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Headspace Analysis: Reproducibility of Syringe Headspace Injections for Aqueous Samples

Headspace Analysis: Reproducibility of Syringe Headspace Injections for Aqueous Samples

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Abstract

This article describes the optimization of residual solvents analysis according USP <467> and Ph.Eur. 2.4.24 using the automated syringe headspace technique on GC-FID and GC-MS systems. The precision of analysis ranged from 4.3 to 7.8 % RSD (GC-FID) for class 2 solvents for all analytes investigated in this study. These values fully comply with USP <467> and Ph. Eur. 2.4.24 requirements.

Keywords

Headspace analysis,
residual solvents,
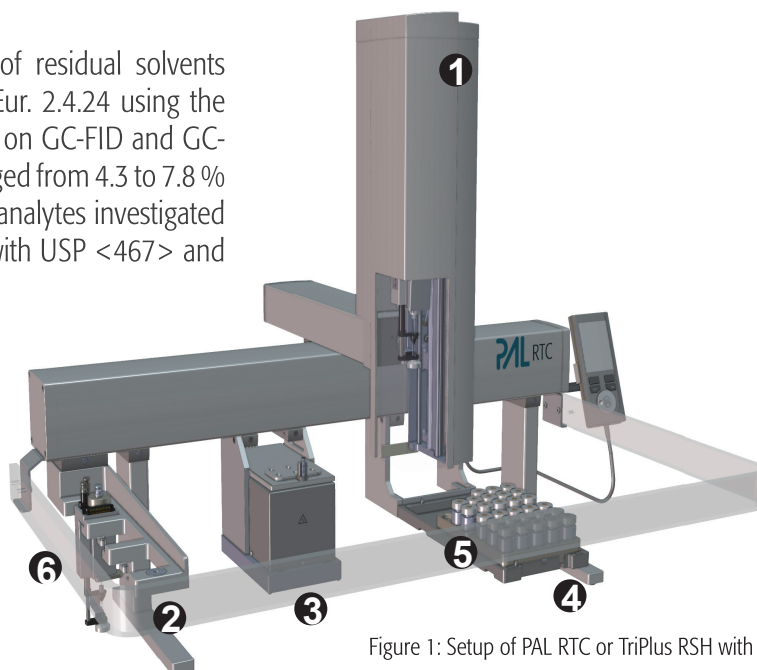USP <467>, Ph. Eur. 2.4.24

Figure 1: Setup of PAL RTC or TriPlus RSH with headspace capability.

Introduction

The equilibrium of volatile substances between a sample matrix (liquid and/or solid) and the gas phase above it in a closed system is the basis for static headspace sampling (ref. 1). Static headspace sampling has been realized with different techniques (syringe injection, sample loop injection and pressure balanced injection). Among these injection techniques, syringe injection is easier to understand and simple to use. A sample is placed in sealed vial and incubated at a given temperature for a given time. After achieving partition equilibrium, volatile substances in the gas phase are transferred by a heated syringe into the GC-system for analysis by FID (flame ionization detector) or MS (mass spectrometer). Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug product (ref. 2). Residual solvent analysis for pharmaceutical products are generally carried out with samples/products being dissolved or dispersed in an adequate amount of water. Water vapor from the matrix can generate problems during injection and analysis, leading to poor precision.

To ensure adequate precision, syringe headspace parameters were optimized for the analysis of residual solvents. Several critical steps to minimize vapor effects were identified.

Instrumentation

PAL RTC /Thermo Scientific TriPlus RSH System	
1	PAL RTC 85/120
2	Park Station
3	Agitator
4	Tray Holder
5	Rack VT15
6	Headspace Tool
7	2.5 mL Headspace Syringe (P/N: SH2500-65-T-23-SP)
System A: GC-FID	
A	PAL RTC system with headspace capability on Agilent 7890B GC-FID. GC Column: Restek Rxi-624Sil MS Column (30 m x 0.32 mmID x 1.8 µm)
System B: GC-MS	
B	Thermo Scientific TriPlus RSH for headspace injection on Thermo Trace 1300 GC-ISQ. GC Column: Restek Rxi-624Sil MS Column (30 m x 0.32 mmID x 1.8 µm)

Chemicals / Vials

1. USP Standards

No.	Standard Name (Catalog Number)	Supplier
1	USP Residual Solvents Class 2 - Mix A (36271)	Restek Corporation

2. Solvents

No.	Compound Name	Supplier	Description
1	Water	J.T. Baker	HPLC grade
2	Dimethyl sulfoxide (DMSO)	J.T. Baker	>99.0%
3	Methanol	J.T. Baker	HPLC grade
4	Ethanol	Acros Organic	96%
5	Acetone	Sigma Aldrich	≥99.5% Reagent grade
6	2-Propanol	J.T. Baker	LCMS grade
7	Acetonitrile	J.T. Baker	HPLC grade
8	Benzene	J.T. Baker	Reagent grade
9	Toluene	J.T. Baker	>99.5%

3. 3. Headspace Vials and Caps
- 20 mL clear glass for headspace, 75.5 X 22.5 mm, DIN crimp neck (PAL System PN: Vial-20-DC20-CG-100)
- Crimp cap for headspace, SPME Fiber and SPME Arrow (PAL System PN: Cap-DC20-St-SP15-100)

Test Solution Preparation

USP Residual Solvents Class 2 - Mix A was diluted with HPLC grade water to obtain the following concentrations (ppm). 6 mL aqueous sample was placed in a headspace vial according to USP <467> (ref. 2) and Ph.Eur. 2.4.24 (ref. 3).

1. For System A (GC-FID):

Compound Name	Concentration, ppm
Methanol	25.02
Acetonitrile	3.42
Methyl chloride	5.00
trans-1,2-Dichloroethene	7.83
cis-1,2-Dichloroethene	7.87
Tetrahydrofuran	5.75
Cyclohexane	32.37
Methylcyclohexane	9.83
1,4-Dioxane	3.17
Toluene	7.42
Chlorobenzene	3.00
Ethylbenzene	3.08
m-Xylene	10.85
p-Xylene	2.55
o-Xylene	1.63

2. For System B (GC-MS):

Compound Name	Concentration, ppm
Methanol	8.34
Acetonitrile	1.14
Methyl chloride	1.67
trans-1,2-Dichloroethene	2.61
cis-1,2-Dichloroethene	2.62
Tetrahydrofuran	1.92
Cyclohexane	10.79
Methylcyclohexane	3.28
1,4-Dioxane	1.06
Toluene	2.47
Chlorobenzene	1.00
Ethylbenzene	1.03
m-Xylene	3.62
p-Xylene	0.85
o-Xylene	0.54

3. Spiked Sample Solution (aqueous)

Compound Name	Concentration, ppm
Methanol	26.37
Ethanol	26.30
Acetone	26.15
2-Propanol	26.17
Acetonitrile	1.310
Benzene	0.07304
Toluene	1.373

Analysis Parameters

	System A (GC-FID)	System B (GC-MS)
	PAL3-RTC	TriPlus RSH
Agitator Temperature	80ºC	80ºC
Syringe Temperature	105ºC	105ºC
Purge Gas Regulator Pressure	1.6 Bar	1.6 Bar
Sample Incubation Time	45 minutes	45 minutes
Pre-Filling	Enable (volume = 2.25 mL)	Enable (volume = 2.25 mL)
Filling Stroke	5 times (volume = 1.2 mL)	5 times (volume = 1.2 mL)
Filling Stroke Delay	30 seconds	30 seconds
Sample Vial Penetration Depth	25 mm	25 mm
Sample Aspiration Speed	6 mL/min	6 mL/min
GC Injector Penetration Depth	45 mm	45 mm
Sample Injection Speed	25 mL/min	25 mL/min
Pre-Injection Dwell Time	3 seconds	3 seconds
Post-Injection Dwell Time	10 seconds	10 seconds
Pre-Injection Purge	Off	Off
Post-Injection Purge	60 seconds	60 seconds

	7890B GC	Trace 1300 GC
GC Inlet Temperature	200ºC	140ºC
GC Carrier Gas	Helium	Helium
GC Column Flow	2.2 mL/min (Constant Flow)	2.2 mL/min (Constant Flow)
Inlet Split Ratio	5:1	20:1
GC Oven Program	Initial 40ºC hold for 20 minutes; Ramp 10ºC/min to 240ºC, hold for 20 minutes	Initial 40ºC hold for 20 minutes; Ramp 10ºC/min to 240ºC, hold for 20 minutes

	7890B FID	
FID Temperature	280ºC	

		ISQ MS
EI Source		70 eV
Transfer Line Temperature		250ºC
Ion Source Temperature		250ºC
Scan Range		m/z 29-150

Table 1: Optimized analysis parameters.

Automated Headspace Workflow

The optimized syringe headspace procedure, which is shown in fig. 2, was performed fully automated on a PAL RTC or TriPlusRSH.
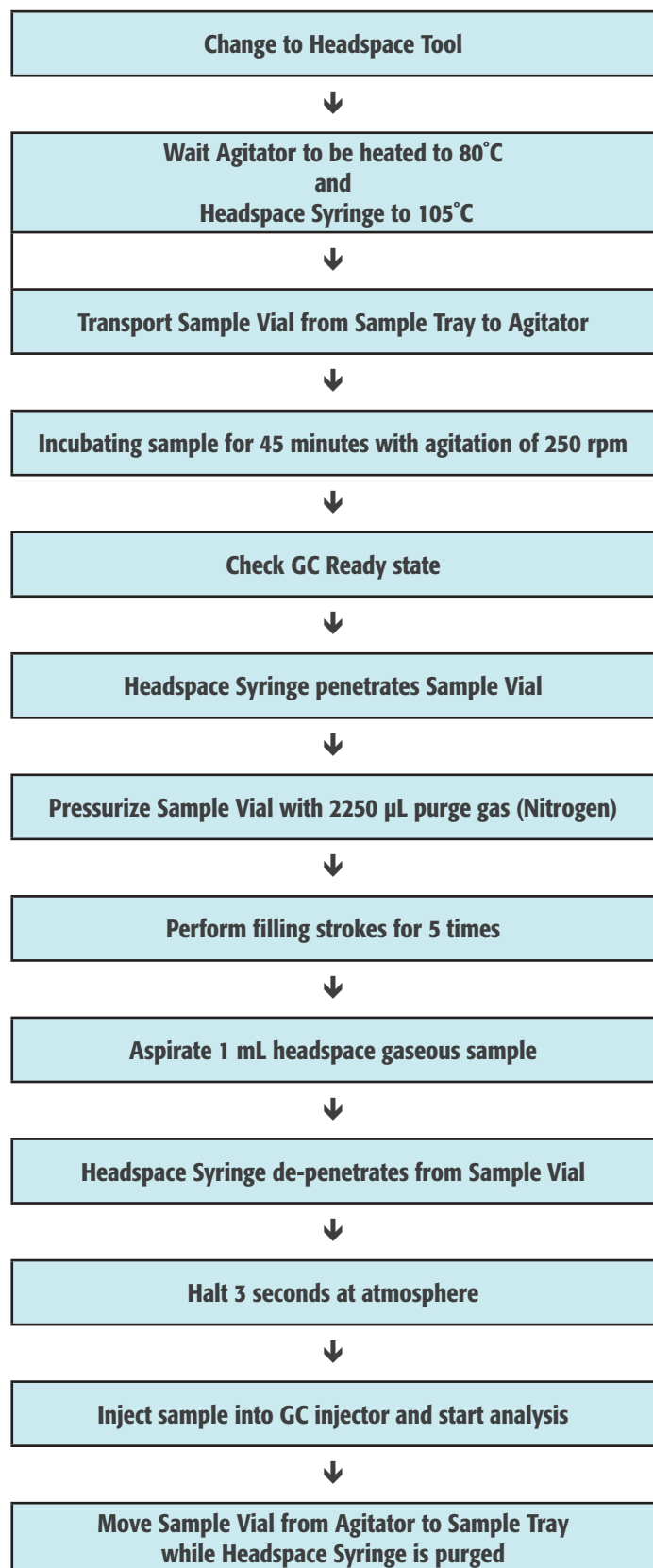
Change to Headspace Tool

↓

Wait Agitator to be heated to 80˚C and Headspace Syringe to 105˚C

↓

Transport Sample Vial from Sample Tray to Agitator

↓

Incubating sample for 45 minutes with agitation of 250 rpm

↓

Check GC Ready state

↓

Headspace Syringe penetrates Sample Vial

↓

Pressurize Sample Vial with 2250 µL purge gas (Nitrogen)

↓

Perform filling strokes for 5 times

↓

Aspirate 1 mL headspace gaseous sample

↓

Headspace Syringe de-penetrates from Sample Vial

↓

Halt 3 seconds at atmosphere

↓

Inject sample into GC injector and start analysis

↓

Move Sample Vial from Agitator to Sample Tray while Headspace Syringe is purged

Figure 2: Syringe headspace procedure optimized for aqueous sample.

Analysis

6 mL of test solution was placed in 20 mL headspace vial and analyzed by System A or System B; instrument settings refers to Analysis Parameters.

Results & Discussions

The following syringe headspace parameters have been optimized:

- Purge gas pressure
- Pre-filling gas volume
- Syringe stroke
- Delay time after syringe stroke

Table 1 shows the list of optimized parameters for the headspace analysis.

Complying with USP <467> and Ph. Eur. 2.4.24 requirements, incubation temperature and syringe temperature were set at 80ºC and 105ºC, respectively. It was found that a purge gas pressure of > 1.6 bar with PAL3-2500µL Headspace Syringe (P/N: SH2500-65-T-23-SP) delivered consistent vial pressurization via pre-filling gas. Additionally, a syringe stroke ensures sufficient stabilization prior to sample aspiration. In combining the optimized workflow (fig.2) with the parameters described in table 1 negative effects from the water matrix could be minimized. Thus, reproducible results were obtained from batch to batch.

Test Solution: USP Residual Solvents Class 2 - Mix A

All analyte compounds were well separated chromatographically except for m-xylene and p-xylene. The co-eluting of m-xylene and p-xylene was matching with Restek Chromatogram Library-Residual Solvents Class 2 — Mix A on Rxi-624Sil MS (G43) by USP <467> (ref.4).

Figure 3 and Figure 4 show chromatograms obtained from System A and System B, respectively.
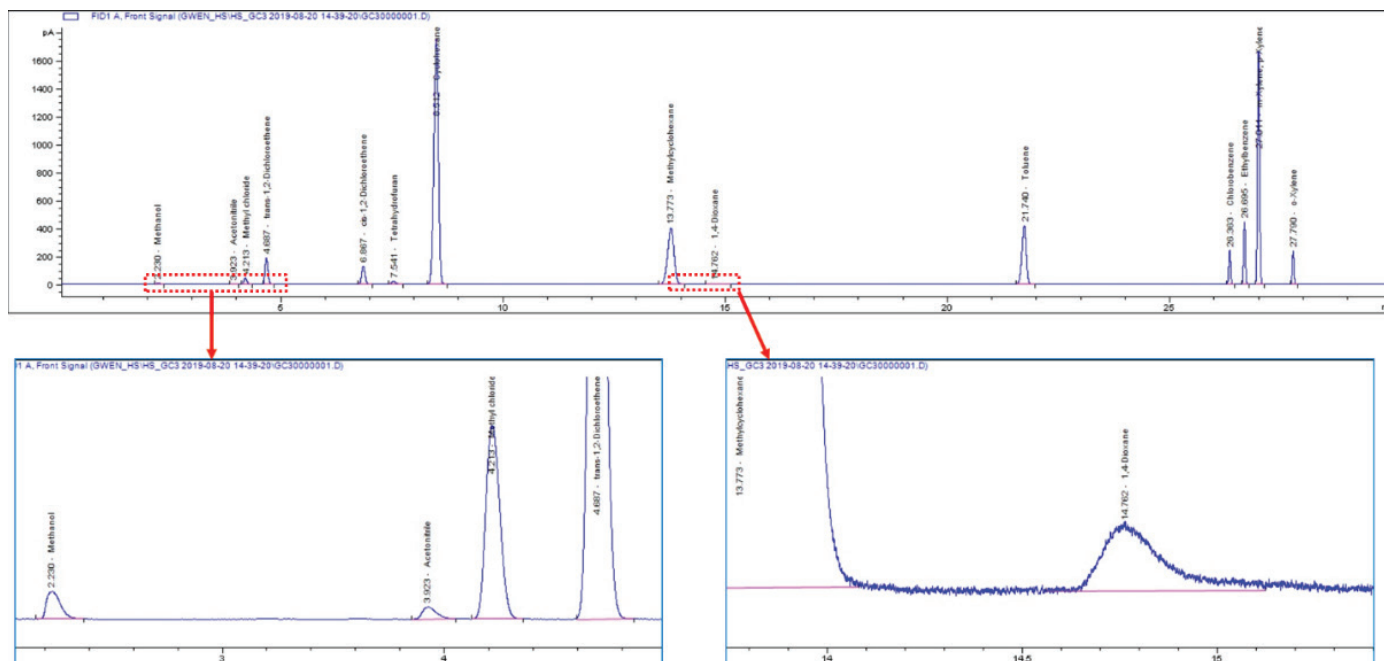

Figure 3: FID chromatogram obtained using Agilent 7890B GC (System A). USP Residual Solvents Class 2 - Mix A diluted with water; concentration described as Test Solution Preparation 1A.
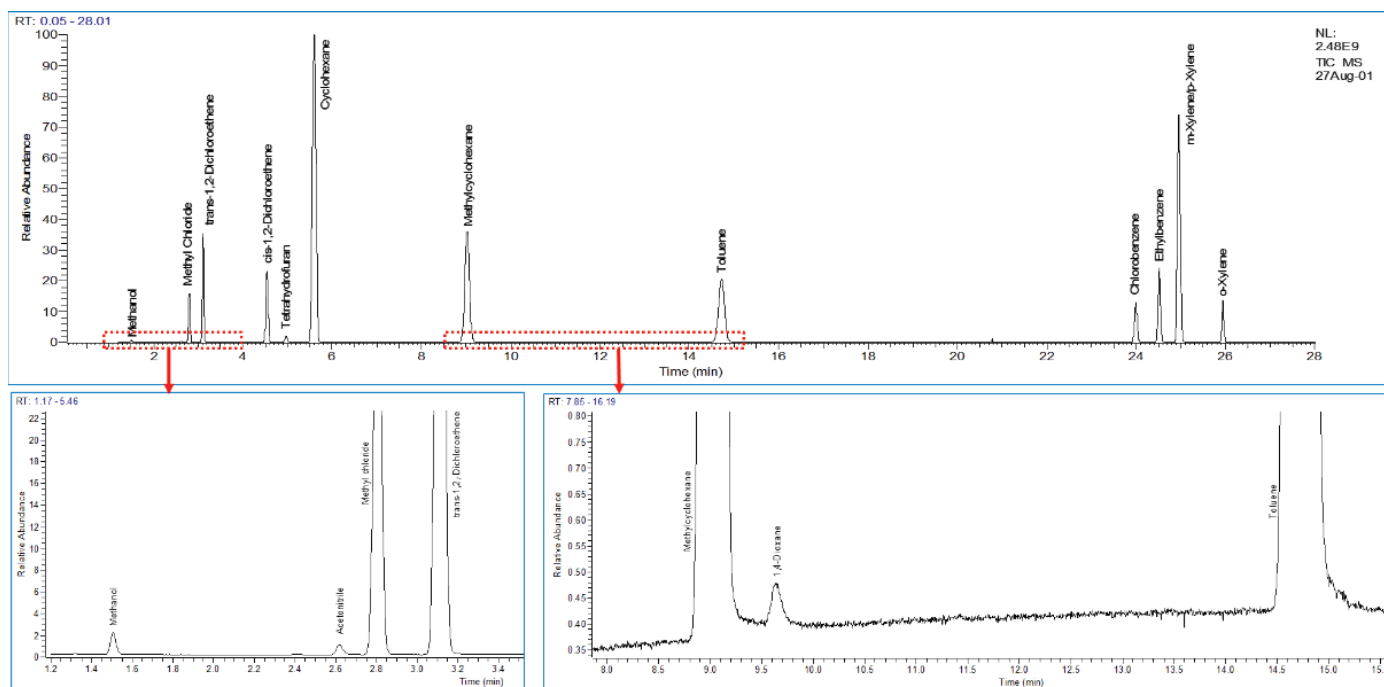
Figure 4: TIC chromatogram obtained using Thermo Trace 1300 GC-ISQ (System B). USP Residual Solvents Class 2 - Mix A diluted with water; concentration described as Test Solution Preparation 1B.

Compound Name	System A		System B	
	Retention Time, min	%RSD (n=20)	Retention Time, min	%RSD (n=20)
Methanol	2.230	4.47	1.503	5.91
Acetonitrile	3.923	4.30	2.609	4.44
Methyl chloride	4.213	4.38	2.792	3.53
trans-1,2-Dichloroethene	4.687	4.73	3.099	4.26
cis-1,2-Dichloroethene	6.867	4.51	4.517	3.70
Tetrahydrofuran	7.541	4.14	4.949	4.26
Cyclohexane	8.512	4.70	5.568	4.09
Methylcyclohexane	13.773	4.71	8.972	3.97
1,4-Dioxane	14.762	7.73	9.601	3.88
Toluene	21.740	4.55	14.632	3.78
Chlorobenzene	26.363	4.49	23.954	4.27
Ethylbenzene	26.695	4.59	24.488	4.27
m-Xylene & p-Xylene	27.011	4.60	24.920	4.51
o-Xylene	27.790	4.51	25.916	3.91

Table 2: Retention time and %RSD for 20 replicates.

Test Solution: Spiked Sample Solution (aqueous)

Further analysis was carried out with larger number of samples. Spiked sample solutions was prepared using HPLC water at concentration described at Test Solution Preparation 2A. Total of 109 samples were analyzed using System A (GC-FID) and 120 samples at System B (GC-MS). 5 batches of replicates have been analyzed with each system.
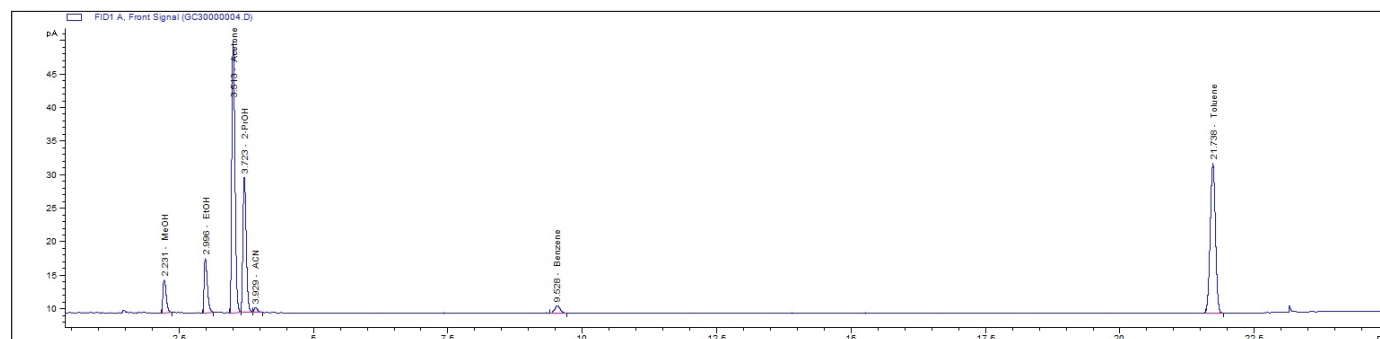

Figure 5: FID chromatogram obtained using Agilent 7890B GC (System A). Simulated Sample Solution (aqueous); concentration described as Test Solution Preparation 2A.
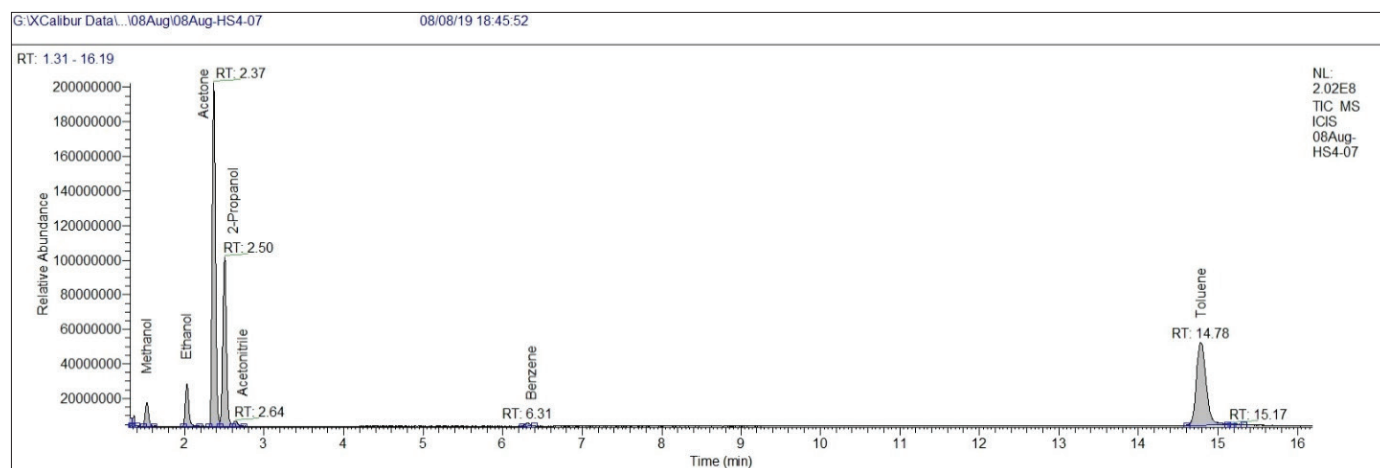

Figure 6: TIC chromatogram obtained using Thermo Trace 1300 GC-ISQ (System B). Simulated Sample Solution (aqueous); concentration described as Test Solution Preparation 2A.

		Compound Name						
		MeOH	EtOH	Acetone	2-PrOH	ACN	Benzene	Toluene
Retention Time, minutes	System A	2.231	2.996	3.513	3.723	3.929	8.528	21.738
	System B	1.527	2.024	2.361	2.497	2.629	6.285	14.720

			Compound Name						
Batch (Replicates)			MeOH	EtOH	Acetone	2-PrOH	ACN	Benzene	Toluene
%RSD of Peak Area, %	System A (Total n = 109)	Batch 1 (n=20)	4.86	3.58	2.02	2.47	3.22	2.03	2.28
		Batch 2 (n=22)	3.26	2.92	2.70	2.67	2.81	2.43	2.60
		Batch 3 (n=25)	3.54	3.34	3.11	3.12	3.39	3.01	2.89
		Batch 4 (n=25)	3.67	3.07	2.56	2.62	3.63	2.72	2.75
		Batch 5 (n=17)	2.38	2.07	1.62	1.61	1.94	1.81	1.80
	System B (Total n = 120)	Batch 1 (n=28)	4.44	5.18	4.96	5.10	6.19	3.04	4.83
		Batch 2 (n=20)	2.86	2.83	3.88	3.86	4.63	6.59	5.17
		Batch 3 (n=30)	4.70	4.75	3.05	4.32	4.89	7.62	7.33
		Batch 4 (n=22)	5.22	5.28	3.92	4.39	4.46	3.57	3.93
		Batch 5 (n=20)	3.84	4.14	2.94	3.43	2.70	4.01	4.65

Table 3: Retention time for each compound and %RSD for each batch of replicates.

Conclusions

Syringe headspace analyses performed with the optimized procedure and parameters as described above deliver reproducible results for aqueous samples. The precision of analysis was < 5 % RSD (GC-FID) for all class 2 solvents investigated in this study, with the exception of 1.4-Dioxane at 7.7% RSD . These values fully comply with USP <467> and Ph. Eur. 2.4.24 requirements (% RSD < 15).

This set of optimized parameters serves as a general reference for headspace analysis of aqueous samples using the syringe headspace technique.

References

1 HJ Huebschmann, Handbook of GC-MS, Wiley VCH, ISBN: 978-3-527-33474-2
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3 European Pharmacopoeia 9.0, Methods of Analysis, 2.4.24. Identification and Control of Residual Solvents.
4 https://www.restek.com/chromatogram/view/GC_PH1175/prod::44538

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