Improving Pharmaceutical Laboratory Throughput in the Analysis of Trace Impurities and Residual Solvents with Liquid/Headspace Unattended Switching and Automated Standard Preparation

[1]Silvia Gemme, [1]Stefano Pelagatti, [2]Dwain Cardona
[1]Thermo Fisher Scientific, Milan, Italy [2]Thermo Fisher Scientific, Austin, Texas

Overview

Purpose: Give a demonstration of the excellent performances of a new robotic autosampler as an effective tool for modern laboratories that will benefit from its automation capabilities in terms of shorter time required for sample preparation, increased results accuracy and reduced chance for errors.

Methods: An innovative robotic autosampler has been used in the analysis of residual solvents according to the USP <467> method exploiting its unique capability to switch automatically from liquid to headspace mode and to perform sample preparation and dilution steps during the same sequence.

Results: RSD% < 5% in HS analysis for Class 2 mix A samples diluted by the autosampler directly into HS vials already capped and crimped. Excellent linearity was displayed for all the components of Class 2 mix A up to the safety concentration limit. All calibration levels were prepared unattendedly by the *Prep Cycle Calibration Dilution* capability of the autosampler.

Introduction

US Pharmacopeia (USP) method <467> details the procedures for the identification, control and quantification of Class 1 and Class 2 residual solvents through the use of headspace gas chromatography. Some pharmaceutical laboratories also have to analyze solvent purity as part of the incoming raw material testing process according to the various solvents' monographs. For this purpose, liquid GC injection is normally chosen.

Because of the limitations of the GC instrumentation currently available, most of these laboratories dedicate one instrument to headspace and another one to liquid injection analyses. This impacts lab productivity and spending.

This poster demonstrates the use of the innovative robotic platform of the Thermo Scientific™ TriPlus™ RSH autosampler with its ability to switch automatically from liquid to headspace mode in the same sequence onto a single GC and also to use different syringe volumes in the same run during the sample preparation phase, thus enhancing overall lab productivity.

Test results of residual solvents quantification, where sample and standard preparation steps according to the USP<467> procedures are carried out by the autosampler prior the headspace analysis, are shown.

Modern laboratories benefit from this automation in terms of shorter time required for sample preparation, increased result accuracy and reduced chance for errors.

FIGURE 1. TriPlus RSH Autosampler and Thermo Scientific TRACE™ 1310 GC

Methods

A TRACE 1310 gas chromatograph equipped with split/splitless injector, an FID detector, and a TriPlus RSH autosampler, controlled by the new Thermo Scientific Dionex™ Chromeleon™7.1 Chromatography Data System, is the instrumental platform for this application.

USP Class 1 Residual Solvent Mixture (Restek™ catalog # 36279) and USP Class 2 mix B Residual Solvent Mixture (Restek catalog # 36272) have been manually diluted following the *Procedure A* for Class1 and Class 2 Water-Soluble articles reported in the US Pharmacopeia (USP) <467> revised method effective from August '11 to the concentrations reported on Table 1.

USP Class 2 mix A Residual Solvent Mixture (Restek catalog # 36271) has been automatically diluted by the TriPlus RSH autosampler to 1/100 of the original concentration (the final concentrations are reported in Table 1) directly into 20 mL HS vials already filled with 5 mL of water and analyzed in HS mode as reported in the US Pharmacopeia (USP) <467> revised method August 2011.

The repeatability tests have been completed on 5 replicates for each class of solvents. Linearity check has been performed on three replicates of the Class 2 mix A calibration levels reported on Table 2 prepared automatically by the TriPlus RSH autosampler, with its proprietary *Calibration Dilution* automated preparation cycle.

Acetonitrile in dimethyl sulfoxide (Restek catalog # 36281) has been injected in liquid mode in the same sequence used to perform the headspace analysis of the different solvent classes as an example of liquid injection.

Methods (cont.)

Column: Thermo Scientific TRACE TR-V1 30 m x 0.32 mm ID x 1.8 µm (P/N 260V339P)
SSL Liner: 4 mm ID straight empty split liner (P/N 453A1295)
TriPlus RSH Autosampler HS mode instrument acquisition method:
Injection volume: 1 mL
Agitator temp: 80 °C; incubation time: 20 min
Syringe temp: 120 °C
Filling speed: 20 mL/min; Injection speed: 50 mL/min;
injection depth: 40 mm; penetration speed: 25 mm/s
TRACE 1310 GC instrument method HS mode
Oven temp: 40 °C (hold for 20 min) to 240 °C @10°C/min (hold for 20 min)
Injector: SSL, 140 °C, split mode (split ratio 1:5)
Detector: FID, 240 °C
Carrier: Helium; constant flow: 2.1 mL/min (35 cm/sec)
TRACE 1310 instrument method liquid mode
Oven temp: 55 °C (hold for 5 min) to 240 °C @ 20 °C/min (hold for 10 min)
Injector: SSL, 210 °C, split mode (split ratio 1:20)
Detector: FID, 240 °C
Carrier: Helium; constant flow: 2.1 mL/min (35 cm/sec).

TABLE 1. Concentrations Prepared and Injected in HS Mode

Class 1 Compounds	analyzed solution conc (ppm)*	Safety Conc Limit (ppm)	
1,1-dichloroethane	4	8	* 1 mL of Class 1 manually diluted solution has been transferred to a 20 mL HS vial already filled with 5 mL of water and analyzed
1,1,1-trichloroethane	5	1500	
Carbon Tetrachloride	2	4	
Benzene	1	2	
1,2-dichloroethane	2.5	5	
Class 2 mix B Compounds	**analyzed solution conc (ppm)***	**Safety Conc Limit (ppm)**	
hexane	14.5	290	* 5 mL of Class 2 mix B manually diluted solution has been transferred to an HS vial with 1 mL of water and analyzed
nitromethane	2.5	50	
chloroform	3	60	
1,2-dimethoxyethane	5	100	
trichloroethylene	4	80	
pyridine	10	200	
2-hexanone	2.5	50	
tetralin	5	100	
Class 2 mix A Compounds	**analyzed solution conc (ppm)***	**Safety Conc Limit (ppm)**	
methanol	150	3000	* 1 mL of Class 2 mix A diluted solution has been directly prepared by the TriPlus RSH in a 20 mL HS vial already filled with 5 mL of water (already capped and crimped) and analyzed
acetonitrile	20.5	410	
dichloromethane	30	600	
trans-1,2-dichloroethane	47	1870	
cis-1,2-dichloroethane	47		
tetrahydrofuran	34.5	720	
cyclohexane	194	3880	
methylcyclohexane	59	1180	
1,4-dioxane	19	380	
toluene	44.5	890	
chlorobenzene	18	360	
ethyl benzene	18.4	2170	
m-xylene	65.1		
p-xylene	15.2		
o-xylene	9.8		

TABLE 2. Class 2 mix A Calibration Levels Prepared by the TriPlus RSH Autosampler with the Prep Cycle Calibration Dilution

Class 2 mix A	Concentration (ppm)				
compound	level 1	level 2	level 3	level 4	level 5
1_ methanol	150	300	750	1500	3000
2_ acetonitrile	20.5	41	102.5	205	410
3_dichloromethane	30	60	150	300	600
4_trans-1,2-dichloroethane	47	94	235	470	940
5_cis-1,2-dichloroethane	47	94	235	470	940
6_tetrahydrofuran	34.5	69	172.5	345	690
7_cyclohexane	194	388	970	1940	3880
8_ methylcyclohexane	59	118	295	590	1180
9_ 1,4-dioxane	19	38	95	190	380
10_ toluene	44.5	89	222.5	445	890
11_ chlorobenzene	18	36	90	180	360
12_ ethyl benzene	18.4	36.8	92	184	368
13_ m-xylene/p-xylene	65.1	130.2	325.5	651	1302
	15.2	30.4	76	152	304
14_ o-xylene	9.8	19.6	49	98	196

TABLE 3. Area and Retention Times Repeatability Results for Each Class of Solvents

	compound	RT (min)	area RSD% (5 replicates)	RT SD (5 replicates)
Class 1	A	3.48	3.1	0.004
	B	8.38	3.3	0.005
	C	8.79	3.1	0.005
	D	9.68	3.5	0.004
	E	10.14	3.3	0.004
Class 2 mix B	a	5.03	3.3	0.008
	b	7.14	2.5	0.011
	c	7.91	1.9	0.012
	d	9.91	2.5	0.012
	e	12.53	3.0	0.015
	f	21.32	3.6	0.010
	g	24.76	0.6	0.003
	h	33.46	1.1	0.001
Class 2 mix A	1	2.31	5.4	0.005
	2	4.32	3.5	0.008
	3	4.47	1.1	0.004
	4	4.87	2.0	0.004
	5	7.22	1.3	0.005
	6	7.89	0.8	0.003
	7	8.48	3.3	0.005
	8	13.45	4.5	0.007
	9	15.45	5.0	0.004
	10	21.91	4.0	0.003
	11	26.43	4.0	0.001
	12	26.72	6.4	0.001
	13	27.02	6.2	0.002
	14	27.83	5.2	0.001

Results

The repeatability for the different classes of residual solvents has been tested at a concentration down to 20 times lower than the Safety Concentration limit stated in the USP <467> method.

Excellent repeatability for both retention time and peak area were achieved as reported in Table 3.

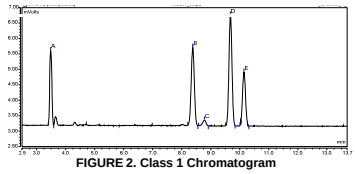


FIGURE 2. Class 1 Chromatogram

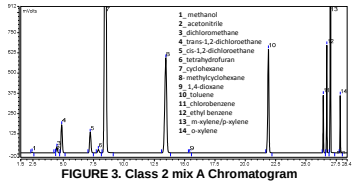


FIGURE 3. Class 2 mix A Chromatogram

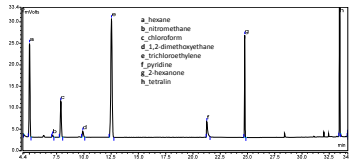


FIGURE 4. Class 2 mix B Chromatogram

Calibration curves in HS mode generated for the components of Class 2 mix A, up to the safety concentration limit for each analyte show outstanding linearity, as summarized in Figure 5 and Table 4.

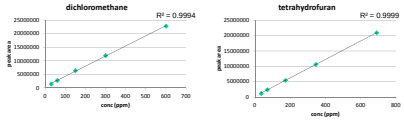
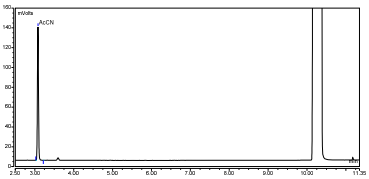


FIGURE 5. Dichloromethane and Tetrahydrofuran Calibration Curves

TABLE 4. R^2 Values for the Calibration Curves of Class 2 Mix A Solvents

compound	R^2
methanol	0.9978
acetonitrile	0.9996
dichloromethane	0.9994
trans-1,2-dichloroethane	0.9987
cis-1,2-dichloroethane	0.9987
tetrahydrofuran	0.9999
cyclohexane	0.9943
methylcyclohexane	0.9938
1,4-dioxane	0.9998
toluene	0.9974
chlorobenzene	0.9947
ethyl benzene	0.9917
m-xylene/p-xylene	0.9910
o-xylene	0.9983

FIGURE 6. Chromatogram of Acetonitrile (2.05 mg/mL, RT 3.08 min) in DMSO, Injected in Liquid Mode



Conclusion

The TriPlus RSH autosampler provides the unique capability of automatic and unattended switching from liquid to headspace mode.

Sample preparation steps according to the USP<467> procedures carried out by the autosampler prior the headspace analysis directly into the 20-mL HS vials already crimped and capped for Class 2 mix A showed good repeatability results (area RSD%< 5%) also if the septum was pierced during the sample preparation prior to the incubation phase.

The proprietary *dilution custom template* feature made it possible to use one device to prepare the samples with liquid syringe operations and then inject the headspace samples in an unattended fashion. This dramatically enhanced the productivity of the lab providing excellent results in terms of precision and accuracy.

Full traceability in a 21 CFR part 11 compliant environment is achieved. Everything from standard preparation to reporting is audited in Chromeleon 7 Chromatography Data System.

References

US Pharmacopeia (USP) <467> revised method effective from August 2011.

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