# GLOBAL ANALYTICAL SOLUTIONS

**GERSTEL**

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# Possibilities for Automation of Sample Preparation Steps prior to HPLC or GC Analysis using a Common Autosampler

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## ABSTRACT

The primary function of an autosampler for a chromatographic instrument is to provide unattended operation and thereby increase instrument utilization and improve sample throughput in the lab. A second important benefit of autosampler use is the improved reproducibility of analytical results compared to manual injections. Automation of sample preparation steps would have similar benefits in improving sampling reproducibility.

This paper describes a software solution to control a common autosampler for HPLC or GC (MPS, GERSTEL GmbH & Co. KG, Germany) that provides flexible sample preparation capability without purchasing expensive laboratory robots. The following example applications illust-

rate the flexibility of this software for custom method generation:

1) Addition of internal standard to samples prior to injection.
2) Sample derivatization (esterification) followed by solvent extraction (hexane) and transfer of the extract into autosampler vials.
3) Online process monitoring using time programmed sampling with a flow cell before HPLC analysis.
4) Hexane extraction of chlorinated pesticides in aqueous samples using a membrane extraction device followed by large volume GC injection.
5) Automated Gerstel Twister solvent back extraction followed by HPLC analysis of PAH's in aqueous samples.

## INTRODUCTION

*Design of the software interface– first simple example.*
The standard software controlling typical GC or HPLC autosamplers allows the user to adjust parameters needed to optimize the sampling and injection into a GC or HPLC injector. Typical parameters controlled include sample incubation and agitation, needle penetration depth, syringe temperature, injection volume and sampling strokes. The actual method steps such as transport of a sample to the agitator, incubation, sampling, injection, and syringe cleaning are fixed and cannot be manipulated.

The new sample preparation software described here is based on the GERSTEL MASter software that controls the MultiPurpose Sampler MPS (Fig.1). The software provides a list of sample handling steps such as transporting a vial to the agitator, injecting a liquid into a defined destination or cleaning a syringe. The user can easily rearrange these single steps to generate a complete sample preparation cycle. This rearrangement

is done in a sequence table that contains all needed steps.

To illustrate the creation of a custom sample preparation method, Figure 2 shows a simple example of a method created to add internal standard from a solvent reservoir to every sample vial, then to mix it in the agitator and inject an aliquot into the GC inlet.
A sample preparation cycle starts with the command >PREP< and ends with the command >END<. Table 1 explains all steps of the sequence table described in figure 2.



**Figure 2.** Sequence table for adding an internal standard into every vial prior to injection of the sample into a GC injector.
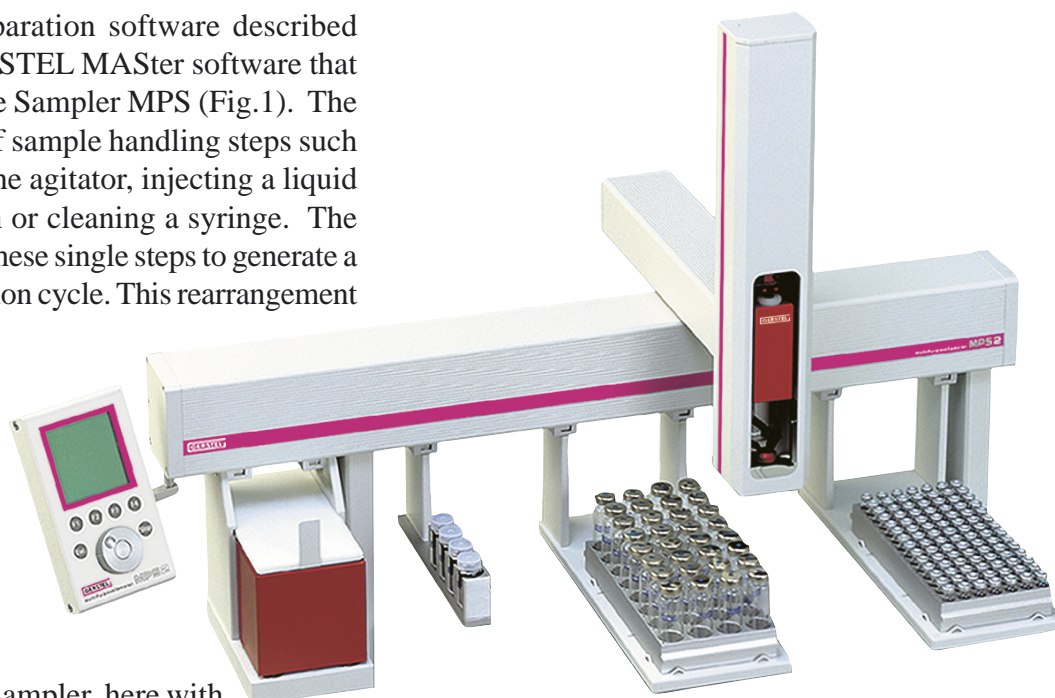


**Figure 1.** MultiPurpose Sampler, here with two sample trays and agitator.

**Table 1.** Commands used in sequence table of figure 2.

| Line | Method | Comment |
|------|--------|---------|
| 1 | >PREP< | This command starts the sample preparation cycle. Setting the parameter "Inj. = 1" in this line instructs the sampler to completely prepare and inject each sample before moving on to the next sample. Parameters "1st Vial =1" and "Last Vial = 10" specify the number of samples to be analyzed. |
| 2 | >ADD< | This command adds a liquid from solvent reservoir1 to the sample vial in tray 4. |
| 3 | >MOVE< | This command moves the sample vial from tray 4 to the agitator. |
| 4 | >MIX< | This command mixes the sample vial in the agitator. |
| 5 | >MOVE< | This command moves the sample vial from the agitator to tray 4. |
| 6 | analyse | This is a normal analysis method which injects an aliquot into a GC inlet. The parameter "Inj. = 2" instructs the sampler to make two injections from each sample vial. |
| 7 | >END< | This command terminates the sample preparation cycle after it has completed all 10 vials. |

Each sample preparation step contains a set of parameters that can be easily edited to customize the step for the desired method. For example, to edit the parameters controlling an ADD-step the appropriate MPS 2 parameter window has to be opened (Fig.3).



**Figure 3.** Parameter editing for an ADD-method.

Some steps contain many editable parameters, while steps like MOVE just need a source and a destination. There are additional commands and steps available like WAIT, WASH or SWITCH that will be explained in the following examples.

## STANDALONE PREPSTATION

*Standalone preparation system for derivatization and liquid-liquid extraction.*

This autosampler can be used as an offline derivatization and extracting robot as shown by the following example for derivatization of pentachlorophenol (PCP) extracted from wood.

This method will require the following steps:

1) Generate a PCP derivative from toluene extracts of wood using acetic acid anhydride under alkaline conditions.
2) Extract the PCP acetate from the aqueous phase with hexane.
3) Dry the extracts with sodium sulphate.
4) Transfer the dried extracts into GC autosampler vials for later analysis on another system.

The instrumental set-up is as follows:
MPS with Agitator and 1 mL syringe
Solvent Reservoir: 1st Pos. n-hexane; 2nd Pos. internal standard; 3rd Pos. acetic acid anhydride
Fast Wash Station: 1st Pos. washing solution; 2nd Pos. aqueous potassium carbonate solution
Tray 1: empty 10 mL headspace vials
Tray 2: toluene wood extracts in 10 mL headspace vials
Tray 3: 10 mL headspace vials with solid sodium sulphate
Tray 4: empty 2 mL GC autosampler vials (tray is cooled)

Table 2 explains the sequence table created to perform this method. The autosampler can be instructed to either perform the entire sample preparation routine on each sample, or to operate in a "batch" mode where each step is performed on a group of samples simultaneously. In this example derivatizations are performed in groups of 6 vials.

This example illustrates how a software solution allows use of a common GC autosampler for complex sample preparation steps.

**Table 2.** Explanation of appropriate sequence for derivatization and liquid-liquid extraction.

| Line | Method | 1st vial | last vial | Inj. | Source | Destination |
|---|---|---|---|---|---|---|
| 1 | >PREP< | 1 | 25 | 6 | | |
| | This command starts the sample preparation cycle. Samples 1 to 25 will be derivatized in groups of 6 vials before injection | | | | | |
| 2 | >ADD< | | | 1 | Fast Wash Station 2 | Tray 1 |
| | This command adds the K2CO3 solution to empty vials on tray 1 | | | | | |
| 3 | >WASH< | | | 1 | | |
| | Washes the syringe only after the 6th sample is prepared | | | | | |
| 4 | >ADD< | | | 2 | Solvent Reservoir 1 | Tray 1 |
| | This command adds internal standard to the K2CO3 solution in vials on tray 1 | | | | | |
| 5 | >WASH< | | | 1 | | |
| | Washes the syringe only after the 6th sample is prepared | | | | | |
| 6 | >ADD< | | | 3 | Tray 2 | Tray 1 |
| | Adds the wood extract or standard solutions to the K2CO3 solution on tray 1. Note: syringe is washed after each sample | | | | | |
| 7 | >MOVE< | | | 1 | | |
| | Moves the prepared reaction solution from tray 1 to the agitator | | | | | |
| 8 | >MIX< | | | 1 | | |
| | Mixes the vials in the agitator to extract the PCP into the aqueous phase as phenolate | | | | | |
| 9 | >ADD< | | | 4 | Solvent Reservoir 3 | Agitator |
| | Adds acetic acid anhydride to the vials in the agitator | | | | | |
| 10 | >WASH< | | | 1 | | |
| | Washes the syringe only after the 6th sample is prepared | | | | | |
| 11 | >ADD< | | | 5 | Solvent Reservoir 1 | Agitator |
| | Adds n-hexane to the vials in the agitator | | | | | |
| 12 | >Wash< | | | 1 | | |
| | Washes the syringe only after the 6th sample is prepared | | | | | |
| 13 | >MIX< | | | 2 | | |
| | Mixes the vials in the agitator to transform PCP into its acetate and to extract it from the aqueous phase into the organic phase | | | | | |

**Table 2.** Explanation of appropriate sequence for derivatization and liquid-liquid extraction (cont.).

| Line | Method | 1st vial | last vial | Inj. | Source | Destination |
|------|--------|----------|-----------|------|--------|-------------|
| 14 | >MOVE< | | | | Agitator | Tray 1 |
| | Transports the vials from the agitator to tray 1 | | | | | |
| 15 | >Wait< | | | 1 | | |
| | Waits a defined time to separate the two phases | | | | | |
| 16 | >ADD< | | | 6 | Tray 1 | Tray 3 |
| | Removes a portion of the organic phase from the vials in tray 1 and transports it to the vials in tray 3 which are filled with solid sodium sulphate. Note: syringe is washed after each sample | | | | | |
| 17 | >WAIT< | | | 2 | | |
| | Waits a defined time to dry the organic phase over sodium sulphate | | | | | |
| 18 | >ADD< | | | 7 | Tray 3 | Tray 4 |
| | Removes the organic phase from drying vials in tray 3 and transports them to the empty GC autosampler vials in tray 4. Note: syringe is washed after each sample | | | | | |
| 19 | >END< | | | | | |

## ONLINE

*Online Sampling from an Industrial Process and Injection into an HPLC System.*

By choosing the special software option "MPS 3 - Online" the autosampler can be used to take samples from a chemical process at defined time intervals using a parameter window that is special for this sampler option. Note: Accurate timing of the sampling interval is given highest priority in this mode. If the sampling interval is very short, the sampler can collect all samples before analyzing any. If the sampling interval is long, then the samples can be analyzed as they are collected.

Here an example is presented where the sampler is used to take online liquid samples using flow cells in front of and behind a filter system. Samples are collected into vials and afterwards injected into an HPLC system.

This method will require the following steps:

1) Before sampling, solenoid valves have to be switched to allow fluids to flow from the filter system through the flow cells.
2) The syringe must be flushed with sample from flow cell 1.
3) Sample is drawn from flow cell 1 and transferred to a vial.
4) The syringe must be flushed with sample from flow cell 2.
5) Sample is drawn from flow cell 2 and transferred to the next vial.
6) The autosampler then performs a standard HPLC injection method from each vial.

Table 3 explains the sequence table created to perform this method.

**Table 3.** Explanation of appropriate sequence for online sampling.

| Line | Method | 1st vial | last vial | Inj. | Source | Destination |
|------|--------|----------|-----------|------|--------|-------------|
| 1 | >PREP< | 1 | 10 | 1 | | |
| | This command starts the sample preparation cycle | | | | | |
| 2 | >SWITCH< | | | 1 | | |
| | The solenoid valves are switched ("1 = on") to allow a flow from the filter system through the flow cells | | | | | |
| 3 | >ADD< | | | 1 | Flow Cell 1 | Waste |
| | Takes a sample from flow cell 1 and discards it to waste | | | | | |
| 4 | >ADD< | | | 2 | Flow Cell 1 | Tray 2 |
| | Takes a sample from flow cell 1 and deposits it into the sample vial on tray 2 | | | | | |
| 5 | ">INC< | " | | | | |
| | Increment the vial counter; this specifies where to deposit the next sample from flow cell 2 | | | | | |
| 6 | >ADD< | | | 3 | Flow Cell 2 | Waste |
| | Takes a sample from flow cell 2 and discards it to waste | | | | | |
| 7 | >ADD< | | | 4 | Flow Cell 2 | Tray 2 |
| | Takes a sample from flow cell 2 and deposits it into the next sample vial on tray 2 | | | | | |
| 8 | >SWITCH< | | | 0 | | |
| | The solenoid valves are switched ("0 = off") to stop the flow from the filter system through the flow cells | | | | | |
| 9 | mps3ol | | | 1 | Tray 2 | GC Injector 1 |
| | This is a normal injection method that is performed when the sampler is finished sampling | | | | | |
| 10 | >END< | | | | | |

The flexible programming of the MPS using this software allows use of special extraction devices. This expands the capabilities of the autosampler, as shown in the following two examples.

## MEMBRANE EXTRACTION

*Automated Membrane-Assisted Solvent Extraction.*
By using the new membrane extraction technique, even dirty aqueous matrices can be studied chromatographically without numerous manual sample preparation steps. The principle of membrane extraction is based on a simple physical mechanism: the distribution between two membrane separated phases is controlled by an exchange through the permeable membrane. To perform good membrane extractions, extraction time must be carefully controlled.
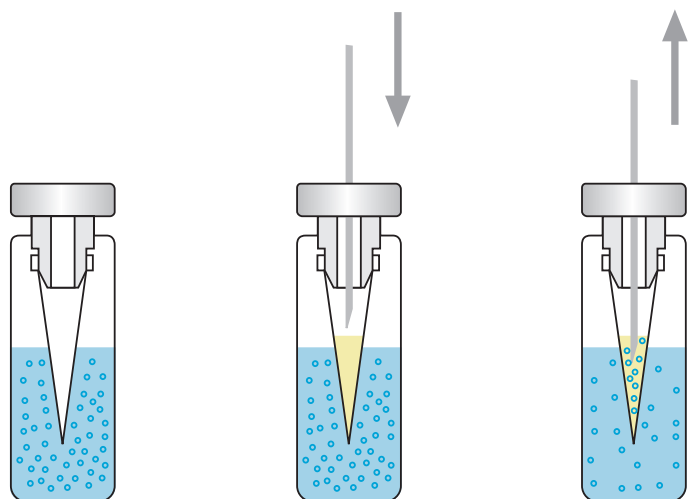


**Figure 4.** Membrane assisted solvent extraction of aqueous samples in 20 mL headspace vials.

This method will require the following steps:

1) Sample is transferred to a 20 mL headspace vial and sealed with a cap containing an integral membrane.
2) The MPS 2 fills the membrane with extraction solution.
3) The sample is heated and agitated for a specific time.
4) Extracted sample is withdrawn from the vial and injected into the GC or HPLC.
5) The MPS 2 is instructed to wait for a ready signal from the GC or HPLC.

When this type extraction is performed, the organic components are enriched in the extraction phase depending on their distribution coefficients. Suspended material or very polar or ionic components can't pass through the membrane and therefore they do not enter the organic phase. The unique feature in the sequence table created to perform this method (Figure 5) is the "Wait" step that forces the MPS 2 to wait until the chromatographic run is done. This step is necessary to assure consistent extraction times.



**Figure 5.** Sequence table for membrane assisted solvent extraction.

## TWISTER BACK EXTRACTION

*Twister Back Extraction for the MPS 3.*

The combination of the Stir Bar Sorptive Extraction (SBSE) with solvent back extraction (acetonitrile/water mixtures) followed by HPLC fluorescence detection allows sensitive and reproducible determinations of PAHs in aqueous samples. The following example shows the first method to automate solvent back extraction of the Twister after sample enrichment to transfer the enriched analytes into a liquid phase for HPLC analysis.

The Twister Back Extraction can also be used for the determination of many other organic compounds, such as pesticides, herbicides and phenols.
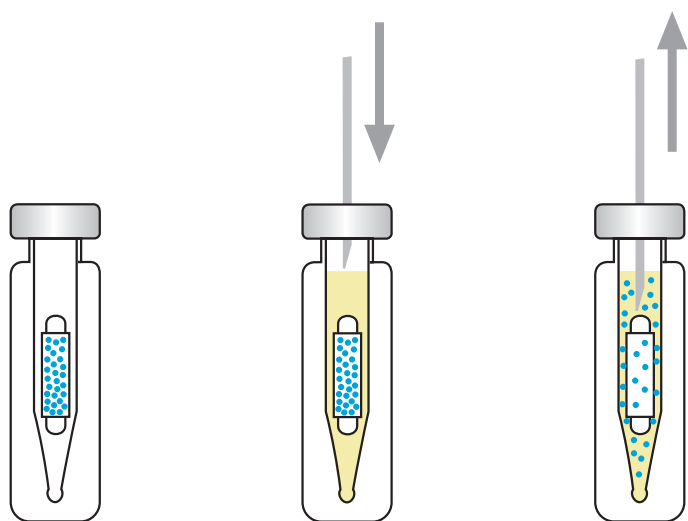


**Figure 6.** Twister Back Extraction using 2 mL autosampler vials with 250 μL inserts.

This method will require the following steps:

1) 10 mL of the water sample is placed in a 10 mL headspace vial containing the Twister.
2) Extract for 60 minutes at room temperature with stirring.
3) Transfer Twister manually to 2mL vial with 250μl conical autosampler vial insert.
4) The MPS 3 fills the insert with extraction solution.
5) The sample is heated and agitated for a specific time.
6) Extracted sample is withdrawn from the vial and injected into the GC or HPLC.
7) The MPS 3 is instructed to wait for a ready signal from the GC or HPLC.

## CONCLUSIONS

The new sample preparation software can be used to automate many manual sample preparation steps, such as standard addition or derivatization. The software is straightforward and requires no macros or programming language.

The software also enables the sampler to interface as an online sampler, or communicate and coordinate operations with external devices.

Samples can be extracted in parallel, automatically processed and analyzed, giving higher sample throughput.

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