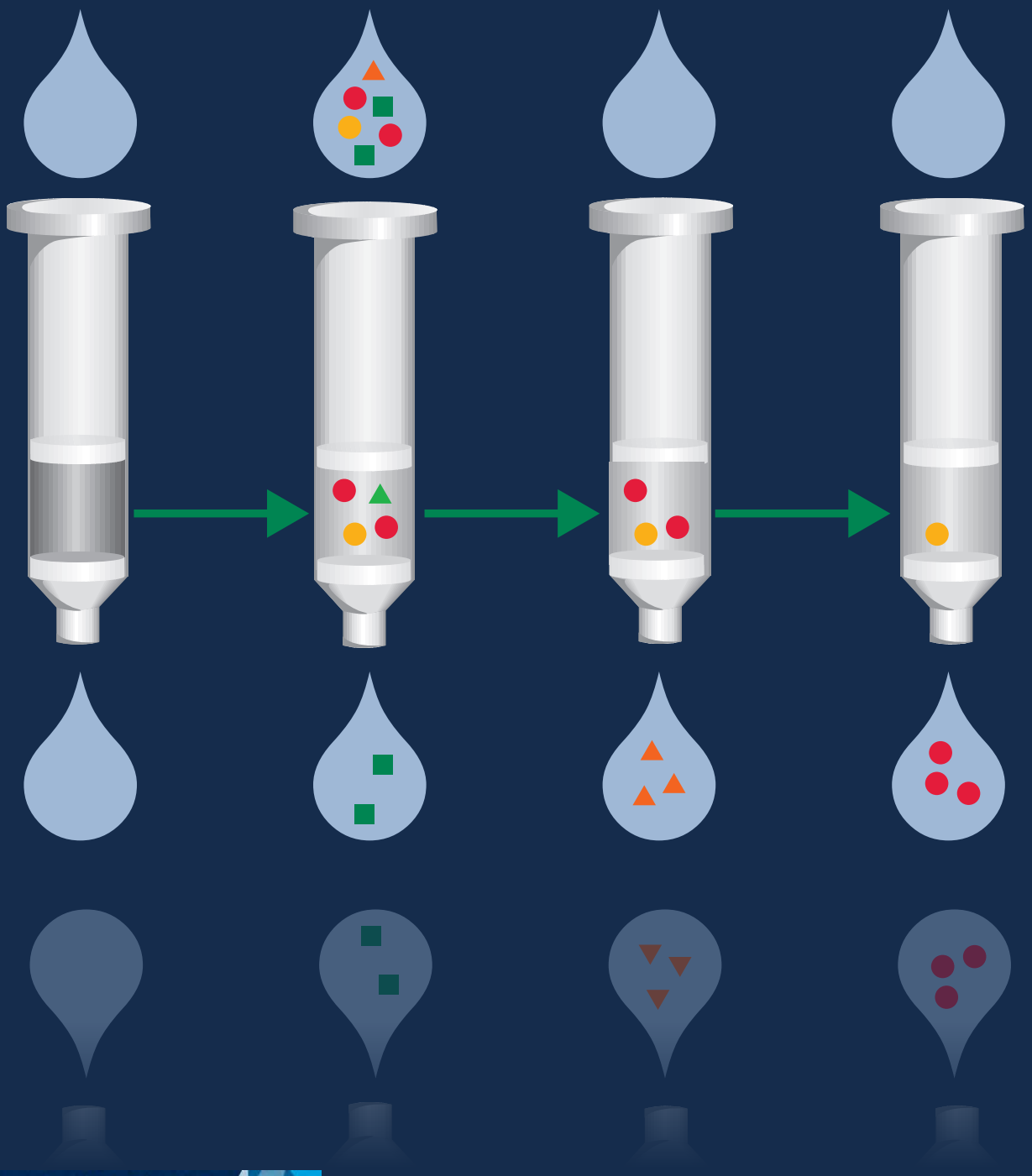
Condition | Load | Wash | Elute

Gilson Guide to SPE Automation



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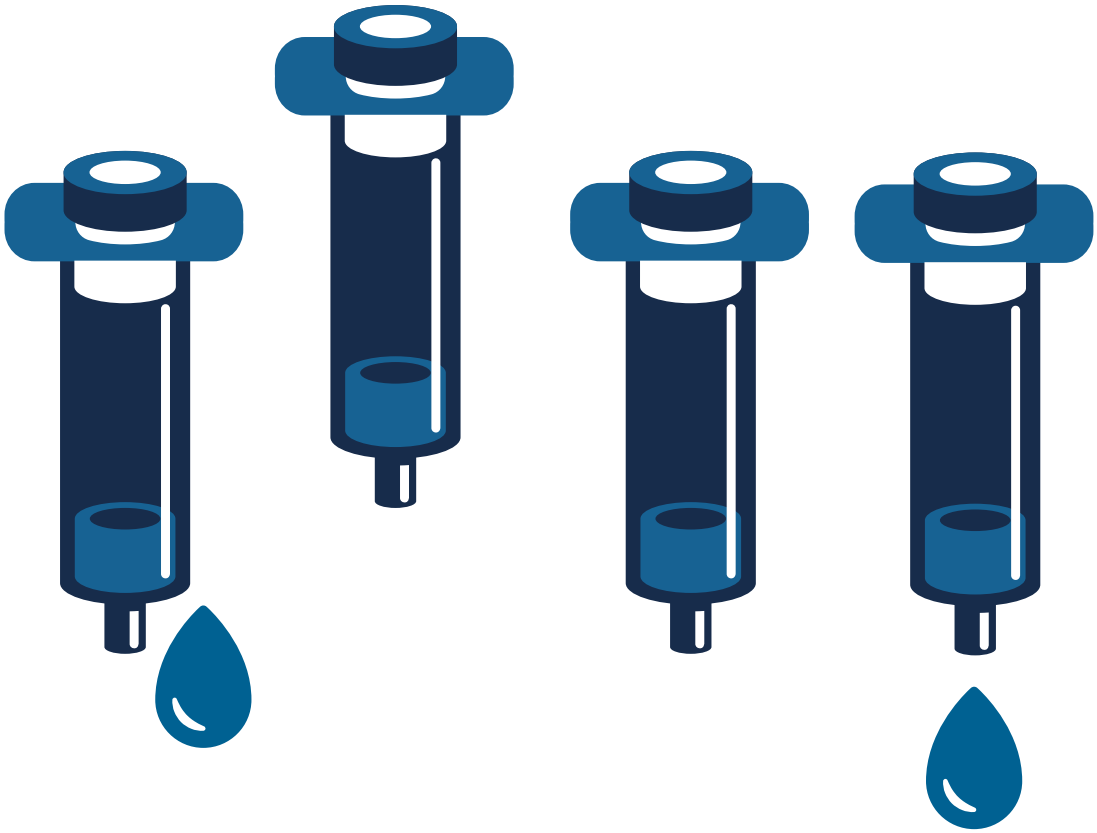
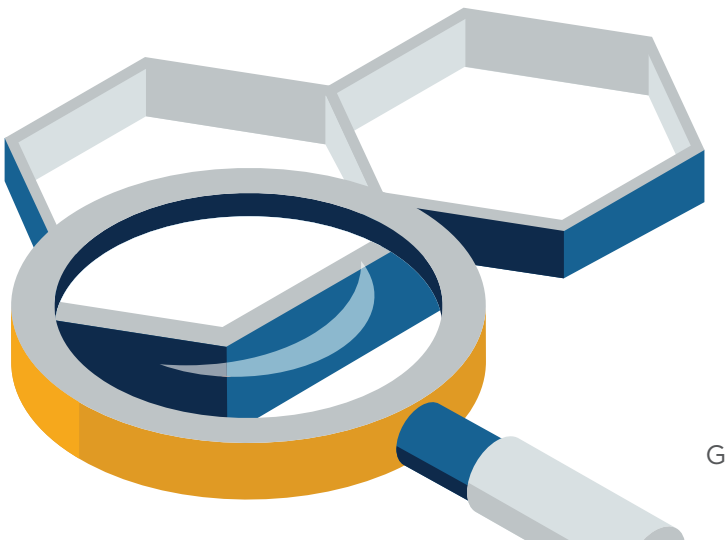
GILSON®

ABOUT THIS GUIDE

This guide provides a background for the technique and explains the science of solid phase extraction (SPE). It also offers practical advice for automating workflows via Gilson SPE systems and software. As you go through the steps of method development or transferring a manual method to your automated system, reference this guide as both a resource and step-by-step troubleshooting handbook. If you are unable to find the information you are looking for within this guide, please contact Gilson Technical Support for assistance.

Contact Gilson Technical Support
techsupport@gilson.com





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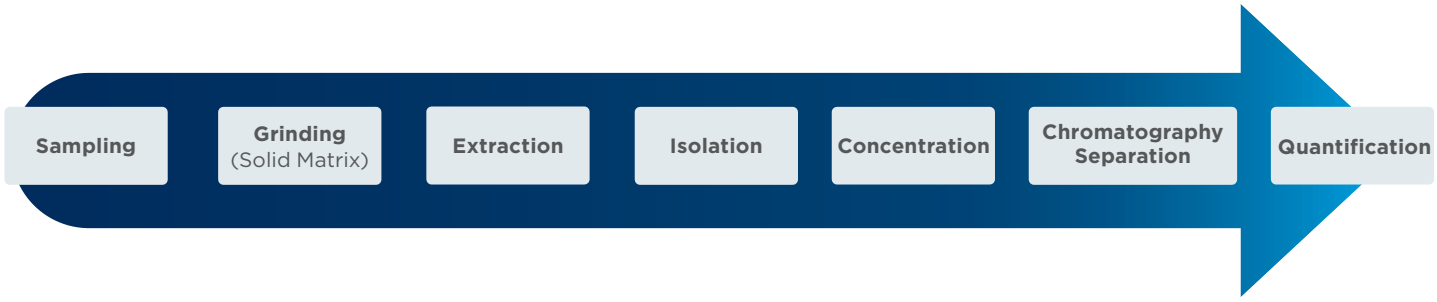
Chapter 1

INTRODUCTION

To protect expensive analytical systems or to improve limits of detection and quantification, a sample preparation step is part of most analytical workflows. Solid phase extraction (SPE) has become the technique of choice for sample Isolation/Clean-up and Trace Enrichment before analysis due to its numerous benefits: versatility, selectivity, and low solvent usage.



Compound and Matrices	Disruption	Extraction	Isolation	Concentration	Analysis Type	Detection
Pesticides in drinking water	N/A	Solid Phase Extraction (SPE)			LC	MS
Acrylamide in fried potatoes	Crush	Liquid extraction	Dual SPE	Evaporate	LC	MS/MS
Fatty acids in macro algae	Cut/Freeze drying	Liquid extraction	SPE fractionation	Evaporate	GG	MS
Amphetamine in human urine	N/A	Solid phase extraction (SPE)		Evaporate	LC	MS/MS
Protein ArgMet in biological matrice	N/A	Precipiation	SPE	Lyophilization	-	NMR

Prior to analysis, samples must go through several steps of extraction and cleanup.

Figure 1
Generic sample analysis workflow

Numerous sorbents are available, making SPE the most universal sample preparation technique, applicable for most types of analytes and liquid matrices.

Matrix effects when analyzing complex solutions is a source of error that can be reduced using the high selectivity of SPE to improve the reliability of the results effectively. At the same time, maintenance and downtime for expensive analytical instruments are shortened by preventing the injection of undesirable substances. The trace enrichment capability of SPE continues to be essential in environmental, food safety, and antidoping control as the required limits of detection are lowered.

In addition to sample preparation, SPE is also used today as a small-scale purification technique, especially in the discovery of new bioactive substances.

Manual SPE procedures can be labor intensive and subject to human error, delays, or sample loss. As such, the optimization of manual SPE methods is often done with a somewhat simplified approach due to the difficulty of controlling key parameters, which limits the outcome from the technology.

These problems have led to the development of instruments by Gilson to automate SPE processes. Controlled by the TRILUTION® LH software, the automated SPE line includes ASPEC® 241, ASPEC® 271, and ASPEC® 274 systems to cover an extensive range of customer needs for capacity, throughput, and flexibility.

TRILUTION® LH

ASPEC® 241

ASPEC® 271

ASPEC® 274

Using interchangeable, disposable extraction cartridge (DEC) racks, the Gilson SPE instruments are designed to automate and optimize SPE to provide more efficient and reproducible sample preparation.

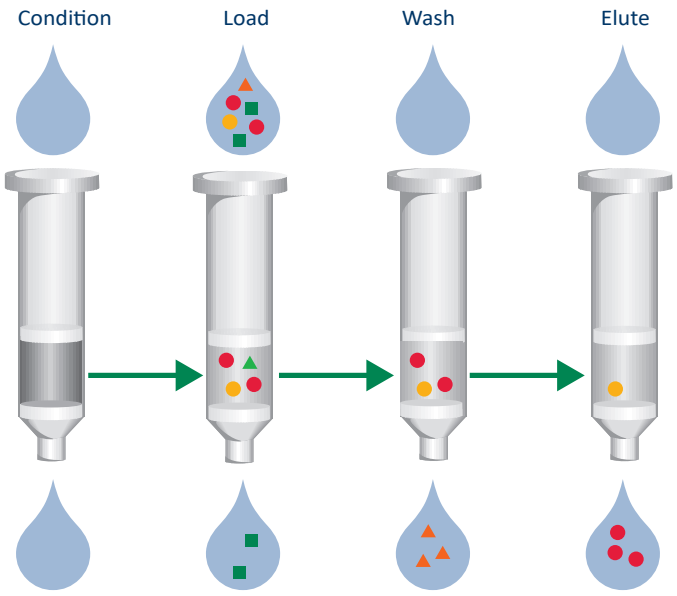


Figure 2
Bind-elute strategy - A typical four-step SPE method

Chapter 2

STEPS OF AN SPE METHOD

The SPE process is based on a selective partition of the compounds of interest between a solid phase, the sorbent, and a liquid phase, the solvent. Two types of strategies can be adopted.

Bind-Elute Strategy

The most common strategy consists of following a "Bind-Elute Strategy". Targeted analytes are first retained on a sorbent while the matrix is discarded with most impurities. Then the analytes are eluted in a pure fraction of solvent. In this case, a typical SPE method is made up of a combination of four common steps or tasks. Each step can occur more than once during the SPE method. A basic introduction to these steps can be found below.

Condition

SPE is usually performed using cartridges packed with dry sorbent. A conditioning step is required first to wet and activate the packing bed by solvation and a second one to equilibrate and promote future retention.

Load

The objective is the quantitative retention of the analyte on the SPE sorbent while matrix interferences are removed.

Wash

After the analyte(s) of interest are retained on the sorbent, washing the packing sorbent should remove the majority of interferences.

Elute

The final step of the process is the quantitative elution of the analyte from the SPE cartridge. This can be performed in one or multiple steps to collect targeted analyte(s) into a pure solvent.

As the volume of elution solvent used is most often less than the loaded sample volume, this strategy also creates a concentration factor.

Non-Retention Strategy

A less frequent alternative is based on a non-retentive extraction.

Condition

Load & Collect

After conditioning, the major impurities are retained during the loading step while compounds of interest are collected.

This strategy can be helpful when the analytes of interest cannot be easily partitioned out of solution onto a sorbent in case of too high solubility in the matrix. However, these two-step protocols prevent any concentration factor.

Key Parameters to Control

Each SPE step takes a specific role and has specific key parameters that need to be considered. Their control is crucial to achieving an accurate and reproducible SPE protocol with high recoveries, sufficient cleanup and optimal analyte concentration.

As we will see throughout this guide, a successful automated SPE method requires the capability to manage precise volumes, accurate and reproducible flow rates no matter the viscosity and volatility of the liquids, avoidance of any channeling, and control of the drying state of the sorbent.

SPE Flow Type

Due to the backpressure created by the sorbent during the loading of the liquids, a flow by gravity is not applicable in most cases. Two approaches exist to overcome this issue.

The majority of manual SPE methods are performed using a vacuum manifold. The SPE cartridges fit into a screw-type valve and are rotated to increase or decrease the vacuum pull. While this type of system may appear simple, in practice, it is highly operator-dependent and requires a great deal of expertise. The chemist must watch for vacuum fluctuation, unevenness, and cartridge drying or channeling while executing the SPE method.

The second option consists of applying a positive pressure into the cartridge. Due to its numerous advantages in terms of flow rate consistency and drying control, this approach is widely used by automated SPE instruments. As the pioneer of this principle, Gilson continues to adopt this option for its automated solutions and its manual SPE system, ASPEC® PPM.

Chapter 3

WHAT CAN ASPEC® SYSTEMS DO FOR YOU

The Gilson ASPEC family can accommodate a wide range of SPE formats, including standard 1, 3, and 6 mL cartridges. By using a combination of different racks on the bed of the instrument, multiple SPE formats can be used, which provides additional flexibility.

Full Automated SPE Protocol

The ASPEC family of automated SPE instruments are based on cartesian X, Y, Z robots. Thanks to their Z-Arm equipped either by a single probe (ASPEC 241 and ASPEC 271 systems) or by four probes (ASPEC 274 system), these instruments have access to different interchangeable racks (tubes, vials, SPE solvent bottles, and SPE cartridges). The interchangeable racks allow the systems to perform automated liquid handling tasks during all SPE steps (Condition, Load or Load & Collect, Wash, Elute) controlled by TRILUTION LH software. The accurate and reproducible liquid transfers are performed thanks to Gilson VERITY® 4X60 syringe pump(s) connected to the pr

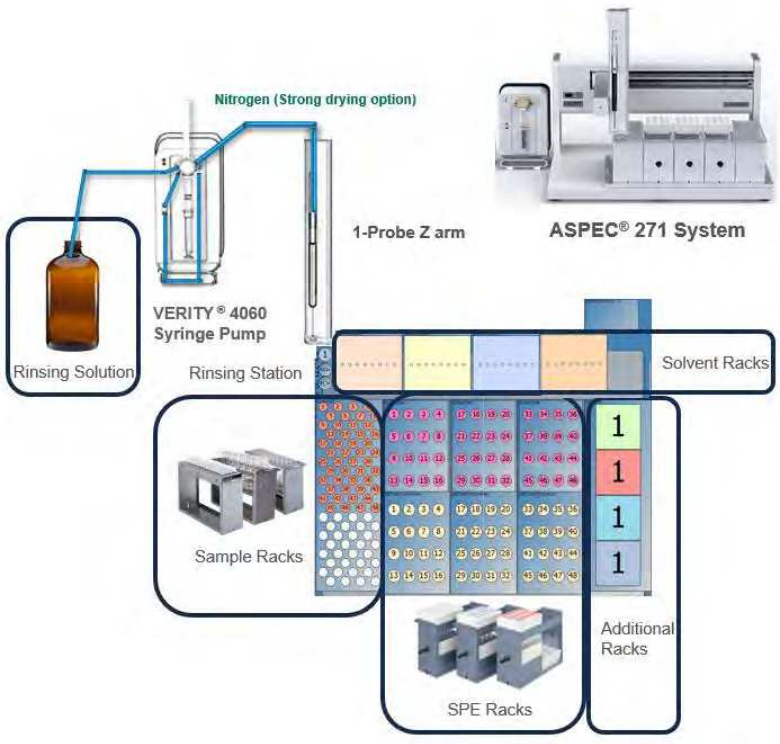


Figure 3
ASPEC® system concept

Automation for More Complex Protocols

Thanks to the mobile rack technology of its SPE racks and the ability to automatically position the SPE cartridge above a waste container or above collection tubes, the ASPEC systems can automate a typical SPE protocol and more complex protocols as summarized below.

Fractionation

The ability to fractionate an elution from one SPE cartridge in multiple collection tubes is a critical step in SPE methods.

Historically, this principle has been employed in method development to determine the breakthrough volume of the SPE cartridge, the optimal strength of wash solvents and to optimize elution volume.

Today, more SPE protocols in routine require fractionation to separate compounds by family. We can cite, for instance, the isolation and separation of acidic, neutral, and basic drugs, the isolation of the extractable petroleum hydrocarbon in soil, and the lipids fractionation.

In natural product discovery and thanks to the improvement of bio-chemometrics to accelerate research, a more high-throughput screening (HTS) approach is emerging. This approach of automated SPE fractionation is used to pre-fractionate crude extracts before the bio-assays screening, dereplication, and identification of new bioactive substances.

Fractionation is very tedious to manage manually as collection vials must be replaced without losing compounds. Automation simplifies this process by using mobile rack technology, allowing the liquid handler to easily control the positioning of the cartridges above collection tubes and move them above fresh tubes to do multiple collections.

Gilson ASPEC systems and the user-friendly TRILUTION LH software simplify and secure this complex process compared to manual SPE.

Multi-Dimensional SPE

Two extraction series using two different cartridges may be required during a clean-up process to improve its selectivity or to dry the eluate before derivatization and injection in GC. For instance, the isolation and concentration of nitrosamines from water using a charcoal SPE column can be followed by drying eluates on a sodium sulfate SPE column.

With open access to the collection tubes and the ability of the ASPEC systems to use different SPE cartridges (sorbent and formats) in the same run, 2D-SPE is easily implemented.

Method & Development

TRILUTION LH software allows for easy and fast design of automated experiment plans using multi-variables parameters. This is a valuable tool for developing a new SPE clean-up method or rapidly transferring an existing manual SPE protocol to an automated one.

SPE cartridges, volumes, flow rates, and solvent mixtures can be tested at each step of the protocol. Combined with the traceability ensured by its software, ASPEC systems simplify and accelerate the SPE optimization phase.

Flexibility Without Limits

Scalability

ASPEC systems present a high scalability at low costs. Just by changing a sample rack(s) or SPE rack(s), it is easy and fast to adapt the system configuration to a new SPE protocol. That is particularly useful, for instance, when doing contaminants impact studies from the food network up to human health because the volume of sample and the SPE cartridge format often vary in function of the studied matrices.

Gilson SPE automated systems can be easily adapted to deal with a few hundred µL of biological sample up to liters of environmental sample (off-bed sample large volume option).

Different SPE Protocols in a Single Run

The large capacity of the ASPEC 271 and ASPEC 274 systems combined with the flexibility of TRILUTION LH allow for running a sample list with different SPE methods. It also allows for use of the instrument at full capacity to improve the guarantee to achieve a fast Return on Investment (ROI).

Other Liquid Handling Operations

Sample Treatment Operations, Solvent Mixture Preparation and Reactions

A strong selling point for the ASPEC system family compared to systems dedicated only to SPE is their ability to handle not only SPE tasks, but also standard liquid handling tasks.

Dilution and homogenization of highly viscous samples (e.g., honey), serial dilution, internal standard addition, sample tube rinsing, automatic preparation of solvent mixtures into test tubes, and derivatization can also be performed by the ASPEC systems.

Automated Injection

SPE is commonly followed by an injection step for quantitation purposes. SPE techniques that don't require a solvent exchange or dry-down of the eluent may be amenable to automated injection with a Gilson single probe instrument (ASPEC 271 system).

Preservation of Samples and Eluates

Maintain Samples at the Right Temperature

ASPEC systems are compatible with thermostated racks to maintain temperature of thermo-sensitive samples.

Use of Closed Vials

During isolation of semi-volatile and volatile compounds, the sample vials have to be closed by septa to avoid the risk of analyte losses by evaporation or sample contamination by ambient air. In this case, ASPEC systems can be equipped with septum piercing probes. At the end of the SPE protocol, eluates can also be automatically transferred into closed vials.

Error Management

One of the most important functions to evaluate when selecting an automation system is how easy it is to take control of the system and how the potential errors are handled.

Error handling is important to avoid wasting a complete batch of samples. In SPE, it is important to track the pressure within the SPE cartridge to detect the potential plugging and to have the ability to automatically skip unfinished samples, especially during night runs. All these can be recorded for later audits.

TRILUTION LH software answers this need of traceability by recording all the actions and alerts that occur during a run.

Each syringe of the VERITY 4X60 pumps is equipped with a pressure sensor monitored with software intelligence that helps your system make the right decision: stop the run or stop the current sample and move on to the next one.

To minimize the risk of errors, TRILUTION LH allows you to simulate the run of a sample list before running real samples. The simulation checks for any programming errors and provides an estimated time for the run.

Increase Throughput and Optimize Productivity

Due to the fact that SPE mechanisms require a minimum contact time between the liquid and the solid phase to achieve an efficient selective partitioning of the compounds, an automated method will generally have a similar run time to the equivalent manual method.

One of the most significant reasons to purchase **reliable complete** SPE automation, as provided by Gilson SPE automated instruments, is the ability to run samples overnight or in multiple shifts. This increases a lab's throughput and optimizes productivity in the lab.

Other Sample Preparation

The versatility of the Gilson ASPEC systems allows for automation of other sample clean-up techniques in addition to the SPE.

Sample Preparation Techniques Using Cartridge Format

Even though SPE is one of the universal sample preparation techniques before analysis, in some cases, other sample preparation techniques can be used in place of SPE according to selectivity and sensitivity requirements, matrices, and volumes. Some use the same cartridge format as SPE and can be easily automated with Gilson ASPEC systems, as summarized below.

Protein Precipitation and Filtration

Protein Precipitation and Filtration are only used to prepare small volumes of biological liquids. This type of sample preparation is faster and simpler than SPE but less selective and sensitive. The complete precipitation process occurs in a filtration cartridge by adding an organic solvent to the sample. After protein precipitation, the samples are directly filtered by applying a positive pressure on the disposable cartridges with our Gilson SPE systems.

Supported Liquid Extraction (SLE)

Compared to Protein Precipitation & Filtration, SLE gives a better selectivity but the applicability is limited. Only lipophilic compounds can be extracted from a small volume of aqueous matrices.

The aqueous sample is loaded on a cartridge that contains diatomaceous earth. The water is adsorbed on this solid support to constitute the liquid stationary phase. After waiting five to ten minutes, the lipophilic analytes are eluted by 3–5 times the sample volume with an immiscible organic solvent with water. This clean-up technique is easily amendable to automation.

Immuno-Affinity

This clean-up technique is highly selective and well adapted to isolate a specific target from complex matrices. This type of cartridge is widely used for mycotoxins isolation from food matrices and is described in numerous AOAC methods. An immunoaffinity cartridge contains a gel suspension of an antibody specific to the antigen of interest (e.g., Aflatoxin). The antibody retains any antigen present in the sample within the gel suspension. A washing step removes unbound material, and then the antigen is released following elution with solvent. Highly sensitive to loading flow rates and contact time variability, this clean-up technique can be highly human-dependent in manual mode. Automation can play a crucial role in case of reproducibility issues.

Molecular Imprinted Polymer (MIP) Technology

The MIP cartridge sorbent is based on the polymer with a “memory” of the shape and the functional groups of the target molecule to extract it from complex matrices. This technique is very selective and also well adapted to food matrices. We can cite, for instance, the extraction of patulin from apple extract and the isolation of glyphosate from red wine. Compared to conventional Immuno-Affinity Cartridges (IAC), MIP cartridges appear more robust thanks to stable materials and require limited method development. Since the MIP workflow follows the same four main steps as a standard SPE protocol, ASPEC systems can fully process it.

Additional Sample Preparation Techniques Using Specific Tools

Small scale liquid/liquid extraction can be performed by using an orbital shaker fully controlled by the TRILUTION LH software.

Table 1

Gilson’s automated SPE instruments

GILSON ASPEC® SYSTEMS	LIQUID HANDLING	SOLID PHASE EXTRACTION 1 ML, 3ML, 6ML							BASED COLUMN SAMPLE PREPARATION				ORBITAL SHAKING
Fully Automated SPE Under Positive Pressure Control	Dilution, Reagent Addition, Mixing…	Standard SPE	Fractionation	Multi-Dimensional SPE	Method Development	Large Sample Volume Option	Injection	Different SPE Protocols in a Single RUN	PP	SLE	IAC	MIP	LLE
ASPEC® 241 System	Limited			–	Limited	Limited	–	–	✓	✓	✓	✓	–
ASPEC® 271 System					✓			✓	✓	✓	✓	✓	✓*
ASPEC® 274 System					✓		–	✓	✓	✓	✓	✓	✓*

*Only with 3 200 Tray Locator

Chapter 4

WHY AUTOMATE SPE?

Sample preparation is generally considered a labor-intensive and error-prone step in an analytical method; thus, automating it presents some clear benefits:

- Increased throughput
- Improved reproducibility
- Reduced personal exposure to hazardous materials
- Free laboratory technician time
- Traceability of users and data

Considerations should be made relative to the cost associated with a technician's time (OPEX); versus the cost of an automated instrument (CAPEX). This brings us to the selection of the automation approach, which must balance the throughput (number of samples processed by unit of time), the capacity (number of samples processes per run), and the price (or total cost of ownership of the system).

In compliance with this principle and to be able to propose the right automated system, Gilson has developed three automated SPE systems, as shown in Table 2.

Increased Throughput and Productivity

Automation does not reduce the time required to process an individual sample, but rather the automated system works a full day and allows laboratory personnel to focus their efforts and time on other projects. Therefore the overall productivity of the laboratory increases.

A multiple-probe instrument like the ASPEC 274 can process four samples in parallel which noticeably increases the number of samples processed per day, as shown in Table 2.

Table 2
SPE throughput comparison

		MANUAL SPE	AUTOMATED SINGLE PROBE SYSTEM		AUTOMATED FOUR PROBES SYSTEM
		Vacuum	ASPEC® 241 System 	ASPEC® 271 System 	ASPEC® 274 System 
Manual Steps		For each individual sample: • Sample preparation • Setup • Condition • Control flow to avoid drying • Load sample • Adjust vacuum • Wash column • Setup collection tubes • Elute analyte	For a batch of samples: • Setup of SPE cartridges • Put samples in rack • Put solvents in rack • Run written application		
Capacity SPE (Columns /Run)	1 mL	12-24	36 (1 x code 371 rack)	108 (3 x code 371 rack)	108 (3 x code 371 rack)
	3 mL	12-24	20 (1 x code 373 rack)	60 (3 x code 373 rack)	60 (3 x code 373 rack)
	6 mL	12-24	16 (1 x code 386 rack)	48 (3 x code 386 rack)	48 (3 x code 386 rack)
Minimum SPE Time		10 min/sample	10 min/sample	10 min/sample	10 min/sample
Troughput Max. # samples per day		48 (2 batches during 8 hours per day)	72 (36 during 8 hours per day + 36 overnight)	135 (27 during 8 hours per day + 108 overnight)	276 (168 during 8 hours per day + 108 overnight)

Enhanced Performance

Accurate and Reproducible Control of SPE Key Parameters

The main points to consider when performing SPE methods are the ability to control volumes, flow rates, the drying state of the packing bed according to the requirements of each SPE step.

For instance, it is crucial to :

- Keep the packing material moist during the initial conditioning steps, especially with silica-based sorbents.
- Load the sample with a sufficiently low flow rate to enable good interaction between the sorbent and the analyte of interest.
- Elute the analyte(s) of interest efficiently while using the smallest volume of elution solvent.
- Create consistency and reproducibility from sample to sample for these key parameters.

Vacuum Manifold

Manual SPE is primarily performed using vacuum manifolds.

SPE cartridge are placed in the manifold cover, and liquids are manually pipetted onto each cartridge.

Liquid flow rates through the cartridge are controlled using a vacuum pump and adjusted manually by gradually opening each valve connected to each cartridge.

The difficulty of this approach is to make adjustments with sufficient accuracy and reproducibility to positively impact the consistency of the results.

Below are a list of reasons why manual SPE can lead to poor reproducibility:

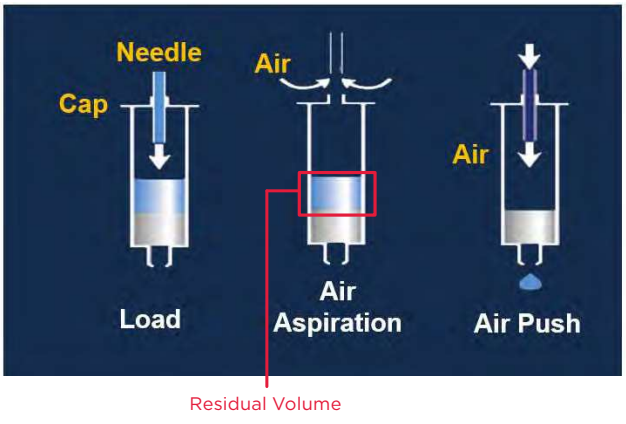
- Flow rate relies exclusively on a visual approach (number of drop/s) highly based on the expertise of the operator
- The multiple repetitive manual valve adjustments at each SPE step are highly prone to errors and generate different flow rates from sample to sample
- Small volumes are challenging to control, and fast flow rates can cause sorbent channeling

Positive Pressure

Full automated SPE using Positive Pressure performed by Gilson automated ASPEC systems constitutes a great alternative approach to overcome these difficulties.

Whatever the Gilson automated system, the pressurization of the cartridge follows the same principle as illustrated in Figure 4.

Following this principle :



To ensure pressurization, the SPE cartridge must be tightly closed by a specific cap to provide a seal with the probe. When the required volume of liquid is delivered through the probe, the pressure increases on the top of the cartridge as the available volume of air decreases. When the pressure exceeds the back pressure created by the sorbent, the solvent volume is pushed through the solid phase at a constant, reproducible, and accurate flow rate thanks to the Gilson VERITY® syringe pump. Then, at the equilibrium, the residual volume of the solvent above the sorbent is pushed through the cartridge, applying a defined volume of air with the syringe pump or using an external gas (N2).

Figure 4
Positive pressure SPE

- High-precision syringe pumps control the liquid, air flow, and volumes.
- Precise liquid handling allows liquid sorbent interactions to be optimized throughout the SPE process with excellent sample-to-sample reproducibility without human dependency.
- The delivered air push volume controls the state of the packing bed. Depending on the programmed volume, it is possible to maintain a wet packing or push the residual volume completely through the sorbent.
- In the latter case, pushing the residual volume at a constant flow rate prevents any phenomena of acceleration and fast flows as can happen at the end of the step with the SPE under vacuum.

Table 3
CV Comparison between manual and automated SPE systems

MANUAL	% Recovery	
	Chlorpromazine	Thioridazine
MEAN (%)	89.0	81.1
% CV	7.1	6.1
AUTOMATED	**% Recovery**	
	Chlorpromazine	Thioridazine
MEAN (%)	92.7	81.6
% CV	4.2	3.6

NOTE Observe the decrease in CV for the automated SPE instrument in Table 3.

Extend SPE Capabilities with Fractionation

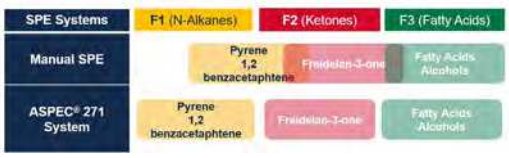
Whatever your objective, method optimization, troubleshooting diagnostic, or recovery of analytes by the family of compounds, one of the most difficult operations to proceed in Manual SPE is the fractionation:

- With a long and meticulous process, the human contact to hazardous materials is emphasized in SPE.
- The split of elution in different tubes increases the risk of error.

Automating a long and complex SPE protocol makes even more sense, especially if extended liquid handling capabilities are included, as illustrated in Figure 5.

Full automation of a long and tedious complex of SPE protocol

Reproducibility improvement



Running overnight and increase throughput & saving time

	Manual SPE	ASPEC® 271 System
Protocol SPE Duration/Sample (min)	62	92
Max Throughput During Working Hours (8h)	8	5
Max Throughput Overnight	0	9
Max Throughput/Day	8	14
Technician Saving Time (h)	0	21,23

Fractionation of Total Lipid Extracts (TLE) Biomarkers from Soil performed by Manual SPE or ASPEC 271

Experiment:
Four soil extract aliquots (15, 20,25 and 30 mg) in triplicates (n=12) followed by an manual or an automated SPE fractionation using a silica gel column to separate compounds in three family of compounds : F1 (n-alkanes), F2 (aldehydes and ketones) and F3 (fatty acids and alcohols).

Results:
Manual SPE : pyrene and 1,2 benzacetnaphtene (PAHs) are present in F1 (n=4) or present in F2 (n=8)
freidelan-3-one is present both in F2 and in F3 with no consistency in term of repartition (n=12)
271 ASPEC : All family of compounds are perfectly separated and collected in the right tubes (n=12)

Figure 5
Long and complex SPE protocol : automated vs manual

NOTE
Add sample homogenization & solvent mixtures preparation to SPE automation increase saving time and decrease human contact with hazardous materials.
Automate SPE fractionation yields better reproducibility.
Automate run overnight increases drastically the throughput despite of a longer duration of automated protocol.

Automated Method Development

Gilson's SPE instruments make it easy to perform multiple levels of automated method development and offer significant advantages, including:

- Easy transfer of existing manual method to an automated one.
- Optimization of SPE key parameters that are difficult to implement manually (flow rates, contact time).
- Complete automation of the development of a new SPE method, from the selection of the SPE sorbent to the optimized method, to avoid a long and tedious manual process.

Better Regulatory Compliance and Documentation

- Automation of SPE facilitates the transfer of a validated methodology.
- Automated SPE offers greater reliability than manual SPE.
- Assistance with compliance is enabled by utilizing TRILUTION LH Software functions such as audit trails (log files or validation of the methodology directly on the automated SPE system) and ERM—electronic records management— (full tracking of samples).
- Error handling methods allow automated systems to run unattended by monitoring unexpected errors.
- Installation Qualification (IQ) and Operational Qualification (OQ) procedures are available for Gilson's automated SPE instruments.
- Routine preventive maintenance (PM) adds to the overall compliance of an automated SPE instrument.

Chapter 5

HOW TO AUTOMATE SPE

Mobile Rack Technology

In manual SPE, solvents are collected in waste vessels during the conditioning, loading, and washing steps. At the same time, collection tubes are used during load and collect (non-retention strategy), elution, or fractionation steps. Full automation of SPE integrates this functionality.

Our Gilson ASPEC system automation is based on the mobility of a rack dedicated to SPE (DEC Rack) consisting of three parts: a mobile SPE cartridge holder, a drain cuvette, and a collection rack where collection tubes are positioned. Thanks to the Z-arm of the instrument, the SPE cartridge holder is automatically moved over either a waste position or collection position depending on which step is being performed. Avoiding the use of a sophisticated tool such as a gripper, this smart solution is robust, simple, and economical.

Mobile Rack Position: Elution, Fractionation

Mobile Rack Position: Condition, Load, Wash Steps

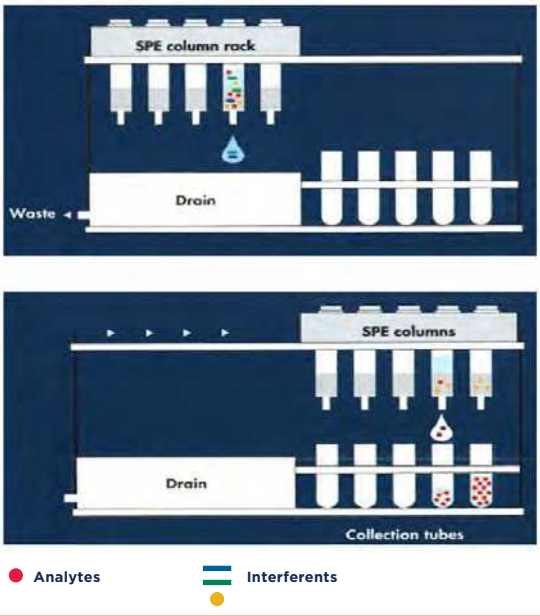


Figure 6
Gilson SPE rack

Access and Transfer of Liquids

Low flow rates are usually used to perform SPE tasks to ensure accuracy.

All the transfer of liquids are ensured by our Gilson VERITY 4X60 syringe pumps to provide volumetric accuracy: ± 2% (10%–90% syringe capacity, water). Their flow rate ranges are listed in Table 4. Optimal flow rates will depend upon sample viscosity, the accuracy required, and the syringe size.

Table 4

Minimum and recommended maximum flow rates (water) for VERITY® syringe pumps

Syringe size (µL)	100 µL	250 µL	500 µL	1 mL	5 mL	10 mL	25 mL
Minimum Flow Rate (mL/min)	0.001	0.001	0.01	0.01	0.1	0.1	0.1
Maximum Flow Rate (mL/min)	4	10	20	40	100	100	100

These specifications are consistent with the flow rate range used when performing SPE tasks, typically between 0.5 and 20 mL/min depending on the SPE cartridge format (1, 3, or 6 mL) and protocol specificity.

The 6-way valve of the VERITY 4X60 syringe pump is connected to the probe of the ASPEC platform so that liquid can be aspirated or distributed to any area of the tray of the ASPEC instrument: solvent bottles, sample tubes, or SPE cartridges.

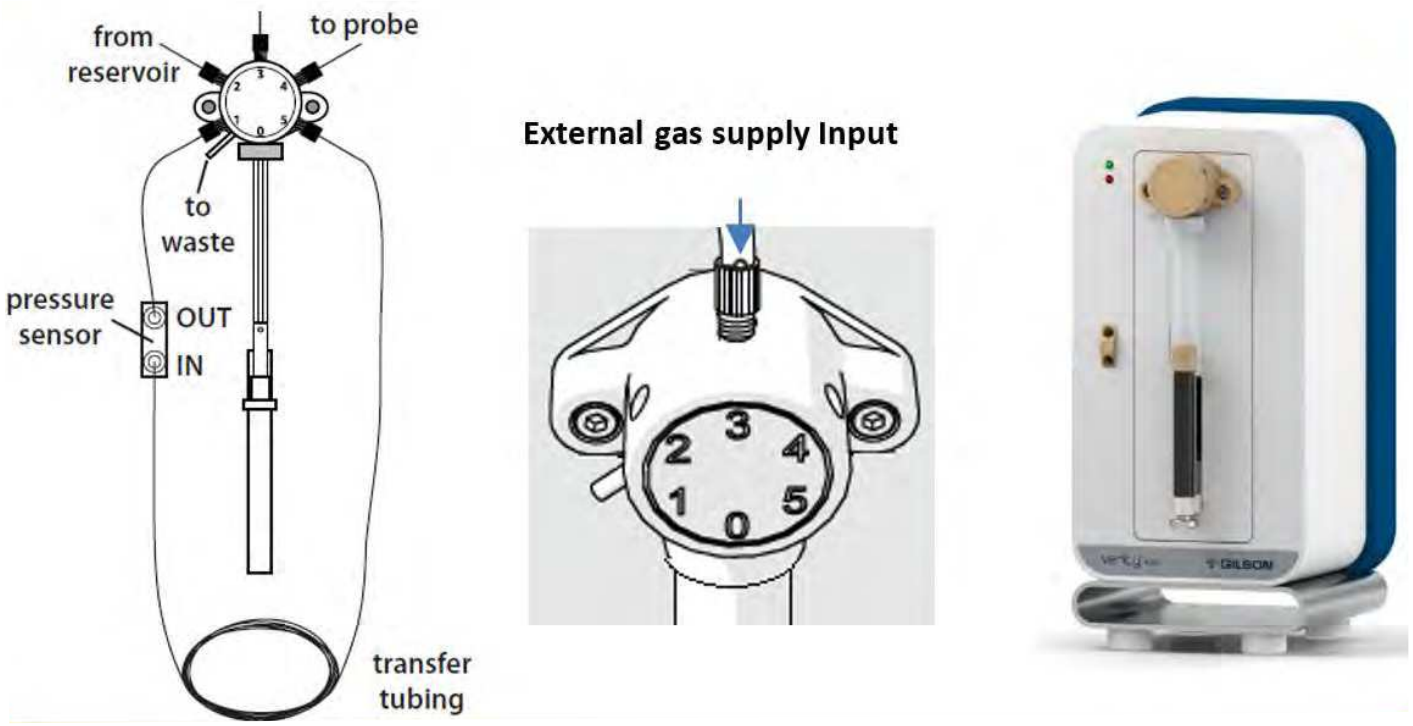


Figure 7

VERITY® 4060 syringe pump connection

By automated switching of this valve, the syringe can aspirate liquid from the reservoir side which contains the system rinsing solution (e.g. Deionized water) and then dispense the liquid through the probe side. This functionality allows not only to make automatic rinsing steps throughout the SPE protocol but also to use the reservoir liquid as an additional SPE solvent.

The optional connection to an external gas supply allows a strong drying of the solid phase if required.

The available source of liquid on the ASPEC systems can be categorized as below:

- Liquid from reservoir: rinsing solution (or SPE solvent).
- Liquid from tray: samples or solvent containers accessible from racks positioned on the ASPEC instrument.
- Liquid from transfer ports: specific to the ASPEC 274 system, two transfer port stations give access to two additional solvent bottles placed outside the tray of the Liquid Handler.

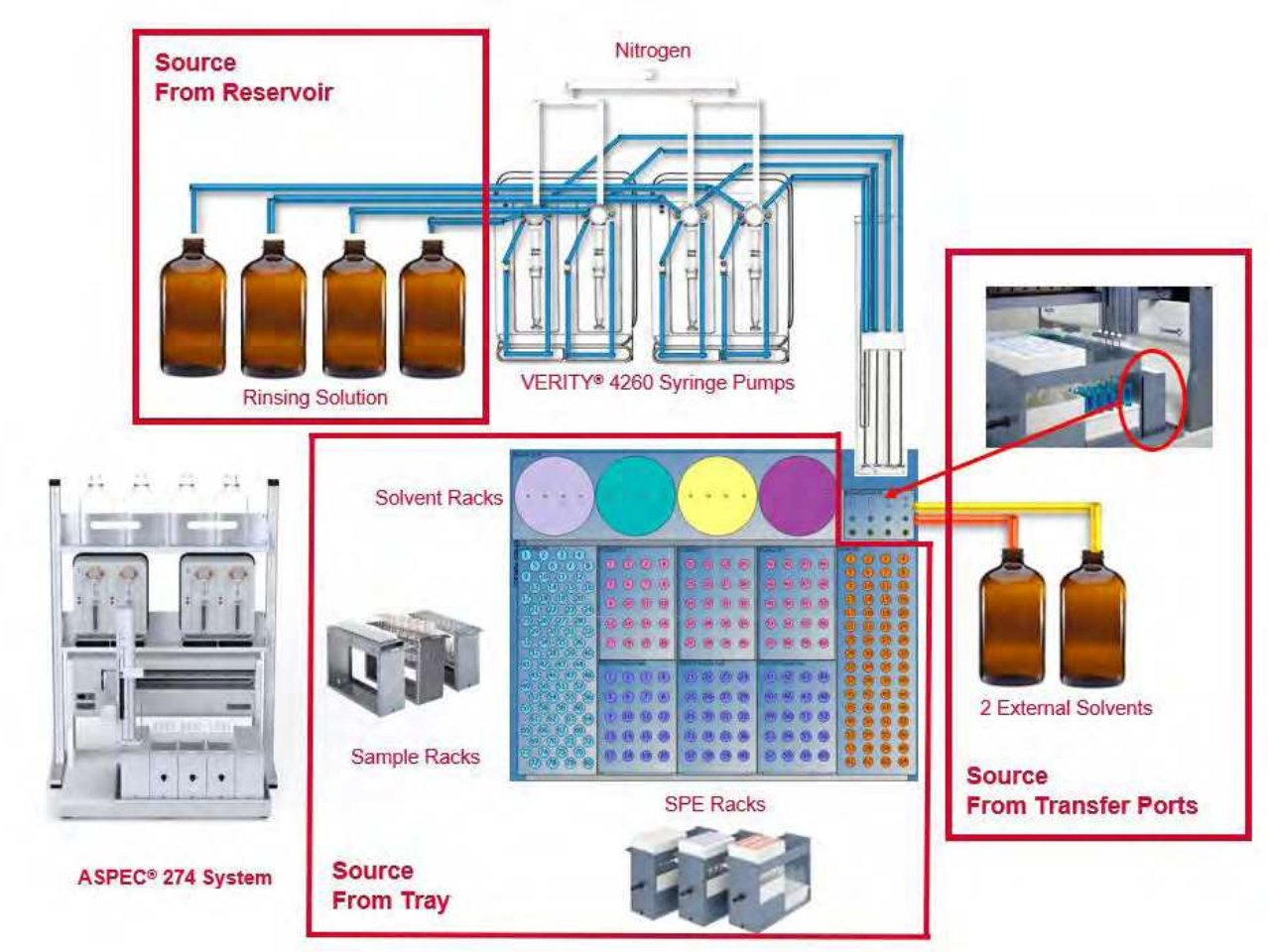


Figure 8
ASPEC® systems - Source type categorization

TRILUTION LH Software is a comprehensive software package for seamless automation of all liquid handling (LH) and solid phase extraction (SPE) methods. Its intuitive interface, simple drag-and-drop method creation, and application simulation combine flexibility, and ease of use.

TRILUTION LH Software Overview

Our goal through this guide is to give some tips on SPE automation. All features of the TRILUTION LH software will not be covered here. Detailed information is available in the online help of the software.

Briefly, an easy-to-use interface leads the user through the software, enabling quick and efficient software navigation. The main menu guides the user who can select the creation of a new SPE protocol (Method Builder) or the creation of a sample list (Application) to run (see Figure 9).

With the automated tracking of any changes, actions, the ability to create user access with password and specific privileges with the possibility to sign a validated method, TRILUTION LH ensures optimal traceability of automated SPE experiments in a secure environment (see Figure 10).

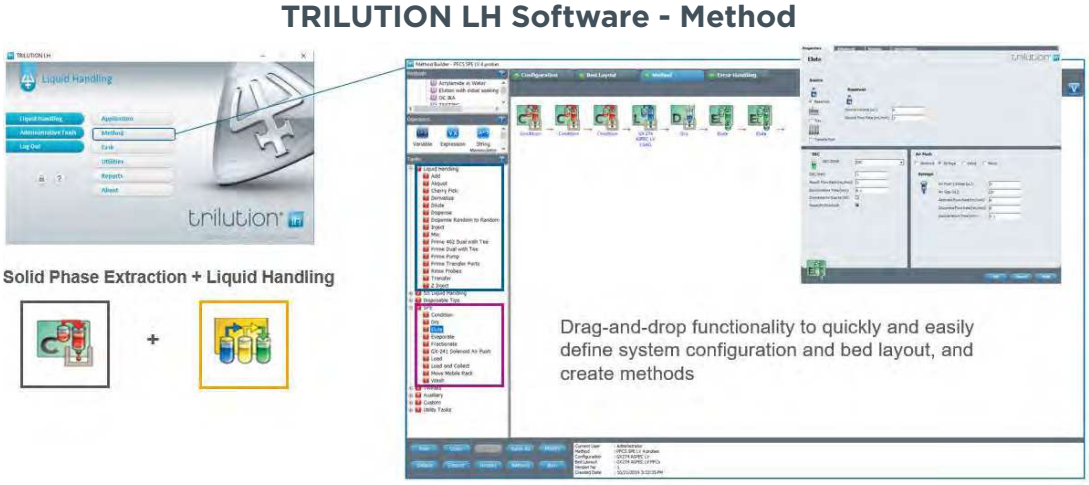


Figure 9
TRILUTION® LH – Easy to use interface

Confidence on your results - TRILUTION LH Software

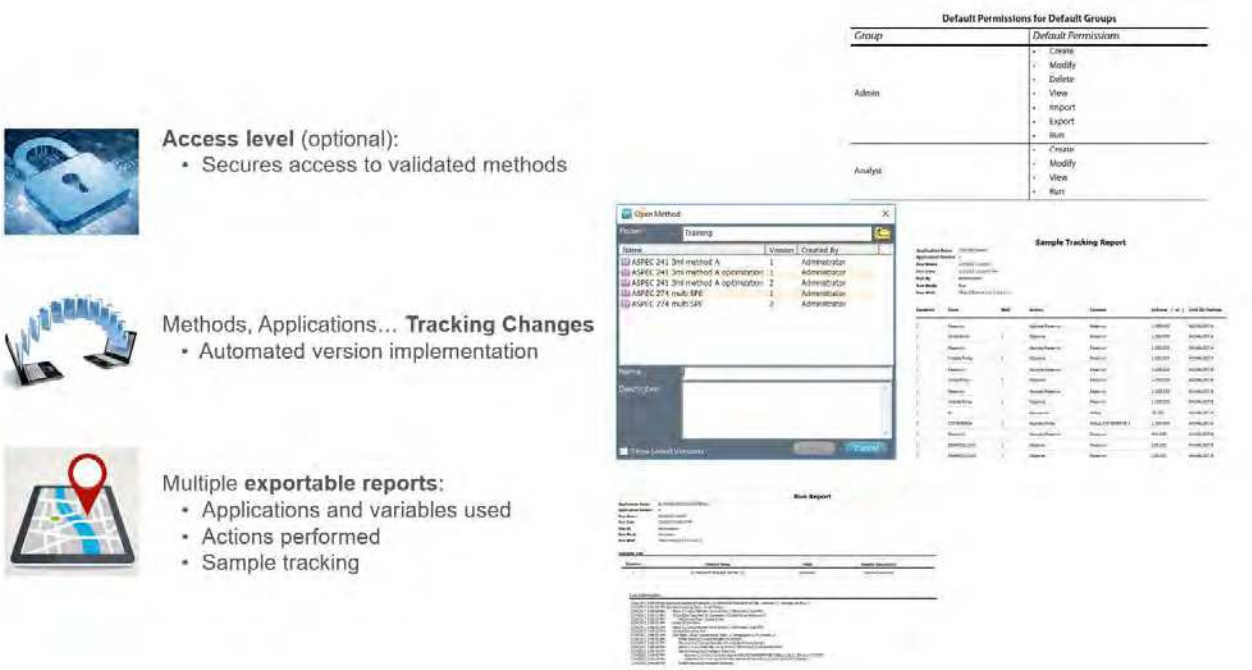


Figure 10
TRILUTION® LH – Access management and traceability

How to Easily and Rapidly Create and Run an SPE Protocol

To avoid any degradation of the numerous benefits inferred by automated SPE in term of productivity and performance, the associated software should be simple and easy to use while maintaining a full control of the SPE parameters. In an increasingly competitive world, in order to accelerate research, laboratory staff no longer have time to program long and complex sequences using a computer language.

That's why Gilson developed the TRILUTION LH software, designed by scientists for scientists.

TRILUTION LH SPE Method Creation

SPE methods are created in TRILUTION LH Method Builder following these four steps:

1. **Configurate the hardware** of the SPE system by defining its different elements (liquid handler and syringe pump(s)...) and their specifications (names, serial numbers, syringe volume...)
2. **Create the bed layout** to position racks and zones
3. **Create the protocol** as a sequence of successive tasks. This is done by dragging and dropping predefined SPE tasks (CONDITION, LOAD or LOAD & COLLECT, WASH, DRY, and ELUTE or FRACTIONATE) or basic liquid handling tasks (MIX, TRANSFER, ADD...)
4. **Create error handling management** to secure the application run

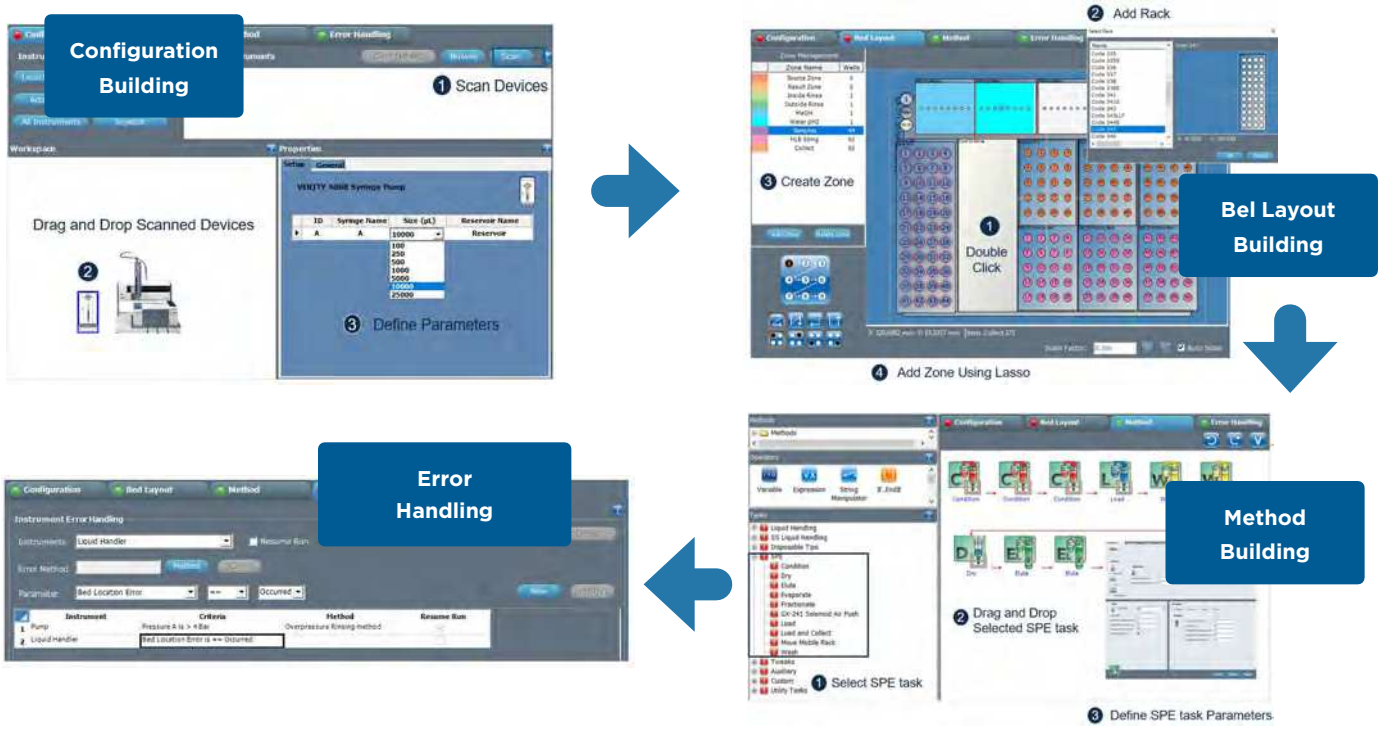
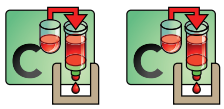


Figure 11
TRILUTION® LH – Method builder workflow

Typical SPE Method Sequence

The typical SPE method consists of one or two CONDITION steps to prepare the retention of targeted analytes on the sorbent, one or more LOAD steps to percolate the sample, one or two WASH steps to remove co-extracted interferents, and finally, one or two ELUTE steps to recover extracted analytes.

Step 1: Condition



Conditioning is most often divided into two steps. The first step in conditioning is usually named the solvation step. This step reactivates the functional groups of the dry sorbent material and eventually removes any trace of impurities. The solid phase must be properly soaked not only to properly reactivate but also to avoid channeling, which can lead to poor reproducibility of compound retention. This initial conditioning step is usually performed with an organic solvent (e.g., methanol).

A second conditioning step, usually named the equilibration step, is required to remove the excess of the solvation solvent and to prepare the retention environment before loading the sample. A solvent quite similar to the sample matrix in terms of pH, ionic strength, or solvent ratio is used.

It's essential to keep in mind that the time between conditioning and the sample loading has to be short enough to avoid the drying out of the sorbent bed or loss of solvation due to evaporation.

Step 2: Load

The sample is introduced to the SPE cartridge and passes through the sorbent material. The main goal in this step is to ensure that the analytes of interest are quantitatively retained by the sorbent with limited loses while major impurities pass through the cartridge. During the loading, the mass transfer of analytes from the matrix to the sorbent must have enough time to occur, and binding interactions must be maximized. To ensure a sufficient contact time, it is important to use a slow and constant flow rate.

Other criteria must be considered to achieve high recoveries: the choice of sorbent, its loading capacity, and potentially a required sample pre-treatment.

Step 3: Wash

Contaminants may be rinsed off once the analyte of interest is strongly retained on the sorbent. It is common practice to employ two wash steps. A first wash is usually performed to eliminate non-retained matrix impurities remaining after sample loading. A second wash is realized with a stronger solvent to remove co-extracted interferences without eluting analytes of interest. The wash steps are crucial in the overall recovery and exclusion of interferences associated with the elution step.

Drying Step:

At the end of the washing, the remaining solvent must be eliminated from the sorbent not to disturb the next elution step. The use of an air push volume may be sufficient. In case of not immiscibility between washing and elution solvents or to avoid any trace of water in the eluate that would disturb the post SPE steps (e.g. evaporation to dryness, analysis by GC), a strong drying with external gas (e.g. nitrogen) may be required.

Step 4: Elute

SPE cartridges are positioned above the collection tubes. The smallest possible elution volume is advised because smaller volumes decrease the dilution of the final extract and minimize the need for evaporation.
A slow and constant flow rate is highly recommended to maximize unbinding interactions. In the same way, a single large elution volume is often split into two smaller volumes to increase contact time, resulting in greater recovery.

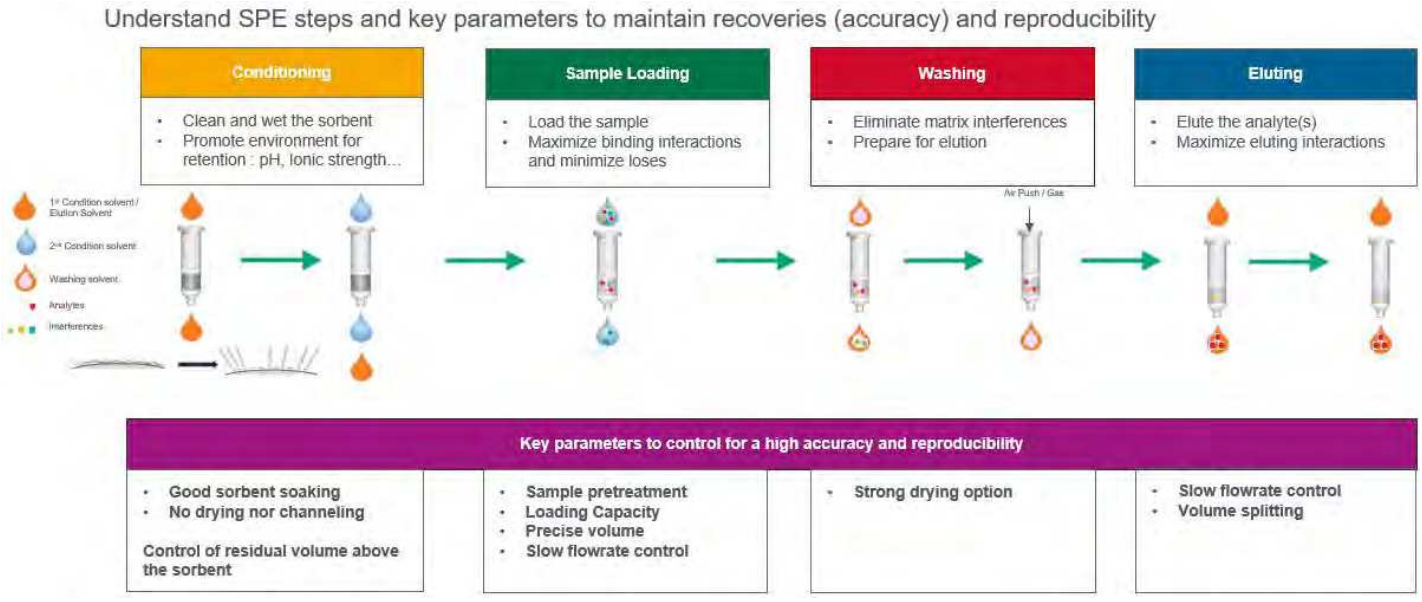


Figure 12
Typical SPE method sequence & key SPE parameters to control

TRILUTION Method - Examples

All the SPE processes can be easily translated within the TRILUTION method by dragging and dropping the corresponding tasks successively in the method workspace, as illustrated in Figure 13.

More complex SPE protocols, such as multi-dimensional SPE or protocols with fractionation, can be easily written thanks to the high flexibility of TRILUTION LH in terms of working zones and bed layout creation (see Figures 14 and 15).

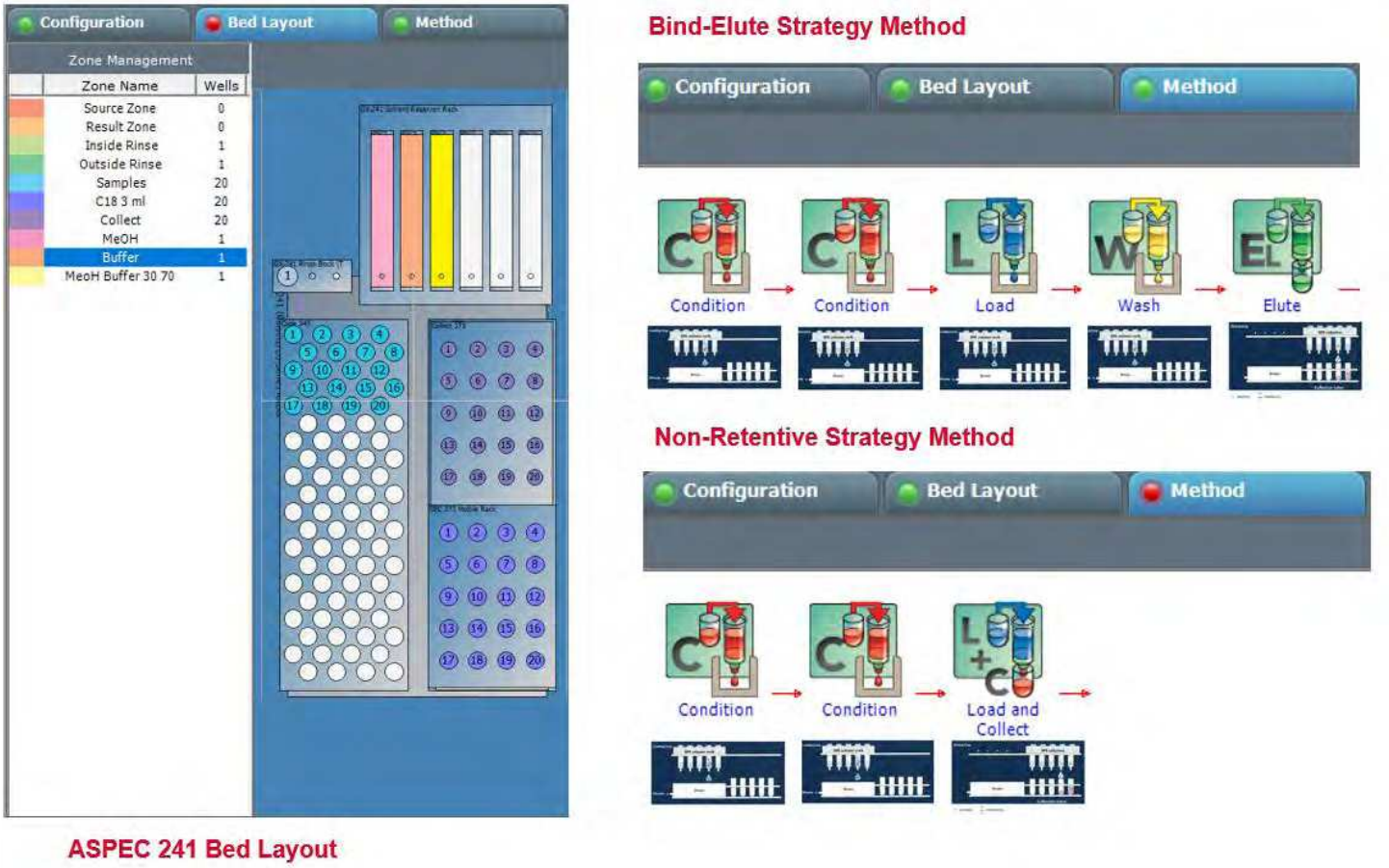


Figure 13
TRILUTION® LH – Examples of SPE methods following bind-elute or non-retentive strategy

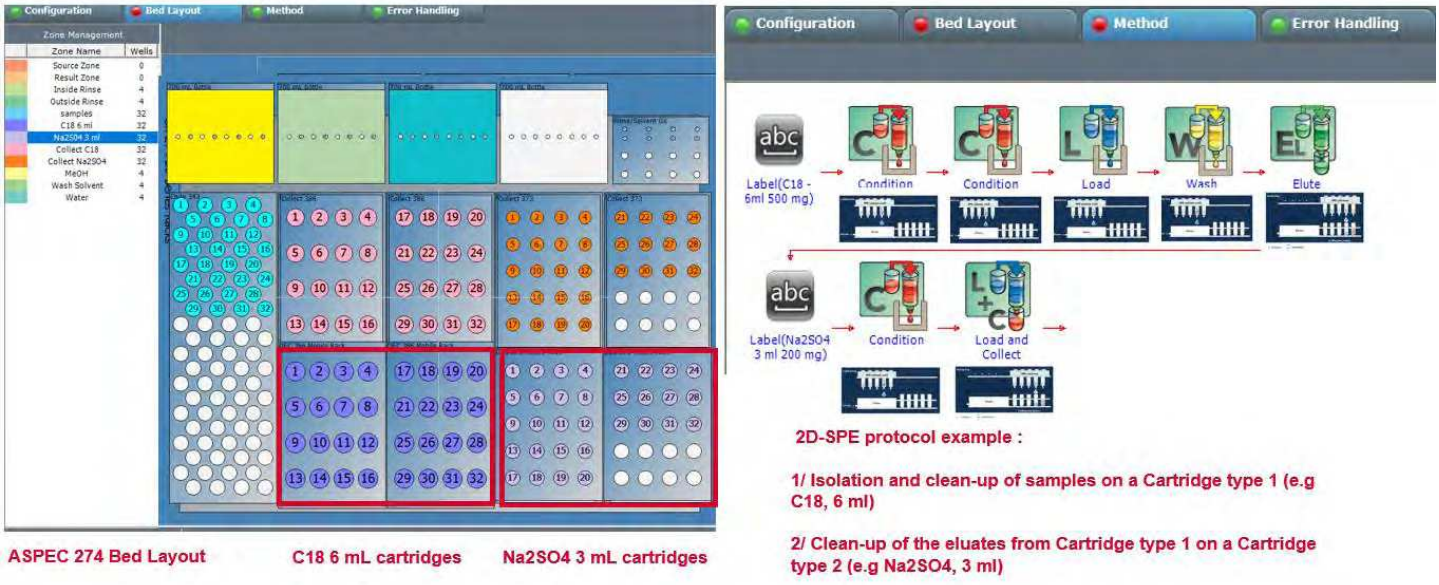


Figure 14
TRILUTION® LH – Example of 2D-SPE method

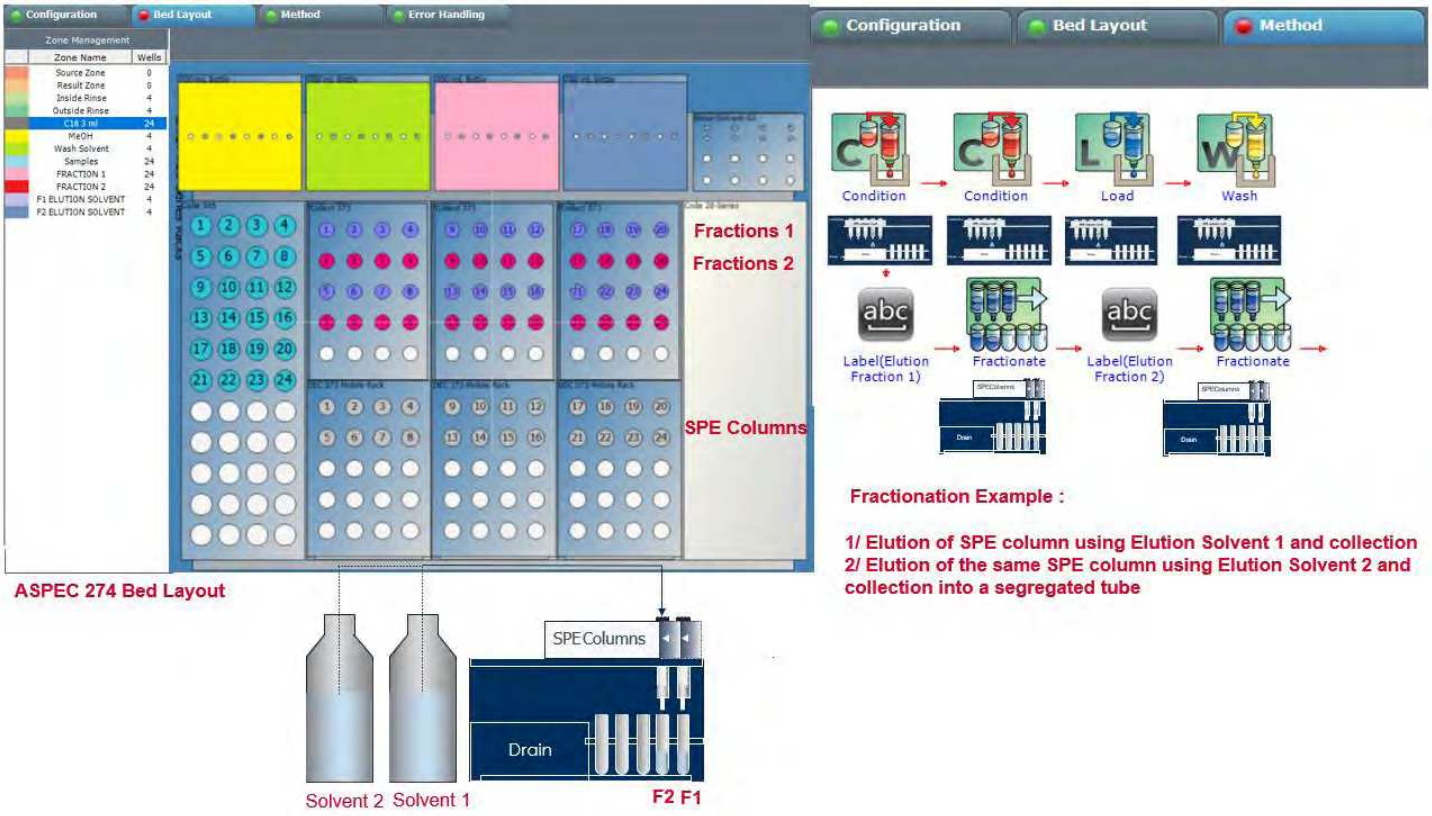


Figure 15
TRILUTION® LH – Example of SPE fractionation method

TRILUTION LH - Error Handling

During an automated SPE protocol, it is crucial to track the pressure within the SPE cartridge and automatically take the right action in case of overpressure: stop the current sample and continue to process the next ones, or stop the run definitively.

Figure 16
TRILUTION® LH method – Error handling of overpressure

TRILUTION LH - SPE Task

Main Parameters of the SPE Task

Each TRILUTION LH SPE task (CONDITION, LOAD, LOAD & COLLECT, WASH, ELUTE...) is constructed in the same manner and briefly requires the definition of main parameters in the properties tab of the TRILUTION LH task:

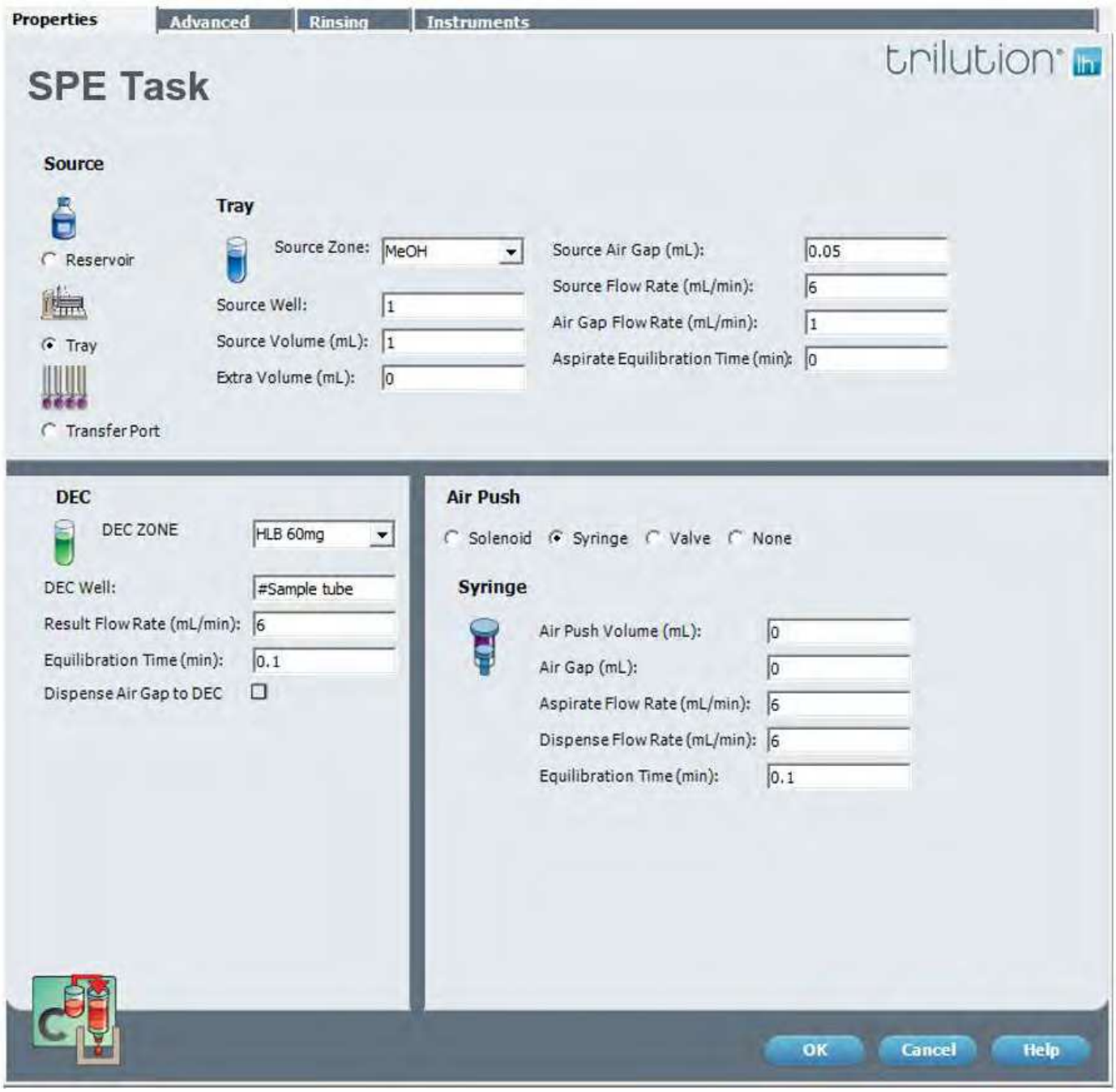


Figure 17
TRILUTION® LH SPE task – Properties tab

These main parameters are divided into three groups:

Source: contains the parameters related to the aspiration of the Sample/Solvent such as the source type (from the reservoir, from the tray, or from the transfer port), the source zone name, the source well, the volume, and the aspiration flow rate.

DEC (Disposable Extraction Cartridge): contains the parameters related to the dispensing of the Solvent/Sample through the SPE cartridge and allows to control Liquid/Solid interactions (soaking, binding, or eluting) using suitable values of dispense flow rate and equilibrium time.

Air Push: contains the parameters related to the control of sorbent material state at the end of the current SPE task: dried or not dried using a volume of air (or an external gas) defined according to the objective.

SPE Task Sequence

In case of source selection from the tray or transfer port, the SPE task sequence can be summarized as follows:

SAMPLE/SOLVENT ASPIRATION

1. Move mobile rack (DEC Zone) over the drain position (CONDITION, LOAD, WASH) or over the collection position (ELUTE, FRACTIONATE)
2. Aspirate an air gap volume

NOTE	Source Air Gap: a volume of air that separates the sample from the reservoir liquid when the sample/solvent is in the probe or tubing during a transfer. It is important to have a well-defined air gap in order to prevent mixing of the two liquids and to maintain good accuracy on the liquid transfers. The air gap volume is defined in order to have a length of air separation around 10 mm in the transfer tubing. In case of a 10 mL transfer tubing a typical value of 38 µL can be used. To ensure a consistent separation, a low air gap aspiration flow rate is used (max 1 mL/min).

3. Move the probe into the source well of the source zone

NOTE	Source Zone: one of the zone names defined on the Bed Layout (set of tubes, vials, bottle(s)…) Source Well: the well of the selected zone to process. In case of an aspiration from a Sample Zone, a well type variable (e.g. “Source well”: #Sample Tube) is required. The sample well to run will be defined later in the sample list of the TRILUTION LH application. See Figure 18. In the same way, a Source well variable (e.g. “Source well”: #Solvent well) is also required in case of an aspiration from a Solvent Zone using an (ASPEC 274 system See Figure 19).

4. Aspirate an extra volume (if defined)

NOTE	An Extra Volume of Solvent/Sample can be aspirated for enhanced volumetric accuracy or to increase the separation with the reservoir liquid. The Extra Volume will be discharged into the rinsing station after distributing Solvent/Sample volume in the cartridge.

5. Aspirate the defined source volume (solvent/sample volume)

NOTE	Moderate Source Aspiration Flow Rates are used to ensure sufficient volume accuracy: liquid aspiration flow rate is typically 6 mL/min in the CONDITION and WASH tasks whereas they are reduced to 3 mL/min during the LOAD, ELUTE and FRACTIONATE steps. However, these usual values will have to be reduced in case of high volatile solvent (e.g. chloroform) or high viscous samples (e.g. oil).

6. Wait an aspirate equilibrium time and move the probe up

NOTE	Aspiration Equilibrium Time: the time the probe will remain inside the liquid before moving up. This time allows the stabilization of the liquid volume inside the tubing. It is recommended to use a value of 0.01 min especially during the LOAD, ELUTE, and FRACTIONATE tasks. In case of high viscous samples, a value of 0.05 min will be more appropriate.

SOLVENT/SAMPLE DISPENSING

7. Move probe into the DEC Zone well

NOTE	DEC Zone: one of the DEC Zones defined on the Bed Layout (a set of SPE cartridges). DEC Well: the cartridge of the selected DEC Zone to process. The probe moves into the selected SPE cartridge. A well variable type is required (e.g. “DEC well” : #Sample Tube) and will be defined in the sample list of the Application. See Figures 18 and 19.

8. Dispense the solvent/sample volume through the DEC

NOTE

Result Flow Rate: the values of dispense flow rates depends on the SPE step and the format of SPE cartridge used (1, 3, 6 mL) as presented in Table 5. To maximize interactions, slow result flow rates are used in the LOAD, LOAD & COLLECT, ELUTE and FRACTIONATE tasks while moderate flow rates are implemented in the other ones.

9. Wait an equilibrium time

NOTE

Equilibrium Time: the time the probe remains in the column after Solvent/Sample dispensing to allow pressure normalization in the DEC. This time is critical to avoid sealing cap spurting when the probe is raised out of the SPE column.
A value of 0.1 min is usually required to overcome any issue. Note that higher values can be also used to increase the contact time between the sorbent and the liquid.

10. Move up the probe up and dispense the air gap volume (and extra volume if defined) into the inside rinse station

NOTE

Dispense Air Gap to DEC: this option is only effective if no extra volume is defined. In case of selection, the small volume of air gap is directly dispensed into the cartridge instead of the rinsing station.

AIR PUSH VOLUME TO CONTROL THE DRYING STATE OF THE SORBENT

11. Move up the probe up and aspirate an air push volume (selection of syringe option)

NOTE

Air Push Volume: the amount of air to be aspirated and dispensed into the SPE column to push the residual solvent volume present above the sorbent after the liquid dispensing. According to the defined volume, the residual Volume of Solvent/Sample is fully flowed through the sorbent material or only up to the top frit of the SPE cartridge to prevent any drying during CONDITION steps. Some guidance of Air Push Volume to use are given in Figure 30 and Table 5.

NOTE

Air Gap: same principle as the air gap used for liquid.
Air Push Aspirate flow rate: 6 mL/min is a standard value.

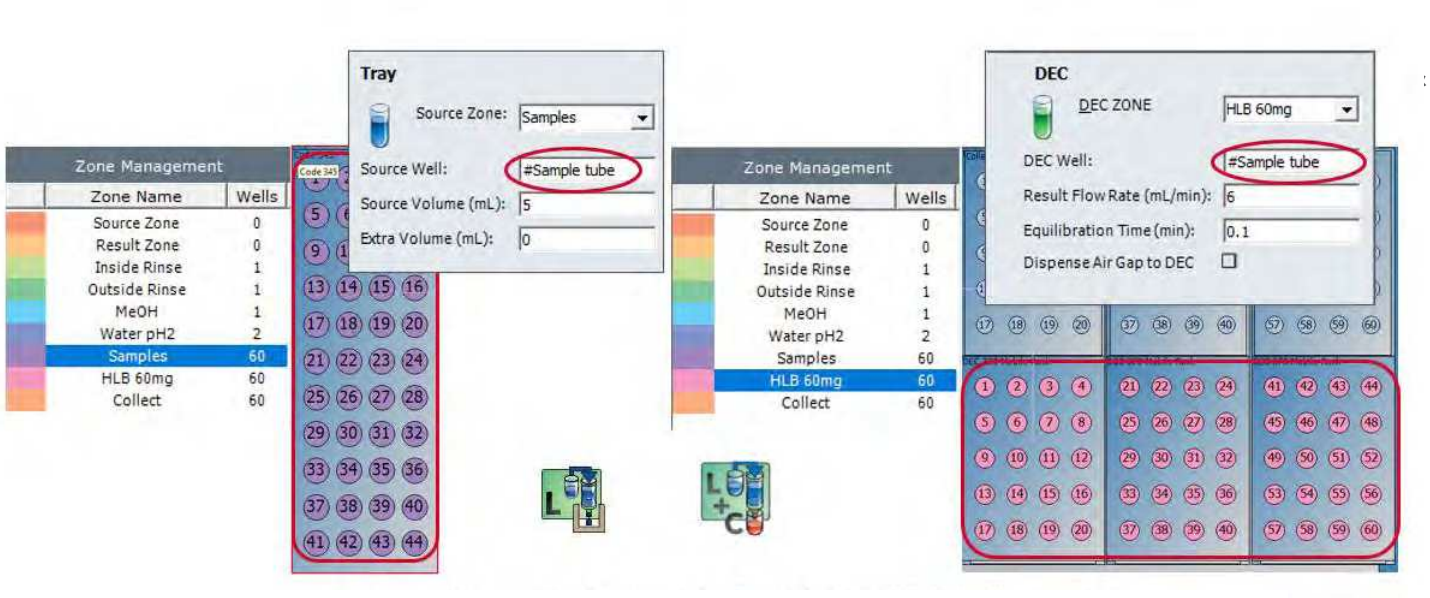
12. Move probe into the DEC well and dispense air push volume

NOTE

Air Push Dispense Flow Rate: the same flow rate used for liquid dispensing is usually implemented in order to push the residual volume of liquid through the cartridge in the same manner as the rest of the volume.

RINSING OF THE PROBES

13. Rinse successively the inside and outside of the probe in the rinsing station. In the case of reservoir source selection, the sequence is the same except for the air gap volume and extra volume, which are not required.



The use of the same variable name for Source Well and DEC Well means that Sample Tube 1 will be loaded in the DEC 1, Sample Tube 2 in the DEC 2 and so on.

Figure 18
TRILUTION LH SPE task – Source Well & DEC Well settings (LOAD, LOAD & COLLECT)

Figure 19
TRILUTION® LH SPE task – Source Well & DEC well settings (CONDITION, WASH, ELUTE, FRACTIONATE)

Advanced/Rinsing Parameters of the SPE Task

Rinsing is a key consideration when automating any liquid handling procedure. If the method utilizes two immiscible solvents back to back, it is essential to provide adequate rinsing with a mutually miscible solvent in between to prevent undesired interactions. Ensuring adequate rinsing will significantly reduce the risk of any carryover from step to step. Typically a rinse is one to two times the volume of the liquid transferred at a relatively high flow rate.

Reducing carryover risk can also be achieved by limiting the probe's contact with the samples. Limiting the immersion depth of the probe in the samples can be done by activating the level detection and monitoring the liquid level during the sample aspiration process.

An optional peristaltic rinsing pump can also be used to increase the efficiency of the outside rinse of the probe.

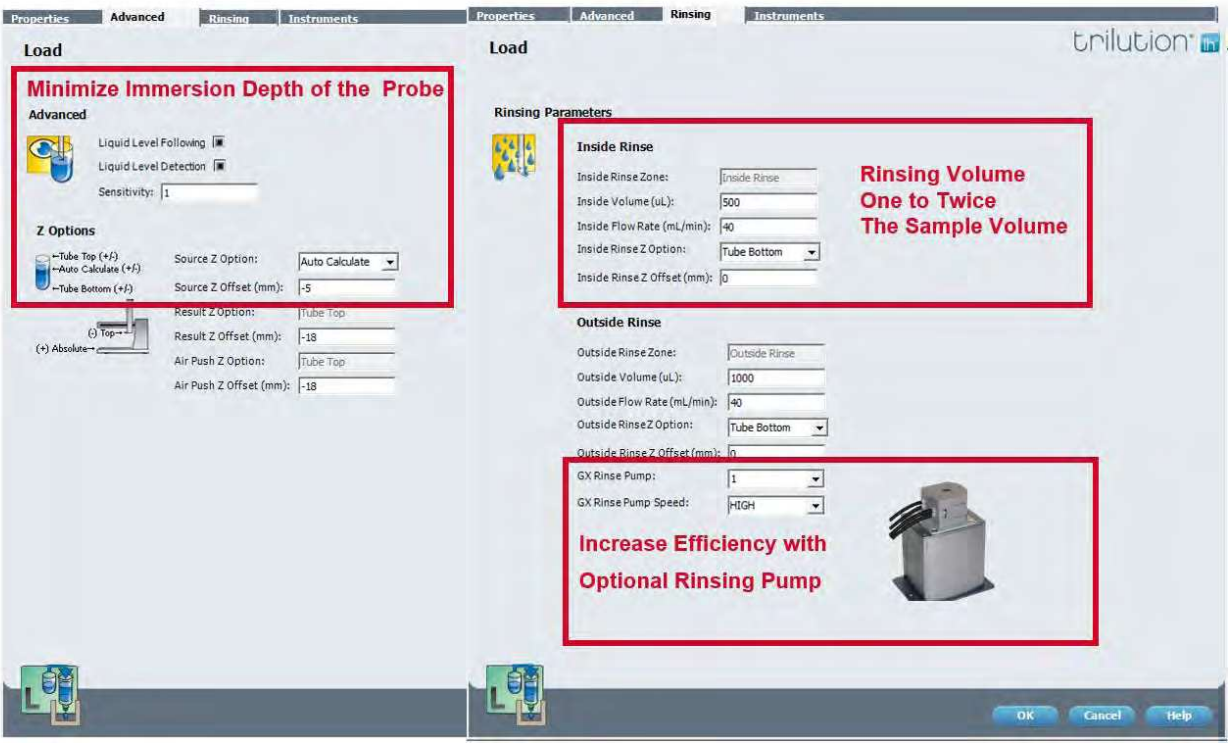


Figure 20
TRILUTION® LH SPE Task – Advanced and rinsing parameters to minimize risks of carryover

TRILUTION LH Application : Create and Run a Sample List

The writing and run of a sample list within TRILUTION LH Application Builder follow four successive steps:

1. Select the desired working tray and method
2. Fill in the sample tubes to process
3. Define if the method will be processed in Sequential Mode or in Batch Mode
4. Save and Run (or simulate a run)

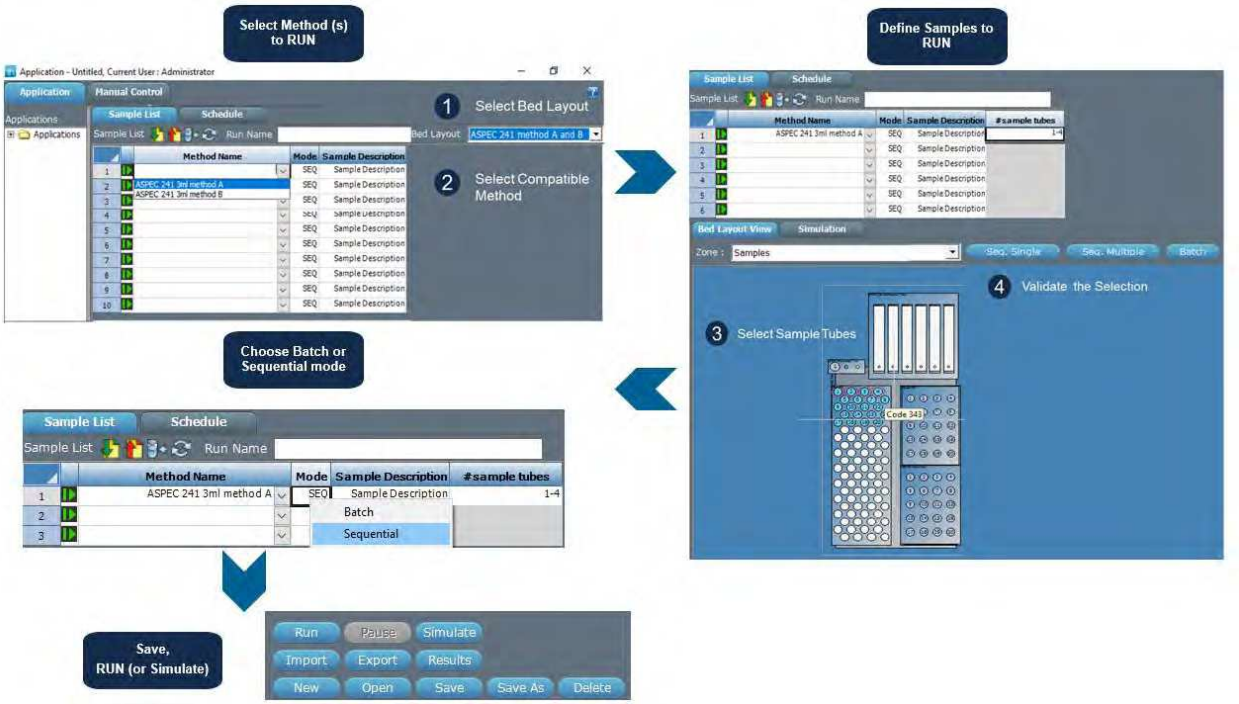


Figure 21
TRILUTION® LH – Application builder workflow

The steps 1, 2, 3 can be repeated several times in case of multiple methods to run.

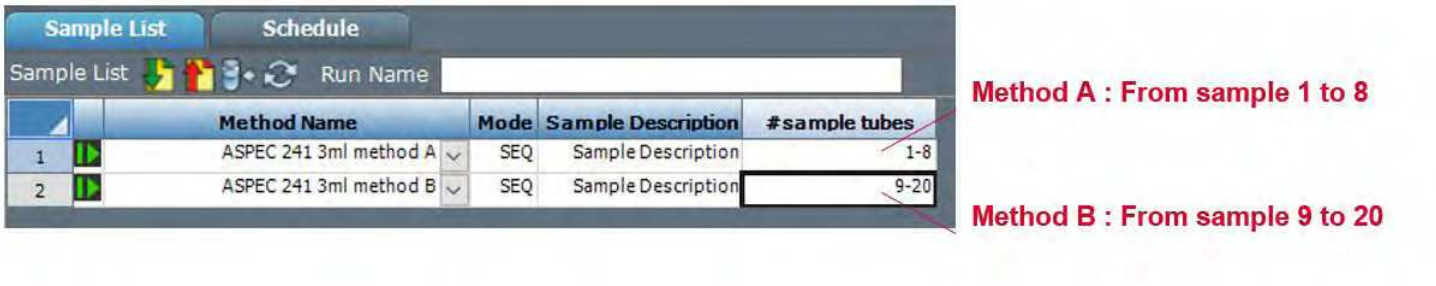


Figure 22
TRILUTION® LH application – Multi method example

Sequential Versus Batch Mode

Gilson's versatile TRILUTION LH allows for complete control of the automated method, including whether the tasks are run in a series or in parallel for each sample. The two modes are "Sequential Mode" and "Batch Mode."

Sequential Mode

A series of tasks/steps are performed on one sample and then repeated for the following sample.

Batch Mode

Every sample is exposed to a single task/step before advancing onto the next step.

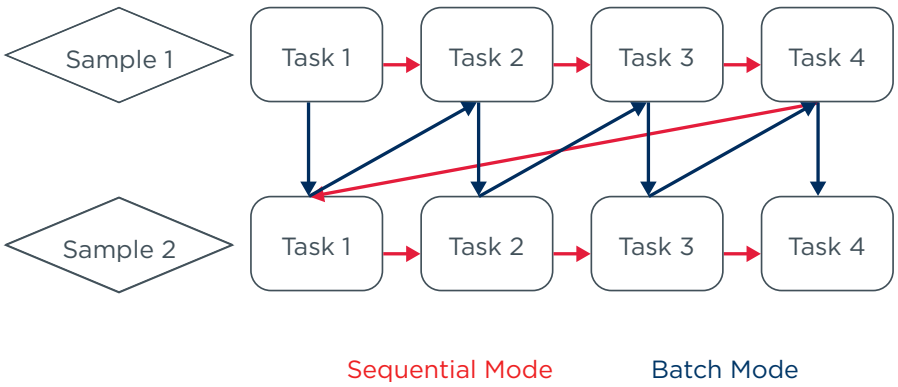


Figure 23
TRILUTION® LH – Sequential versus batch mode diagram

The mode choice will depend on the sample stability, the required throughput, and the total run time.

Chapter 6

AUTOMATED METHOD DEVELOPMENT

The major goal of method development is to optimize the extraction efficiency (maximizing the recovery of the target analyte(s) and minimizing the co-eluted interferences). These conditions must provide not only reproducible (low variance) results but also a procedure that is simple and economical.

Optimizing a method is finding a balance between recovery, purity, time, and cost. If the recovery needs to be higher, can the high purity be sacrificed to do so? Is it more important to complete it faster or recover more samples? These questions need to be kept in mind as the method is optimized. Also, remember the following hints when trying to adjust parameters:

- Optimum Volume is the smallest volume that can fully elute the analyte or interferants. The smaller the volume, the less time and cost go into the step.
- Optimum Flow Rate is the fastest flow rate that can be implemented without compromising recovery.

At first, SPE method development may appear somewhat overwhelming, but with the use of automation, the process can be easily rationalized. Moreover, impacts of parameters such as volume, flow rate, and equilibrium time can be easily studied compared to manual method development.

Strategy for Developing a New Automated SPE Method

Optimizing an SPE Method Through Automated Fractionation

Gilson SPE instruments allow automated method optimization and development through stepwise movement of the SPE cartridges across a series of collection tubes (refer to Figure 24). This allows the load, wash, and elute steps to be collected and analyzed specifically for sample breakthrough, recovery, and interferences. In addition, variations to the wash and elute steps can be evaluated and optimized without manual intervention. Automation allows for complete evaluation and optimization of every step in the SPE method, which is usually cost and time prohibitive if done manually.

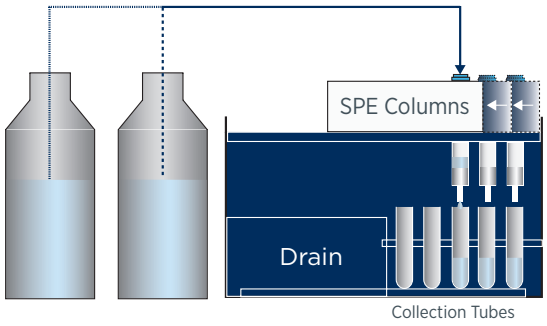


Figure 24
Automated fractionation

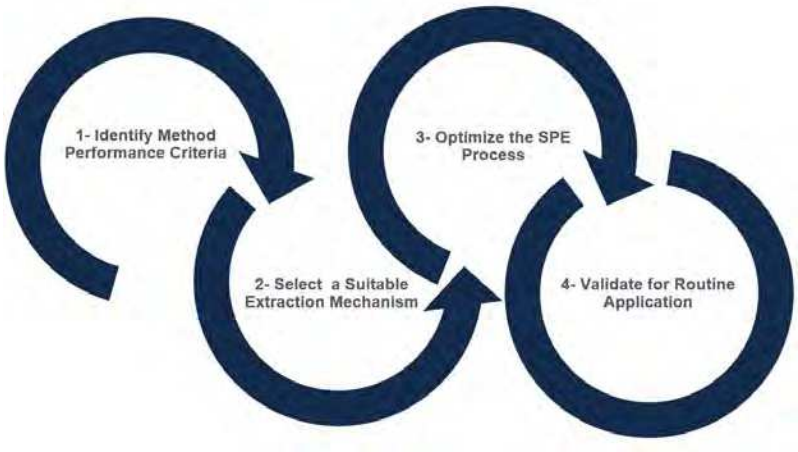


Figure 25
New Method development – General strategy

Automation of an Existing Manual SPE Method

In case of the transfer of an existing validated manual SPE method to an automated one, the automated development workflow starts directly at the optimization (step 3).

Using our ASPEC systems makes this transfer easy and fast to process since only a few parameters need to be optimized: mainly liquid dispense flow rates concerning required bind/elute interactions and air push parameters concerning the required drying state of the sorbent.

Starting Parameters of a New SPE Method Using ASPEC Systems

The volume and flow rate of solvents at each step in an SPE method can vary greatly from application to application. Although this is something that needs to be optimized in the final SPE method (step 3), general starting points can be found in Table 5, which provides some guidance on the critical SPE parameters used in the early stages of development with our ASPEC systems. Other less minor parameters can most often be kept at their default values in the TRILUTION LH software and are summarized in Table 6.

Table 5

Typical SPE cartridge values during the SPE process (Most critical parameters are highlighted in red). (*Strong drying required in case of solvent miscibility issues)

1 mL SPE Columns (100 mg)	Condition	Load	Wash	Elute
Liquid Volume (mL)	1.00	1.00	1.00	2.00
Liquid Dispense Flow (mL/min)	5.00	1.00	3.00	2.00
Air Push Volume (mL)	0 (No Drying)	0.50	0.75	1.00
External Gas (min)*	NA	NA	5.00-15.00 (Strong Drying)	NA
3 mL SPE Columns (500 mg)	**Condition**	**Load**	**Wash**	**Elute**
Liquid Volume (mL)	2.00	1.50	1.50	1.50
Liquid Dispense Flow (mL/min)	8.00	2.50	6.00	3.00
Air Push Volume (mL)	0.30 (No Drying)	1.50	2.00	2.00
External Gas (min)*	NA	NA	10.00-20.00 (Strong Drying)	NA
6 mL SPE Columns (1000 mg)	**Condition**	**Load**	**Wash**	**Elute**
Liquid Volume (mL)	3.00 to 6.00	2.00 to 20.00 or more	2.00 to 6.00	2.00 to 3.00
Liquid Dispense Flow (mL/min)	10.00	5.00	10.00	5.00
Air Push Volume (mL)	0.50 (No Drying)	2.00	4.00	4.00
External Gas (min)*	NA	NA	15.00-30.00 (Strong Drying)	NA

Table 6

TRILUTION® LH SPE tasks default values of minor parameters (**Air Gap Vol. enough to have 10-15 mm in transfer tubing)

SPE Columns	Condition	Load	Wash	Elute
Air Gap Volume (µL)**	50.00	50.00	50.00	50.00
Liquid Asp. Flow (mL/min)	6.00	3.00	6.00	3.00
Liq. Eq. Time (min)	0.10	0.10	0.10	0.10
Air Push. Asp. Flow (mL/min)	6.00	6.00	6.00	6.00
Air Push. Disp. Flow (mL/min)	6.00	6.00	6.00	6.00
Air Push. Disp. Flow (mL/min)	0.10	0.10	0.10	0.10

STEP 1. IDENTIFICATION OF METHOD PERFORMANCE CRITERIA

The starting point when developing a method is to define its objectives .

Objectives	Details
Analytical Range	The interval between the upper and lower analyte levels for which the calibration relationship is correct.
Limit of Detection (LOD)	The lowest quantity of analyte that can still be distinguished from the background noise. LOD is sometimes defined as the signal/noise ratio, which should be greater than three.
Limit of Quantification (LOQ)	The lowest concentration of analyte that can be determined with sufficient precision and accuracy.
Accuracy (ISO Analytical definition)	The closeness of agreement between the result and the accepted reference value.
Precision (ISO Analytical definition)	The closeness of agreement between independent test results obtained under prescribed conditions. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. higher imprecision is reflected by a larger standard deviation.
Throughput	The throughput indicates the number of samples processed (SPE preparation) per day.

STEP 2. SELECTION OF EXTRACTION MECHANISM

After identifying the performance criteria, it is vital to conduct background research on your analyte to determine which sorbent and solvents would result in the best separation. Sometimes an SPE method already exists for the analyte of interest or a compound of similar structure, functionality, and polarity. This existing method can provide an excellent starting point and usually, after some optimization, can lead to an ideal SPE method.

Chromatographic data from HPLC methods of similar compounds (functional groups, polarity, pKa) can suggest the behavior of an analyte during the separation process and offer insight as to the choice of the SPE mechanism. Indeed, it is helpful to keep in mind that SPE is another form of chromatography. If SPE is based solely on the binding-eluting principle (retention factor k'>>1000 or <<0.001), the mechanisms available are the same as those encountered in HPLC: normal phase (NP), reversed-phase (RP), and ion exchange (IEX) mechanisms as illustrated in table 7.

Extraction Mechanisms vs Analyte/Matrices Properties

In any case, the choice of mechanism is geared towards maximizing the partitioning out of analytes from the matrix using a suitable solid phase. It is essential to take into account the chemical structure of the analyte and the nature of the matrix:

- **Functional group of the Analyte:**

  The presence of nonpolar groups (alkyl chains, aromatic rings, double bonds) suggests the potential for retention by nonpolar interactions. Alternatively, analytes containing polar groups (hydroxyls, amines) are good candidates for retention by polar mechanisms. The presence of ionic functions (amines, carboxylic acids) means that ion exchange sorbents will probably retain the analyte. Ion exchange sorbents can be selected according to the pKa of ionizable groups of the analyte to best meet retention and subsequent elution requirements.

- **Matrix Nature:**

  The properties of the matrix from which the analyte is extracted must be considered when choosing an extraction mechanism. Analytes containing nonpolar or ionic functional groups in aqueous samples can usually be extracted with nonpolar or ion exchange sorbent, respectively; whereas polar analytes in oily samples or in nonpolar solvents would require extraction by polar interactions. Table 7 indicates a general rule of applicable SPE mechanisms versus Analyte/Matrix properties.

Table 7

Common SPE mechanisms vs Analyte/Matrix nature

Analyte Functional groups	Matrix Type	Mechanism Type	Sorbent Type	Examples
Non-Polar i.e., Alkyl, Aromatics	**Polar Solution** i.e., Water, Aqueous buffers	**Reversed Phase (RP) Extraction** Non-Polar Hydrophobic Interactions (Van der Waals forces or dispersion forces)	**Non-Polar** i.e., C18,C8,DVB	Pharmaceuticals from plasma Antibiotics from blood Drugs of abuse from urine Hormones from cell culture Pesticides from water
Polar i.e., Amine, Hydroxyl	**Non-polar solution** ie. Hexane extract, Oils	**Normal Phase (NP) Extraction** Polar Hydrophilic interactions (hydrogen bonding, pi-pi, dipole or induced dipole interactions)	**Polar** i.e., Silica, Alumina	Lipid separation Toxins from peanut butter Steroid vitamins from plasma Fatty acids from cell culture
Ionisable or charged	**Polar solution with Low Ionic Strength**	**Ion Exchange (IEX) Extraction**	**Ionized or Ionizable**	
Anion / Negatively charged i.e., Ionic form of acids	i.e., Water, Aqueous buffers (<100 mM)	Anion attraction on positively charged sorbent	**Anion Exchanger** i.e., SAX, WAX *	Organic acids from urine Neurotransmitters from urine Ribonucleosides from cell culture
Cation / Positively charged i.e. Ionic form of bases		Cation attraction on negatively charged sorbent	**Cation Exchanger** i.e., SCX, WCX *	Catecholamines from plasma Pharmaceuticals from serum Herbicides from soils Antibiotics in food

Sorbent Leads Selection

As presented in Figure 26, after selecting an extraction mechanism considering properties of the analyte and sample/matrix, a range of potential sorbents are available. To develop an effective SPE method, it is essential to choose the unique properties of each sorbent relative to specificity and selectivity.

The information is useful when selecting likely sorbent candidates for the initial screening experiments.

- Significant factors for normal phase SPE sorbents include relative polarity, surface area, surface coverage, surface pH, and water content loss on drying.
- Reversed-phase SPEsorbents significant factors are: surface area, carbon loading, surface coverage, end-capping efficiency, and pore size.
- Significant factors for ion exchange SPE sorbents include their titration behavior and pKa, total ion exchange capacity, surface chemistry, and counter ion content.

Each of these properties must be consistent from lot to lot to obtain reproducible results.

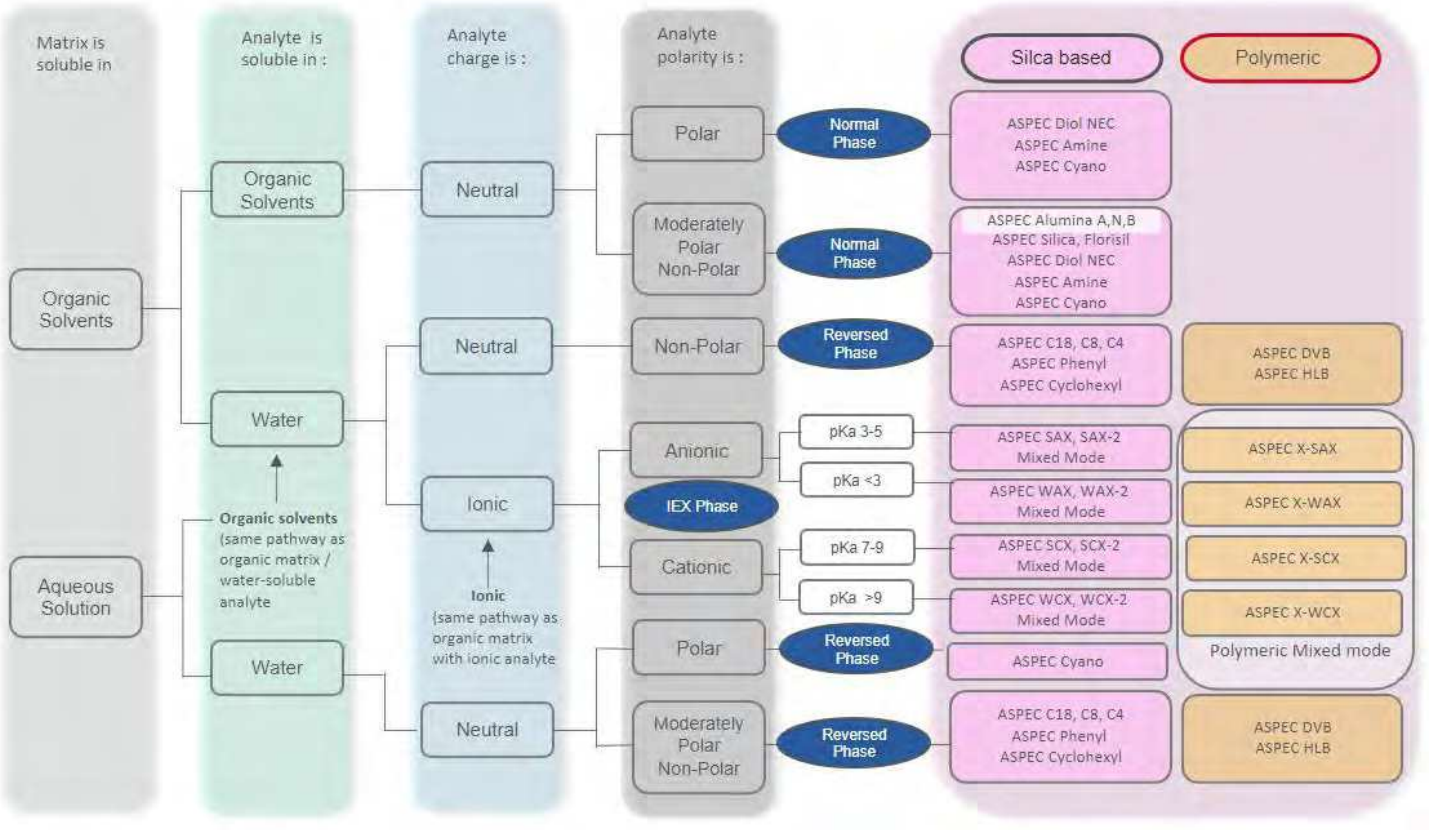


Figure 26
SPE sorbent selection guide

Solvent Miscibility and Properties Tables on page 49 contain detailed information on the relative polarity and hydrophobicity of common SPE sorbents.

SPE Column Format/Mass of Sorbent Selection

Once the extraction mechanism and potential sorbent chemistry have been defined, choosing the sorbent mass is next. Proper sorbent mass for extraction is defined as the amount that provides sufficient capacity to retain both the analyte and any interferences that may also be retained during the loading step.

The volume of the sorbent bed is dictated by the sorbent binding capacity and the analyte concentration range required in the final extract according to the performance criteria of the method.

Typical Loading Capacity:

The loading capacity of the sorbent depends on the nature of the solid support, silica or polymeric, the type of bonding chemistry, and the manufacturing.

Typical values are:

- 1-5 mg / 100 mg phase (silica)
- 10-20 mg / 100 mg phase (polymer)
- 0.3 to 0.6 milliequivalent / g (IEX)

Table 8
Common SPE mechanisms vs analyte/matrix nature

Sorbent Mass of Common SPE Cartridge Sizes					
1 mL		3 mL		6 mL	
Silica Based	Polymeric	Silica Based	Polymeric	Silica Based	Polymeric
50 mg 100 mg	30 mg	200 mg 500 mg	60 mg	500 mg 1 g 2 g	100 mg 200 mg 500 mg
Number of Cartridges or Plate by Box					
100		50	50	30	

If the SPE cartridge retains compounds other than the analyte of interest, the capacity of the analyte is reduced proportionally to the number of competitors present. An excellent place to start is with an analyte mass of less than half the SPE cartridge capacity

Typical volumes of SPE column versus sorbent mass are mentioned in Table 8. Gilson automated SPE systems accommodate 1 mL, 3 mL, or 6 mL cartridges.

Sample Pre-Treatment

Depending on the mechanism selected, it may be necessary to introduce pre-treatment steps when some compounds may interfere with the extraction process or when the sample must be placed under specific conditions to promote analyte retention.

- **Oils, fats, lipids, and unsaturated hydrocarbons**

In polar extraction, oils, fats, and related compounds tend to absorb on the sorbent, reducing the capacity for analyte retention. Liquid-liquid extraction with a nonpolar solvent (hexane, Ch2Cl2) can be used to remove them. Cooling the resulting solution will precipitate fats. Gel permeation chromatography (GPC) clean-up is another option for the removal of lipids from a complex matrix.

- **Inorganic salts**

Inorganic salts, which are present in many samples, are ionic in solution and interfere with analyte retention by ion exchange. This problem is overcome by desalting the sample through a nonpolar sorbent before ion exchange (double extraction).

- **Surfactants**

Surfactants exhibit multiple chemical characteristics therefore they can be retained by various SPE mechanisms. Prior to SPE, removal of the detergent can be accomplished by “trapping” the detergent onto an ion exchange or mixed bed column (double extraction).

- **Carbohydrates and high molecular weight polysaccharides**

Carbohydrates and high molecular weight polysaccharides are highly polar and soluble in polar solvents only. The addition of organic solvent to such molecules often results in a dramatic increase in sample viscosity. Pre-diluting the sample with water or buffer will help decrease the interference in extracting these types of compounds.

- **Proteins**

Compounds that are bound to proteins by non-covalent interactions behave differently than expected. To disrupt protein binding, different strategies may be considered:

  - Modifications of the matrix pH
  - Addition of chaotropic agents (protein denaturing)
  - Addition of protein precipitation agents

- **Acids and bases**

Depending on the pH of the sample solution, acids and bases can be in an ionic form, neutral form, or both forms. It is crucial that it is in the right form to ensure good retention.

Using a reversed-phase (i.e., C18) to extract a weak acid from an aqueous solution implies that the analyte is only present in its neutral form. This involves adjusting the pH of the sample solution two units below the pKa of the acid. Conversely, using a cation exchange phase (i.e., SCX), a pH adjustment of two units above the pKa is required so that the analyte is only present in its ionic form.

- **Viscous and concentrated samples**

Viscous samples with volumes less than 1 mL may yield higher recoveries if diluted before loading onto the SPE cartridge. Dilute analytes bind more efficiently to the active sites on the SPE cartridge because the mass transfer to the stationary phase is improved. Plasma samples require the dilution to also be tested and manually validated. This ensures that the plasma sample does not illicit clotting by diluting the anticoagulants or protein precipitation.

Sorbent Screening

At this stage, SPE sorbent candidates should be evaluated. After conditioning the sorbent, a known volume of standard solution is passed through the different columns. Each eluent is collected and analyzed. The best choice is indicated by the least amount of analyte.

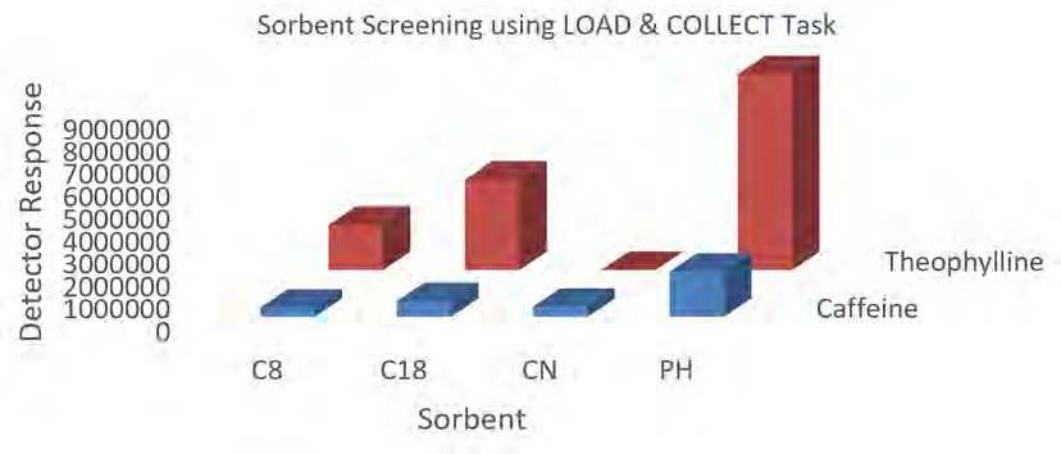


Figure 27
Automated sorbent screening using ASPEC® systems

This procedure is simple and quick, especially in the case of automation with our ASPEC systems.

Solvent Selection

Once the analyte's properties have been analyzed, and a sorbent has been chosen, solvent selection seems to fall into place fairly quickly. Refer to Table 9 to find common solvent types for each SPE mechanism.

Table 9
Solvent suggestions for SPE steps

Mechanism	Condition Step	Wash Step	Elute Step
Non-polar extraction (RP)	1. Water miscible organic solvent (i.e. MeOH) 2. Aqueous, buffers (water or buffer)	Aqueous or buffered solutions with 5 to 50% polar organic solvent	Polar or non-polar organic solvent(s) with or without water, buffer and/or strong acid or base
Polar Extraction (NP)	1. Isopropanol or MeOH 2. Low polar organic (i.e. Hexane)	Non polar organic solvents with 1 to 5% moderate polar organic solvent (i.e. Isopranol in Hexane)	Mixture of polar organic solvent and non-polar solvent (50 to 95%) (i.e. Isopropanol in Hexane)
Ion Exchange (IEX) : Anion or Cation Exchange	1. Polar organic solvents pH adjusted with 5% strong acid (HCO_2H) or strong base (NH_4OH) in Acetonitrile or Methanol 2. Low ionic strength buffers	Aqueous buffers of low to intermediate salt concentrations with or without organic solvent	Polar organic solvent at adjusted pH with acids or bases

Analytical workflow considerations:

It is important to keep in mind that SPE is rarely the last step for a sample, and subsequent testing can significantly impact the elution solvent choice. The Gilson SPE instruments can be used to prepare samples before various analytical techniques (e.g., HPLC, GC, RIA, LC/MS, etc.) The choice of analytical method can limit the options available for elution solvents to avoid an intermediate dry-down step or irremediable incompatibility with the analytical system (i.e., high salts concentration and MS detector). Purity requirements of the extracted analyte also depend on the analytical technique employed. A less selective detector (UV) requires a more efficient extraction process.

Conditioning Solvents

Typically there are two conditioning steps performed before loading the sample. The first one most commonly uses methanol to ensure sorbent solvation and rinse any possible contaminants off the cartridge. The second one will likely be chemically similar to the sample matrix as it will prepare the sorbent to receive the sample.

Elute and Wash Solvents

To aid in the selection of solvents for the WASH and ELUTE steps, there are a few tests that can be performed:

1. **Selection of the elution solvent**
   Standards are applied on the SPE cartridges, and different elution solvents are tested. The best solvent elutes the greatest amount of analyte in the smallest volume.

2. **Evaluation of interfering matrix components**
   A blank matrix is applied to the column, and elution is performed with the previously selected solvent. If interfering peaks are detected, the washing step should be implemented.

3. **Selection of the washing solvent**
   Spiked sample matrix are passed through SPE cartridges, and different washing solvents are tested. The best solvent will remove a maximum of interferences without eluting the analytes of interest.

If the analyte recovery in the spiked sample is different from that of the standard solutions, this indicates that the analyte interacts with matrix components.

NOTE

All these steps can be easily automated using Gilson ASPEC systems as Illustrated by the example in Figure 28:

- The use of standard liquid handling tasks allows the automated preparation of solvent mixtures in test tubes, while homogenization is produced by mixing via liquid asp/disp cycles or by air bubbling.
- Each Solvent or Solvent mixtures can be tested using an automated experiment plan created in the TRILUTION LH Application.

Analyte solubility:

Analyte solubility helps in the selection of the elution solvents (in which analyte solubility is high) and the washing solvents (in which analyte solubility is poor).

Miscibility of solvents:

Following this analyte solubility approach, it is not rare to have miscibility issues between solvents used one after other. In this case, any trace of the first non-miscible solvent should be removed from the sorbent before using the second one to avoid any undesired interactions. This removal is achieved by a strong drying step using an external gas (e.g., nitrogen) for a sufficient period. A general solvent miscibility chart can be found in Solvent Miscibility and Properties Tables on page 49.

Analyte stability:

The stability limitations of analytes in certain solvents and narrow pH ranges should also be taken into account.

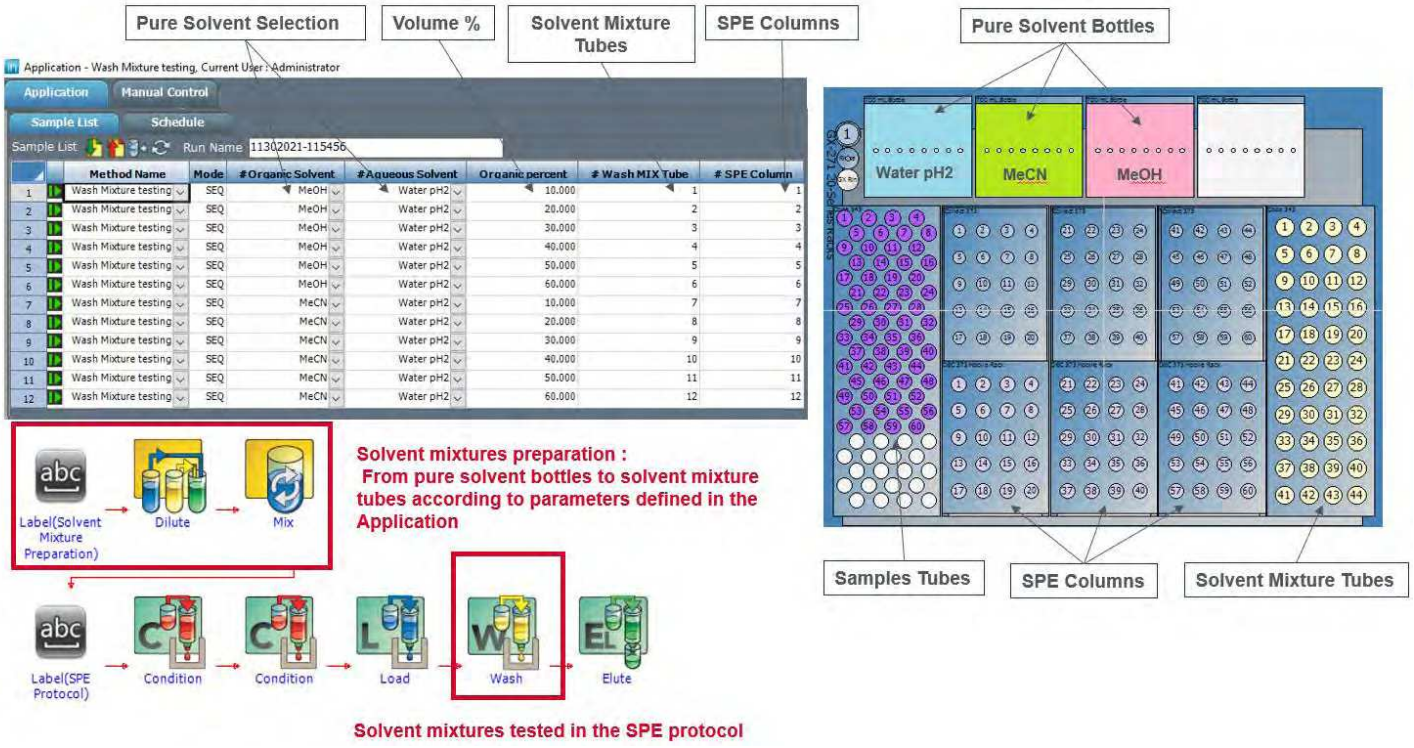


Figure 28
Automated wash solvent composition testing using Gilson ASPEC® systems

STEP 3. OPTIMIZATION AND PRACTICAL STRATEGY

After a first run using default values (Table 5) and observing any problems (e.g., low recovery, interference, or carryover), one set of parameters should be optimized at a time. It is recommended to change only a single variable within a single experiment to observe the effect of that change. Following the flow chart below helps to ensure that no aspect of method optimization is overlooked.

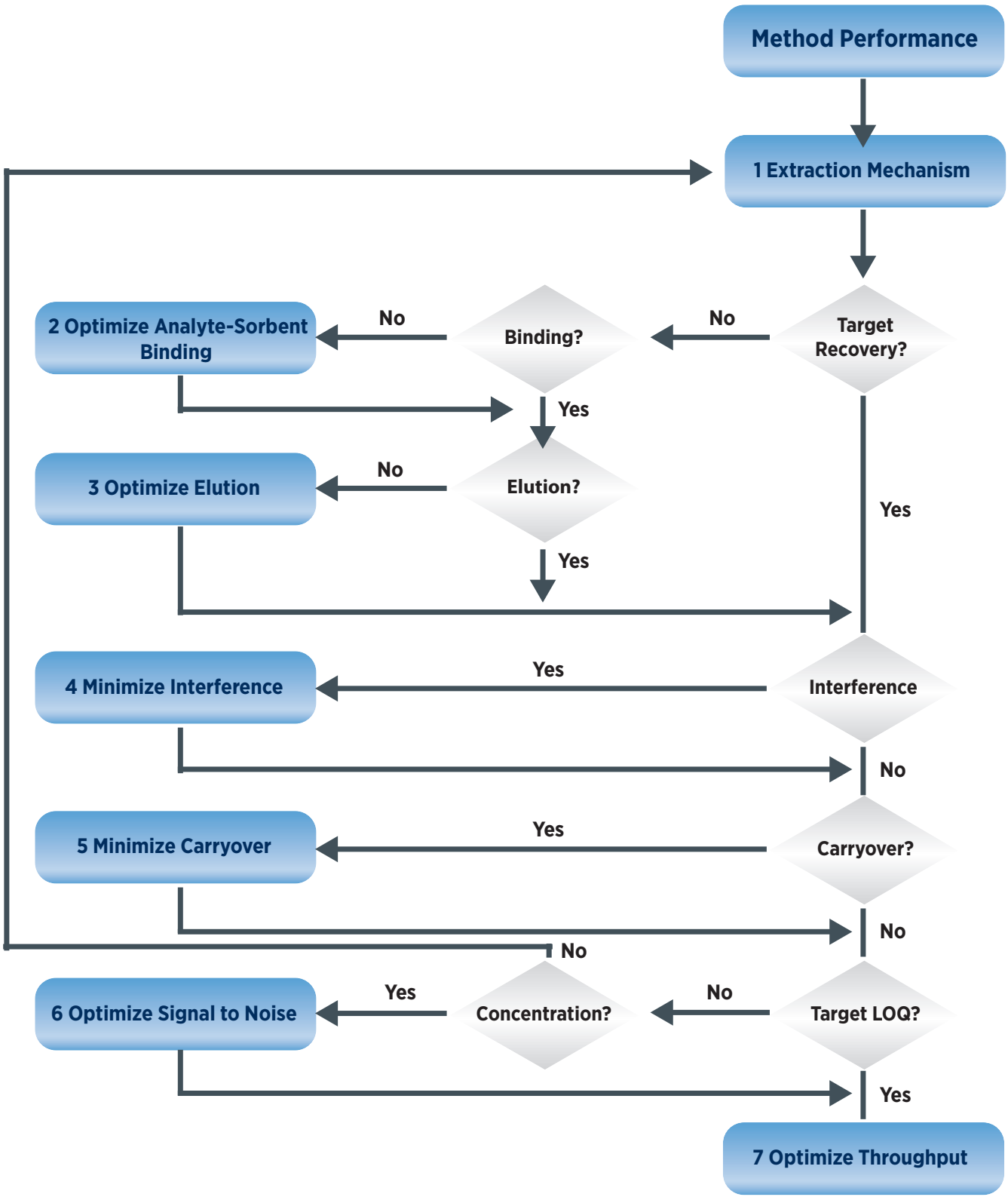


Figure 29
SPE optimization flow chart

Optimizing Analyte-Sorbent Binding

Conditioning Air Push Volume

The positive pressure generated when a volume of air is dispensed onto the SPE cartridge is responsible for flow rate reproducibility, optimum recoveries, and precise results. Since the SPE cartridge should not be allowed to dry during the conditioning step, this air push volume should be selected according to the conditioning solvent, the SPE cartridge size, the nature of the solid phase (silica or polymeric), and the mass of sorbent. This helps to maintain a sufficient level of liquid above the sorbent (1-2 mm). Figure 30 gives an idea of the air push volumes that should be used for different solvent volumes according to SPE column size.

Conditioning Flow Rate

Conditioning is weakly influenced by the flow rate. Relatively high values can be set; however, keep in mind that excessive flow rates can cause channeling.

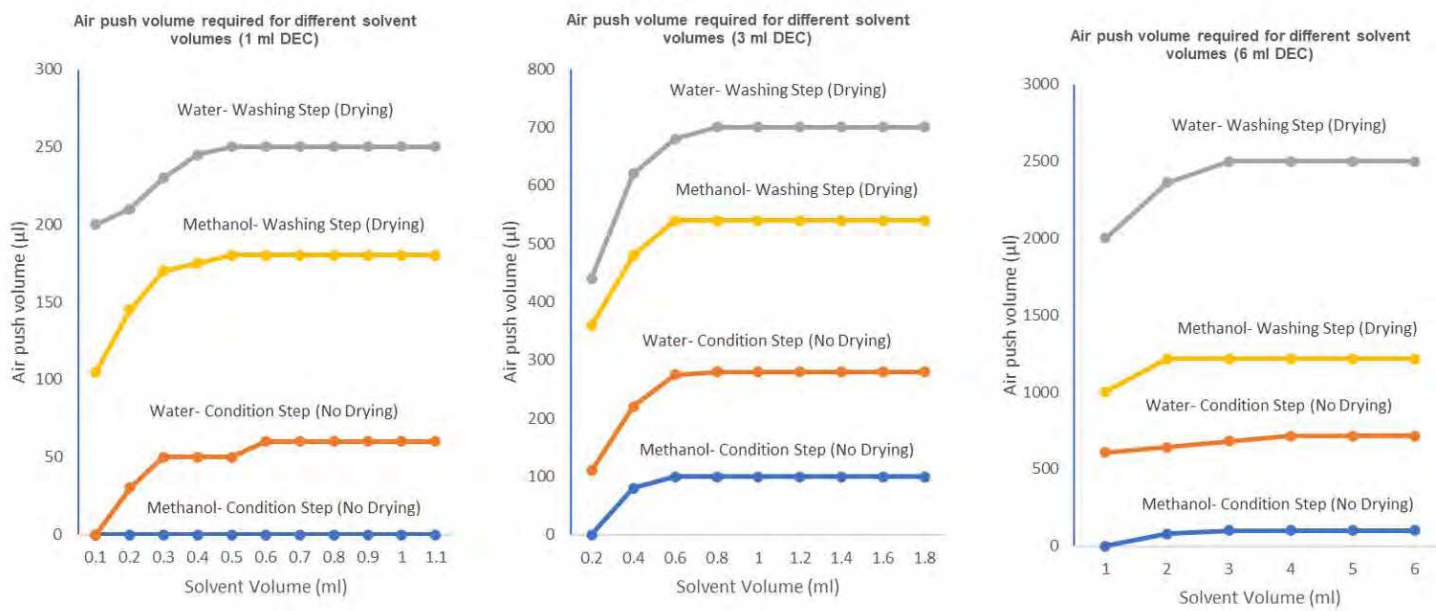


Figure 30
Conditioning and washing air push volumes for different volumes of solvent and SPE column formats

Loading Flow Rate

Loading flow rate is a critical parameter since the load step involves the mass transfer between the liquid sample and the sorbent. A flow rate should be selected which is slow enough to allow a complete mass transfer. The optimum flow rate is defined as the fastest flow rate that can be employed without compromising recovery. According to the SPE column manufacturer's application sheet, it can be determined by automatically processing samples using our ASPEC systems at different flow rates and comparing recoveries.

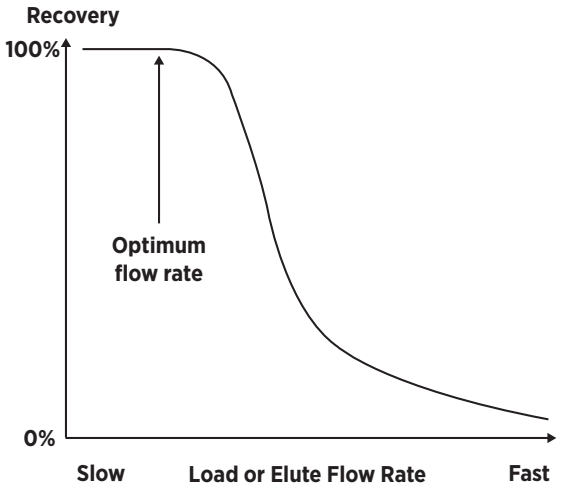


Figure 31
Relationship between recovery and loading flow rate

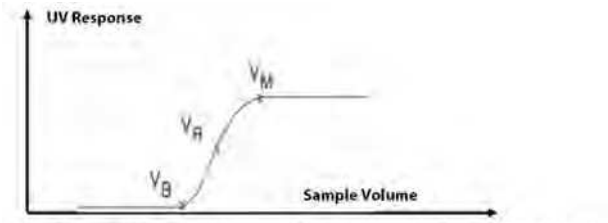
Loading Air Push Volume

This parameter must be optimized in order to ensure the introduction of the total sample to the SPE cartridge. Positive pressure provides a constant flow rate through the SPE cartridge, and every cartridge is acted upon in the same manner.

Loading Volume

Although some precautions were taken during the previous stages of method development when selecting the sorbent mass, it is fundamental to keep in mind never to exceed the breakthrough volume of the cartridge in the working concentration range of the method.

This breakthrough volume (VB) can be determined using a real sample matrix spiked at the maximum level of the working concentration range.



V_B = The volume where analyte first appears (~ 1% maximal value)

V_R = The retention volume

V_M = The volume where maximal analyte breakthrough has occurred (~ 99% of maximal value)

Figure 32
Breakthrough volume determination

NOTE

ASPEC systems and their multi-collect capability can load successive aliquots of sample to an SPE cartridge while collecting each waste into segregated collection tubes which allows you to determine rapidly the breakthrough volume after the analysis of each of the collection tubes.

Optimizing Analyte-Sorbent Elution

Strong Drying of Cartridge before Performing Elution

Strong drying of the SPE cartridge may be required when two immiscible solvents are used in sequence. This can be observed between the wash and elution solvents in most cases. The gas application time must be long enough to ensure proper drying. This time depends on the type of sorbent, its mass, the size of the SPE cartridge, and the washing solvent's nature. Initially, 15 to 20 minutes can be adopted for a polymeric solid phase, while 30 to 40 minutes are generally required for a silica-based cartridge.

Due to the impact of the drying step on the total duration of the SPE workflow, running a series of samples with various drying times is recommended to determine the shortest drying time required to achieve maximum recovery.

Elution Flow Rate

The elution step involves mass transfer between the sorbent and the solvent; the flow rate should be slow enough to allow a complete mass transfer.

Determination of this flow rate is available from the SPE manufacturer's application sheet or by processing samples at different flow rates and comparing recovery using an automated experiment plan. Elution flow rate should not exceed 6 mL/min.

Elution Air Push Volume

This is the air volume required to fully remove the residual elution solvent from the solid phase to optimize analyte recovery.

Elution Volume

The elution volume is the volume required to elute the analyte from the SPE cartridge. This volume should be as small as possible to avoid analyte dilution and optimize throughput. The multi-collect functionality of our ASPEC systems is also an excellent tool in this case: successive aliquots of the eluting solvent can be applied to an SPE column while recovering each fraction into segregated collection tubes. As shown in Figure 33, after each fraction is analyzed, an elution volume profile per compound can be established to determine an optimized elution volume quickly. In this example, an elution volume of 2 mL can be chosen due to a compromise between recoveries and the concentration factor (a 2-3% of loss of Phenytoin is accepted to use only 2 mL of elution solvent instead of 2.5 mL).

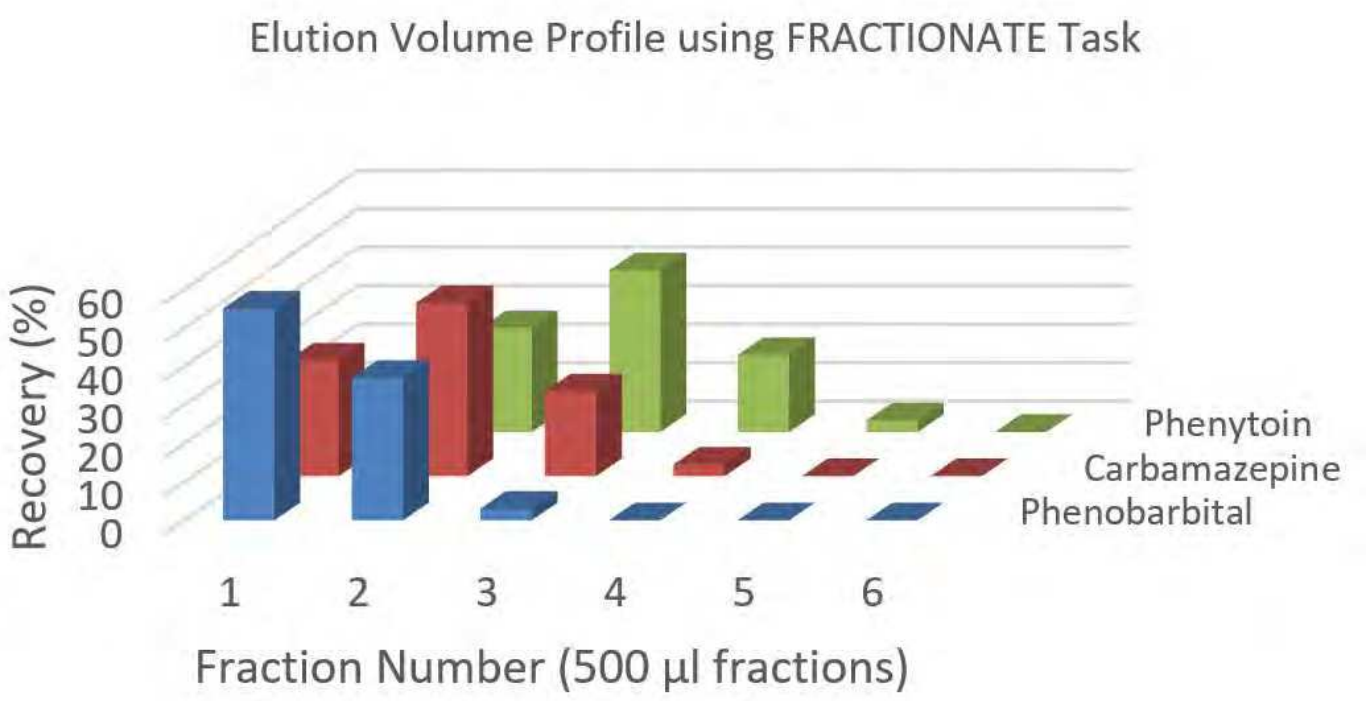


Figure 33
Elution volume profile using ASPEC® systems

Minimizing Interference

Washing Flow Rate

The washing step associated with SPE is only moderately influenced by flow rate; however, interferants may not be adequately removed if the flow rate is too high. Therefore diffusion of the wash solvent with the packing material is improved with lower flow rates. The default values given in Table 5 should be correct.

Washing Volume

Wash volumes that are insufficient will result in interfering compounds in the eluent. If interfering compounds are detected in the eluent, wash volumes should be increased.

Washing Solvent

It is not unusual to test different solvents for the wash step. In general, the solvent needs to be strong enough to remove interfering compounds without affecting the analyte of interest.

For example, if an SPE protocol elutes the compound of interest with 40% solvent strength, use 5-10% solvent strength for the washes.

Washing Air Push Volume

Sufficient Air Push Volume should be used between washing steps and before the elution step to remove the remaining solvent from the sorbent before loading a new one to avoid disturbance from the previous solvent (solvent composition variability) and to eliminate remaining interferents. Some guidance is given in Figure 30.

As already mentioned, in case of non-miscibility issues, an additional strong drying should be performed using an external gas.

Minimizing Carryover

By design, Gilson's automated SPE instruments minimize carryover.

If Rinsing Parameters can be optimized within each SPE task to eliminate carryover, particular attention should be taken after sample loading to minimize carryover

If carryover is observed:

- Increase the rinsing flow rates and choose a higher flow rate to create more effective rinsing.
- Increase the rinsing volume. The minimum recommended value is a volume equal to x1.5 the sample volume.
- Increase the number of rinsing steps and rinse sites.
- Possibly adding 10%–20% organic (ACN, MeOH) to the rinsing solvent reservoir.
- Implement the use of a flow-through rinse station with a peristaltic pump.
- Implement Liquid Level detection and Liquid Level Following.

STEP 4. VALIDATION

Validation of an automated SPE method requires that the experiments are designed to suit the requirements of the laboratory. Validation requirements may vary; these should be discussed on an individual basis with laboratory personnel responsible for the validation.

Four Important Validation Rules

1. Validate the complete and optimized analytical method
2. Validate over the entire working range of concentration
3. Validate over the entire working range of matrix
4. Validate over several days

APPENDICES

Troubleshooting

Problem	Possible Cause	Corrective Action	TRILUTION LH SPE Task
Over-pressurization	Matrix interference: • Inorganic salts • Surfactants • Oils • Carbohydrates • Proteins • Solid particles	• Double extraction (desalting) • Double extraction • LLE , GPC • Dilute the sample • Modify pH, Protein Precipitation • Filtration	LOAD & COLLECT and LOAD LOAD & COLLECT and LOAD DILUTE ADD, LOAD LOAD & COLLECT
	Viscous sample	• Dilute the sample • Decrease Flow Rates	DILUTE LOAD, LOAD & COLLECT: • Decrease Liq. Disp. Flow rate • Decrease Air. Disp. Flow rate
	Improper conditioning	Do not allow the sorbent to dry	CONDITION: • Air Push Vol. Decrease
	Immiscible reagents	• Dry the SPE sorbent between solvents using external gas. • Increase Drying Time	DRY (with external gas) Within SPE task : • Select Air Push Valve • Increase Air Push Time
Carryover	Inadequate probe rinsing	• Rinse after each liquid handling step • Optimize the rinsing step • Minimize surface contact between liquid and probe	Within SPE task(s): • Increase Rinsing flow rates and Volumes • Select LLD and LLF
	Inadequate rinsing solvent	• Add extra-rinsing solvents • Select a different rinsing solvent	RINSE
Interference	Poor washing efficiency	• Optimize mass transfer • Increase solvent volume • Optimize washing solvent	WASH: • Increase Air Push Vol. • Decrease Disp. Flow rate WASH: • Increase volume FRACTIONATE: • Collect multiple washes
LOQ not reached	Eluent too dilute	• Minimize the elution volume • Concentrate sample by evaporating to a smaller volume	ELUTE, FRACTIONATE: • Collect with various volumes of eluant to optimize elution volume EVAPORATE

Problem	Possible Cause	Corrective Action	TRILUTION LH SPE Task
Low Throughput	Time consuming tasks	Optimize protocol	Decrease solvent volume / Increase flow rate without compromising recoveries
	Unnecessary steps	Optimize protocol	Remove unnecessary rinsing steps
	Improper methodology	• Reconsider the extraction mechanism and the sorbent • Eliminate evaporation step	
Poor Recovery	Improper conditioning	• Do not allow sorbent to dry	CONDITION: Air Push Vol. Decrease
	Initial diagnostic tests	• Determine where breakthrough or loss may be occurring	FRACTIONATE, allows for the collection of each SPE step into its own collection vessel for further testing
	Poor binding	• Consider SPE mechanism and required sample pre-treatment • Optimize mass transfer • Do not exceed the breakthrough volume • Decrease the mass loaded • Increase mass of sorbent • Test different sorbents, test for binding capacity	LOAD: decrease Disp. Flow / increase Air Push Volume FRACTIONATE : Breakthrough volume verification LOAD: decrease sample volume FRACTIONATE: breakthrough volume verification LOAD & COLLECT, FRACTIONATE
	Improper washing	• Decrease the elution strength of the washing solvent while maintaining acceptable clean-up	FRACTIONATE: Collect multiple washes
	Poor Elution	• Optimize column drying at the end of washing step to maintain elution solvent composition integrity • Optimize mass transfer • Increase elution solvent volume • Optimize nature of elution solvent	WASH: increase Air Push Volume or Drying time ELUTE, FRACTIONATE: decrease liquid dispense flow, increase air push volume FRACTIONATE: Collect multiple fractions to define optimum volume (Elution Volume Profile) ELUTE: Collect with varying eluent solvents
	Evaporation	• Prevent from losses of semi-volatile compounds by evaporation	TRANSFER: transfer eluates into sealed vials
Poor Reproducibility	Improper conditioning	• Refer to the SPE manufacturer's recommendation	CONDITION
	Incomplete aspiration of sample	• Reduce aspiration flow rate • Increase Asp. Eq. Time • Dilute sample (viscous sample)	LOAD DILUTE
	Improper mixing of the eluent before injection	• Mix the eluent before injection	MIX, increase number of cycles, change mixing height

ASPEC SPE Cartridges – Retention Mechanism Selection

Analyte is soluble in			Analyte polarity	Recommended Phases (SILICA BASED - POLYMERIC)
Aqueous	Sample Matrix Type	Aqueous	Non polar	ASPEC C18, ASPEC C8, ASPEC C4, ASPEC PHENYL, ASPEC CYCLOHEXYL, **ASPEC DVB**
			Non polar/ Moderately polar	ASPEC C18 NEC, ASPEC C8, ASPEC C4, ASPEC CYANO, **ASPEC HLB**
			Polar	ASPEC CYANO, ASPEC ACT. CARBON
		Aqueous/Organic	Cationic/Basic	ASPEC SCX, ASPEC SCX-2, ASPEC WCX
			Cationic/Basic (Neutral)	**ASPEC X-SCX**, **ASPEC X-WCX**
			Anionic/Acid	ASPEC SAX, ASPEC SAX-2, ASPEC WAX, ASPEC WAX-2
			Anionic/Acid (Neutral)	**ASPEC X-SAX**, **ASPEC X-WAX**
		Organic	Non polar	ASPEC C18, ASPEC C8, ASPEC C4, ASPEC PHENYL, ASPEC CYCLOHEXYL, **ASPEC DVB**, **ASPEC HLB**
Organic		Aqueous	Non polar	ASPEC C18, ASPEC C8, ASPEC C4, ASPEC PHENYL, ASPEC CYCLOHEXYL, **ASPEC DVB**, **ASPEC HLB**
			Cationic/Basic	ASPEC SCX, ASPEC SCX-2, ASPEC WCX
			Cationic/Basic (Neutral)	**ASPEC X-SCX**, **ASPEC X-WCX**
			Anionic/Acid	ASPEC SAX, ASPEC SAX-2, ASPEC WAX, ASPEC WAX-2
			Anionic/Acid (Neutral)	**ASPEC X-SAX**, **ASPEC X-WAX**
		Organic	Moderately polar	ASPEC SILICA, ASPEC FLORISIL, ASPEC ALUMINA, ASPEC CYANO, ASPEC DIOL NEC
			Polar	ASPEC CYANO, ASPEC DIOL NEC

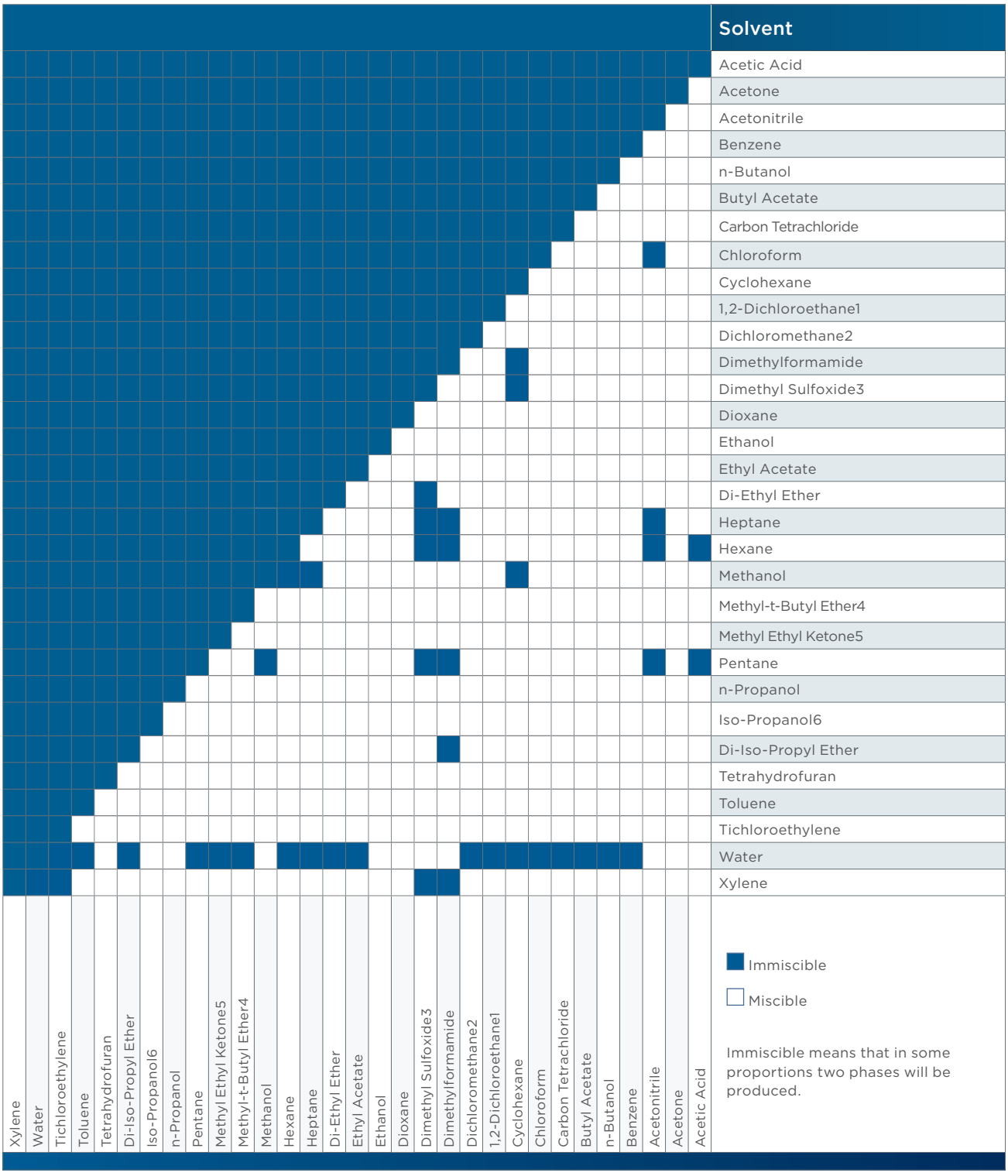
Additional information available on our web site: https://www.gilson.com/default/shop-products/lab-system-consumables/spe-cartridges.html

Solvent and Sorbent Polarity Chart

Relative Polarity	Compound Formula	Chemical Group	Representative Solvents	Eluting Strength (ε0)
Nonpolar	R-H	Alkanes	Petroleum ether	0.0
			Ligroin	0.0
			Hexane	0.0
			Haptane	0.0
			Isooctane	0.01
			Cyclohexane	0.03
	Ar-H	Aromatics	Toluene	0.22
			Benzene	0.27
	R-X	Alkyl Halides	Carbon Tetrachloride	0.11
			Chloroform	0.31
			Methylene chloride	0.32
	R-O-R	Ethers	Tetrahydrofuran	0.35
			Diethyl ether	0.38
			Dioxane	0.49
	R-CO-R	Ketones	Methyl ethyl Icetone	0.39
			Acetone	0.43
	R-CO-OR	Esters	Ethyl acetate	0.45
	R-CN	Nitriles	Acetonitrile	0.50
	$R-NR_2$	Amines	Pyridine	0.55
			Triethylamine	0.73
	R-OH	Alcohols	Isopropanol	0.63
			Ethanol	0.70
			Methanol	0.73
	$R-CO-NR_2$	Amides	Dimethylformamide	0.73
	R-COOH	Carboxylic Acids	Acetic Acid	>0.73
Polar	H_2O	Water	Water	>0.73

Polarity Index (P')	Water Miscible	Water Solubility (% W/W)	Sorbent Polarity
-	No	-	SDB Polymers
-	No	-	-
0.06	No	0.001	C18 (EC)
0.2	No	0.0003	-
-	No	-	C18 (Non-EC)
0.0	No	0.01	-
2.4	No	0.051	C8/Octyl
3.0	No	0.18	-
1.6	No	0.08	PH/Phanyl
4.4	No	0.815	-
3.4	No	1.6	CN/Cyano
4.2	Yes	100	-
2.9	Slight	6.89	Si/Silica
-	Yes	100	-
4.5	Slight	24	NH2/Amino
5.4	Yes	100	-
4.3	Slight	8.7	FI/Florisil
6.2	Yes	100	-
5.3	No	-	Al/Aluminum
-	Yes	100	-
4.3	Yes	100	SCX/Aromatic
-	Yes	100	Sulfonic acid
6.6	Yes	100	-
-	Yes	100	SAX/Quaternary
6.2	Yes	100	Amine
10.2	Yes	100	-

Solvent Miscibility and Properties Tables



Solvent	Polarity Index	Refraction Index @20°C	UV(nm) Cut-off @1AU	Boiling Point (°C)	Viscosity (cPoise)	Solubility in water (%w/w)
Acetic Acid	6.2	1.372	230	118	1.26	100
Acetone	5.1	1.359	330	56	.032	100
Acetonitrile	5.8	1.344	190	82	0.37	100
Benzene	2.7	1.501	280	80	0.65	0.18
n-Butanol	4.0	1.394	254	125	0.73	0.43
Butyl Acetate	3.9	1.399	215	118	2.98	7.81
Carbon Tetrachloride	1.6	1.466	263	77	0.97	0.08
Chloroform	4.1	1.446	245	61	0.57	0.815
Cyclohexane	0.2	1.426	200	81	1.00	0.01
1,2-Dichloroethane1	3.5	1.444	225	84	0.79	0.81
Dichloromethane2	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide3	7.2	1.478	268	189	2.00	100
Dioxane	4.8	1.422	215	101	1.54	100
Ethanol	5.2	1.360	210	78	1.20	100
Ethyl Acetate	4.4	1.372	260	77	0.45	8.7
Di-Ethyl Ether	2.8	1.353	220	35	0.32	6.89
Heptane	0.0	1.387	200	98	0.39	0.0003
Hexane	0.0	1.375	200	69	0.33	0.001
Methanol	5.1	1.329	205	65	0.60	100
Methyl-t-Butyl Ether4	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone5	4.7	1.379	329	80	0.45	24
Pentane	0.0	1.358	200	36	.23	.004
n-Propanol	4.0	1.384	210	97	2.27	100
Iso-Propanol6	3.9	1.377	210	82	2.30	100
Di-Iso-Propyl Ether	2.2	1.368	220	68	0.37	
Tetrahydrofuran	4.0	1.407	215	65	0.55	100
Toluene	2.4	1.496	285	111	0.59	0.051
Tichloroethylene	1.0	1.477	273	87	0.57	0.11
Water	9.0	1.333	200	100	1.00	100
Xylene	2.5	1.500	290	139	0.61	0.018

Synonym Table

[1] Ethylene Chloride
[2] Methylene Chloride
[3] Methyl Sulfoxide
[4] tert-Butyl Methyl Ether
[5] 2-Butanone
[6] 2-Propanol

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NOTES

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