

A Fully Automated Workstation for SPE Applications

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Abstract

We describe a unique liquid handling platform, based on a Tecan EVO, specifically designed for the fully automated processing of solid phase extractions (SPE). The platform utilizes a combination of an off-the-shelf software (Tecan Gemini 4.2) and a custom-designed user-interface for method definition and processing. The system convinces with robust and reproducible quality and a very easy, intuitive user interface. All security requirements as per FDA 21 CFR part 11 were considered, when programming the software bundle.

Introduction

Solid phase extraction (SPE) in a 96 well format is an indispensable technique in chromatographic methods. For cleaning up analytes from complex matrices and for concentrating by elution with less volume than the initial sample volume, SPE allows for robust and reproducible analytical results, regardless whether the measurement is later performed using HPLC or LC-MS/MS.

There are two ways to effectively get the matrix or solvents through the solid phase. Either by vacuum, pulling the liquid from below into the collection vessels, or as so-called positive pressure (PP), applying a constant gas flow from top. Many laboratories prefer the latter one, since it significantly reduces the risk of splashing and cross-contamination. Therefore, the integration of a PP-unit into a liquid handling robot was also our choice.

Standard SPE applications require at least conditioning of the columns, applying the sample, one or more wash steps until all is drained into the liquid waste, and a final elution into a fresh sample collection plate. In manual approaches, these steps are performed by either using single channel or multi-channel pipettes, with a manual transfer onto a stand-alone PP-unit. This of course is, depending on the method, rather laborious and occupies the lab technician for hours.

A fully automated SPE workup, allowing for multiple plates processing, is therefore a valuable support in a high throughput laboratory. Moreover, the underlying software safety features are in full compliance with FDA 21 CFR part 11 requirements, allowing for operation in regulated laboratory environments (1, 2).

System Description

The robotic platform consists of a 100cm Freedom EVO (Tecan Group Ltd., Männedorf, Switzerland) equipped with an 8-channel liquid handling arm (LiHa), an eccentric gripper arm (RoMa) and

one PP-unit (amplius GmbH, Rostock Germany), as presented in Figure 1. Up to eight different solvents can be handled per method (being an extraordinary high number, normally, not more than four solvents are required).

Gemini 4.2 was chosen as the robotic software administering the deck, labware and liquid classes, and a custom-made user interface (Fornax Technologies GmbH und Co KG), administering the methods (volumes, times) and driving the Gemini to execute the steps. The software package runs on Windows 7. A full computerized system validation was applied (3, 4) before setting the system into productive usage.

Software Description

The Gemini 4.2 software (Tecan) controls the robotic workstation and the peripheral equipment, based on a standard deck layout (Figure 2) that differentiates between source and destination locations plate and liquid transfer. For setting up a method, the interface gets connected with the appropriate Gemini script (*.gem), to allow for the usage of different labware, e.g. SPE plates with different heights. Eight troughs are reserved for assay specific solvents, identified by text overlay from the entry fields and the deck layout. The user can easily tick, whether an aqueous or organic solvent is used, choosing the appropriate liquid class automatically. From this list of solvents, the user then selects those required for conditioning, sample dilution (if required), washing and elution. The LiHa will pick and drop the solvent specific tips from fixed positions and reuse those for the same solvent, if required, repeatedly during the process. For sample transfer of up to two sample plates, two sets of tips are available, being used once only and discarded right after usage.

With all information entered to the interface, a method will be saved using a unique identifier, and a description (e.g. SOP title) will be entered in a header. Apart from the specific SPE methods, a couple of supporting maintenance scripts have been predefined, which allows the system to also handle simple plate to plate transfers and liquid-liquid extraction with subsequent sampling of optional upper or lower liquid phases.

Regarding data security, access levels and user rights define which user can create or modify a method within SPIKE and Gemini, and who is limited to executing methods. Electronic signatures and audit trail round the software security requirements as per FDA 21 CFR part 11 (5).

Results

Meanwhile multiple SPE methods have been established and validated with the described fully automated SPE workstation. Depending on the complexity of the method and the volumes being required for equilibration and washing, a method allows for walk-away of the lab technician between 30 minutes and three hours, when two sample plates are processed sequentially in one run. Consequently, the lab personnel can invest this free time in either preparing the next samples, or setting up the LC-MS/MS system, while these tasks could not be done in parallel when the SPE is performed manually.

Method validation using the SPE robots showed fully acceptable results with regard to sensitivity and specificity, accuracy and precision and of course also full exclusion of cross contamination.

Figure 1. System consisting of Tecan EVO 150cm, multiple reagent troughs¹, 8-channel LiHa using conductive 1000 µL tips², PP-unit with source³ and destination⁴ position and pressure part⁵. The transfer of source, SPE and elution plate is performed using the RoMa⁶.

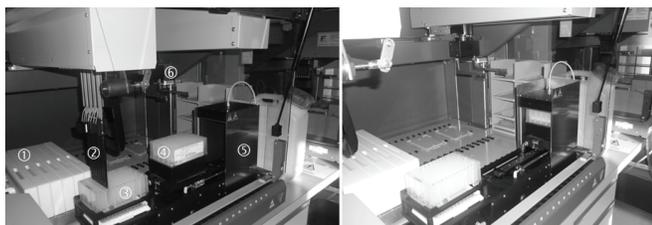


Figure 3. Example frame for entering method specific parameters into the user interface, including solvent name, volume, waiting time (before applying pressure) and duration of the pressure. In the current method, the solid phase gets eluted twice.

Example Method

- Global -		- Konditionieren -		- Dilute -		- Proben -		- Waschen -		- Eluieren -		- Commands -	
Solvent		Vol	Wait	SPE									
1.5% NH4 Acetone		400	120	20									
<input checked="" type="checkbox"/> 2x eluieren													

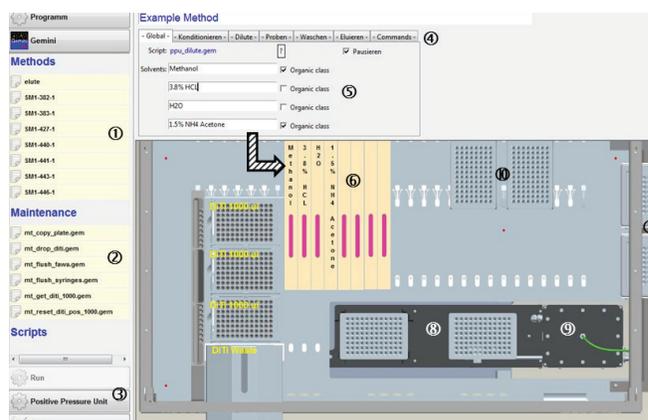
Discussion

We described a custom made, fully automated SPE workstation, which combines flexibility, throughput and technical robustness with software security requirements as per FDA 21 CFR part 11. Compared to the very few commercially available SPE workstations, our system is superior, as it does not have the throughput limitations and software security shortcomings as the Biotage Extrahera™, nor the volume and solvent limitations as the Zephyr G3 SPE Workstation (Perkin Elmer) and is much smaller and cheaper than the Resolvex™ positive pressure workstation (Tecan) embedded in a Tecan EVO (with a minimum size of 150cm, rather 200cm).

A throughput of two plates per run is considered sufficient for LC-MS/MS methods, being equivalent or superior to the above mentioned commercial off-the-shelf systems.

Figure 2. User interface for method specific parameters:

- ¹ Display of all bioanalytical methods,
- ² Display of supporting scripts,
- ³ Direct control panel for PP-unit,
- ⁴ Frames to set up method specific volumes, dilutions and pressure times,
- ⁵ List of solvents used in the process (names get transferred to the layout below),
- ⁶ Storage location for source, SPE and elution plate,
- ⁷ Pipetting location with source left and destination right
- ⁸ Pressure unit,
- ¹⁰ Final location of plates after assay



References

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