:

1. Methanol
2. 1,1-Dichloroethene
3. Acetonitrile
4. Dichloromethane
5. *trans*-1,2-Dichloroethene
6. Hexane
7. Nitromethane
8. *cis*-1,2-Dichloerethene
9. Tetrahydrofuran
10. Chloroform
11. Cyclohexane/1,1,1-Trichloroethane
12. Benzene
13. 1,2-Dichloroethane/1,2-Dimetoxyethane
14. Trichloroethene
15. Methylcyclohexane
16. 1,4-Dioxane
17. Toluene/Pyridine
18. 2-Hexanone
19. Chlorobenzene
20. Ethylbenzene
21. *m*-Xylene/*p*-Xylene
22. *o*-Xylene
23. Cumene
24. Tetralin

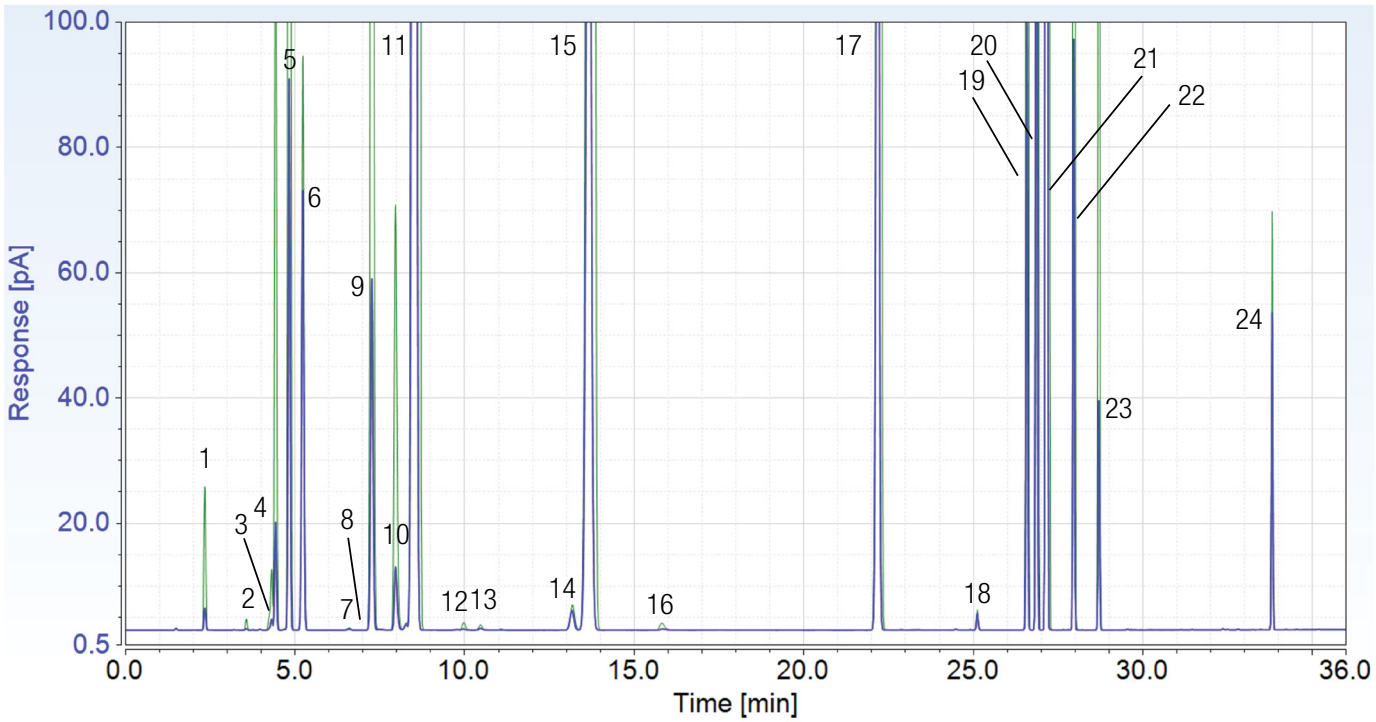

Figure 8. Comparison between peak profiles obtained for spiked aspirin solution (green chromatogram) and standard test solution (blue chromatogram). Class 1, Class 2A, and Class 2B peaks in the spiked sample solution showed higher responses compared to standard test solution.

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 3. Peak area %RSDs obtained from n=18 consecutive injections using water and DMSO as diluents for the concentrated standard solutions. Average %RSDs for the assessments are <3% for all residual solvent classes.

Compound Name	%RSD (n=18)	
Class 1	**Diluent: water**	**Diluent: DMSO**
1,1-Dichloroethene	1.5	0.7
1,1,1-Trichloroethane	1.0	0.8
Carbon Tetrachloride	4.9	2.9
Benzene	0.8	0.9
1,2-Dichloroethane	1.6	1.0
Average %RSD	**2.0**	**1.3**
Class 2A	**Diluent: water**	**Diluent: DMSO**
Methanol	0.7	1.4
Acetonitrile	0.8	1.6
Dichloromethane	3.1	0.7
trans 1,2-Dichloroethene	4.0	1.2
cis 1,2-Dichloroethene	3.4	0.8
Tetrahydrofuran	0.9	1.4
Cyclohexane	3.6	2.8
Methycyclohexane	3.0	2.4
1,4-Dioxane	1.3	1.9
Toluene	3.6	0.8
Chlorobenzene	3.3	0.7
Ehylbenzene	3.4	0.9
m-Xylene/*p*-Xylene	3.3	0.9
o-Xylene	3.1	0.8
Average %RSD	**2.7**	**1.3**
Class 2B	**Diluent: water**	**Diluent: DMSO**
Hexane	1.2	0.8
Nitromethane	2.9	1.5
Chloroform	0.9	1.0
1,2-Dimethoxyethane	1.4	0.9
Trichloroethylene	1.9	0.7
Pyridine	0.8	1.4
2-Hexanone	0.6	0.4
Tetralin	0.9	0.6
Average %RSD	**1.3**	**0.9**

System linearity

System linearity was assessed by serially diluting the stock solutions for Class 1, Class 2A, and Class 2B residual solvents as described in the USP <467> method (procedure C for water-insoluble pharmaceutical products). This way, four calibration levels were obtained: at 12.5%, 25%, 50%, and 100% of the concentration limit. Prior to analysis, 1 mL of each calibration solution was added to 5 mL water corresponding to 50 mg real sample. Each calibration level was prepared and analyzed in triplicate. Residual solvents showed good linear responses with an average coefficient of determination R^2=0.998 as reported in Table 4. Moreover, the relative standard deviation (%RSD) of the residuals across each calibration level was <8% indicating good linearity.

Table 4. Correlation coefficients (R^2) and relative standard deviation of residuals (%RSD) obtained over four calibration levels at 12.5, 25, 50, and 100%. Data analyzed in triplicate.

Compound Name	Concentration Range (µg/g)	Correlation Coefficient (R^2)	Residuals Standard Deviation (% RSD)
Class 1			
1,1-Dichloroethene	1.0–8.0	1.00	2.0
1,1,1-Trichloroethane	187.5–1500	0.999	2.9
Carbon Tetrachloride	0.5–4.0	0.997	6.9
Benzene	0.3–2.0	0.999	3.4
1,2-Dichloroethane	0.6–5.0	0.999	2.4
Class 2A			
Methanol	375–3000	1.00	1.4
Acetonitrile	51.3–410	1.00	1.7
Dichloromethane	75–600	0.998	4.2
trans 1,2-Dichloroethene	233.8–935	0.999	2.9
cis 1,2-Dichloroethene	233.8–935	0.998	5.0
Tetrahydrofuran	90–720	1.00	2.2
Cyclohexane	422.5–3880	0.999	3.0
Methycyclohexane	147.5–1180	1.00	2.5
1,4-Dioxane	47.5–380	1.00	1.5
Toluene	111.3–890	0.997	5.6
Chlorobenzene	45–360	0.995	6.5
Ehylbenzene	46.1–369	0.997	5.3
m-Xylene	162.8–1302	0.996	6.0
p-Xylene	162.8–1302	0.996	6.0
o-Xylene	24.4–195	0.997	5.6
Class 2B			
Hexane	36.3–290	0.998	5.8
Nitromethane	6.3–50	0.998	4.8
Chloroform	7.5–60	0.997	5.6
Thrichloroethene	10–80	0.999	2.9
2-Hexanone	6.3–50	0.992	7.8
Tetralin	12.5-100	0.999	3.0

Examples of calibration curves for benzene (Class 1), methylcyclohexane (Class 2A), and trichloroethene (Classs 2B) are shown in Figure 9.

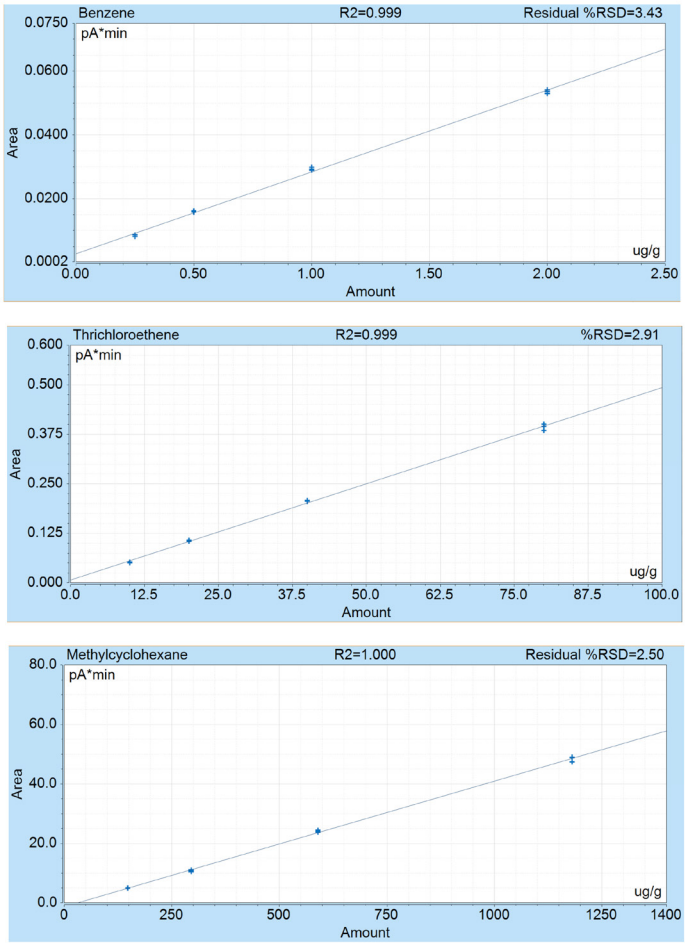

Figure 9. Examples of calibration curves for benzene, methylcyclohexane, and trichloroethene. Linearity is shown from 0.01 to 0.1 µg/mL (corresponding to 0.25–2.0 µg/g* in 50 mg pharmaceutical product) for benzene, at 7.4 to 59 µg/mL corresponding to 147–1180 µg/g*) for methylcyclohexane, at 0.5 to 4.0 µg/mL (corresponding to 10–80 µg/g*) for tricholoethene. For each calibration level n=3 replicates.

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 the 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 outstanding repeatability and precision in routine analysis. This was demonstrated by excellent peak area response obtained (average peak area %RSDs for n=18 consecutive injections was <3%).
- Sensitive compound detection can be easily achieved with the Instant Connect FID. Moreover, the TraceGOLD TG-624 column allowed to easily meet and exceed USP <467> method resolutionrequirement (Rs ≥1.0), delivering expected chromatographic separation.
- 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.
- Chromeleon CDS software (compliant with the Title 21 CFR Part 11 requirements) ensures sample integrity, traceability, and effective data management from instrument control to the final report.

Overall these results demonstrate that the TriPlus 500 HS autosampler provides unparalleled levels of performance making it a reliable and robust analytical solution for routine laboratories.

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Learn More Today

For more information about the TriPlus 500 Headspace Autosampler, to request a demo, or to discuss your GC/GC-MS needs, please contact your Thermo Scientific representative today.

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