Investigation of Key Parameters for a Smooth Method Transfer to a New Optimized Valve and Loop Headspace Autosampler

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ABSTRACT

This paper describes how to efficiently transfer a method from an existing valve and loop headspace autosampler to the new Thermo Scientific™ TriPlus 500 HS.
The four key instrument parameters of a headspace method that may affect the quality of analytical results, that is system temperatures, vial shaking, vial and loop pressurization, are investigated.
The results obtained on different valve and loop instruments are compared and recommendations for migrating a method to the new TriPlus 500 HS provided. This information can be used as guidelines in case a re-validation process is required.
The new TriPlus 500 HS introduces some technological advancements and new features aimed to simplify the method development and parameter settings. However, the core valve and loop technology remains unchanged so that the method transfer from an existing instrument is straightforward and effective.
As an example above all, the method for the analysis of <USP467> Class 2A residual solvents[1] was transferred to the TriPlus 500 HS from two different sources and results compared to those obtained with the original valve and loop instrumentation.
Data show legacy methods can be efficiently transferred from an existing system to the TriPlus 500 HS producing similar or even better results.

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 Pharma industry. A method transfer can be required for example to move the method from a development laboratory to the QA/QC routine laboratory or, as described in this paper, to move a validated method from an old platform to a new instrument.
To prepare a smooth and streamlined method transfer from an existing valve and loop headspace sampler to a different equipment, it is fundamental to asses how the key parameters affect the performance and demonstrate the existing method applied to a new instrument produces equivalent or better results.
Based on the valve and loop sampling technology, the new TriPlus 500 HS introduces advanced features such as a proprietary pneumatic circuit design, a direct column interface and a exclusive vial shaking, delivering superior reliability, high data quality and a simplified method set tip.

TriPlus 500 HS KEY FEATURES

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. This 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 vial pressure 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

The new proprietary design of the vial shaking during the incubation produces a larger exchange surface between the liquid and the vapor phase producing a faster and more homogeneous migration of the analytes in the headspace thus improving the system productivity and repeatability.

SYSTEM TEMPERATURES

The optimization of the vial incubation temperature is important to maximize the concentration of the analytes in the gas phase at the equilibrium. The incubation oven of the TriPlus 500 HS guarantees an accurate oven temperature control: during a method transfer, setting the same incubation temperature produces the same results with an unmatched quantitative precision for all classes of compounds.

The TriPlus 500 HS is equipped with a short and direct GC interface instead of a long external transfer line: a more efficient and accurate temperature control, a shorter sample path and reduced dead volumes, minimize the risk of carryover and sample degradation and optimize the sample transfer to the column.

An effective heating of the entire sample path is obtained with a single thermal control, from the sampling valve to the GC column. Therefore, when migrating a method from a conventional headspace sampler to the new TriPlus 500 HS, the transfer line temperature parameter can be simply ignored, while maintaining the incubation and loop temperatures unchanged (Table1). In addition, as the column is directly connected to the interface into the GC oven, the injector is not heated and there is no need to set any injector temperature parameter.

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

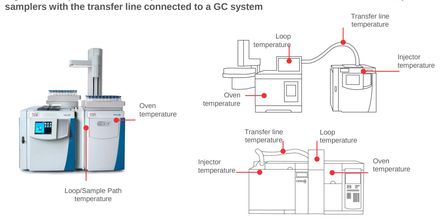


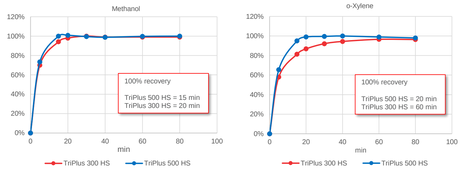
Table 1. System temperatures migration

	HS Sampler with transfer line	TriPlus 500 HS
Incubation Temperature (°C)	85	85
Loop/Sample path Temperature (°C)	85	85
Transfer Line Temperature (°C)	100	-

VIAL SHAKING

An effective agitation of the vial during sample incubation results in a faster reaching of the equilibrium thus providing a reduction of the incubation time and an improved extraction repeatability.
The TriPlus 500 HS implements the new proprietary "Quick Spin Shaking": three shaking levels are available, optimized to provide the user with the right combination of flexibility and system's simplicity.
A comparison between the TriPlus 500 HS and the Thermo Scientific™ TriPlus 300 HS was performed incubating a test solution containing Methanol, THF, Toluene and o-Xylene in water at the 85 °C at increasing incubation times at the maximum shaking level.
Results for Methanol and o-Xylene, selected to represent compounds with different polarity and partition coefficient, show that the recovery on the TriPlus 500 HS is higher than that obtained on the TriPlus 300 HS with the same incubation time for both Methanol and o-Xylene (Figure 2).
This means a shorter incubation time can be set on the TriPlus 500 HS to reach the maximum headspace concentration thus increasing the sample throughput and productivity.
This demonstrates the "Quick Spin Shaking" is more effective than a conventional shaking and that transferring the same shaking level when migrating to the TriPlus 500 HS guarantees similar or even higher recovery, depending on the compound.

Figure 2. Comparison of the recovery at increasing incubation time and maximum shaking level for Methanol and o-Xylene. As expected, the difference observed for compounds with a lower partition coefficient is higher.



VIAL AND LOOP PRESSURE

Vial Pressurization

In the valve and loop technology, a certain amount of inert gas is added to the vial to generate an overpressure and transfer the gas phase into the loop. The vial pressure parameter is the pressure into the vial before the loop filling and its optimization is very critical since directly impacts both sensitivity and repeatability.

The TriPlus 500 HS implements a proprietary and advanced pneumatic circuit which provides an accurate and precise control of the pressure in the vial.
A built-in and optimized strategy to reach the target vial pressure produces the best results extremely simplifying the method development.

In the default mode "Pressure", the user sets the vial pressure and the autosampler automatically regulates an optimized pressure rate and time to reach the desired setpoint.
The migration of the pressure parameter from an existing valve and loop headspace sampler to the TriPlus 500 HS is extremely simple: when the existing method includes a vial pressure parameter, it is enough to set the same pressure setpoint. In case the method parameter is a pressurization time, the pressure setpoint will be the pressure value regulated by the pressure regulator on the auxiliary gas line.

To compare the effect of the pressurization, the test solution was analyzed on the TriPlus 500 HS in the default pressurization mode and on the TriPlus 300 HS in Pressure and Standard mode as reported in Table 2. The results show the pressurization of the new TriPlus 500 HS produces equivalent area counts demonstrating that the method porting is effective and there is no impact on the analytical results, even when compared to the Standard pressurization mode.

Table 2. Comparison of different pressurization mode. All other parameters are constant (incubation 40 min at 85 °C). The Loop Pressurization mode is always Pressure to exclude any possible contribution to this test.

	TriPlus 300 HS (Pressure)	TriPlus 300 HS (Standard)	TriPlus 500 HS
Vial Pressurization mode	Pressure	Standard	Pressure
Vial Pressure	130kPA	-	130kPa
Aux Gas Pressure	-	130kPa	-
Pressurization Time	-	0.2min	-
Equilibration Time	1min	1min	1min
Loop Pressurization mode	Pressure	Pressure	Pressure
Loop Pressure	70kPa	70kPa	70kPa
	Area Counts (pA*min)		
MeOH	0.32	0.29	0.48
THF	1.38	1.22	1.50
Toluene	32.18	28.22	33.93
o-Xylene	4.79	4.17	5.00

Loop Pressurization

The loop pressure is a crucial parameter to improve method sensitivity and repeatability. Setting the loop pressure above the ambient pressure 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 analytes).
Through a proprietary and patent pending control of the pressure, the TriPlus 500 HS delivers an accurate and precise loop filling in any conditions. Similarly to the vial pressure, the system doesn't add complexity to the method development as it only requires to set the desired loop pressure.

In the event of a method transfer to a TriPlus 500 HS and the existing method includes a loop filling pressure, setting the same loop pressure conditions will produce comparable results.
In case the parameter is a loop fill time and the loop is filled at ambient pressure, the corresponding loop pressure setting will be 0.

Data in Table 3 show that the TriPlus 500 HS produces results comparable to those obtained on the TriPlus 300 HS setting the loop pressure at the same setpoint, either with the loop at ambient pressure or in overpressure, making the transfer of this parameter smooth and results reliable.

Table 3. Comparison of different loop pressurization modes. All other parameters are constant (incubation 40 min at 85 °C). The Vial Pressurization mode is always Pressure to exclude any possible contribution to this test.

	TriPlus 300 HS (Standard)	TriPlus 500 HS (Amb Press)	TriPlus 300 HS (Pressure)	TriPlus 500 HS (Pressure)
Vial Pressurization mode	Pressure	Pressure	Pressure	Pressure
Vial Pressure	130kPa	130kPa	130kPa	130kPa
Equilibration Time	1min	1min	1min	1min
Loop Fill mode	Standard	Pressure	Pressure	Pressure
Loop Pressure	-	0kPa	70kPa	70kPa
Loop Fill Time	0.2min	-	-	-
Loop Equilibration Time	0.2min	0.2min	0.2min	0.2min
	Area Counts (pA*min)			
MeOH	0.23	0.38	0.33	0.48
THF	0.65	1.01	1.39	1.50
Toluene	13.27	18.97	32.19	33.93
o-Xylene	1.96	2.78	4.80	5.01

<USP467> METHOD TRANSFER

Transfer of Method Parameters from the Agilent Application note 5990-7625EN

The method reported in the Agilent Application note 5990-7625EN[2] for the analysis of USP<467> Class 2A compounds was transferred to a TriPlus 500 HS coupled to a Thermo Scientific™ TRACE™ 1310 GC.
Based on the previous considerations, the method was easily converted to the new instrument with a lower number of parameters and no substantial differences in the operating conditions so that a complete method revalidation might not be required.

Table 4. <USP 467> Method from Agilent Application Note 5990-7625EN transferred to the TriPlus 500 HS autosampler. Standard solutions and samples prepared according to the <USP 467> method. GC parameters set as in the Agilent method. Chromatogram shown on Figure 3.

Headspace Parameters	App Note 5990-7625EN		TriPlus 500 HS
Incubation Temperature	85°C	→	Same
Incubation Time	40min	→	Same
Valve/Loop Temperature	85°C	→	Same
Transfer Line	100°C	→	-
Shaking Level	2	→	Low
Vial Fill Mode/Vial Pressure	Default (Flow to Pressure)	→	Pressure
Vial Pressure	103kPa	→	Same
Loop Fill Mode/Loop pressure	Custom	→	Pressure
Loop Pressure	69kPa	→	Same
Vial Pressure Equilibration Time	1min	→	Same
Loop Equilibration Time	0.05min	→	Same
Injection mode	Standard	→	Same
Injection Volume	1mL	→	Same
Injection Time	0.5min	→	Same

Table 5. Results obtained with the TriPlus 500 HS and the method parameters reported on Table 4.

	Area Counts (pA*min)	RSD% (n=10)
Methanol	1.57	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

Data reported in Table 5 for the USP<467> Class2A mixture compounds show that, even using a method migrated from an Agilent system, the TriPlus 500 HS coupled with TRACE 1310 GC-FID system produces outstanding results in terms of repeatability on the entire compound range producing an average RSD% = 1.6.

Despite the two instruments use different pressure control modes, setting the temperature and pressure setpoints suggested in the Agilent Application Note is enough to obtain excellent results, fully in line with the expectations thus confirming the method porting is very effective.

Transfer of Method Parameters from a Thermo Fisher application

The method adopted on a TriPlus 300 HS coupled to a TRACE 1310 GC for the analysis of USP<467> Class 2A compounds was transferred to a TriPlus 500 HS with the same GC.
The TriPlus 300 HS was used in Standard mode to replicate the behavior of the previous generation headspace samplers where there is no control of the filling pressure and the loop is always at ambient pressure. Despite the different control of the vial and loop pressure, the core technology on the two systems is the same so porting a method from a previous generation sampler doesn't require substantial changes in the parameters (Table 6).

Figure 3. Overlay of the chromatograms obtained from 10 repeated analysis of the <USP 467> Class 2A test mixture according to conditions reported in Table 4.

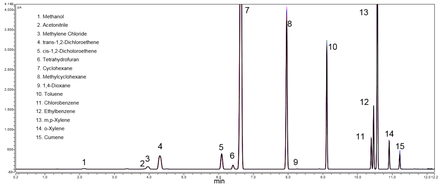


Table 6. <USP 467> Residual Solvent Method transferred from a TriPlus 300 HS in Standard mode to the TriPlus 500 HS autosampler. Same GC parameters set on both systems. Standard solutions and samples prepared according to the <USP 467> method.

Headspace Parameters	TriPlus 300 HS (Standard mode)		TriPlus 500HS
Incubation Temperature	80°C	→	Same
Incubation Time	40min	→	Same
Valve/Loop Temperature	90°C	→	Same
Transfer Line	100°C	→	-
Shaking level	Medium	→	Medium
Pressurization Mode	Standard	→	Pressure
Aux Pressure	130 kPa	→	-
Aux Time	0.2 min	→	-
Via Pressure	-	→	130 kPa
Pressure Equilibration Time	1 min	→	Same
Injection Time	1 min	→	Same
Injection Mode	Standard	→	Same
Loop Filling Mode	Standard	→	Pressure
Loop Filling Time	0.2min	→	-
Loop Pressure	-	→	0 kPa

Table 7. Results obtained with the TriPlus 300 HS in standard mode and the TriPlus 500 HS with the method parameters reported on Table 6.

	TriPlus 300 HS (Standard mode)		TriPlus 500 HS	
	Area Counts	RSD%	Area Counts	RSD%
Methanol	0.22	4.12	0.31	5.84
Acetonitrile	0.06	6.52	0.09	3.77
Methylene Chloride	0.54	5.18	0.68	1.53
trans-1,2-Dichloroethene	2.8	5.17	3.63	1.72
cis-1,2-Dichloroethene	2.3	5.36	2.97	1.54
Tetrahydrofurane	0.55	5.68	0.76	1.64
Cyclohexane	35.45	5.21	45.73	1.94
Methylcyclohexane	12.94	5.38	16.67	1.99
1,4-Dioxane	0.03	4.46	0.04	2.58
Toluene	9.34	5.57	12.41	1.60
Chlorobenzene	2.05	5.59	2.68	1.49
Ethylbenzene	4.17	5.78	5.54	1.67
m,p-Xylene	17.71	5.98	24.01	1.62
o-Xylene	1.87	5.86	2.46	1.56
Average RSD%		5.42		2.18

Data in Table 7 show that porting the parameters from the TriPlus 300 HS to the TriPlus 500 HS was successful.

In particular, the TriPlus 500 HS actively controls the loop pressure even when venting the loop at ambient pressure and produces a significant improvements in the repeatability compared to the TriPlus 300 HS used in Standard mode.

Moreover, average higher responses are obtained with the TriPlus 500 HS, this potentially being explained by the more efficient vial shaking.

These results confirm that the method transfer is easily applied also in case of legacy instruments with loop at ambient pressure.

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 Pharma industry where the equivalence of the results is a must.
- Recommended method settings for the key parameters to successfully transfer an existing method to the TriPlus 500 HS are provided. The transfer of two existing methods for the determination of <USP 467> Class2A compounds was also tested and comparative data show similar or better results are obtained. This information can be used as guidelines in case a re-validation process is required.
- The advanced features and design of the TriPlus 500 HS offer reliable and high quality data. Moreover, it requires a simplified set of parameters, making the method development and optimization much easier and quicker.

REFERENCES

1. General Chapter USP <467> Organic Volatile Impurities, Chemical Tests, United States Pharmacopeia
2. Firor, R. L. Analysis of <USP 467> Residual Solvents with Improved Repeatability Using the Agilent 7697A Headspace Sampler. Agilent Appl. Note 5990-7625EN (2012)

TRADEMARKS/LICENSING

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