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## 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 set-up, 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
	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)
	MIX	Allows the MPS to control a programmable agitator
	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
	WASH	Instructs the MPS to wash the syringe with a user defined wash program
	DILUTE	Instructs the MPS to use the dilutor option to add liquid to a user defined vial
	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
	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
	VALVE	Allows the MPS to control the position of a serial valve drive
	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

### INTEGRATED ANALYTICAL BALANCE

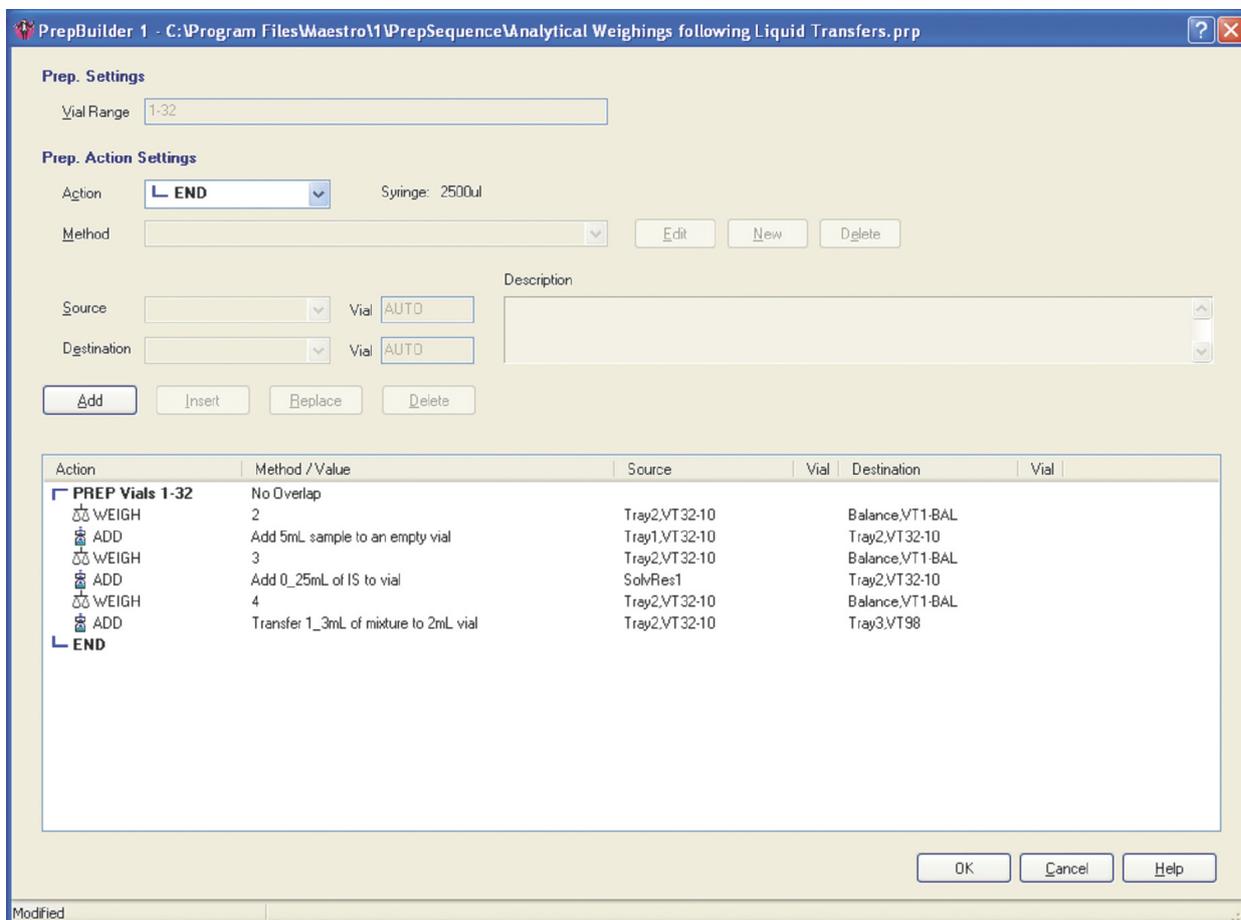


**Figure 1.** Sartorius balance option for MPS 2 autosampler.

- 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.



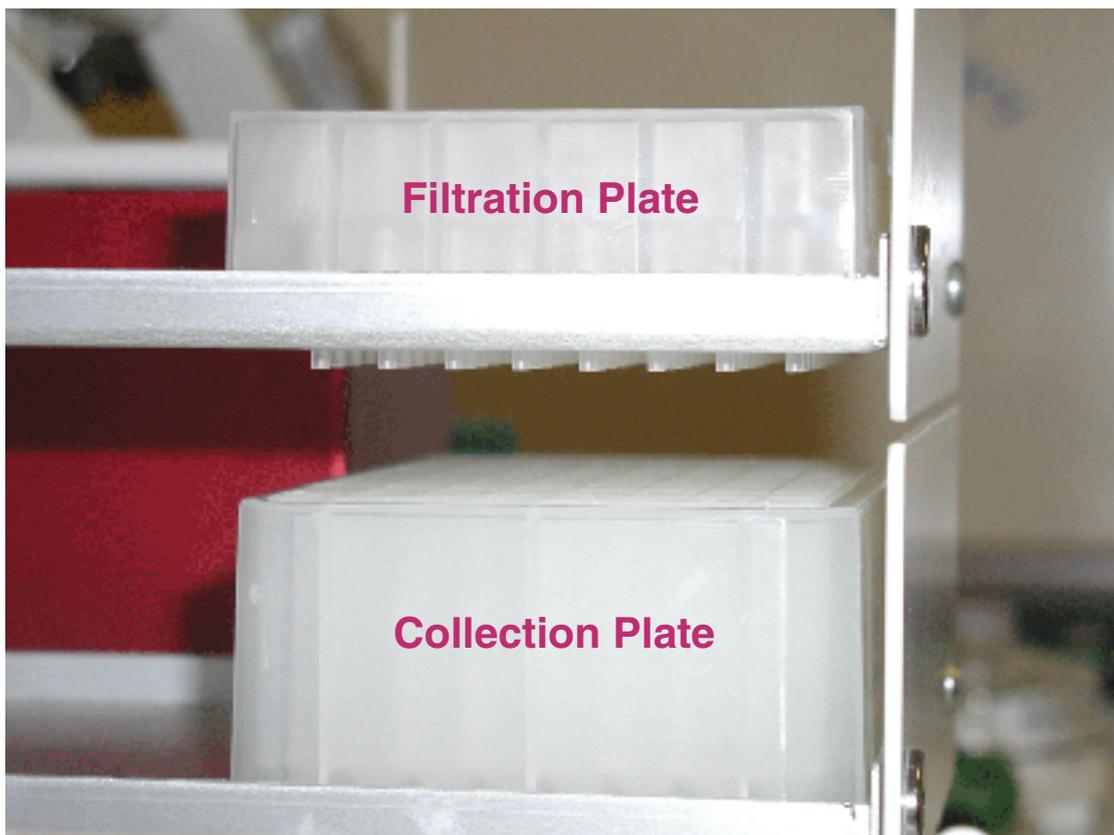
**Figure 2.** MAESTRO PrepSequence for automated liquid delivery followed by analytical balance weighing.

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

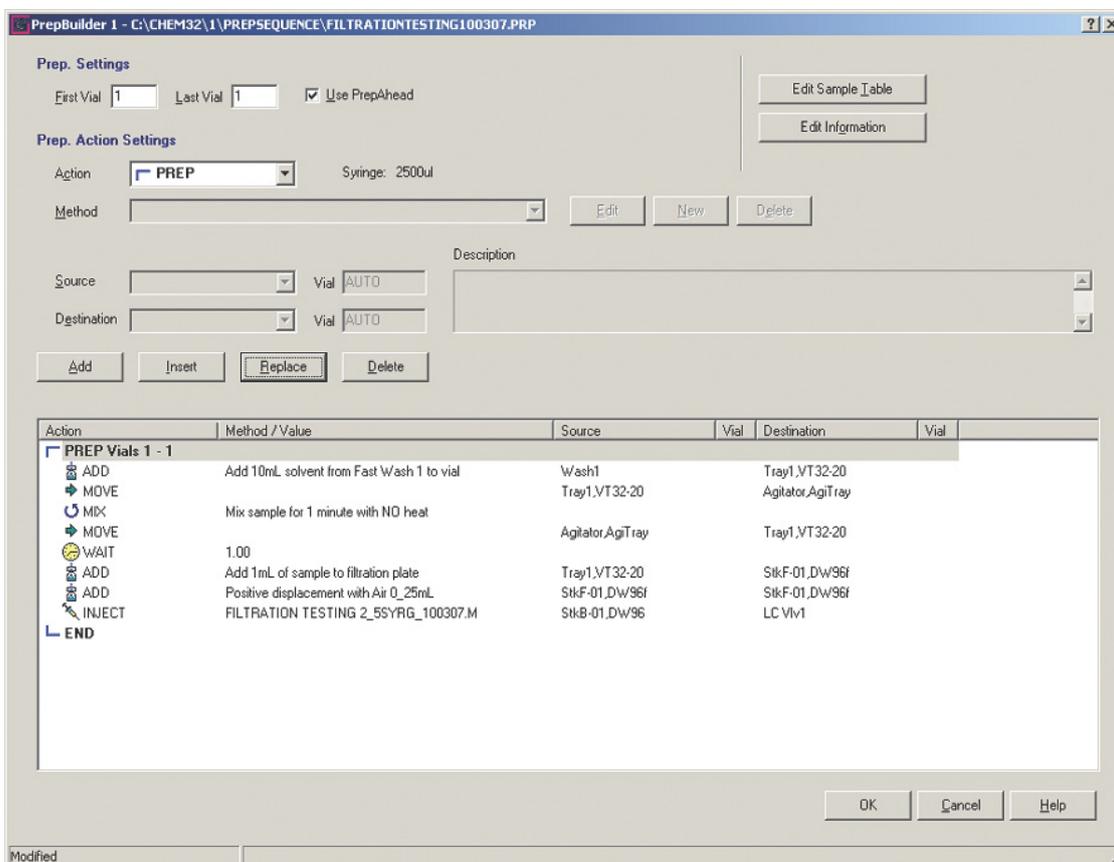
**Table1.** Automated analytical balance data obtained following automated liquid delivery.

Sample	Empty	Sample Addition	IS 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

## AUTOMATED FILTRATION



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



**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

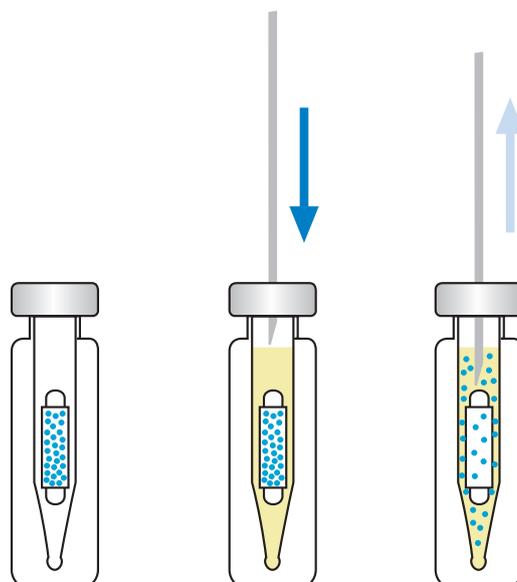


Figure 5. Automated Twister back extraction.

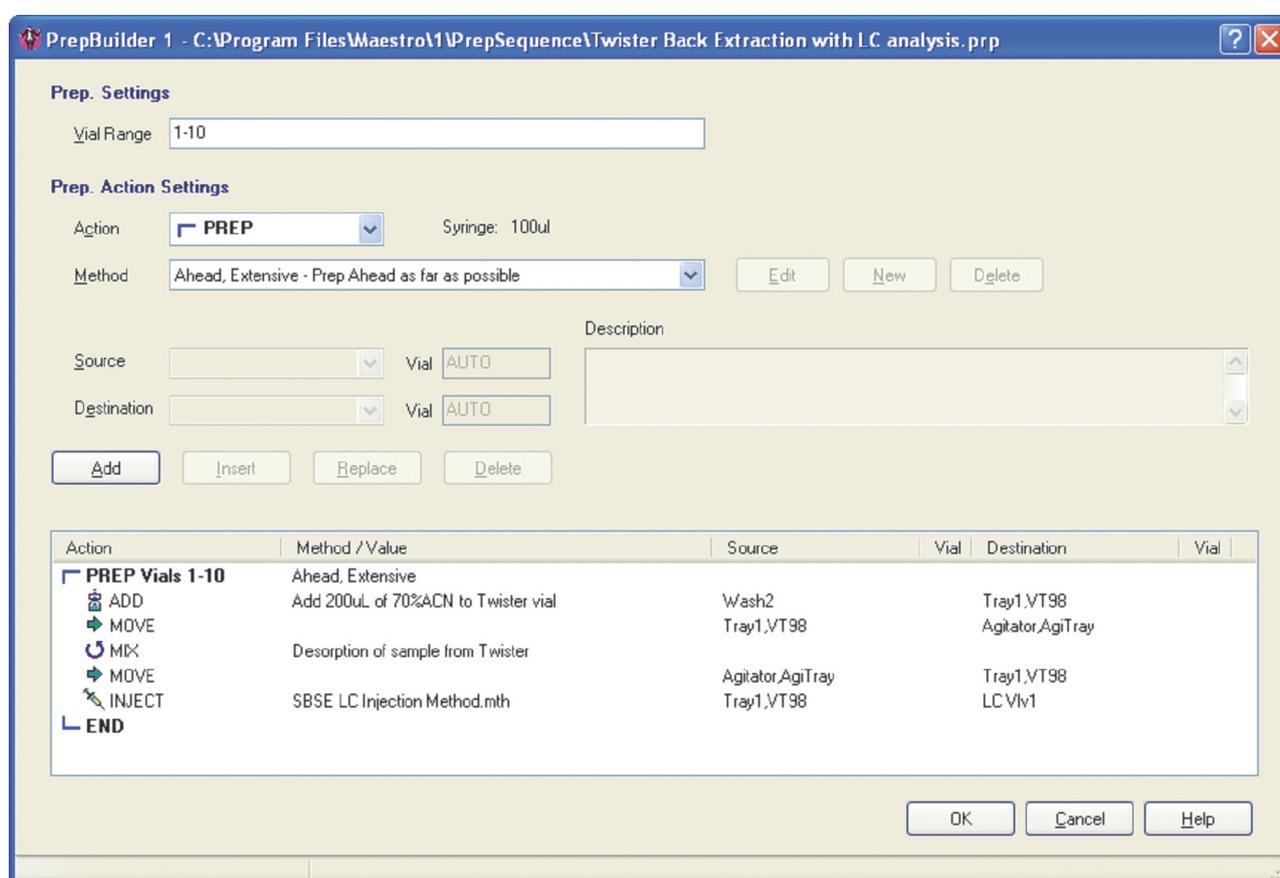


Figure 6. MAESTRO prep-sequence for Twister back-extraction followed by LC analysis.

- 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  $\mu$ L conical insert. This vial is crimp-sealed using a magnetic cap.
  2. The MPS fills the insert with 200  $\mu$ 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.

PrepBuilder 1 - C:\Program Files\Maestro\1\PrepSequence\Automated SPE with sample loop flush prior to LC.prp

**Prep. Settings**  
 Vial Range: 1-32

**Prep. Action Settings**  
 Action: PREP Syringe: 2.5mLX  
 Method: Ahead, Extensive - Prep Ahead as far as possible [Edit] [New] [Delete]

Source: [ ] Vial: AUTO  
 Destination: [ ] Vial: AUTO

[Add] [Insert] [Replace] [Delete]

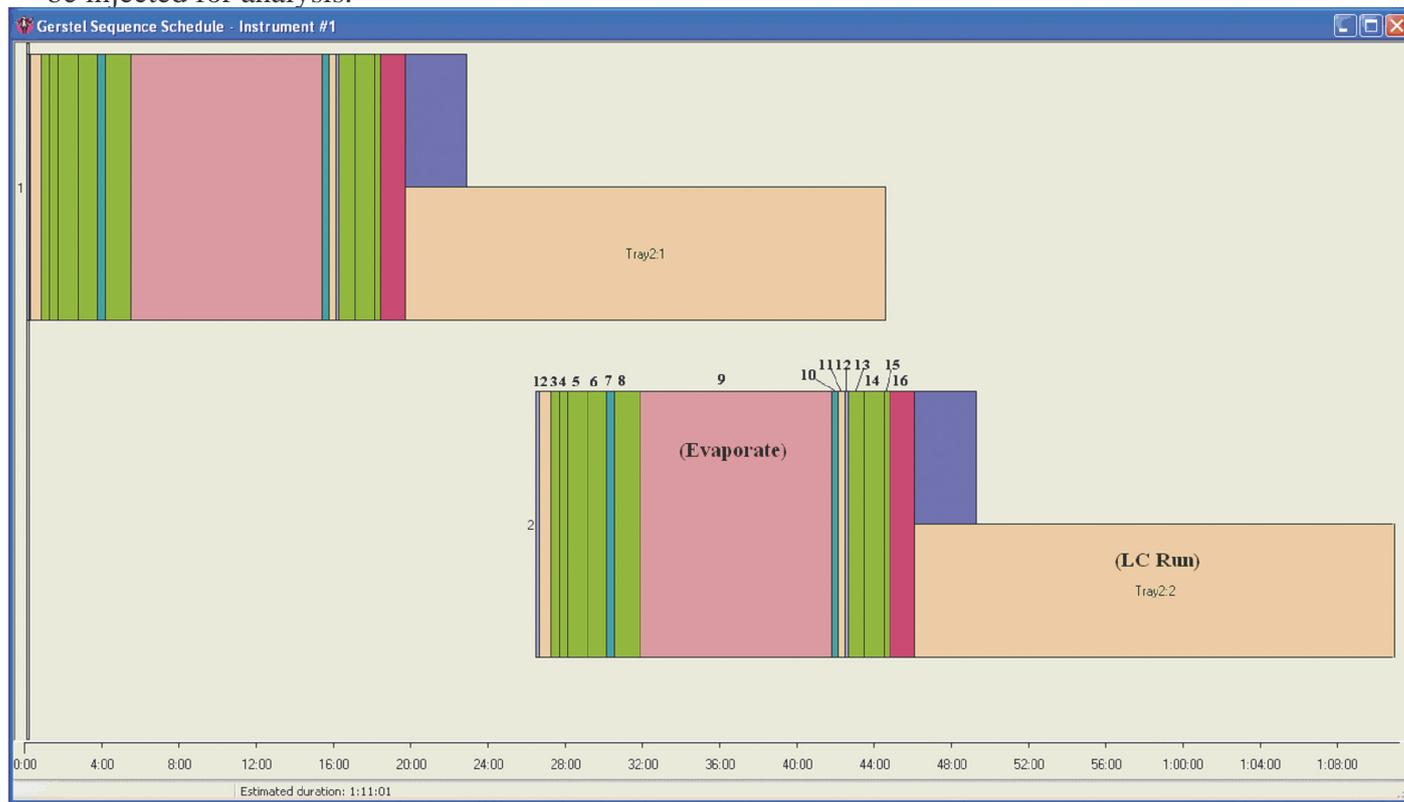
Action	Method / Value	Source	Vial	Destination	Vial
PREP Vials 1-32	Ahead, Extensive				
1 MOVE		Tray2.VT98		SPESampl.VTSPEV	
2 MOVE		SPETray2.VT98spe		SPEWaste	
3 ADD	Condition SPE using 1mL MeOH	SFSWash1		SPEWaste	
4 ADD	Condition SPE using 1mL H2O	SFSWash2		SPEWaste	
5 ADD	Add 1mL of sample to SPE	Tray2.VT98		SPEWaste	
6 ADD	Wash using 1mL of 5% MeOH	SFSWash3		SPEWaste	
7 SPE SHIFT	WASTE -> VIAL				
8 ADD	Elute with 1mL of MeOH	SFSWash1		SPEVial	
9 SPE EVAPORATE	Evaporate Eluent				
10 SPE SHIFT	VIAL -> WASTE				
11 MOVE		SPEWaste		CritWaste.VTSPEW	
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 Vlv1	
15 ADD	Add 250uL of MeOH to sample loop for flush	SFSWash1		LC Vlv1	
SWITCH INJ	Standby			LC Vlv1	
16 INJECT	LC Injection Method.mth	Tray2.VT98		LC Vlv1	
END					

[OK] [Cancel] [Help]

Modified

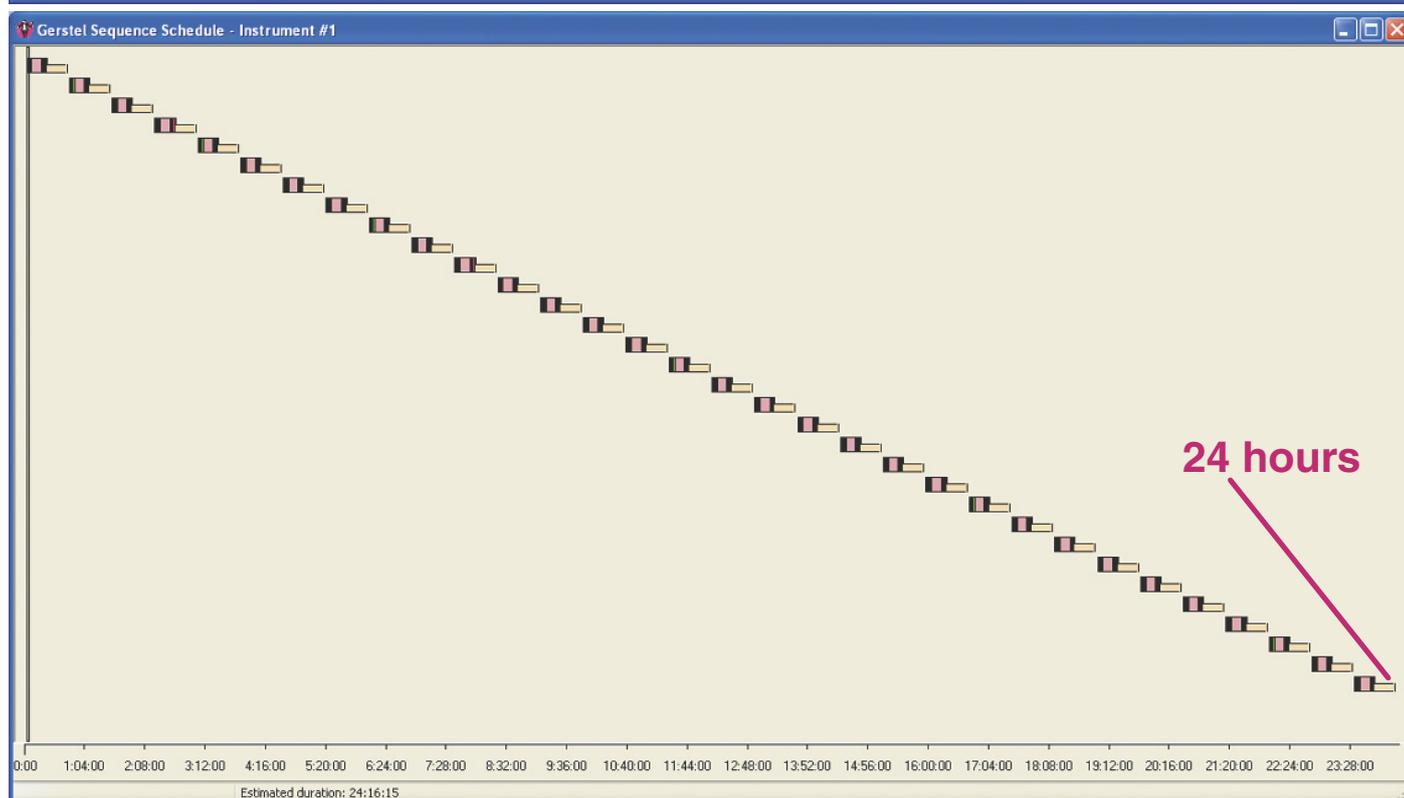
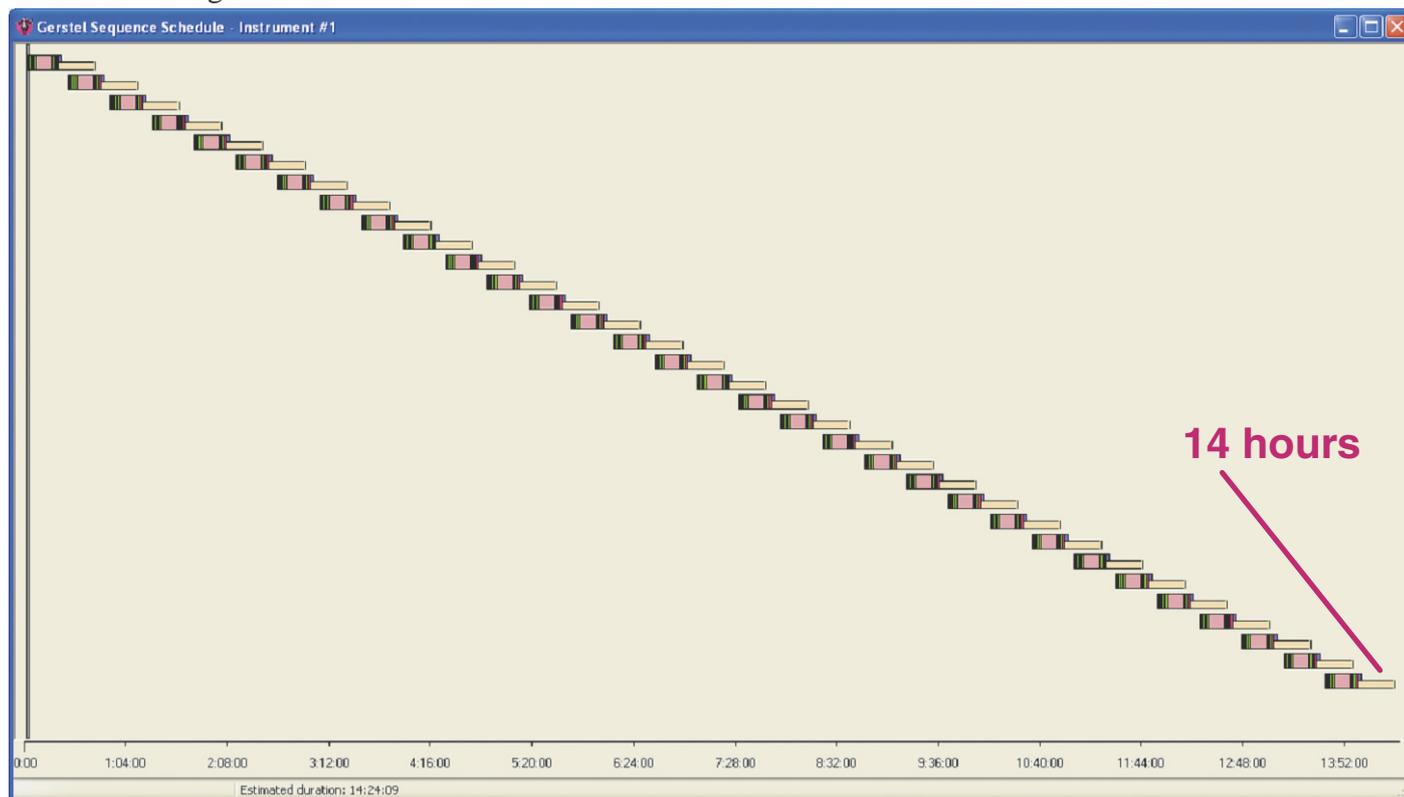
Figure 8. MAESTRO prep-sequence for automated SPE with sample loop flush prior to LC injection.

- 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 SAPONIFICATION AND ESTERIFICATION

**Figure 11.** Benchtop workstation for automated saponification/esterification.



PrepBuilder 1 - C:\Program Files\Maestro\1\PrepSequence\Auto SaponificationEsterification 32 samples.prp

**Prep. Settings**  
Vial Range: 1-32

**Prep. Action Settings**  
Action: PREP Syringe: 5000ul  
Method: Ahead, Bounded - Prep Ahead within this loop [Edit] [New] [Delete]

Source: [ ] Vial: AUTO Description: [ ]  
Destination: [ ] Vial: AUTO

[Add] [Insert] [Replace] [Delete]

Action	Method / Value	Source	Vial	Destination	Vial
PREP Vials 1-32	Ahead, Bounded				
ADD	NaOH MeOH	SFSWash1		Tray2,VT32-20	
END					
WASH	Water wash 1				
WASH	MeOH wash 1				
PREP Vials 1-32	Ahead, Bounded				
MOVE		Tray2,VT32-20		HeatTray,VT32-20h	
WAIT	35.00				
ADD	BF3 MeOH	SFSWash2		HeatTray,VT32-20h	
WAIT	60.00				
MOVE		HeatTray,VT32-20h		Tray2,VT32-20	
END					
WASH	MeOH wash 1				
WASH	Water wash 1				
PREP Vials 1-32	Ahead, Bounded				
ADD	NaCl	SolvRes1		Tray2,VT32-20	
END					

**Figure 12.** MAESTRO prep-sequence for automated saponification and esterification using heated 32-position tray.

Action	Method / Value	Source	Vial	Destination	Vial
WASH	Water wash 1				
ADD	IPA wash 1	SolvRes3		Waste	
ADD	IPA wash 1	SolvRes3		Waste	
ADD	IPA wash 1	SolvRes3		Waste	
WASH	Hexane wash				
PREP Vials 1-32	Ahead, Bounded				
ADD	Hexane	SFSWash3		Tray2,VT32-20	
MOVE		Tray2,VT32-20		Agitator,AgiTray	
MIX	Vortex				
MOVE		Agitator,AgiTray		Tray2,VT32-20	
END					
PREP Vials 1-32	Ahead, Bounded				
ADD	Transfer hexane	Tray2,VT32-20		Tray1,VT42sp1	
END					
ADD	IPA wash 1	SolvRes3		Waste	
ADD	IPA wash 1	SolvRes3		Waste	
ADD	IPA wash 1	SolvRes3		Waste	
WASH	MeOH wash 1				

[OK] [Cancel] [Help]

- 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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