

Automation of Sample Preparation Steps using a Robotic X-Y-Z Coordinate Autosampler with Software Control

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KEYWORDS

Automation, sample preparation, filtration, weighing, SPE, saponification, esterification

ABSTRACT

Today's analytical chemist faces the task of analyzing an increasing number of samples and maximizing throughput while ensuring that the highest quality in the resulting data is achieved. There is therefore an increasing need for automated sample preparation. Many instruments are available that have been created to automate specific sample preparation techniques. As a result, a number of different instruments are needed, and each of these systems has its own controlling software.

A robotic X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The MAESTRO software enables control of an expanded list of sample preparation techniques such as derivatization, addition of an internal standard, saponification, esterification, analytical weighing, filtration, and solid phase extraction. In addition to ease of use and intuitive windows-based setup, the software includes tools to automate and optimize timing parameters ensuring efficient sequence creation and maximum sample throughput. The sampler can be configured as part of a GC or LC system or can be configured as a benchtop workstation.



In this paper, we discuss various sample preparation techniques available when using the robotic autosampler in conjunction with the MAESTRO software. Examples of automating techniques such as solid phase extraction, Twister solvent back extraction for HPLC, and steps such as sample weighing are shown.

INTRODUCTION

In addition to providing unattended operation as an autosampler for a chromatographic instrument, the use of the MultiPurpose Sampler (MPS) robot in combination with the MAESTRO software enables full automation of a number of sample preparation steps. MAESTRO software control provides flexible sample preparation capability while helping to improve sample throughput in the lab.

Some of the sample preparation steps actions available within the MAESTRO software are listed below.

	ADD	Used to add a liquid from a source location to a destination location
$\overline{\mathbf{i}}$	WAIT	Allows the MPS to wait for a defined time period
\$	MOVE	Allows the MPS to move a vial from a tray to a user specified destination (e.g., move a vial to the agitator in order to mix a solution)
J	MIX	Allows the MPS to control a programmable agitator
1	INJECT	Instructs the MPS to inject sample into the chromatographic system using a user defined injection method
 \$	NEWLINER	Allows the MPS to replace a GC inlet liner with a new liner
	HZAW	Instructs the MPS to wash the syringe with a user defined wash program
ا	DILUTE	Instructs the MPS to use the dilutor option to add liquid to a user defined vial
27	WEIGH	Instructs the MPS to open the draft shield of an analytical balance, move a specified vial onto the balance, capture the weight of the vial and transfer the data to an Excel spreadsheet, move the vial back to its original location and then closed the draft shield of the analytical balance
4	OUTPUT	Instructs the MPS to send an output signal that could be used to start/stop additional accessories within the chromatographic system
₽₹	FLUSH	Used with an MPS headspace syringe to deliver clean gas through the syringe for cleaning or sample vial purging
3	VALVE	Allows the MPS to control the position of a serial valve drive
ī ¢	SPE SHIFT	Instructs the MPS to position the solid phase extraction unit either in the waste position or over a collection vial
₽₹	SPE EVAPORATE	Allows the eluent from a solid phase extraction to be evaporated for solvent exchange or concentration using inert gas flow through the SPE syringe

NEW FEATURES



- The MPS 2 XL autosampler can be configured as a bench top workstation including a Sartorius balance to prepare trays of samples for GC or HPLC analysis.
- With MAESTRO software control, samples can be moved to the balance, weighed, and returned to the sample tray. Weights are written to an Excel spreadsheet.

The basic procedure shown in Figure 2, is as follows:

- 1. An empty 10 mL headspace vial with magnetic cap is moved to a ME614S Sartorius balance, the vial is weighed, and the data is transferred to an Excel spreadsheet.
- 2. 5 mL of a sample is added to the 10 mL vial using a 2.5 mL syringe.
- 3. The vial is re-weighed and the data is transferred to the Excel spreadsheet.
- 4. 0.250 mL of an internal standard is added to the 10 mL vial.
- 5. The vial is re-weighed and the data is transferred to the Excel spreadsheet.
- 6. Finally, 1.3 mL of the final solution is transferred to a 2 mL autosampler vial for subsequent analysis.

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Modified							

Figure 2. MAESTRO PrepSequence for automated liquid delivery followed by analytical balance weighing.

Figure 1. Sartorius balance option for MPS 2 autosampler.

Table 1 shows the results of the liquid transfers and weighing, illustrating precision and accuracy of the transfers.

Sample	Empty	Sample	IS
		Addition	Addition
Tray2,01	12.347	4.938	0.240
Tray2,02	12.311	4.947	0.240
Tray2,03	12.305	4.941	0.240
Tray2,04	12.451	4.947	0.240
Tray2,05	12.350	4.945	0.240
Tray2,06	12.277	4.947	0.240
Tray2,07	12.397	4.945	0.240
Tray2,08	12.350	4.947	0.240
Tray2,09	12.441	4.943	0.240
Tray2,10	12.221	4.946	0.240
Tray2,11	12.423	4.947	0.241
Tray2,12	12.444	4.945	0.240
Tray2,13	12.390	4.944	0.240
Tray2,14	12.409	4.946	0.240
Tray2,15	12.376	4.948	0.240
Tray2,16	12.374	4.945	0.240
Tray2,17	12.404	4.948	0.240
Tray2,18	12.418	4.947	0.241
Tray2,19	12.282	4.938	0.241
Tray2,20	12.287	4.946	0.240
Tray2,21	12.416	4.947	0.240
Tray2,22	12.199	4.945	0.241
Tray2,23	12.498	4.950	0.239
Tray2,24	12.602	4.952	0.240
Tray2,25	12.607	4.930	0.239
Tray2,26	12.613	4.898	0.240
Tray2,27	12.503	4.927	0.240
Tray2,28	12.566	4.935	0.240
Tray2,29	12.526	4.919	0.240
Tray2,30	12.548	4.926	0.240
Tray2,31	12.608	4.950	0.240
Tray2,32	12.527	4.933	0.240
mean		4.941	0.240
SD		0.011	0.000
%CV		0.224	0.181

Table1. Automated analytical balance data obtained following automated liquid delivery.

AUTOMATED FILTRATION



Figure 3. Automated Filtration accessory using 96-well filtration plates.

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Figure 4. MAESTRO prepSequence for automated filtration using 96-well filtration plates.

• When used in conjunction with the MAESTRO software, the MPS can be configured with 96 well filtration and collection plates, to automate the extraction, filtration, and injection of sample extracts.

TWISTER BACK EXTRACTION



Figure 5. Automated Twister back extraction.

🏶 PrepBuilder	I - C:\Program Files\Maestro\1\PrepSequence\Twister Back Extr	traction with LC analysis.prp 🔹 🥐	
Prep. Setting	:		
<u>V</u> ial Range	1-10		
Prep. Action	Settings		
Action	PREP Syringe: 100ul		
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		OK <u>C</u> ancel <u>H</u> elp	

Figure 6. MAESTRO prep-sequence for Twister back-extraction followed by LC analysis.

- The basic procedure shown in Figure 6 requires the following steps:
- 1. After extraction, the Twister is manually transferred to a 2 mL vial with a 250 μ L conical insert. This vial is crimp-sealed using a magnetic cap.
- 2. The MPS fills the insert with 200 μ L of desorption solvent.
- 3. The sample vial is moved to the agitator and the Twister is back extracted at user defined temperature, agitation speed and duration.
- 4. The sample vial is moved back to its original sample tray and the sample extract is withdrawn from the vial and injected into the HPLC.

SOLID PHASE EXTRACTION



Figure 7. Automated solid phase extraction accessory.

repBuilder 1 - C: Progr	am FilesWaestro\1\PrepSequence\Auto	mated SPE with sample loop fl	lush prior to LC.prp	?
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Vial Range 1-32				
rep. Action Settings				
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Action	Method / Value	Source	Vial Destination	Vial
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2 🌩 MOVE		SPETray2,VT98spe	SPEWaste	
3 🚡 ADD	Condition SPE using 1mL MeOH	SFSWash1	SPEWaste	
4 📓 ADD	Condition SPE using 1mL H20	SFSWash2	SPEWaste	
5 📓 ADD	Add 1mL of sample to SPE	Tray2,V198	SPEWaste	
	Wash using TML of 5% MeUH	5F5Wash3	SPEWaste	
9 \$ ADD	WASTE -> VIAL Flute with 1ml of MeDH	CECW/ash1	SPEVial	
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12 🌩 MOVE		SPESampl, VTSPEV	Tray2,VT98	
13 🗟 ADD	Add 250uL reconstitution solution to extract	SFSWash4	Tray2,VT98	
14 🗟 ADD	Mix extract via syringe	Tray2,VT98	Tray2,VT98	
SWITCH INJ	Active		LC VIv1	
15 🖀 ADD	Add 250uL of MeOH to sample loop for flush	SFSWash1	LC VIv1	
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			OK Cancel	Help

Figure 8. MAESTRO prep-sequence for automated SPE with sample loop flush prior to LC injection.

- Using MAESTRO software, a Prep Sequence can be created to automate the entire solid phase extraction of samples.
- Following elution, evaporative concentration, and reconstitution of the evaporated extract, the sample can be injected for analysis.



Figure 9. MAESTRO sequence scheduler associated with automated SPE method.

• The sequence schedule preview graphically displays all method steps included in the Prep Sequence as well as the duration of each step. The methods labeled in Figure 9, correspond to the Prep Sequence method lines labeled in Figure 8.



Figure 10. Automated SPE batch completion time comparison: "with prep ahead feature" vs. "without prep ahead feature".

- The use of the MAESTRO "Prep Ahead" feature enables the preparation of the following sample in a batch during GC or LC analysis of the current sample, maximizing sample throughput.
- The sequence schedule preview graphically displays all method steps included in the Prep Sequence with their relevant duration. The methods labeled in Figure 9, correspond to the Prep Sequence method lines labeled in Figure 8.

AUTOMATED **S**APONIFICATION AND **E**STERIFICATION

Figure 11. Benchtop workstation for automated saponification/esterification.



			IcationEsternication	32 samp	les.prp		
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- END							
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🤗 WAIT	35.00						_
	BF3 MeOH		SFSWash2		HeatTray,VT32-20h		
MOVE	80.00		HeatTray.VT32-20h		Trav2.VT32-20		
- END							
📥 WASH	MeOH wash 1						
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Action Action	Ahead, Bounded NaCl1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane	_	SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SolvRes3 SolvRes3	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 AniPatro AniTray	Vial	
PREP Vials 1-32 a ADD END Action ≪WASH a ADD a ADD a ADD a ADD a ADD w WASH PREP Vials 1-32 a ADD c MOVE C MIX	Ahead, Bounded NaCl1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex		SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SolvRes3 SolvRes3	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 Agitator,AgTray	Vial	
PREP Vials 1-32 à ADD END Action ♦ WASH à ADD à ADD à ADD ♦ WASH PREP Vials 1-32 à ADD ♦ MOVE ♥ MOVE	Ahead, Bounded NaC11 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex		SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SolvRes3 SFSWash3 Tray2.VT32:20 Agitator.AgiTray	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 Agitator,AgTray Tray2,VT32-20	Vial	
PREP Vials 1-32 à ADD END Action ♦ WASH à ADD à ADD à ADD à ADD b WASH PREP Vials 1-32 à ADD ♥ MOVE C MIX ♥ MOVE END	Ahead, Bounded NaCI1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex		SolvRes1 Source SolvRes3 SolvRes4 SolvRes5 SolvRes5 SolvR	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 Agitator,AgiTray Tray2,VT32-20	Vial	
PREP Vials 1-32	Ahead, Bounded NaCi1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex Ahead, Bounded Tracific Ibauana		SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SolvRes3 SrSWash3 Tray2.VT32:20 Agitator.AgiTray	Vial	Tray2,VT32-20 Destination Waste Waste Tray2,VT32-20 Agitator,AgiTray Tray2,VT32-20 Tray2,VT32-20	Vial	
PREP Vials 1-32	Ahead, Bounded NaCl1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex Ahead, Bounded Transfer hexane		SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SrSWash3 Tray2.VT32-20 Agitator.AgiTray	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 Agitator,AgTray Tray2,VT32-20 Tray2,VT32-20	Vial	
PREP Vials 1-32 a ADD END END Action ▲ WASH a ADD a ADD	Ahead, Bounded NaCl1 Method / Value Water wash 1 IPA wash 1 IPA wash 1 IPA wash 1 IPA wash 1 Hexane wash Ahead, Bounded Hexane Vortex Ahead, Bounded Transfer hexane IPA wash 1		SolvRes1 Source SolvRes3 SolvRes3 SolvRes3 SolvRes3 SFSWash3 Tray2,VT32-20 Agitator,AgiTray Tray2,VT32-20 SolvRes3	Vial	Tray2,VT32-20 Destination Waste Waste Waste Tray2,VT32-20 Agitator,AgTray Tray2,VT32-20 Tray1,VT42sp1 Waste	Vial	
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Figure 12. MAESTRO prep-sequence for automated saponification and esterification using heated 32-position tray.

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- With a total of 999 lines available, a single, detailed and comprehensive sample preparation procedure can be created for every conceivable task.
- Sample preparation steps, like reagent addition, digestions, standard addition, derivatization, extraction and transfers are created by mouse-click in an MS® Windows® environment with no need for macro programming..
- Using the heated 32-position tray in conjunction with the "Prep Ahead" feature, the software enables maximum sample throughput by overlapping sample preparation steps, i.e. by performing these in parallel. The number of samples that can be prepared in parallel depends on the method parameters.
- For a sample set of 32 samples, Prep Ahead reduced total preparation time from 55 hours to 6 hours.

CONCLUSIONS

Novel PrepFunctions in the GERSTEL MAESTRO software provide increased versatility for automated control of sample preparation including special functions without the need for macro programming. New accessories for the MPS (integrated balance option; 96-well filtration plate; 32-position heated tray) further expand the range of automation capabilities controlled by the MAESTRO software.



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