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Investigation of key parameters for a smooth method transfer to the new Thermo Scientific TriPlus 500 Headspace Autosampler

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Goal

Demonstrate how to efficiently transfer an analytical method from an existing valve and loop headspace autosampler to the new Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace (HS) Autosampler.

Introduction

The transfer of an analytical method from one instrument to another is a challenging task which assumes particular relevance in those laboratories that operate in regulated environments such as the pharmaceutical industry.

A method transfer can be required for various reasons: it may be needed to move a method from a development laboratory to the QA/QC routine laboratory or, as described in this paper, to move from an existing platform to new equipment.

The TriPlus 500 HS autosampler represents an evolution of the previous generation headspace samplers. Characterized by a compact design and fully integrated with the Thermo Scientific™ TRACE™ 1300 Series Gas

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Chromatograph, the TriPlus 500 HS autosampler is capable of offering maximum productivity and scalability to accommodate sample growth over time due to its modular architecture.

In addition to the compact and modular design, the TriPlus 500 HS autosampler introduces some technological advancements and new features, such as a proprietary design of the pneumatic circuit, direct column interface, original vial shaking, to provide superior reliability, high data quality and a simplified method setting.

In this white paper, the key parameters of the valve and loop headspace sampling technique are reviewed and some practical suggestions on method optimization and transfer are provided.

These guidelines were successfully applied to transfer the United States Pharmacopeia (USP) <467> method for the residual solvents determination in pharmaceutical products from an Agilent 7697A HS and from a Thermo Fisher Scientific TriPlus 300 HS to the TriPlus 500 HS autosampler. As these platforms are based on the same core valve and loop technology, the transfer of the method is straightforward and can be made without the need of a complete re-validation. Data show that the TriPlus 500 HS autosampler is able to provide similar or even better results by transferring the existing method with almost no change in instrument parameters.

Technological advancements of the TriPlus 500 Headspace autosampler

The TriPlus 500 HS autosampler introduces three main technological advancements for outstanding system reliability and performance:

- **Direct GC column interface:** a short inert interface directly connects the sampling system to the chromatographic column in place of the typical longer transfer line. The direct connection provides a more efficient and accurate temperature control, a shorter sample path and no dead volumes, minimizing the risk of condensation of high-boiling compounds and sample degradation and optimizing the sample transfer to the column.
- **Proprietary pneumatic control:** the innovative control of the vial and loop pressure during the sampling phase delivers excellent repeatability of the sample amount injected into the gas chromatograph, while an effective purging of the vent line assures minimal to no carryover.
- **Quick Spin Shaking (QSS):** the new proprietary design of the vial shaking during the incubation produces a larger exchange surface between the liquid and the vapor phases, ensuring a faster and more homogeneous migration of the analytes in the headspace and improving system productivity and repeatability.

Although these improvements introduce some differences in the method parameters, the core technology of the valve and loop sampling remains unchanged, thus, limiting the need of time consuming and expensive re-validation in the case of method transfer from instrument to instrument. Each laboratory has the responsibility to document these changes and demonstrate the suitability of the new headspace GC system.

Key method parameters

For the comparative study on the key method parameters, a water solution containing Methanol (150 ppm), Tetrahydrofuran (40 ppm), Toluene (50 ppm) and o-Xylene (10 ppm) was used. The selected compounds cover a representative range of polarity and volatility.

System temperatures

The vial incubation temperature is one of the most important parameters to optimize during the development of a headspace method. The higher the temperature, the lower the partition coefficient, which means a higher concentration of the analytes in the gas phase at the equilibrium[1]. When choosing the incubation temperature, it is important to consider the sample matrix to avoid excessive overpressure in the vial or, as in case of water, an undesired excess of moisture in the gas phase injected into the gas chromatograph.

There are no special considerations concerning the incubation temperature when migrating a method, other than setting the same temperature of the existing method. The oven of the TriPlus 500 HS autosampler guarantees an accurate and uniform temperature control of the vial during the incubation, providing an unmatched quantitative precision for all classes of compounds.

The loop and sample path temperature must be set high enough to avoid the condensation of analytes and solvent, preventing contamination of the system and carryover.

For headspace samplers of the previous generation, the temperature of the long external transfer line is typically set 10-20 °C higher than the incubation and loop temperatures to avoid sample condensation.

One of the main achievements of the TriPlus 500 HS autosampler's compact design is an improved and simplified sample path. Instead of a long external transfer line, the TriPlus 500 HS is equipped with a short and integrated GC interface: as the sampling loop is closer to the chromatographic column, the sample path is shorter and, therefore, a single thermal control from the sampling valve to the GC column is implemented, providing a more efficient and accurate heating of the line. The absence of possible cold spots along with no dead volumes of the direct column connection, minimizes the risk of carryover and sample degradation, optimizing the sample transfer to the column.

The single thermal control eliminates the need for a specific temperature setting for the external transfer line, simplifying the method setup. The TriPlus 500 HS autosampler contains just two temperature parameters: the Incubation Oven and the Loop/Sample Path parameters. Therefore, when migrating a method from a conventional headspace sampler to the new TriPlus 500 HS autosampler, the transfer line temperature parameter can be simply ignored while maintaining the oven and loop temperature unchanged.

Table 1 shows an example of the temperature settings used on a conventional headspace sampler with transfer line and the corresponding setting on the TriPlus 500 HS autosampler.

In addition, as the column is directly connected to the interface into the GC oven, the injector is not used and there is no need to set any injector temperature parameter, further simplifying the method set up and optimization. A comparison between the temperature zones of the TriPlus 500 HS autosampler and other headspace samplers with transfer line is showed in Figure 1.

Table 1. Method porting: example of temperature settings

	Conventional HS Autosampler with Transfer Line	TriPlus 500 HS Autosampler
Oven Temperature	85 °C	85 °C
Loop / Sample Path Temperature	85 °C	85 °C
Transfer Line Temperature	100 °C	-

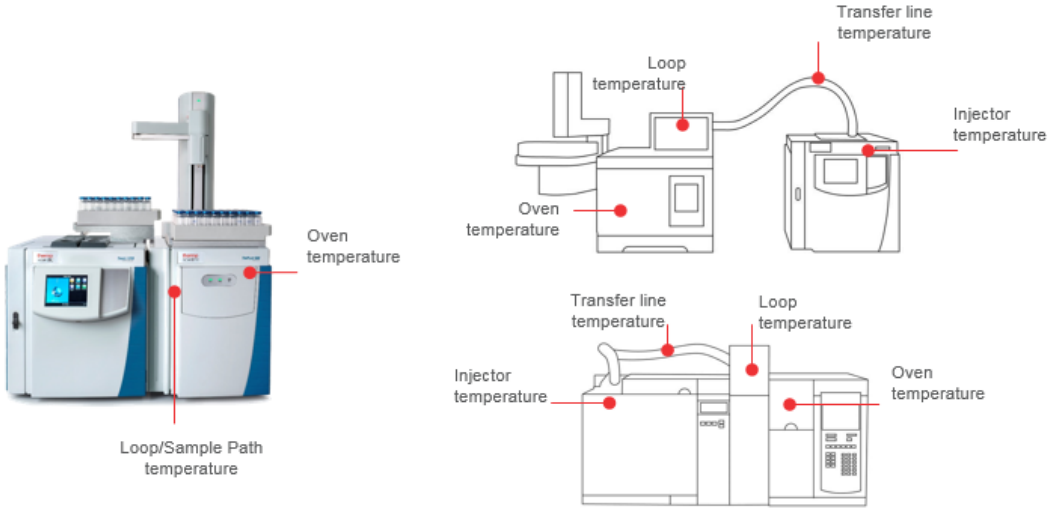

Figure 1. Comparison of the heated zones between a TriPlus 500 HS autosampler and headspace samplers with external transfer line connected to a GC system

Vial shaking

Thanks to the new proprietary Quick Spin Shaking (QSS), the agitation of the TriPlus 500 HS autosampler produces a larger exchange surface between liquid and gas phases and is more effective than conventional vial agitation. The result is a faster equilibration and a reduced vial incubation time, shortening the overall cycle time for increased instrument throughput and efficiency. Moreover, the shaking produces a more homogeneous distribution of the analytes in the headspace which typically results in an improved extraction repeatability.

Three vial shaking levels (Slow, Medium and Fast) have been optimized to provide flexibility and simplicity to the user. If the agitation is not required, the shaking can be turned off.

To show the effect of the vial shaking, the test mixture was incubated, with and without shaking, for 15 minutes at 85 °C using the TriPlus 500 HS autosampler.

Table 2. Effect of shaking on sample recovery

	MeOH	THF	Toluene	o-Xylene
Incubation Time 15 min – Shaking Off				
Average Area (pA*min)	1.327	4.467	68.488	9.482
RSD% (n=10)	1.760	4.187	4.713	4.937
Incubation Time 15 min – Fast Shaking				
Average Area (pA*min)	1.367	5.138	107.596	16.200
RSD% (n=10)	1.006	1.277	1.032	0.923

The incubation without shaking provides a limited extraction of the analytes and poor repeatability. Applying the vial shaking, the peak areas increase significantly, especially for the compounds with low partition coefficient. Moreover, the repeatability improved from 4 to 1 RSD% for all the compounds, confirming the positive effect of the shaking on sensitivity with shorter cycle time and data quality (Table 2).

To show the significant effect of the vial shaking on precision, another example is reported in Table 3. The results were obtained equilibrating the aqueous standard solution for 40 minutes applying the same incubation conditions (temperature and shaking) described above.

The average peak area RSD% shows a significant improvement, confirming the positive effect of the vial shaking on the precision.

To show the effect of the vial shaking on the analyte recovery, the aqueous standard solution was analyzed by progressively increasing the incubation time at 85 °C and applying the highest shaking level. This test was performed comparing the Quick Spin Shaking of the TriPlus 500 HS to the shaking approach of the previous model TriPlus 300 HS.

The compound recovery for Methanol and o-Xylene versus the incubation time, expressed as percentage of the maximum value at equilibrium, is reported as an example in Figure 2. Methanol and o-Xylene were selected to show the effect on compounds with different polarity and partition coefficient.

The effective shaking of the TriPlus 500 HS autosampler allows for the maximum recovery of Methanol and o-Xylene in shorter incubation times compared to the TriPlus 300 HS autosampler: the incubation time is reduced from 20 minutes to 15 minutes and from 60 minutes to 20 minutes, respectively.

Table 3. Effect of vial shaking on repeatability

	MeOH	THF	Toluene	o-Xylene
Incubation Time 40 min – Shaking Off				
Average Area (pA*min)	1.355	5.047	80.160	11.636
RSD% n=10	1.582	6.509	14.753	15.634
Incubation Time 40 min – Fast Shaking				
Average Area (pA*min)	1.388	5.292	110.891	16.682
RSD% n=10	1.05	0.908	0.709	0.690

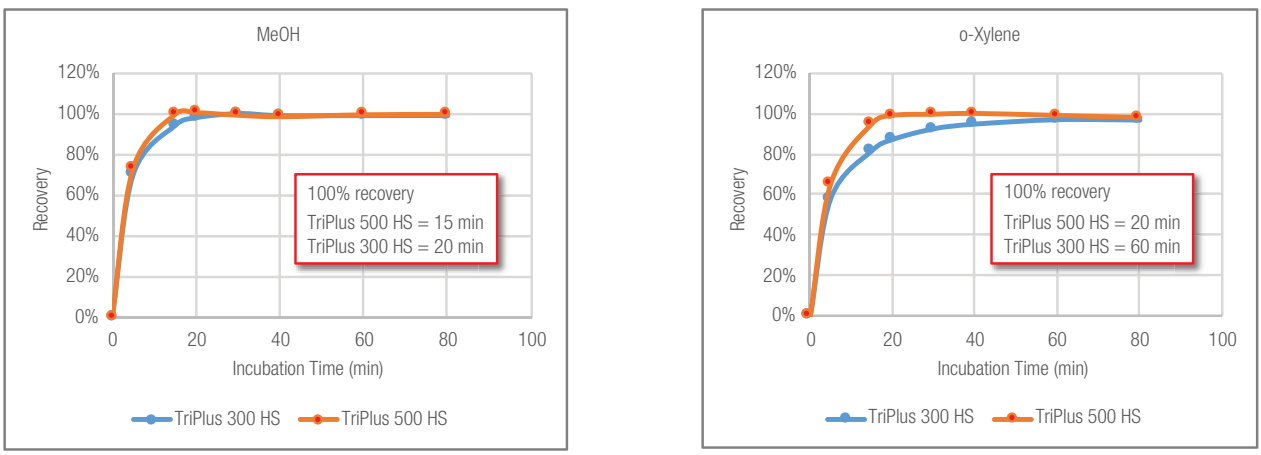

Figure 2. Comparison of the shaking effect between the TriPlus 500 HS and TriPlus 300 HS autosamplers

The Quick Spin Shaking approach is demonstrated to be more effective than conventional shaking as it produces the maximum recovery in a shorter incubation time, thus, increasing the sample throughput and productivity.

Moreover, it shows that transferring the same shaking level when migrating from the TriPlus 300 HS autosampler to the TriPlus 500 HS autosampler guarantees similar or even higher recovery, depending on the compound.

A comparison between the shaking of the TriPlus 500 HS autosampler and the one of the Agilent 7697A HS sampler was also performed analyzing the test mixture over the three shaking levels of the TriPlus 500 HS autosampler and the nine shaking levels of the Agilent 7697A HS sampler. The incubation time and temperature were set at 15 minutes and 80 °C respectively.

The results reported in Figure 3 for o-Xylene show the relative increase of peak area over the full range of shaking level for the TriPlus 500 HS and the Agilent 7697A. The TriPlus 500 HS autosampler Quick Spin Shaking covers and exceeds the full range of shaking levels, simplifying methods set up and optimization.

As a conclusion, in the event of a method porting from a TriPlus 300 HS autosampler or an Agilent system, the recommended shaking level parameter to migrate from one instrument to the new TriPlus 500 HS autosampler are suggested in Table 4, assuring equivalent or better results.

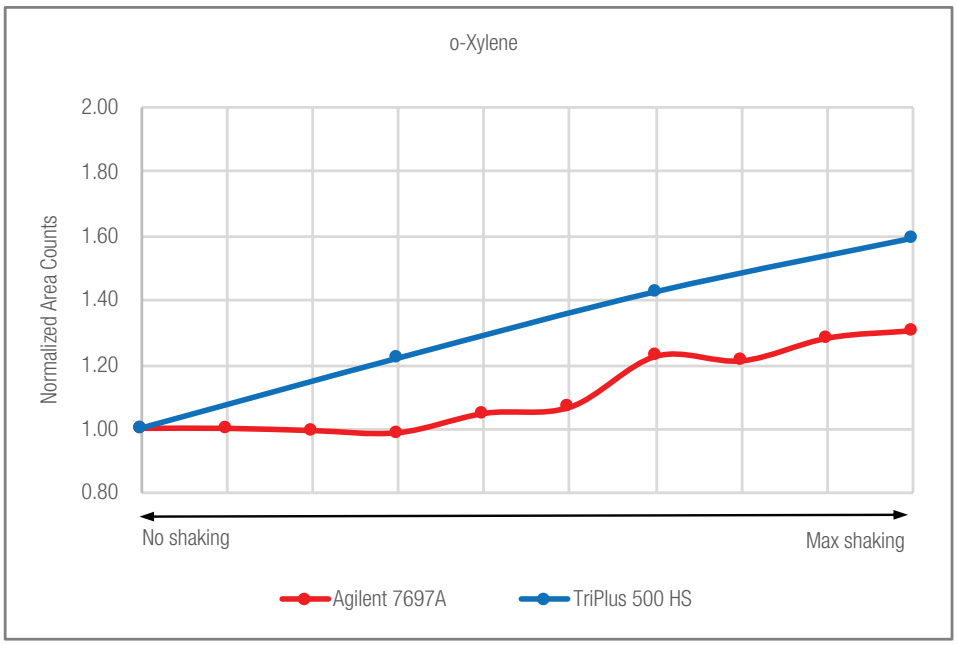

Figure 3. Effect of shaking on recovery for the TriPlus 500 HS autosampler and the Agilent 7697A HS sampler

Table 4. Suggested migration of shaking level parameter

	TriPlus 300 HS Autosampler	Agilent 7697A HS Sampler	TriPlus 500 HS Autosampler
Shaking Level	Low	1-3	Slow
	Medium	4-6	Medium
	High	7-9	Fast

Vial pressurization

In the valve and loop technology, a certain amount of inert gas is added to the vial to generate an overpressure which transfers the gas phase to the loop.

The vial pressure parameter represents the pressure in the vial after the pressurization step and before the loop is filled. Its optimization is very critical since it directly impacts both sensitivity and repeatability. The final vial pressure must be high enough to fill the loop volume and sweep it at least once. Its optimization depends on the desired loop pressure before the injection, which corresponds to the residual vial pressure after filling the loop.

However, the higher the vial pressurization, the greater the dilution of the headspace. When developing a method, this should be considered and vial pressurization limited to the minimum value required to assure the loop is properly filled.

As a rule of thumb for the TriPlus 500 HS autosampler, setting an initial vial pressure 30–60 kPa higher than the desired final pressure in the loop ensures the sample path is repeatedly washed with the sample and the standard loop size (1 mL) properly filled.

Further optimization might be required to maximize the method sensitivity or when using different loop volumes. The formula below can be used to calculate the minimum pressure difference between Vial Pressure and Loop Pressure.

$$\Delta P = 1.5(P_{Loop} + P_{amb})\frac{V_{Loop}}{V_{HS}}$$

Where:

ΔP = difference between the Vial Pressure and the Loop Pressure

P_{Loop} = Loop Pressure/residual pressure in the vial

P_{amb} = Ambient pressure

V_{Loop} = Loop volume in mL

V_{HS} = Headspace volume in mL

Note that the set Vial Pressure cannot be in any case lower than the pressure generated by the sample vapor and gas phase heating during incubation. In the case of water based matrices, the pressure (in kPa) generated by water vapor can be estimated from the graph shown in Figure 4.

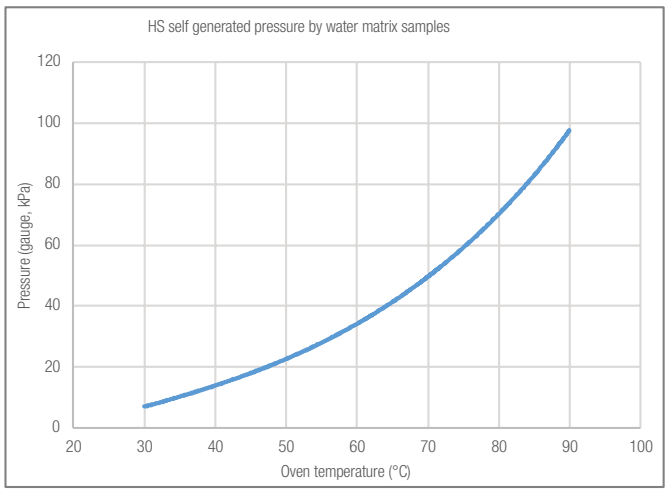

Figure 4. Generated vial pressure vs. incubation temperature for water-based sample

The TriPlus 500 HS autosampler implements a proprietary and advanced pneumatic circuit which provides an accurate and precise control of the pressure in the vial.

The default vial pressurization mode on the TriPlus 500 HS is “Pressure.” With this modality, the user needs to set only the desired vial pressure, while the pressure rate or the time to reach the pressure set point is self-optimized by the autosampler.

“Rate” and “Time” vial pressurization modes are also available to the user to customize the pressure rate or the pressurization time. The user sets the vial pressure and the pressurization rate or time requested to reach the desired pressure set point. In principle, for a more accurate method optimization, a different pressurization rate or pressurization time can be used to accelerate or slow down this step.

However, the default “Pressure” mode represents a good balance between data repeatability and productivity and is recommended for the majority of the applications.

The migration of the pressure parameter from an existing valve and loop headspace sampler to the TriPlus 500 HS autosampler is extremely simple. When the existing method includes a vial pressure setting, it is enough to set the same pressure value. In case the starting method indicates a pressurization time, the pressure setpoint of reference will be the pressure value indicated by the pressure regulator on the auxiliary gas line.

A comparison between the default pressurization mode on the TriPlus 500 HS autosampler and the available pressurization modes for the TriPlus 300 HS autosampler was performed. The sampling conditions and results are described in Table 5. Incubation time and temperature were set at 15 minutes and 85 °C, respectively. Two vial pressurization options (Standard and Pressure) are available for the TriPlus 300 HS autosampler. The "Standard" pressurization mode was used to reproduce the vial pressurization, based on time setting, which is used on legacy equipment where there is no active control of the pressure. To compare the different vial pressurization modes, the loop pressurization mode on the TriPlus 300 HS autosampler was maintained in "Pressure" to exclude any contributions.

The default "Pressure" mode of the new TriPlus 500 HS autosampler produces equivalent peak areas when compared to different vial pressurization strategies, with no impact on the analytical results, as shown in Table 5.

This makes the method porting in all cases extremely simple and effective and increases the possibility to avoid a full method re-validation.

Loop filling and pressurization

The new TriPlus 500 HS autosampler implements a proprietary control of the loop pressure, allowing for accurate and precise loop filling in any conditions through an optimized process. With this simplified system, the user has only to set the desired loop pressure value.

Similar to the vial pressure, some considerations on how to select the correct loop pressure are described:

- Setting the loop pressure above the ambient pressure means to avoid a full discharge of the headspace overpressure during the loop filling step. It increases the amount of molecules transferred to the GC. The higher the loop pressure, the higher the peak area (the increase is linear and depends on the analyte)
- Setting the loop pressure equal or lower than the carrier pressure at the injection is suggested to improve the sample transfer and obtain a better chromatography
- A delta between the desired pressure in the loop and the overpressure in the vial (typically 30-60 kPa) is required to guarantee a complete and efficient loop filling, as described in the previous section

Table 5. Comparison between different vial pressurization modes

	TriPlus 300 HS Autosampler	TriPlus 300 HS Autosampler	TriPlus 500 HS Autosampler
Vial Pressurization Mode	Pressure	Standard	Pressure
Vial Pressure	130 kPa	-	130 kPa
Aux Gas Pressure	-	130 kPa	-
Pressurization Time	-	0.2 min	-
Equilibration Time	1 min	1 min	1 min
Loop Fill Mode	Pressure	Pressure	-
Loop Pressure	70 kPa	70 kPa	70 kPa
	Area Counts (pA*min)		
	A	B	C
MeOH	0.327	0.292	0.482
THF	1.387	1.220	1.503
Toluene	32.188	28.221	33.935
o-Xylene	4.799	4.173	5.006

- In case of high concentrated analytes overloading the column, it is still possible to fill the loop at ambient pressure (loop pressure = 0), that means to fully discharge the vial overpressure to ambient pressure, without compromising the repeatability.

The test mixture was analyzed on the TriPlus 500 HS autosampler applying two different loop pressure conditions: Pressure=0 kPa (corresponding to ambient pressure) and Pressure=70 kPa. Comparable modes on the TriPlus 300 HS were the "Standard" and "Pressure" loop pressurization. The loop pressurization in "Standard" mode on the TriPlus 300 HS autosampler was used to reproduce the loop filling at ambient pressure. In fact, similar to the vial pressure, the previous generation headspace samplers do not have direct control of the loop pressure and the loop filling is performed by completely discharging the headspace overpressure to ambient pressure by setting a loop filling/venting time. To compare the different loop pressurization modes, the vial was pressurized at 70 kPa using the "Pressure" mode to exclude possible contribution to this test. Results and conditions are reported in the Table 6.

Results demonstrate that data produced by the TriPlus 500 HS autosampler with the loop at ambient pressure are comparable to those obtained with the TriPlus 300 HS autosampler in "Standard" mode (Columns A and B).

Results are also comparable between the TriPlus 300 HS autosampler and TriPlus 500 HS autosampler with the same filling mode and loop pressure setpoint (Columns C and D).

It is evident that, in the event of a method transfer to a TriPlus 500 HS autosampler, setting the same loop pressure value of an existing method produces comparable results in terms of area counts. From this perspective, the great advantage of the TriPlus 500 HS autosampler is the unmatched repeatability offered by the exclusive and precise pneumatic control as shown in Table 10.

Table 6. Comparison between different loop pressurization modes on TriPlus 300 HS and TriPlus 500 HS. A-B: Loop at ambient pressure; C-D: Loop maintained at 70 kPa

	A TriPlus 300 HS Autosampler	B TriPlus 500 HS Autosampler	C TriPlus 300 HS Autosampler	D TriPlus 500 HS Autosampler
Vial Pressurization Mode	Pressure	Pressure	Pressure	Pressure
Vial Pressure	130 kPa	130 kPa	130 kPa	130 kPa
Equilibration Time	1 min	1 min	1 min	1 min
Loop Fill Mode	Standard	—	Pressure	—
Loop Pressure	Ambient Pressure	0 kPa	70 kPa	70 kPa
Loop Fill Time	0.2 min	Self Optimized	Self Optimized	Self Optimized
Loop Equilibration Time	0.2 min	0.2 min	0.2 min	0.2 min
Peak Area (pA*min)				
MeOH	0.227	0.379	0.327	0.482
THF	0.651	1.011	1.387	1.503
Toluene	13.268	18.968	32.188	33.935
o-Xylene	1.966	2.780	4.799	5.006

Smooth migration of the USP <467> residual solvents method from the AN 5990-7625EN[2]

The analysis of residual solvents in pharmaceuticals is very important to ensure patient safety.

The determination of the residual solvents content is typically performed by static headspace-gas chromatography being a simple, reliable and robust techniques. Moreover, the valve and loop is the preferred headspace sampling technology in the pharmaceutical market as it produces highly precise injections.

The analytical procedure used worldwide to ensure the quality and the safety of all drug substances, excipients and product follows the USP <467> method.[3] The TriPlus 500 HS autosampler coupled to the TRACE 1300 Series GC fully matches USP <467> method requirements, combining excellent performance with high-throughput operations.

To demonstrate the migration of an existing USP <467> residual solvents method to the TriPlus 500 HS autosampler, the parameters reported in the Application note 5990-7625EN for the Agilent 7697A HS sampler coupled to a 7890B gas chromatograph equipped with a flame ionization detector (FID) were considered, focusing on the analysis of the Class 2A compounds.

Based on the considerations reported in the previous sections, the method was easily converted to the new instrument as the number of parameters is lower and there are no substantial differences in the operating conditions so that a complete method re-validation might not be required.

Sample preparation

According to the procedure for water-insoluble pharmaceuticals, USP <467> Class 2A mixture (Restek P/N 36012) was diluted 1:100 in pure DMSO in a 100 mL flask (Stock solution A).

20 mL clear headspace vials (P/N C4020-20) filled with 5 mL pure water were spiked with 1mL Stock solution A and immediately sealed with crimp caps (P/N 20-MCBC-ST3) with Silicone/PTFE septa.

Method porting

The parameters reported in the AN 5990-7625EN for the Agilent 7697A HS sampler and 7890B GC and the corresponding settings used on the TriPlus 500 HS autosampler coupled with a TRACE 1310 GC are shown in Table 7.

Table 7. <USP 467> Residual Solvents method parameters: migration from Agilent HS-GC system to TriPlus 500 HS autosampler – TRACE 1310 GC

Headspace Parameters	Agilent 7697A HS Sampler		TriPlus 500 HS Autosampler
Incubation Temperature	85 °C	→	Same
Incubation Time	40 min	→	Same
Valve/Loop Temperature	85 °C	→	Same
Transfer Line Temperature	100 °C	→	Not required
Shaking Level	2	→	Slow
Vial Pressurization Mode/ Vial Pressure	Default (Flow to Pressure)	→	Pressure
Vial Pressure	103 kPa	→	Same
Loop Fill Mode/Loop pressure	Custom	→	Not required
Loop Pressure	69 kPa	→	Same
Vial Pressure Equilibration Time	1 min	→	Same
Loop Equilibration Time	0.05 min	→	Same
Injection Mode	Standard	→	Same
Injection Volume	1 mL	→	Same
Injection Time	0.5 min	→	Same

GC Parameters	Agilent 7890B GC		TRACE 1310 GC
Inlet Temperature	140 °C	→	Not required
Inlet Pressure	80 kPa	→	Same
Carrier Gas	Helium	→	Same
Split Ratio	5:1	→	Same
Column Flow	2.5 mL/min	→	Same
Column	TG-624 30 m × 0.32 mm × 1.8 µm	→	Same
Oven Program	40 °C (5 min) to 240 °C (2 min) at 18 °C/min	→	Same
FID Conditions			
Temperature	250 °C	→	Same
Hydrogen Flow	40 mL/min	→	Same
Air Flow	400 mL/min	→	Same
Makeup Flow	40 mL/min	→	Same
Acquisition Rate	25 Hz	→	Same

Results

Data reported in Table 8 for USP <467> Class 2A mixture compounds show that the TriPlus 500 HS autosampler coupled with a TRACE 1310 GC produces outstanding results in terms of repeatability, with an average RSD% = 1.6, when using a method migrated from an Agilent HS-GC system.

Despite the use of different pressure control modes, the TriPlus 500 HS applied the temperature and pressure set points as suggested in the Agilent application note, which is enough to obtain excellent results in accordance with expectations, thus, confirming the method porting is simple and effective. A chromatogram obtained with the TriPlus 500 HS autosampler-TRACE 1310 GC is shown in Figure 5.

Table 8. Peak Area (pA*min) and RSD% obtained on the TriPlus 500 HS autosampler with the method reported in the application note 5990-7625EN.

	TriPlus 500 HS Autosampler	
	Peak Area (pA*min)	RSD% (n=10)
Methanol	1.37	1.41
Acetonitrile	0.42	2.03
Methylene Chloride	5.80	2.00
trans-1,2-Dichloroethene	29.74	1.65
cis-1,2-Dichloroethene	22.25	1.35
Tetrahydrofurane	5.53	1.42
Cyclohexane	492.32	2.43
Methylcyclohexane	158.15	2.48
1,4-Dioxane	0.20	1.52
Toluene	90.51	1.32
Chlorobenzene	19.75	1.14
Ethylbenzene	40.06	1.39
m,p-Xylene	168.28	1.34
o-Xylene	17.93	1.22
Average RSD%		**1.62**

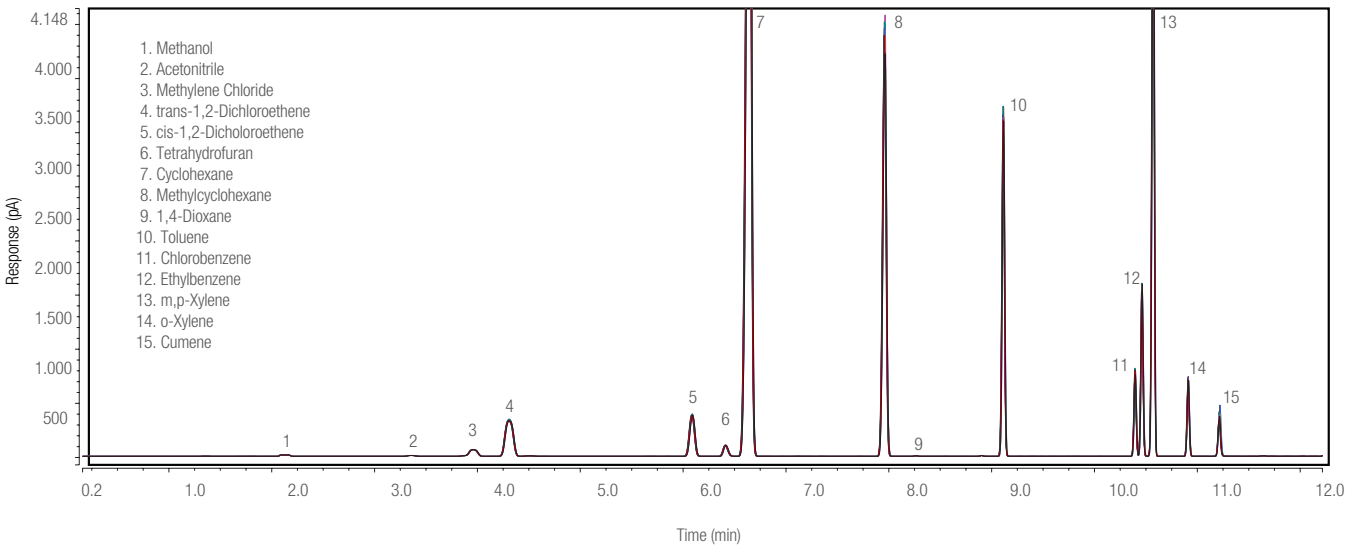

Figure 5. Chromatogram of <USP 467> Class 2A compounds from a TriPlus 500 HS autosampler-TRACE 1310 GC (operating conditions in Table 7)

The USP <467> residual solvents method can be further improved as reported in the Thermo Scientific application note AN10676[4] where operating conditions are optimized to produce the same data quality in a much shorter analysis time, thus, improving the system productivity.

Migration of the USP <467> residual solvents method from the TriPlus 300 HS autosampler operating in standard mode to the TriPlus 500 HS autosampler

As already mentioned, the new TriPlus 500 HS autosampler implements an advanced vial and loop pressure control that produces better performance in terms of sensitivity and repeatability compared to the previous generation headspace samplers.

Nevertheless, in many highly regulated environments such as the pharmaceutical industry, methods are validated for older instruments and must be very rigorously applied to avoid the need of expensive and time-consuming re-validation processes.

In this section, the method for the determination of residual solvents applied on a TriPlus 300 HS autosampler was migrated on the TriPlus 500 HS autosampler and results obtained on the two systems compared. The TriPlus 300 HS autosampler was used in "Standard" mode to simulate a valve and loop headspace sampler that does not implement the active control of the vial and loop pressure. Although this is not the best condition for the TriPlus 500 HS autosampler, the loop filling was performed at ambient pressure to reproduce the same operating conditions.

Sample preparation

According to the procedure USP <467> method for water-insoluble pharmaceutical products, Class 2A mixture (Restek P/N 36012) was diluted 1:100 in pure DMSO in a 100 mL flask (Stock solution A).

20 mL clear headspace vials (P/N C4020-20) were filled with 5 mL pure water, spiked with 1 mL Stock solution A and immediately sealed with crimp caps (P/N 20-MCBC-ST3) with Silicone/PTFE septa.

Method porting

Despite the different control of the vial and loop pressurization, the TriPlus 500 HS and TriPlus 300 HS autosamplers are based on the same valve and loop core technology so the method porting does not require any substantial changes in the parameters.

The Triplus 300 HS autosampler parameters and the corresponding settings for the TriPlus 500 HS autosampler are listed in Table 9.

Table 9. <USP 467> Residual Solvents method parameters: migration from a TriPlus 300 HS autosampler to TriPlus 500 HS autosampler coupled with a TRACE 1310 GC

Headspace Parameters	TriPlus 300 HS Autosampler		TriPlus 500 HS Autosampler
Incubation Temperature	80 °C	→	Same
Incubation Time	40 min	→	Same
Valve/Loop Temperature	90 °C	→	Same
Transfer Line Temperature	100 °C	→	Not required
Shaking Level	Medium	→	Medium
Vial Pressurization Mode	Standard	→	Pressure
Aux Pressure	130 kPa	→	Not required
Aux Time	0.2 min	→	Not required
Vial Pressure	-	→	130 kPa
Vial Pressure Equilibration Time	1 min	→	Same
Injection Time	1 min	→	Same
Injection Mode	Standard	→	Same
Loop Filling Mode	Standard	→	Not required
Loop Filling Time	0.2 min	→	Not required
Loop Pressure	-	→	0 kPa
GC Parameters	TRACE 1310 GC	→	Same
Inlet Temperature	180 °C	→	Not required
Inlet Pressure	75 kPa	→	Same
Carrier Gas	Nitrogen	→	Same
Split Ratio	10:1	→	Same
Column Flow	2.5 mL/min	→	Same
Column	TG-624 30 m × 0.32 mm × 1.8 µm	→	Same
Oven Program	40 °C (5 min) to 240 °C (5 min) at 10 °C/min	→	Same
FID Conditions			
Temperature	260 °C	→	Same
Hydrogen Flow	45 mL/min	→	Same
Air Flow	450 mL/min	→	Same
Makeup Flow	25 mL/min	→	Same
Acquisition Rate	20 Hz	→	Same

Table 10 shows data collected on the TriPlus 300 HS autosampler operating in “Standard” mode and data collected on the TriPlus 500 HS autosampler with the conditions reported above.

Data demonstrate that the porting of the method from the TriPlus 300 HS autosampler to the TriPlus 500 HS autosampler was successful and that the same criteria can be easily applied in the case of legacy instruments with loop filled at ambient pressure.

In particular, the pneumatic control of the TriPlus 500 HS autosampler offers improved repeatability. Moreover, the TriPlus 500 HS autosampler shows an average higher response in the same operating conditions, as a result of more efficient vial shaking.

Conclusions

Instrument-to-instrument headspace method transfer can be challenging: unexpected changes in the results can occur due to differences in the equipment. This concern is especially important in highly regulated environments, such as the pharmaceutical industry, where the equivalence of the results is a must.

In this paper, recommended method settings for the key parameters are provided to successfully transfer an existing method to the Thermo Scientific TriPlus 500 HS autosampler. Reported data also confirm that the TriPlus 500 HS autosampler reaches and exceeds the standards in terms of overall performance, as repeatability, ease of use and productivity in the fields of the headspace analyses.

Table 10. Area counts and RSD% obtained on the TriPlus 300 HS autosampler and TriPlus 500 HS autosampler for the analysis of <USP 467> Class 2A (operating conditions as reported in Table 9)

	TriPlus 300 HS Autosampler		TriPlus 500 HS Autosampler	
	Peak Area (pA*min)	RSD% (n=10)	Peak Area (pA*min)	RSD% (n=10)
Methanol	0.217	4.12	0.308	5.84
Acetonitrile	0.065	6.52	0.096	3.77
Methylene Chloride	0.538	5.18	0.678	1.53
trans-1,2-Dichloroethene	2.814	5.17	3.634	1.72
cis-1,2-Dichloroethene	2.301	5.36	2.975	1.54
Tetrahydrofurane	0.547	5.68	0.756	1.64
Cyclohexane	35.451	5.21	45.727	1.94
Methylcyclohexane	12.944	5.38	16.668	1.99
1,4-Dioxane	0.031	4.46	0.044	2.58
Toluene	9.340	5.57	12.407	1.60
Chlorobenzene	2.047	5.59	2.679	1.49
Ethylbenzene	4.169	5.78	5.539	1.67
m,p-Xylene	17.710	5.98	24.011	1.62
o-Xylene	1.871	5.86	2.465	1.56
Average RSD%		**5.42**		**2.18**

The smooth porting of existing methods for the determination of USP <467> Residual Solvents Class 2A Compounds to the TriPlus 500 HS autosampler demonstrates that the system can run the analytical methods already in use on analytical platforms available in the market, delivering comparable or better performance.

In addition to the reliable and high quality data, the advanced features and design of the TriPlus 500 HS autosampler offer a simplified set of parameters, making the method development and optimization easier and quicker in comparison to conventional headspace instruments.

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