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Pharma

Rapid and cost-effective determination of Class 3 residual solvents in pharmaceutical products by HS-GC with hydrogen as carrier gas

Authors

Giulia Riccardino[1], Daniela Cavagnino[1], Carlo Ceciliato[1], and Daniel Kutscher[2]

[1]Thermo Fisher Scientific, Milan, IT

[2]Thermo Fisher Scientific, Bremen, DE

Keywords

Class 3 residual solvents, USP <467>, valve and loop static headspace, TriPlus 500 headspace autosampler, gas chromatography, GC, TRACE 1610 gas chromatograph, flame ionization detector (FID), mass spectrometry detector, hydrogen carrier gas

Goal

The aim of this study is to demonstrate the suitability of the Thermo Scientific™ TriPlus™ 500 static headspace autosampler in combination to the Thermo Scientific™ TRACE™ 1610 gas chromatograph to run an optimized rapid method for the determination of Class 3 residual solvents in pharmaceutical products.

Introduction

The concept of green chemistry dates back to 1991 when the United States Environmental Protection Agency (EPA) launched the Alternative Synthetic Pathways for Pollution Prevention[1] research program with the objective of reducing/eliminating the use of hazardous substances, including the development of greener solvents and safer chemicals. Solvents play a major role in the manufacturing process of pharmaceuticals, impacting yield, purity, and final product solubility. This led many pharmaceutical companies to further develop their production processes by applying the green chemistry principles to reduce their environmental footprint, solvent waste, and disposal costs. Since solvents do not provide any therapeutic benefit to patients, they should be removed from the final product before commercialization. To ensure patient safety, it is important 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 stated in the United States Pharmacopeia (USP) General Chapter <467>.[2] In accordance with the International Conference on Harmonisation (ICH) guidelines,[3] USP <467> classifies the residual solvents based on their toxicity[2] as follows: solvents with unacceptable toxicity (Class 1), solvents with less severe toxicity (Class 2), and solvents with low toxicity (Class 3). In particular, Class 3 solvents (Table 1) can be considered the preferred choice for the production processes as none are known to pose a hazard to human health at levels normally accepted in pharmaceuticals (<50 mg/day, corresponding to 5,000 ppm or 0.5% w/w).[2] Therefore, they are considered green solvents, environmentally preferable compared to Class 1 and Class 2 solvents.[4,5]

Table 1. Class 3 residual solvents listed in the USP <467> method

Class 3 residual solvents	
Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Triethylamine

Gas chromatography (GC) coupled to the static headspace (HS) sampling technique[6] is the method of choice for the analysis of residual organic solvents in pharmaceutical products. Organic solvents have relatively low boiling points and good thermal stability and can be easily extracted from the non-volatile matrix by heating the sample.

In this study, a fast and cost-effective analytical method was optimized for the determination of Class 3 solvent residues in pharmaceutical products using static headspace sampling. The injected sample was split between a flame ionization detector, used for targeted quantitative analysis, and a mass spectrometer, used for putative identification of target solvents as well as unknown compounds possibly present in the sample. In addition, the proposed method also provides a viable alternative for the determination of Class 1 and Class 2 residual solvents by meeting the USP <467> system suitability requirements. The determination of Class 1 and Class 2 residual solvents in accordance with the official method conditions stated in USP <467> is reported in a previous application note.[7]

Experimental

In this study, a TriPlus 500 valve and loop static headspace autosampler configured for 240-vial capacity was coupled to a TRACE 1610 GC equipped with a Thermo Scientific™ iConnect™ split/splitless injector (SSL). A Thermo Scientific™ Dual Detector Microfluidics device (P/N 19071030) was used to split 1:1 the carrier gas flow from the analytical column between a Thermo Scientific™ iConnect Flame Ionization Detector (FID) and a Thermo Scientific™ TSQ™ 9610 Triple Quadrupole MS System. Please note that the TSQ 9610 GC-MS/MS system was operated in full scan (FS) mode only; therefore, similar performance can be achieved with a single quadrupole system, like the Thermo Scientific™ ISQ™ 7610 GC-MS.

Chromatographic separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-624SilMS, 30 m × 0.32 mm × 1.8 µm column (P/N 26059-3390). This column provided high inertness and thermal stability with maximum temperatures up to 320 °C. The high phase thickness makes this column ideal for volatile organics analysis. In these experiments, hydrogen was preferred as the carrier gas as a renewable alternative to helium combined with favorable properties in terms of separation efficiency and speed. The vial pressurization in the TriPlus 500 headspace autosampler was achieved with nitrogen. Experimental conditions applied for the analysis of Class 3 residual solvents are reported in Table 2.

The TriPlus 500 headspace autosampler is directly connected to the analytical column by-passing the SSL injector, which is used only for the pneumatic control of the carrier gas. The direct connection to the column ensures sample integrity during transfer with highly precise injections and no carryover effects.

Standard and sample preparation

Class 3 residual solvent mix A (5,000 µg/mL in *N,N*-dimethylformamide) was purchased from Restek™ (P/N 36013) and diluted in HPLC-MS grade water (Fisher Scientific P/N W-0112-17) to obtain a standard solution at a concentration of 500 µg/mL. Limit test solution at 5,000 µg/g, corresponding to the maximum accepted intake level of 50 mg/day, was obtained by spiking an aliquot (200 µL) of the standard solution in 2 mL water (corresponding to the preparation of 20 mg real sample).

Table 2. HS-GC and TSQ 9610 triple quadrupole MS analytical parameters used for Class 3 solvent content determination

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)	150
Vial pressure equilibration time (min)	1
Loop size (mL)	1
Loop/sample path temperature (°C)	80
Loop filling pressure (kPa)	70
Loop equilibration time (min)	1
Injection mode	Standard
Injection time (min)	1
Needle purge flow level	2

TSQ 9610 Triple Quadrupole MS parameters	
Ion source	NV-AEI
Transfer line temperature(°C)	280
Source temperature (°C)	300
Ionization mode	EI
Electron energy (eV)	50
Emission current (µA)	10
Acquisition mode	Full Scan (*m/z* 30–400)

TRACE 1610 GC parameters	
Inlet module and mode	SSL, split
Split flow for Class 3 residual solvents (mL/min)	89.2
Split ratio for Class 3 residual solvents	10:1
Split flow for Class 1 and Class 2 residual solvents (mL/min)	44
Split ratio for Class 1 and Class 2 residual solvents	5:1
Septum purge mode, flow (mL/min)	Constant, 5
Carrier gas, carrier mode, pressure (kPa)	H_2, constant pressure, 70
Oven temperature program	
Temperature 1 (°C)	40
Hold time (min)	3
Rate (°C/min):	8
Temperature 2 (°C)	60
Rate (°C/min)	15
Temperature 3 (°C)	95
Rate (°C/min)	15
Temperature 4 (°C)	230
Hold time (min)	2.5
Total GC run time	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	
TraceGOLD TG-624SilMS (P/N 26059-3390)	30 m × 0.32 mm × 1.8 µm

Sample preparation

Dispersive aspirin (acetylsalicylic acid, 75 mg) was purchased at a local prescription counter. A model sample solution was obtained by diluting 1,000 mg (± 0.1) of dispersive aspirin into 100 mL of HPLC-MS grade water. An aliquot (2 mL) of this solution, corresponding to 20 mg, was transferred into a 20 mL crimp cap headspace vial (P/N 6ACV20-1R, caps P/N 6PBCC20-ST3) and seated in the autosampler tray for analysis. The remaining part of the prepared sample solution was used as diluent for the standards used for linearity, MDL, and repeatability assessments.

Calibration solution preparation

Linearity was assessed by diluting the Class 3 standard solution with the sample solution to obtain seven matrix-matched calibration levels ranging from 100 µg/g to 15,000 µg/g (corresponding to 1 mg/day to 150 mg/day). An aliquot (2 mL) of each calibration standard was transferred into a 20 mL crimp cap HS vial. Each calibration level was prepared in duplicate.

Minimum detectable level (MDL) and peak area repeatability were assessed by preparing two sets of HS vials (n=8) containing 2 mL of matrix-matched solutions at 250 and 500 µg/g (2.5 and 5.0 mg/day), respectively.

Data acquisition, processing, and reporting

For the experiments described here, the Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used. The instrument control is fully integrated in the CDS, ensuring a streamlined automated workflow for sample analysis and data review with minimal user intervention. Moreover, with the evolving requirements for data integrity and data security, Chromeleon CDS provides a secure platform for analytical laboratories to comply with modern regulatory guidelines including FDA 21 CFR Part 11 and European Commission (EU) Annex 11. Chromeleon CDS offers an extension package for full method validation based on ICH guidelines. The predefined eWorkflow™ templates allow the user to create a complete sequence in a few mouse clicks as all the associated files and methods are pre-set. The complete data processing can be done in Chromeleon CDS with predefined processing methods, which only require a few user-defined adjustments. Moreover, reporting is quick and easy because results are provided on a single sheet.[8]

Results and discussion

Chromatography

Headspace sampling allows for the extraction of the target volatile analytes in a fast and simple way without the need for time-consuming sample preparation. The microfluidic device was positioned after the separation column and used to split the effluents 1:1 to the FID and the mass spectrometer, ensuring a minimal effect on the retention times (max RT shifts 0.02 min), as demonstrated in Figure 1. The high thermal stability and superior inertness of the TraceGOLD TG-624SilMS column combined with the use of hydrogen as carrier gas, ensured baseline chromatographic separation, with resolution (R_s) > 1.1 in a short analysis time (<11 minutes) for all target compounds with the exception of ethyl formate and 2-propanol, which required a slightly more polar stationary phase to achieve baseline chromatographic separation. The two components could be putatively identified using mass spectrometry detection based on NIST20 spectral library match after full scan data deconvolution using the Thermo Scientific™ Deconvolution Plugin software. An example of the FID and MS chromatograms for the limit test solution at 5,000 µg/g (50 mg/day), demonstrating the achieved chromatographic separation as well as the deconvoluted spectra for ethyl formate and 2-propanol, is shown in Figure 1.

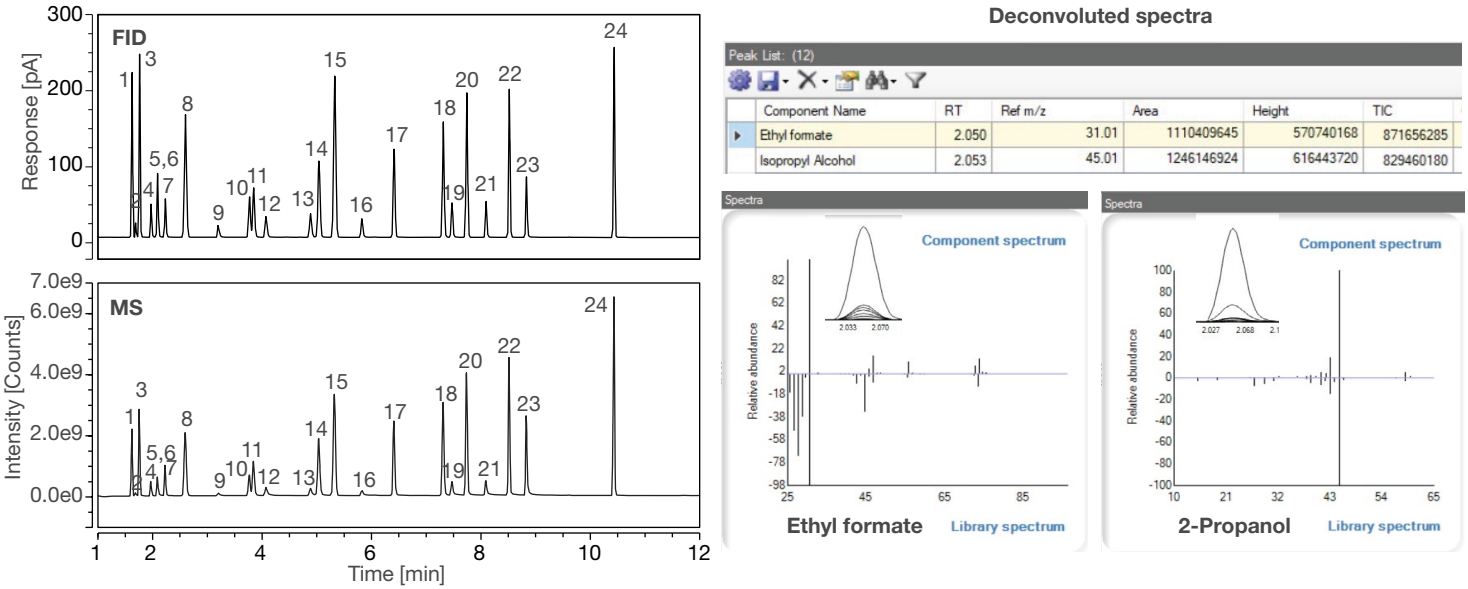
Testing a pharmaceutical product

The Class 3 standard solution at the limit threshold of 5,000 µg/g (or 50 mg/day) and the sample solution were injected into the chromatographic system. Peak responses obtained for the sample solution were compared with the corresponding peaks in the Class 3 standard chromatogram.

A small amount of residual acetone was detected in the sample, and it resulted well below the limit threshold of 5,000 µg/g or 50 mg/day, as demonstrated in Figure 2. The identity of the residual acetone was confirmed by i) RT comparison with the Class 3 standard solution (FID and MS FS traces) and ii) mass spectrum match with NIST20 spectral library (MS FS trace). An unknown peak was also detected in the sample solution at 11.75 minutes. This peak was putatively identified as D-limonene based on NIST20 spectral library match with a search index score (SI) of 897 and reverse index score (RSI) of 901 (Figure 2). This was in agreement with the ingredients declared by the manufacturer in the package leaflet.

Linearity

Linearity was assessed by running a seven-point matrix-matched calibration curve ranging from 100 µg/g to 15,000 µg/g (corresponding to 1 mg/day to 150 mg/day), with each calibration point prepared in duplicate. The calibration curve was plotted using the external calibration function. The investigated solvents showed good linear responses with average coefficient of determination R^2 of 0.997 and residual values (measured as % RSD of the average response factors (AvCF)) <20%, thus confirming the linear trend (Table 3). Examples of calibration curves achieved for some selected compounds across the eluting range are shown in Figure 3.



1=Pentane, 2=Ethanol, 3=Ethyl ether, 4=Acetone, 5=Ethyl formate, 6=2-Propanol, 7=Methyl acetate, 8=tert-Butylmethyl ether, 9=1-Propanol, 10=2-Butanone, 11=Ethyl acetate, 12=2-Butanol, 13=Isobutanol, 14=Isopropyl acetate, 15=Heptane, 16=1-Butanol, 17=n-Propyl acetate, 18=Methylisobutylketone, 19=3-Methyl-1-butanol, 20=Isobutyl acetate, 21=1-Pentanol, 22=Butyl acetate, 23=N,N-Dimethylformammide, 24=Anisole

Figure 1. FID and MS (full scan) chromatograms for Class 3 limit test solution as well as deconvoluted spectra for ethyl formate and 2-propanol (RT=2.050 and 2.053 min, respectively)

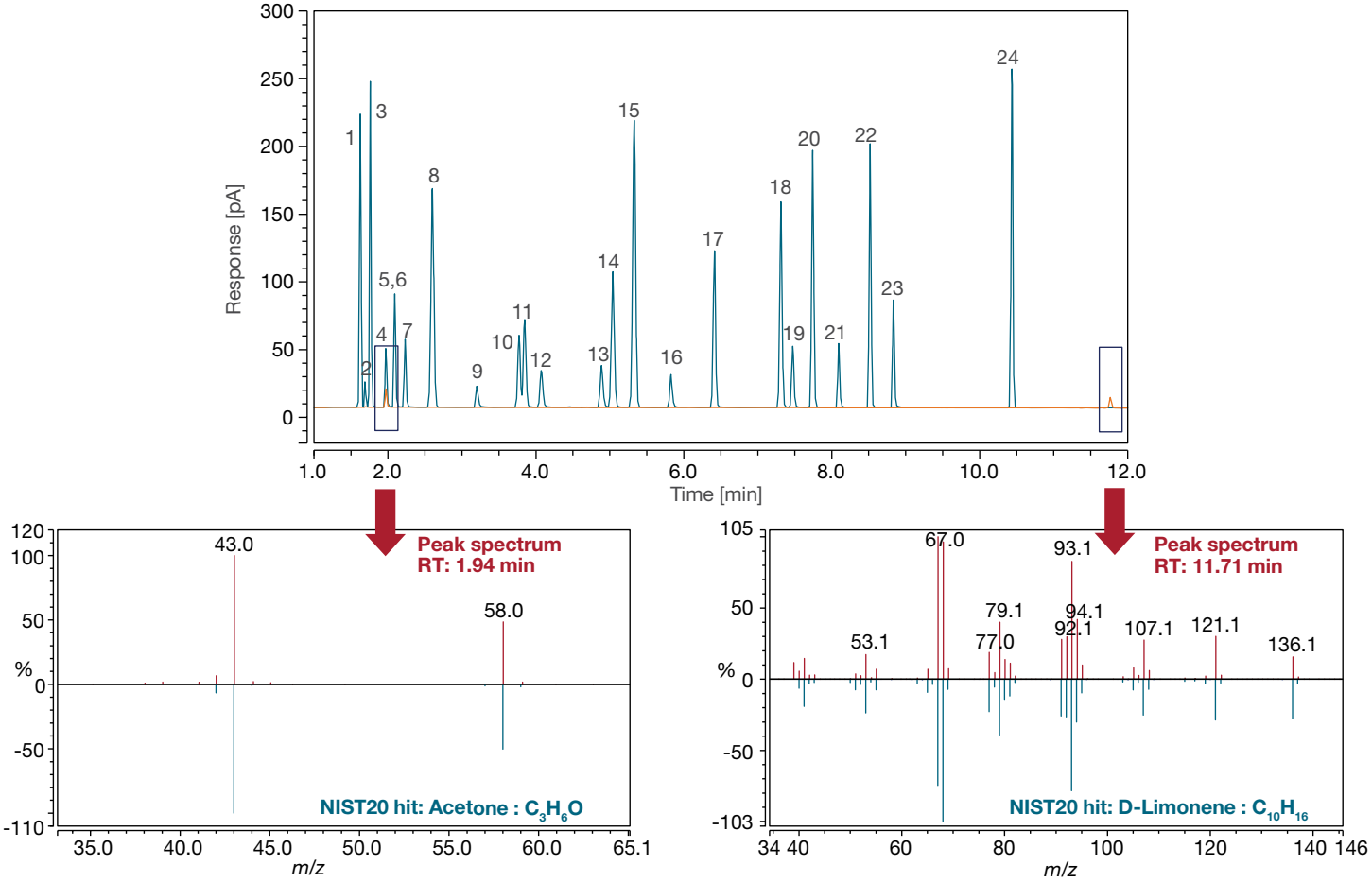


Figure 2. Comparison between Class 3 limit test solution (blue) and acetylsalicylic acid solution (orange). Residual acetone was found to be below the limit threshold of 5,000 µg/g (or 50 mg/day). D-Limonene was also identified in the sample, in agreement with the ingredients declared by the manufacturer.

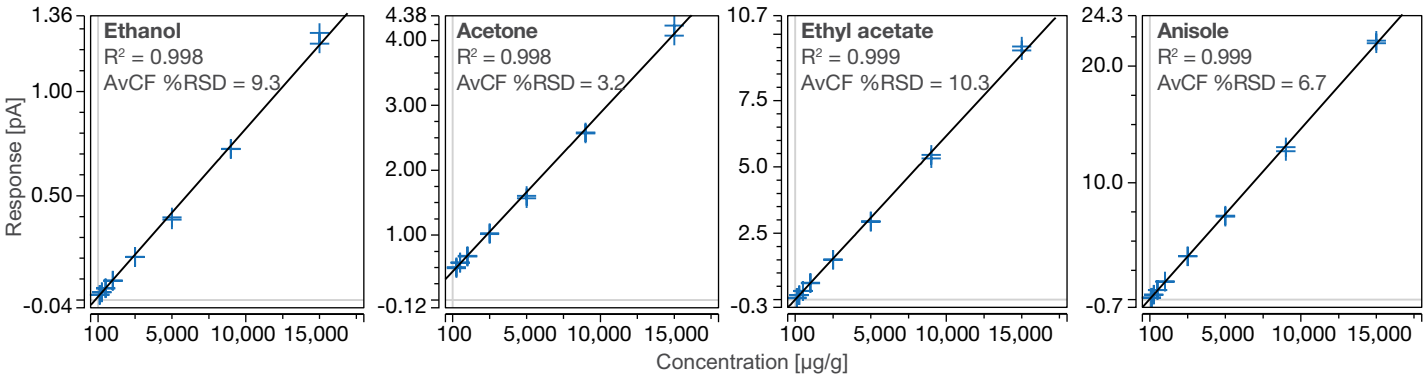


Figure 3. Examples of calibration curves obtained by injecting a seven-point matrix-matched calibration curve ranging from 100 µg/g to 15,000 µg/g (corresponding to 1 mg/day to 150 mg/day). Each calibration point was prepared in duplicate.

MDLs were calculated by injecting two sets (n=8 vials each) of sample solution spiked with the solvent standard solution at a final concentration of 250 and 500 µg/g (2.5 and 5.0 mg/day), respectively.

Calculated MDLs were <100 µg/g (or 1.0 mg/day) for all investigated compounds, with the exception of pentane, acetone, and heptane for which the calculated MDLs were 180, 140, and 140 µg/g, respectively. The average peak area repeatability at the levels used for MDL assessment showed an average RSD of 2.6%. The calculated concentrations were within 20% of the spiked amount with a recovery ranging from 85 to 110% (Table 3).

Table 3. Calibration ranges as well as calculated R^2, AvCF %RSD, calculated MDLs, peak area %RSD, and %recovery obtained for the investigated green solvents

						Calculated MDL			
Peak name	Ret. time (min)	Calibration range (µg/g)	Coefficient of determination (R^2)	AvCF %RSD	Level spiked for MDL (µg/g)	µg/g	mg/day	Peak area %RSD (n=8)	%Recovery
Pentane	1.60	250–15,000	0.992	13.9	500	180	1.8	6.4	85
Ethanol	1.66	100–15,000	0.998	9.3	250	40	0.4	3.1	94
Ethyl ether	1.74	100–15,000	0.999	9.9	250	10	0.1	0.7	110
Acetone	1.94	250–15,000	0.998	3.2	500	140	1.4	2	109
Ethyl formate*	2.05	100–15,000	0.998	13.0	250	30	0.3	4.2	89
2-Propanol*	2.05	100–15,000	0.999	6.2	250	70	0.7	6.5	92
Methyl acetate	2.20	100–15,000	0.999	10.2	250	10	0.1	1.7	105
tert-Butylmethyl ether	2.56	100–15,000	0.998	13.4	250	10	0.1	0.6	109
1-Propanol	3.15	100–15,000	0.999	12.6	250	20	0.2	4.1	104
2-Butanone	3.72	100–15,000	0.999	10.3	250	10	0.1	1.4	105
Ethyl Acetate	3.79	100–15,000	0.999	10.3	250	10	0.1	1.4	107
2-Butanol	4.02	100–15,000	0.999	9.4	250	20	0.2	2.5	106
Isobutanol	4.83	100–15,000	0.999	8.8	250	30	0.3	3.3	105
Isopropyl acetate	4.98	100–15,000	0.998	12.1	250	10	0.1	1.3	107
Heptane	5.27	250–15,000	0.994	16.5	500	140	1.4	9.7	91
1-Butanol	5.77	100–15,000	0.999	10.4	250	20	0.2	2.5	105
n-Propyl acetate	6.36	100–15,000	0.999	11.4	250	10	0.1	0.7	106
Methylisobutylketone	7.26	100–15,000	0.999	9.8	250	10	0.1	0.8	106
3-Methyl-1-butanol	7.43	100–15,000	0.999	7.2	250	20	0.2	2.6	104
Isobutyl acetate	7.70	100–15,000	0.998	12.3	250	10	0.1	0.6	106
1-Pentanol	8.05	100–15,000	0.999	7.3	250	20	0.2	2.4	105
Butyl acetate	8.48	100–15,000	0.998	12.1	250	10	0.1	0.8	106
Anisole	10.39	100–15,000	0.999	6.7	250	10	0.1	1.1	106

*Values derived from MS deconvoluted data due to peak co-elution: Ethyl formate quantitation ion: *m/z* = 74, 2-Propanol quantitation ion: *m/z*=59

Method suitability for analysis of Class 1 and Class 2 residual solvents

The optimized chromatographic conditions applied for Class 3 were also tested for Class 1 and Class 2 residual solvents. The USP General Notices and Requirements[9] allows for the use of alternative methods, but they shall be validated and must be shown to give equivalent or better results compared to the USP standard methods. Class 1 standard and system suitability solutions as well as Class 2 standard solution were prepared according to the procedure stated in the USP <467> method. Then, the chromatographic conditions optimized for the analysis of Class 3 solvents were applied.

As demonstrated in Figure 4, USP <467> system suitability requirements were met for both Class 1 and Class 2 residual solvents with:

- Peak-to-peak signal-to-noise ratio (S/N) for 1,1,1-trichloroethane in Class 1 standard solution >5:1
- Peak-to-peak S/N of each peak in Class 1 system suitability >3
- Resolution between acetonitrile and dichloromethane in Class 2 standard solution >1.0

The use of optimized chromatographic conditions allows for a >4x improvement in Class 1 and Class 2 residual solvent analysis speed without compromising on resolution and method performance.

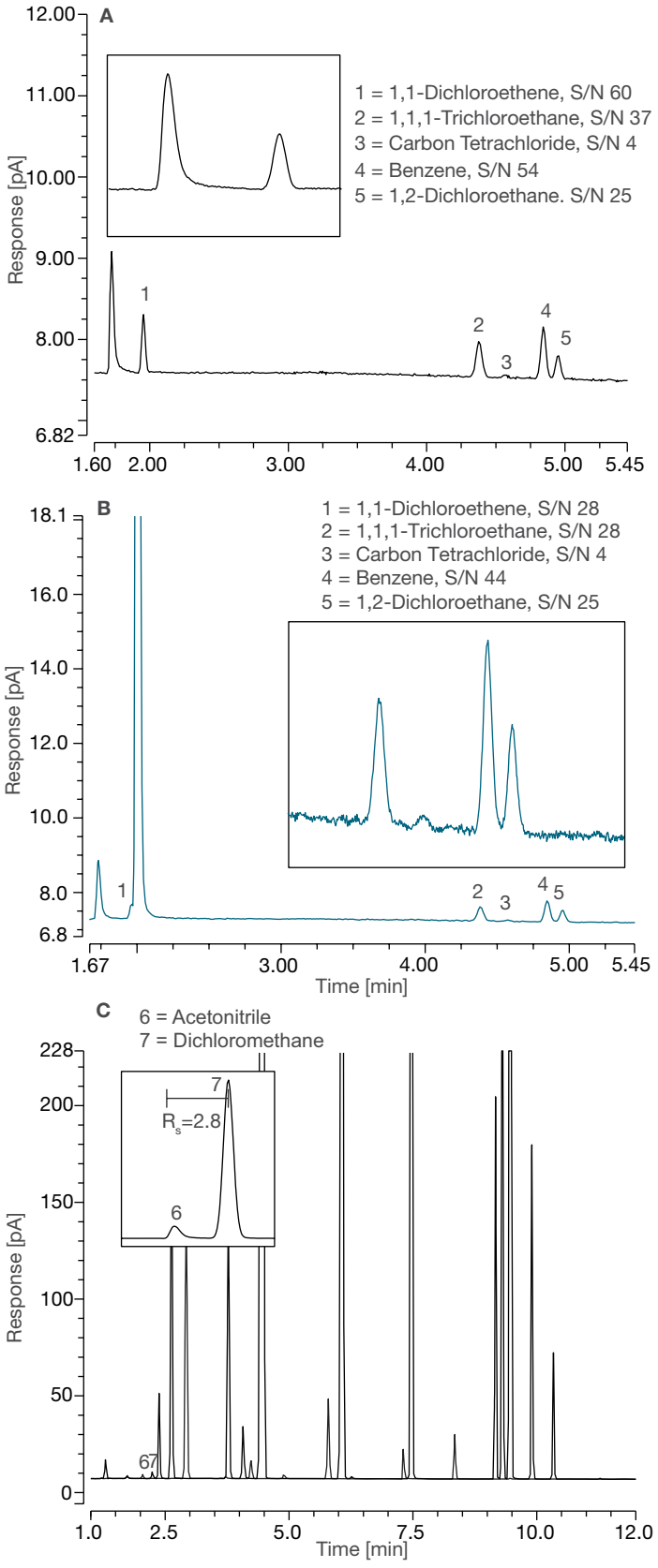


Figure 4. USP <467> system suitability requirements were met for both Class 1 and Class 2 residual solvents with: peak-to-peak S/N for 1,1,1-trichloroethane in Class 1 standard solution >5:1 (A); peak-to-peak S/N of each peak in Class 1 system suitability >3 (B); and resolution between acetonitrile and dichloromethane in Class 2 standard solution >1.0 (C)

Conclusions

The TriPlus 500 headspace autosampler in combination with the TRACE 1610 GC system offers QA/QC laboratories a quick method for testing residual solvents in pharmaceutical products, ensuring confident quantitative and qualitative results for Class 1, 2, and 3 solvents.

- The high column efficiency combined with the use of hydrogen as the carrier gas allowed for a fast GC oven ramp, maintaining adequate chromatographic separation ($R_s \geq 1.1$) for all analyzed compounds, thus supporting shorter analysis time and high sample throughput to easily meet the needs of QA/QC laboratories.
- The dual detector configuration FID/MS increased the confidence in compound identification and quantitation in case of co-elution of analytes, thanks to deconvoluted MS data. Putative identification of unknown peaks possibly occuring in the analyzed samples is also achieved through comparison with the NIST20 spectral library.
- The efficient pneumatic control during sampling and injection, high inertness of the sample path, and consistent analyte transfer through the headspace direct interface with the analytical column ensured reliable performance in terms of linearity (average R^2 of 0.997 and AvCF %RSD < 20%), sensitivity (average calculated MDL < 40 µg/g (or 0.4 mg/day)), % recovery (ranging from 85 to 110%), and peak area repeatability (average % RSDs of 2.6 for n=8 consecutive injections).
- Chromeleon CDS (compliant with the FDA 21 CFR Part 11 requirements) ensured data integrity, traceability, and effective data management from instrument control to the data review with minimal user intervention.
- The optimized chromatographic conditions presented in this study for Class 3 solvents had proven successful to meet the system suitability criteria specified in the USP <467> method also for analysis of Class 1 and Class 2 solvents, therefore representing a rapid, cost-effective, and high-throughput alternative to classical conditions stated in the USP <467> for analysis of residual solvents.

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