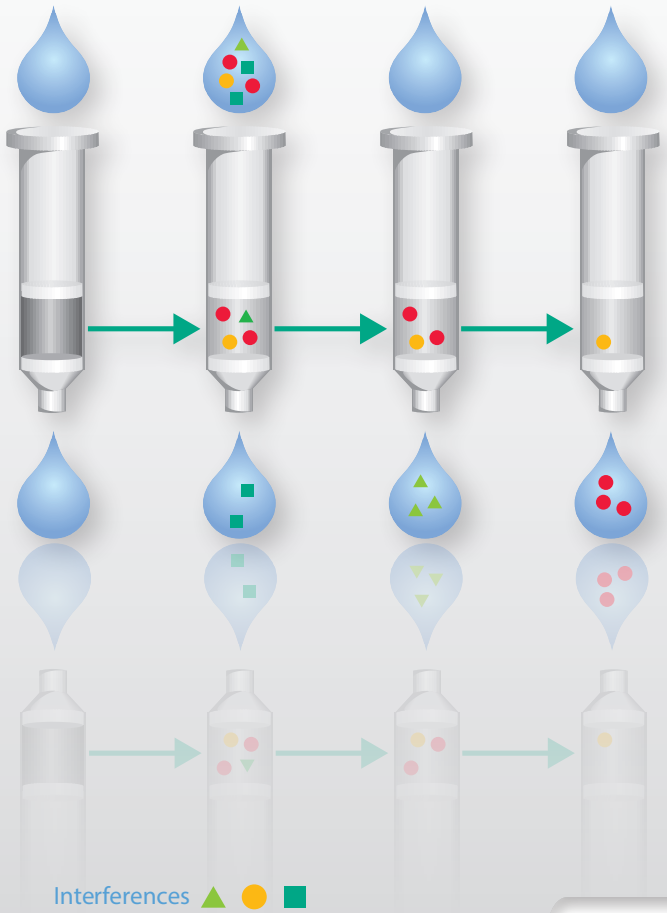
Gilson Guide to SPE Automation

Condition | Load | Wash | Elute



GILSON®

About This Guide

This guide provides a background for the technique and science of solid phase extraction (SPE), as well as tips for automating it 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, the Gilson Technical Service Department is available for assistance.

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Solid phase extraction (SPE) has become the technique of choice for sample clean-up and trace enrichment. Some of the benefits of performing SPE are: versatility, selectivity, speed, and low solvent usage. Manual SPE procedures can be labor intensive, and subject to resultant human error, delays, and sample loss. These problems have led to the development of instruments by Gilson to automate the process.

The Gilson line of automated SPE instruments includes: GX-241 ASPEC™, GX-271 ASPEC™, and GX-274 ASPEC™, but Gilson's flexible disposable extraction cartridge (DEC) racks allow any Gilson liquid handling system containing a syringe pump to perform SPE techniques. The Gilson SPE instruments are designed for the automation and optimization of SPE, in order to provide more efficient and reproducible sample preparation, in addition to on-line injection.

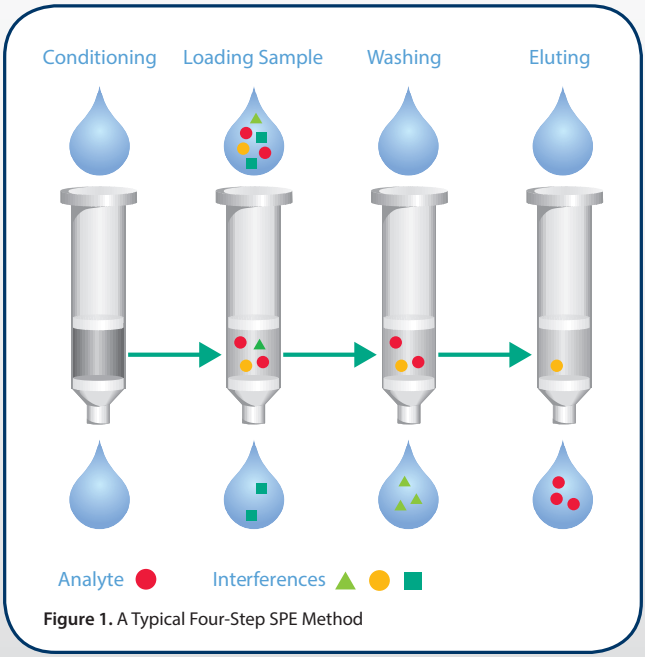
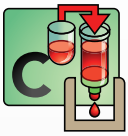


Figure 1. A Typical Four-Step SPE Method

Steps of an SPE Method

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. Conditioning is required to wet and activate the packing bed by solvation.

Load

The objective is the quantitative retention of the analyte on the SPE cartridge while matrix interferences are removed. An alternative, which is less frequently used, is the retention of interferences with the collection of the analyte of interest.

Wash

After the analyte(s) of interest is 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 a single analyte or multiple analytes into separate collection tubes.

Types of SPE

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. Anywhere from 1–24 cartridges can be processed simultaneously on the manifold. The chemist must watch for vacuum fluctuation and unevenness and must be very vigilant to watch for cartridge drying or channeling while executing the SPE method.

Although the vacuum technique for SPE has been automated, Gilson utilizes a positive pressure approach to the method. The probe(s) on the instrument goes through the DEC cap, forming a seal. As the instrument dispenses either liquid or air, pressure builds up inside pushing the liquid through the sorbent bed and out the cartridge. Since the SPE method is completely controlled through Gilson's TRILUTION® LH software, the chemist is free to perform other lab activities while the software dictates the flow and collection of liquid.

What Can Be Automated

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

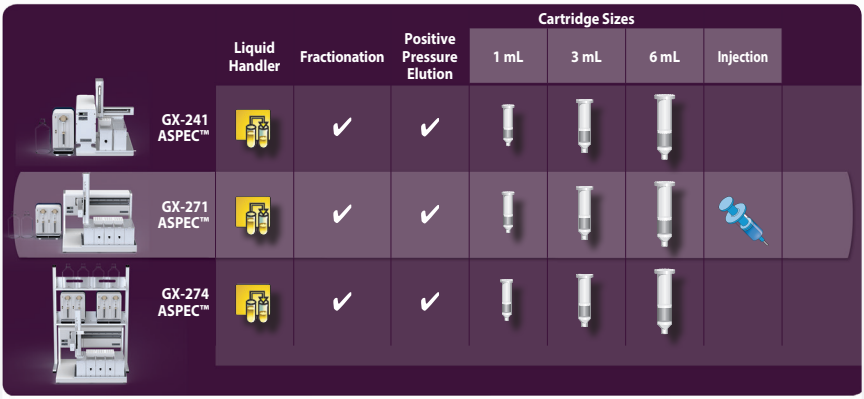


Figure 2. Gilson's Automated SPE Instruments

Sample Pre-treatment

Gilson's automated SPE instruments can also automate other manual liquid handling techniques. Liquid can be added and mixed with the sample, or with other reagents, in addition to internal standard addition. Dilutions can be performed, and larger volumes are easily handled. Mixing is performed either by air/gas or by multiple aspirating and dispensing cycles of the liquid.

Automated Injection

SPE is commonly followed by an injection step for quantitation purposes. Gilson SPE instruments are capable of automating the transfer of liquids to other vessels, permitting the samples to be manually placed on the final analytical instrument (spectrophotometer, GC, MS, RIA, etc.) for determination. SPE techniques not requiring a solvent exchange or dry-down of the eluent may be amenable to automated injection. Gilson single probe instruments are able to automate the injection step as well as the entire HPLC process using Gilson LC instrumentation with TRILUTION® LC software, thus eliminating another task for the lab technicians.

Why Automate SPE?

The advantages of automating sample preparation, generally considered the most labor-intensive and error-prone step in an analytical method, are clear: increased throughput, improved reproducibility, minimal personal exposure to hazardous materials, and increased productivity for the laboratory technician. Considerations should be made relative to the cost associated with a technician's time (continuous); versus the cost of an automated instrument (one-time). In addition, automated method development capabilities are available on the Gilson SPE instruments which enhances the efficiency and precision for the method and method transfer between labs.

Increased Throughput

The time required to process an individual sample by manual or automated SPE (approximately 10 min) usually remains the same. However, the automated system works a full day, 20–24 hours/day, and laboratory personnel can focus their efforts and time on other projects while the automated SPE instrument is operating, increasing their overall productivity. A multiple-probe instrument like the GX-274 ASPEC can process four samples in parallel. With automation there is a noticeable increase in samples processed per day as seen in Table 1.

		Single Probe	Single Probe	Four Probe
	Vacuum Manifold (non-Automated Procedure)	GX-241 ASPEC	GX-271 ASPEC	GX-274 ASPEC
	SPE cartridge	SPE cartridge	SPE cartridge	SPE cartridge
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 reagents in rack • Run written application		
Time for SPE	10 min/sample	10 min/sample	10 min/sample	10 min/sample
Number of Samples/day	50 (8 hr/day), with a 10 SPE manifold	120 (20 hr/day)	120 (20 hr/day)	480 (20 hr/day)

TABLE 1. SPE Throughput Comparison

Enhanced Performance

When performing SPE methods, there are some main factors to consider related to optimal performance:

- Keep the packing material moist during the initial conditioning steps.
- Load the sample with a sufficiently low flow rate to enable a good interaction between the sorbent and the analyte of interest.
- Elute the analyte(s) of interest in an efficient manner for high recovery by using as small of an amount of elution solvent as possible.
- Consistency and reproducibility from sample to sample.

Vacuum Manifold

- Liquid flow rates through the columns are controlled using a vacuum pump.
- Optimizing vacuum for sufficient accuracy and reproducibility is difficult.
- Small volumes are difficult to control.
- Fast flow rates cause sorbent channeling.
- Different flow rates occur across the manifold and from sample to sample.

Positive Pressure

- Liquid flow rates through the column are controlled by a push of either air or carrier gas (house air/N_2).
- Pushing the liquid across the sorbent packing occurs at a constant flow rate.
- Each cartridge is acted upon equally and independently via software control of the syringe or air push.
- A special polyethylene DEC cap provides a seal between the instrument's probe and the SPE cartridge.
- See Figure 3 for pictures of how Gilson's positive pressure SPE is achieved.

SPE uses the technique of positive pressure where sample or solvents are applied to an SPE cartridge with a probe. The probe is either withdrawn to aspirate air and then inserted back into the cap to push the liquid, or the gas valve is switched on and a stream of carrier gas pushes the liquids through the sorbent.

Figure 3. Gilson's Positive

The liquid and air flow on Gilson instruments are controlled by high-precision syringe pumps. The valve on the dilutor pump controls access to the carrier gas. The gas regulation valve is set to a common range (7-15 psi). Most SPE applications run perfectly setting the regulator at 7 psi for a 1 mL cartridge and up to 15 psi for a 6 mL cartridge. Precise liquid handling allows liquid sorbent interactions to be mastered throughout the SPE process with excellent sample-to-sample reproducibility. Note the decrease in CV for the automated SPE instrument in Table 2 below.

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

Data from Application Note 213 (www.gilson.com).

TABLE 2. CV Comparison between Manual and Automated SPE Systems

Easy Transfer of Manual Methods

Manual SPE is usually performed with a vacuum source and disposable columns. During the conditioning, loading and washing steps, solvents are collected in waste vessels, while collection tubes or microplates are used for the elution step. The experimental steps of a manual SPE application can be directly translated into an automated method. Gilson's DEC racks move to place the SPE cartridge over either a waste drain or collection tube depending on which step is being performed. Automation of manual SPE applications also allows for continued optimization and improvement of the current process. Optimization can lead to better overall consistency with sample handling from the source tube through the final elution step. Further considerations and guidelines for transferring manual SPE applications to an automated system can be found in later sections of this guide.

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 the system for unexpected errors. Installation Qualification-Operational Qualification (IQ/OQ) procedures are available for Gilson automated SPE instruments. Routine preventative maintenance (PM) adds to the overall compliance of an automated SPE instrument.

How to Automate SPE

The typical SPE method consists of one or two CONDITION steps, one or more LOAD steps, one or two WASH steps, one or two ELUTE steps, and optionally an INJECT step.

Step 1: CONDITION

Conditioning is performed to solvate the dry sorbent material. Solvation creates an environment suitable for analyte retention. Initial conditioning is usually performed with Methanol. A second conditioning may be required to remove the excess of the first conditioning solvent, activate the interaction with the analyte, or prevent precipitation of the sample matrix.

Step 2: LOAD

The sample is introduced to the SPE cartridge and passes through the sorbent material. In many instances where a large sample load is required, it is helpful to introduce the crude sample to the SPE material in two steps instead of one. This allows additional time for the sample and the SPE media to interact while facilitating analyte(s) absorption.

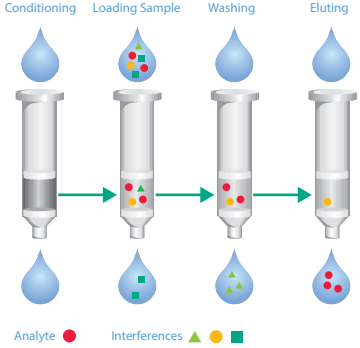


Figure 4. A Typical Four-Step SPE Method

Step 3: WASH

Once the analyte of interest is strongly retained on the sorbent, contaminants may be rinsed off. If analyte-sorbent interaction is weak, care must be taken not to elute the analyte during the wash steps. It is common practice to employ two wash steps: the first wash is usually consistent with the load matrix in solvent strength and the second wash is usually a stronger solvent to remove additional interferences. The wash steps are often overlooked in SPE method development, however they are very important determinants in the overall recovery and exclusion of interferences associated with the elution step, see the *Minimizing Interference* section, on page 25.

Step 4: ELUTE

SPE cartridges are positioned above the collection tubes/microplates. The smallest possible elution volume is recommended because smaller volumes decrease dilution of the final extract and minimize the need for evaporation. Ideally the elution solvent is compatible with the analytical conditions of the HPLC system, allowing for direct injection and optimum throughput.

A second elution step is often recommended. A single large elution volume can be split into two smaller volumes, which can result in greater recovery.

Step 5: INJECT (optional)

Inject the eluent onto the analytical column. Many times the elution solvent is too strong for direct injection onto the analytical column, identified by peak splitting and chromatographic anomalies. To overcome this phenomenon, the eluent is diluted with a solvent to decrease its strength. However, this can affect the signal-to-noise (S/N) ratio. An alternative approach is to evaporate the eluent to dryness and redissolve the analyte in the mobile phase.

The key to automating SPE is the ability to dictate what is collected and what is sent to waste. Gilson utilizes its mobile SPE racks in order to accomplish this. When the columns are conditioned, loaded or washed, the DEC rack is positioned above the drain. During elution, the DEC rack is moved above the collection rack, and the SPE cartridges are eluted into collection vials. The SPE process using Gilson mobile racks is shown in Figure 5.

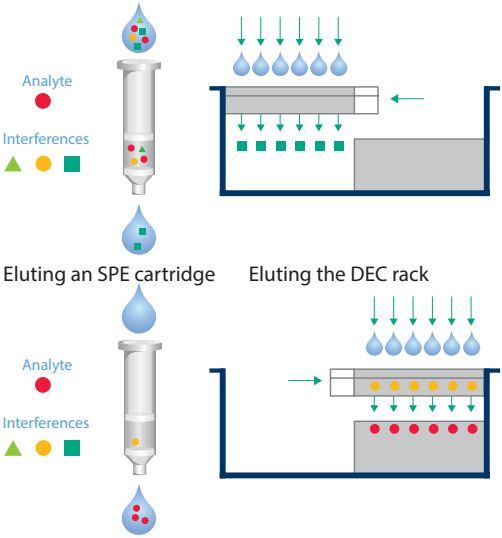


Figure 5. Gilson DEC Rack

Basic Liquid Handling Parameters

When automating an SPE method, parameters of the basic liquid handling steps must also be taken into consideration. The values for air gap, flow rate, and rinsing parameters must be defined for each of the SPE steps.

An air gap is a volume of air that separates the sample from the reservoir liquid when the sample 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.

Flow rates for loading sample or solvent onto the SPE cartridge are typically quite low, but other portions of the task or method can use higher flow rates. The maximum flow rates for Gilson's line of SPE instruments are listed in Table 3. Low flow rates are usually used when performing SPE tasks to ensure accuracy. Optimal flow rates will depend upon sample viscosity, the accuracy required, and the syringe size.

Rinsing is, of course, a key consideration when automating any liquid handling procedure. If the method utilizes two immiscible solvents back to back, it is especially important to provide adequate rinsing with a mutually miscible solvent in between in order to prevent undesired interactions. Ensuring adequate rinsing will also eliminate the need to worry about any carryover from step to step. Typically a rinse is one to two times the volume of the liquid transferred at a relatively fast flow rate. For more rinsing suggestions, please see the *Solvent Selection* section on Page 17 or the *Minimizing Carryover* section on page 25.

Sequential Versus Batch Mode

Gilson's versatile TRILUTION® LH software allows for complete control of the automated method, including whether the tasks are run in series or 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 next sample.

Batch Mode: every sample is exposed to a single task/step before advancing onto the next step.

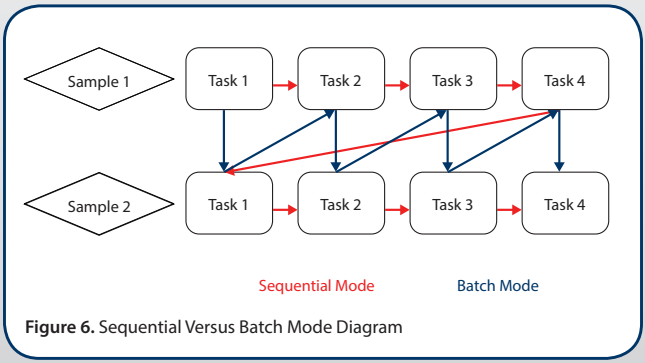


Figure 6. Sequential Versus Batch Mode Diagram

The choice of mode will depend on the sample stability, the required throughput, and the SPE process itself. When an injection step is included in the automation, the method is often run in sequential mode as the HPLC run is frequently longer than the SPE method.

The following table shows the minimum and maximum flow rates for the VERITY® 4060 Syringe and 4260 Syringe Pumps.

VERITY® 4000 Series Syringe Pumps							
Syringe Size (µL)	100	250	500	1000	5000	10000	25000
Minimum Flow Rate (mL/min)	0.0001	0.001	0.001	0.01	0.01	0.01	0.1
Maximum Flow Rate (mL/min)	4	10	20	40	100	100	100

TABLE 3. Minimum and Maximum Flow Rates for VERITY® Syringe Pumps

Method Development Criteria

The major goal of method development is to optimize the extraction efficiency (maximize recovery of the target analyte(s) and minimize the amount of co-eluted interferences). These conditions must provide not only reproducible (low variance) results, but also a procedure that is simple and economical. Solid phase extraction method development may at first appear somewhat overwhelming, but, with use of automation, the process can be easily rationalized. The general strategy for developing a new method can be summarized as follows, with specifics for each strategy outlined in the subsequent sections:

1. Identify Performance Criteria
2. Research
   - **a.** Analyte
   - **b.** Sorbent
   - **c.** Solvents
3. Optimize
4. Validate

Step 1. Identify Performance Criteria

The starting point when developing a method is to define the objectives in terms of:

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 3.

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. Research

After the performance criteria have been identified, it is important to perform some background research on your analyte in order 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. Unfortunately this is not always the case. If an existing method is not available, some basic research must be completed.

Analyte/Sample Properties: The structure and chemistry (solubility, stability, etc.) of the analyte will affect which sorbent and method solvents are chosen. The sample matrix can also force the choice in a different direction. Whether any sample preparation is necessary should also be investigated at this stage.

Sorbent Selection: Matching the correct SPE mechanism with the analyte of interest is a critical step in finding an SPE method that is successful. Once a mechanism has been identified, a specific sorbent must be chosen, and then cartridge size and sorbent mass selected.

Solvent Selection: The analyte's solubility and the choice of SPE mechanism will indicate what solvents should be used for the SPE method. In order to perform the SPE method, solvent volumes, flow rates and strengths must be decided upon.

Further information can be found in the *Method Development & Research* section on page 13.

Step 3. Optimize

It is always the goal of a separations chemist to get as much of the pure sample in as little time as possible. The method developed after thorough research may be a good starting point; however, in order to develop a selective, reproducible, and robust SPE method it is necessary to optimize the retention and elution of the analyte of interest and to minimize interference and carryover.

Further details and information can be found in the *Optimization* section on page 22.

Step 4. Validate

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

Method Development & Research

Analyte Properties

The structure and properties of the analyte plays an important role in the SPE process.

Nature of the Functional Group

The presence of non-polar groups (alkyl chains, aromatic rings, double bonds) suggests 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 the analyte will probably be retained by ion exchange sorbents. Ion exchange sorbents can be selected according to the pKa of ionizable groups of the analyte to best meet retention and subsequent elution requirements.

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).

Analyte Stability

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

Protein Binding

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

- Modifications of the matrix pH
- Addition of chaotropic agents 14 (protein denaturing)
- Addition of protein precipitation agents

Similar Compounds

Chromatographic data from HPLC methods of similar compounds (functional groups, polarity, pKa) can suggest an analyte's behavior during the separation process and offer insight as to the choice of SPE mechanism.

Nature of the Matrix

The properties of the matrix from which the analyte is to be extracted must be considered when choosing an extraction mechanism. Analytes containing non-polar or ionic functional groups in aqueous samples can usually be extracted with non-polar or ion exchange sorbent, respectively. Whereas, polar analytes in oily samples or in non-polar solvents would require extraction by polar interactions. Table 4 on the next page indicates a general matrix compatibility with each sorbent type.

Mechanism	Sorbent	Analyte	Matrix	Examples
Non-polar extraction (RP)	SDB[1], C18, C8, C2, PH, CH, CN	Non-Polar functional groups (alkyl, aromatic)	Polar solution Aqueous buffers	Pharmaceuticals from plasma Antibiotics from blood Drugs of abuse from urine Hormones from cell culture Pesticides from water
Polar Extraction (NP)	Si, 2OH, CN, NH_2, Florisil, Alumina	Polar functional groups (amine, hydroxyl)	Non-polar solvents Oils	Lipid separation Toxins from peanut butter Steroid vitamins from plasma Fatty acids from cell culture
Cation Exchange	SCX, WCX, PRS, CBA	Positively charged groups (amines)	Aqueous low ionic strength	Catecholamines from plasma Pharmaceuticals from serum Herbicides from soils
Anion Exchange	SAX, WAX, NH_2, PSA, DEA	Negatively charged groups (organic acids)	Aqueous low ionic strength	Organic acids from urine Neurotransmitters from urine Ribonucleosides from cell culture

[1]Polymeric-based resin, typically composed of highly cross-linked polystyrene divinylbenzene, contrast to bare silica SDB is non-polar and capable of strong hydrophobic and π - π interactions.

TABLE 4. Common Mechanism-Analyte Pairs

Sorbent Chemistry

In order to develop an effective SPE method, it is very important to choose the appropriate sorbent. Judicious sorbent selection requires consideration of the physical nature of the sample/matrix, the chemical structure of the analyte, and the unique properties of each individual sorbent relative to specificity and selectivity. Sorbent specificities are subdivided into three categories: normal phase, reverse phase, and ion exchange. Table 4 contains information that correlates general analyte and matrix types to an SPE mechanism. 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.
- Significant factors for reversed phase SPE sorbents include surface area, carbon loading, surface coverage, endcapping 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 in order to obtain reproducible results.

Sorbent Screening

After selecting an extraction mechanism, a range of potential sorbents 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 presence of the least amount of analyte.

Appendix B (page 29) contains detailed information on the relative polarity and hydrophobicity of common SPE sorbents.

Sorbent Mass

Once the extraction mechanism and sorbent chemistry have been defined, the next step is to choose the sorbent mass. Proper sorbent mass for an 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 as well as by the analyte concentration required in the final extract.

Most column manufacturers supply a wide choice of SPE cartridges with volumes ranging from 0.5 to 100 mL. The source of silica, carbon loading, bonding chemistry, and endcapping chemistry affect the surface characteristics of the bonded phase. These are the variables that lead to performance discrepancies between manufactures. See Figure 8 for additional information.

Analyte capacity for bonded silica sorbents (C18, C8, CN, etc.) is estimated to be 10–20 mg of analyte/g of packing. Capacity for bonded silica ion-exchange sorbents is typically 0.5–1.5 mg/g.

If compounds other than the analyte of interest are retained by the SPE cartridge, the capacity of the analyte is reduced proportionally to the amount of competitor present. Approximately 5% by weight of the packing material is retained in the cartridge.

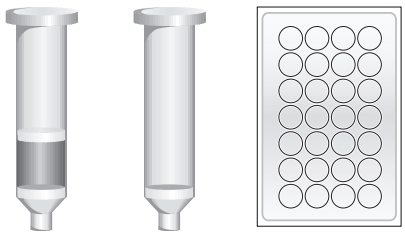
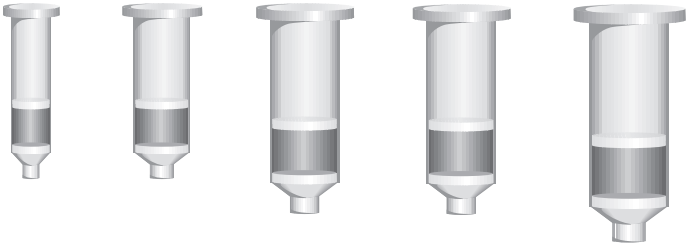


Figure 7. Various SPE Formats: Packed Columns, Columns with Disk, and 96-well Microplate.

Disk columns, in which small particles of sorbent are embedded in a glass-fiber or Teflon-fiber supports, offer higher efficiency with reduced channeling. In addition, disk columns have small void volumes and small elution volume, producing a more concentrated eluent. The Gilson family of automated SPE instruments accommodates, 1 mL, 3 mL, or 6 mL cartridges as well as 96-well formats.

Sorbent Mass/Column Volume				
50 mg/1 mL	100 mg/1 mL	200 mg/3 mL	500 mg/3 mL	1 g/6 mL
Retention Capacity* up to				
2.5 mg	5.0 mg	10 mg	25 mg	50 mg
Minimum Elution Volume:				
125 µL	250 µL	500 µL	1.2 mL	2.4 mL



*Approximate Values: Capacity (mg) – 5% of sorbent mass
Bed Volume – 120 µL/100 mg of sorbent

Figure 8. Capacity and Elution Volumes for Common SPE cartridge Sizes

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. Reference Table 5 to find common solvent types for each SPE mechanism.

Mechanism	Condition Step	Wash Step	Elute Step
Non-polar extraction (RP)	1. Solvation-polar organic solvents (Methanol) 2. Equilibration-aqueous, buffers (water or buffer)	Aqueous buffers with 5 to 50% polar organic solvent (Methanol: Water, 1:9)	Polar or non-polar organic solvents, with or without Water, buffer, and/or strong acid or base (Methanol, ACN, Water, HCl 4:4:2)
Polar Extraction (NP)	1. Solvation-polar organic solvents (Methanol) 2. Equilibration-sample/ matrix solvent	Non-polar organic solvents with a low concentration (1 to 5%) of moderate to low polarity organic solvents (Hexane with 1% THF, ethyl acetate, acetone, ACN or IPA)	Non-polar solvents containing higher concentrations (5 to 50%) of a moderate to high polarity organic solvent (Hexane with 10% THF, Ethyl Acetate, Acetone, ACN or IPA)
Cation Exchange	1. Conditioning-polar organic solvents (Methanol) 2. Equilibration-low ionic strength buffers, pH adjusted (25 mM Tris-OAc, pH 7.0)	Aqueous buffers of low to intermediate salt concentrations with or without organic solvent (50 mM Tris-OAc, pH 7.0 plus 50 mM NH_4Cl plus 20% Methanol)	Neutralize the charge on the weak cation, increase the ionic strength and counter ion, add a strong displacer (50 mM Tris-OAc, plus 200 mM NH_4Cl, pH 2 plus 20% Methanol)
Anion Exchange	1. Conditioning-polar organic solvents (Methanol) 2. Equilibration-low ionic strength buffers, pH adjusted (25 mM Tris-OAc, pH 7.0)	Aqueous buffers of low to intermediate salt concentrations with or without organic solvent (50 mM Tris-OAc, pH 7.0 plus 50 mM NH_4Cl plus 20% Methanol)	Neutralize the charge on the weak cation, increase the ionic strength and counter ion, add a strong displacer (50 mM Tris-OAc, plus 200 mM NH_4Cl, pH 2 plus 20% Methanol)

Information provided for Condition, Wash and Elute steps is reprinted from the Phenomenex SPE Reference Manual & User's Guide, part #AA0-6067.

TABLE 5. Solvent Suggestions for SPE Steps

Typically there are two conditioning steps performed prior to loading the sample. The first CONDITION step most commonly uses Methanol to ensure sorbent solvation and rinse any possible contaminants off the cartridge. The second CONDITION solvent will likely be chemically similar to the sample matrix as it will prepare the sorbent to receive the sample. To aid in the selection of solvents for the WASH and ELUTE steps, there are a few tests that can be performed using standards.

- Selection of the correct elution solvent can optimize the automation process. Standards are applied on the SPE cartridges, different elution solvents are tested. The best solvent elutes the greatest amount of analyte in the smallest volume.

Keep in mind that SPE is rarely the last step for a sample, and subsequent testing can have a significant impact on the elution solvent choice. The Gilson family of automated SPE instruments can be used to prepare samples prior to various analytical techniques (e.g., HPLC, GC, RIA, LC/MS, etc.). The choice of analytical method can limit the options available for elution solvents in order to avoid an intermediate dry down step. Purity requirements of the extracted analyte also depend on the analytical technique employed. A less selective detector (UV) requires a more efficient extraction process.

- Selection of the washing solvent is an important factor in developing methods. Standards 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. It is beneficial to use two different wash solvents prior to elution. In general, the first wash solution is usually a mixture of low organic and high aqueous or buffer to remove more water soluble interferences. The second wash solution is based on the following guideline:

If the compound(s) elutes with 40% strength solvent, use 5 to 10% solvent strength for the washes.

- Evaluation of interfering matrix components should be performed. In this process, a blank matrix is applied to the column and elution is performed with the previously selected solvent. If interfering peaks are detected, further washing steps should be implemented and retested.

Solvent Compatibility

After solvents have been determined, it is important to examine whether solvents used back to back have miscibility issues. A general solvent miscibility chart can be found in Appendix C. Though this is not a concern with manual methods as separate pipettes are typically used for each solvent, in an automated system the solvents will use the same probe and tubing. Extra rinsing with a mutually miscible solvent should be completed in order to avoid any undesired interactions.

Solvent Parameters

The volume and flow rate of solvents at each step in an SPE method can vary greatly from application to application. Although it is something that needs to be optimized in the final SPE method, general starting points can be found in Tables 6-7.

1 mL SPE cartridge	CONDITION	LOAD	WASH	ELUTE
Liquid Volume (mL)	1.0	1.0	1.0	1.0
Liquid Dispense Flow (mL/min)	5.0	1.0	1.3	2.0
Air/Gas/Vacuum Time (min)	0.1	0.3 – 0.5	0.3 – 0.5	1.0

3 mL SPE cartridge	CONDITION	LOAD	WASH	ELUTE
Liquid Volume (mL)	2.0	1.5	1.5	1.5
Liquid Dispense Flow (mL/min)	8.0	2.5	6.0	3.0
Air/Gas/Vacuum Time (min)	0.1	0.5 – 0.75	0.5 – 0.6	1.0 – 2.0

6 mL SPE cartridge	CONDITION	LOAD	WASH	ELUTE
Liquid Volume (mL)	3.0 – 6.0	2.0 – 20.0	2.0 – 6.0	2.0 – 3.0
Liquid Dispense Flow (mL/min)	10.0	5.0	10.0	5.0
Air/Gas/Vacuum Time (min)	0.1	0.5 – 1.0	0.5 – 1.0	2.0 – 4.0

TABLE 6. Typical SPE cartridge Values During the SPE Process (critical parameters are highlighted in purple)

	CONDITION	LOAD	WASH	ELUTE
Liquid Aspiration Flow Rate	High	Medium	High	Medium
Air Gap Aspiration Flow Rate	High	Medium	High	High
Air Gap Volume (µL)	Enough so that air gap is at least 10 mm in transfer tubing.			
Air/Gas/Vacuum Time	Low	Medium	Medium	High

TABLE 7. SPE cartridges: Default range of minor parameters

Sample Preparation

Some compounds will interfere in the extraction process, requiring the implementation of a pre-treatment step.

Oils, Fats, Lipids, and Unsaturated Hydrocarbons

In polar extraction, oils, fats. and related compounds have a tendency to absorb on the sorbent, reducing the capacity for analyte retention. Liquid-liquid extraction with a non-polar solvent (hexane, CH_2Cl_2) can be used to remove them. Cooling the resulting solution will precipitate fats. Gel permeation chromatography (GPC clean-up) is another option for removal of lipids from a complex matrix.

Inorganic Salts

Inorganic salts, which are present in a wide variety of samples, are ionic in solution and interfere with analyte retention by ion exchange. Desalting the sample by passing it through a non-polar sorbent prior to ion exchange (double extraction) overcomes this problem. Other alternatives for removing inorganic salts are dialysis, gel filtration, or membrane desalting.

Surfactants

Surfactants exhibit multiple chemical characteristics, therefore they can be retained by a variety of different SPE mechanisms. Removing the detergent can be accomplished by "trapping" the detergent onto an ion-exchange or mixed bed column (double extraction), prior to SPE.

Carbohydrates and High Molecular Weight Polysaccharides

Carbohydrates and high molecular weight polysaccharides are highly polar and soluble in polar solvents only. 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 the extraction of these types of compounds.

Proteins

Protein, the major component in physiological samples, often bind to analytes (e.g., drugs). Proteins are large molecules which tend to be unretained on sorbents, therefore the drugs will be carried through the sorbent bed by the protein instead of being retained.

The key is to disrupt the protein analyte binding:

1. Modify the pH: protein binding is often pH dependent.
2. Protein denaturation: addition of chaotropic agents (e.g. urea, guanidine hydrochloride 8M) or organic solvents (e.g. ACN in 1:1 ratio).
3. Protein precipitation: Addition of TFA, sulfosalycilic acid, ACN, or perchloroacetic acid.
4. Competition for analyte-binding site: addition of competitor molecules which are close in structure to the analyte of interest, analytes can be released from the binding sites.

The following represents the power of soft acids to disrupt protein binding.

		Chlorpromazine		Thioridazine	
Soft Acid Concentration		Recovery	CV	Recovery	CV
Acid	(%)	(%)	(%)	(%)	(%)
None	0	88	3.0	71	4.0
TFA	0.03	92	1.1	79	1.7
Acetic	0.03	91	2.4	73	2.8
Acetic	0.05	95	1.7	86	1.6
H_3PO_4	0.03	98	1.7	83	1.8
H_3PO_4	0.05	90	2.3	76	1.8

TABLE 8. Effect of Soft Acids on Recovery of Analytes in a Protein Matrix

Viscous and Concentrated Samples

Viscous samples with volumes less than 1 mL may yield higher recoveries if diluted before being loaded 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.

Automating Sample Preparation

Serial dilution, derivatization, double extractions, and the addition of reagents, solvents, or internal standards are just a small sampling of tasks that can be fully automated on the Gilson family of instruments. With the addition of various racks and accessories, almost any task involving the movement of liquid is able to be automated. These tasks can be accomplished within the original sample vial or in an intermediate vial to which all components are added. A homogeneous sample is produced by mixing via liquid, air, or an orbital shaker (vortexing).

To determine if sample preparation is necessary, measure the analyte recovery from a spiked sample matrix. If the analyte recovery in the spiked matrix is lower than that from the standard solution, the analyte is most likely interacting with the matrix components.

Optimization

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 in order to do so? Is it more important to have it completed faster or to recover more sample? These are the questions that 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. Extreme flow rates do not save time if it requires twice as many extractions to recover the same amount of material.

Optimizing SPE Method Through Automated Fractionation

The Gilson line of automated SPE instruments allow automated method optimization and development through stepwise movement of the SPE cartridges across a series of collection tubes (see Figure 9). This allows for 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.

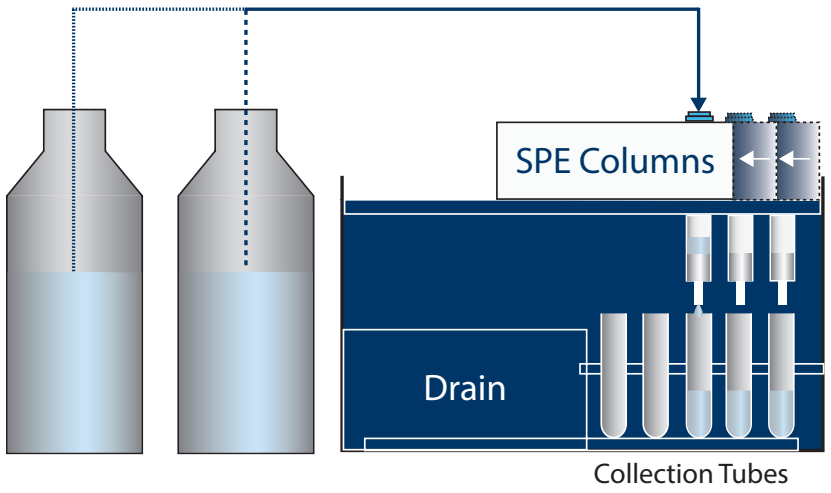


Figure 9. Gilson Automated Fractionation

Practical Development Strategy

After a first run using default values 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 in order to observe that change's effect. Following the flow chart below helps to ensure that no aspect of method optimization is overlooked.

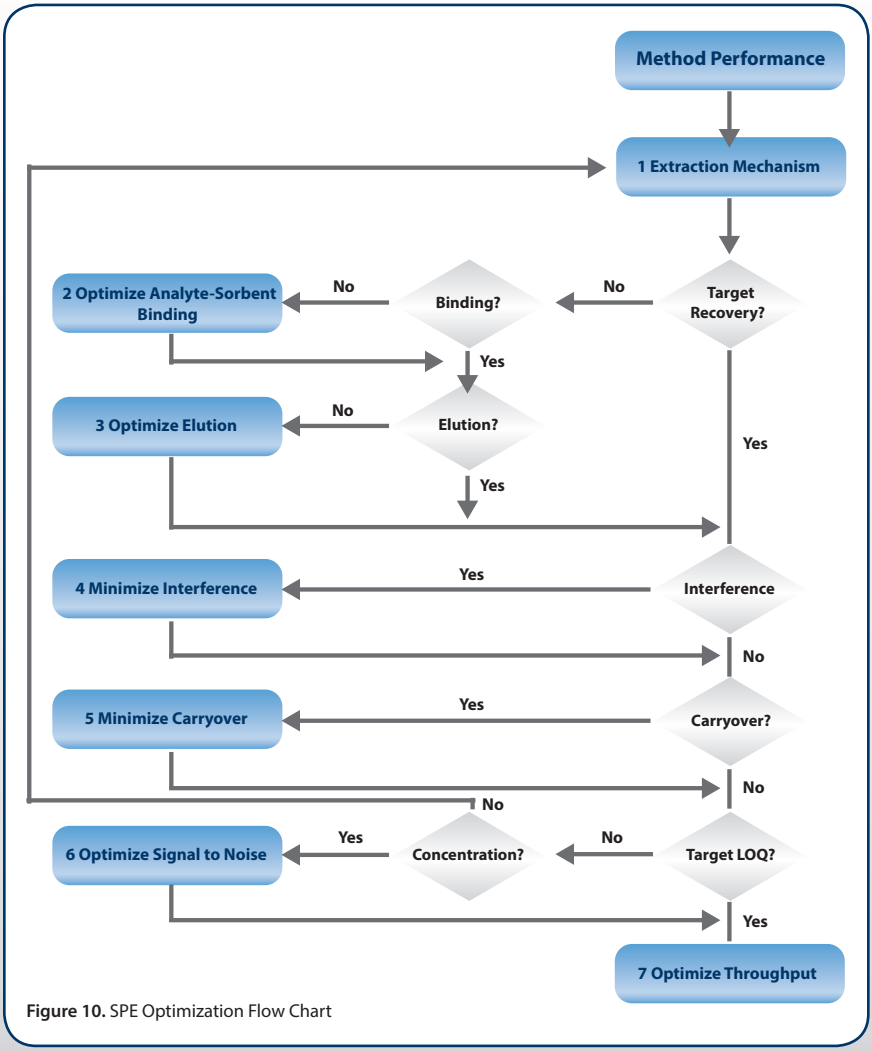


Figure 10. SPE Optimization Flow Chart

Optimizing Analyte-Sorbent Binding

CONDITION Air Push

Since the SPE cartridge should not be allowed to dry during the conditioning step, this push should be selected according to the conditioning solvent and SPE cartridge size. Do not allow the column to dry out during this conditioning step.

CONDITION 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.

LOAD Volume

Breakthrough is the point at which the SPE sorbent becomes saturated and can no longer retain additional analytes. One should not work beyond the breakthrough volume. A good place to start is with an analyte mass of less than half the SPE cartridge capacity. If the sample is viscous the liquid should be aspirated with a low flow rate (<3 mL/min) ensuring reproducible results.

LOAD Flow Rate

This is a critical parameter. The LOAD involves the mass transfer between the liquid sample and the sorbent. Select a flow rate that is slow enough to allow complete mass transfer. Sample dispensing flow rates should not exceed 6 ml/min and often times the flow rate is reduced to 3 mL/min to ensure maximum recovery and reproducibility.

LOAD Air Push

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

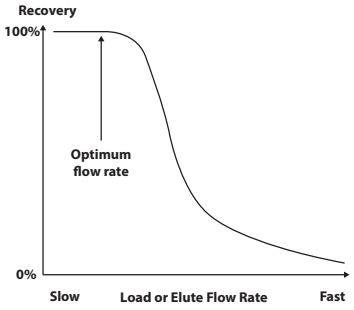


Figure 11. Relationship between sample recovery and flow rate in SPE

Optimizing Analyte Elution

ELUTE Volume

The ELUTE 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.

ELUTE Flow Rate

The ELUTE step involves mass transfer between the solvent and the sorbent; the flow rate should be slow enough to allow complete mass transfer. Determination of this flow rate is available from the SPE manufacture's application sheet or by processing samples at different flow rates and comparing recovery. Elution flow rates should not exceed 6 mL/min.

ELUTE Air Push

This is the amount of time that is applied to optimize analyte recovery. At this point it is not necessary to be concerned with drying the column, hence it is better to have a longer time than too short of a time.

Column Drying

Drying of the SPE can be necessary when two immiscible solvents are used in sequence (DUA, steroid analysis) or when a series of wash solvents could result in the elution of the analyte of interest. The amount of time that the gas is applied should be long enough to ensure proper drying. Running a series of samples with varied drying times would determine the shortest drying time required to achieve maximum recovery.

Minimizing Interference

WASH Flow Rate

The washing step associated with the SPE is only moderately influenced by flow rate; However, if the flow rate is too high, interferants may not be adequately removed. Therefore diffusion of the wash solvent with the packing material is improved with slower flow rates.

WASH Volume

WASH volumes that are insufficient will result in the presence of interfering compounds in the eluent. If interfering compounds are detected in the eluent the wash volumes should be increased.

WASH Solvent

It is not unusual to test different solvents for the WASH step. Rule of thumb, the solvent needs to be strong enough to remove interfering compounds without affecting the analyte of interest. For example, if a SPE protocol elutes the compound of interest with 40% solvent strength use 5–10% solvent strength for the washes.

Column Drying

Drying the sorbent between wash steps may be required if two immiscible solvents are used to avoid any mixing with sequential solvents.

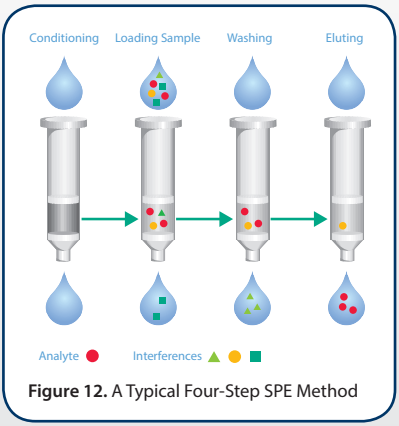


Figure 12. A Typical Four-Step SPE Method

Minimizing Carryover

By design, Gilson's automated SPE product line minimizes carryover. The only liquid that comes in contact with the syringe of the syringe pumps is the reservoir solvent, which is usually the probe rinsing solvent. The liquid transferred from the bed space of the liquid handler mainly comes in contact with the probe and the transfer tubing. The probe and transfer tubing are cleaned very efficiently by "RINSE tasks". Parameters can be optimized within the task to eliminate carryover, and additional solvents can be accessed.

If carryover is observed:

- Increase the rinsing flow rates and choose a higher flow rate to create more effective rinsing.
- Increase the rinsing volume.
- Increase the number of rinsing steps and rinse sites.
- Consider another rinsing solvent, possibly adding 10 to 20% organic (ACN , Methanol) to the rinsing solvent reservoir.
- Implement the use of a flow-through rinse station with a peristaltic pump.

Optimizing Throughput

Only optimize sample throughput after the recovery and carryover requirements have been met.

Setting Throughput Goals

In sequential mode (*see page 9*) there is no advantage to be gained by further decreasing the sample preparation time below the analytical run time, e.g., GX-271 ASPEC with on-line HPLC.

Consider employing the use of a GX-274 ASPEC. This Gilson instruments has multiple probes and therefore substantially increase throughput.

Solvent Volumes

Optimize SPE procedures by implementing the use of the smallest effective solvent volumes.

Flow Rates

Set the highest flow rates possible when the volumetric accuracy is not critical, e.g., rinsing the probes or aspiration of conditioning solvent.

Unnecessary Steps

Rinsing the probe(s) after every step may not be required. Evaluate the automated SPE procedure and remove unnecessary steps (e.g., rinsing the probes between consecutive conditioning steps).

Extraction Mechanism

Consider changing to a smaller sorbent mass or smaller SPE cartridge if it is an option. This will reduce both the sample and solvent volumes. In addition, the use of a more efficient sorbent packing will allow the use of higher flow rates and possibly the reduction of washing steps (consult your SPE manufacturer).

Recovery and Carryover

If any or some of these suggestions have been implemented, reconfirm that the automated SPE method is still operating as before by comparing recovery and carryover to previous analysis.

Appendix A: SPE Troubleshooting

Problem	Possible Cause	Corrective Action	Gilson TRILUTION® LH SPE Task
Over-pressurization	• Matrix	• Double extraction, dialysis	• LOAD & COLLECT
	• Inorganic salts	• Double extraction	• LOAD & COLLECT
	• Surfactants • Oils	• Double extraction, LLE (liquid-liquid extraction) • Gel Permeation Chromatography (GPC)	• LOAD & COLLECT
	• Carbohydrates	• Dilute the sample	• DILUTE
	• Proteins	• Modify the pH, protein precipitation, protein degradation	• ADD
	• Viscous sample	• Dilute the sample • Decrease Flow Rates	• DILUTE
	• Improper conditioning	• Do not allow sorbent to dry during conditioning	• CONDITION, decreased gas/air/vacuum
	• Immiscible reagents	• Dry the SPE sorbent • Decrease flow rate between solvents	• DRY, increase gas/time
Poor recovery	• Improper conditioning	• Do not allow sorbent to dry during conditioning	• CONDITION, decrease the air/gas/time
	• Poor binding	• Do not exceed the breakthrough volume	• LOAD, decrease sample volume
	• Improper washing	• Optimize mass transfer	• LOAD, decrease sample dispense flow, increase the air/gas push time
	• Poor Elution • Overloading column	• Collect the load, wash, and elute steps to determine where breakthrough or loss may be occurring. • Try different sorbents, test for binding capacity • Optimize column drying between successive washing solvents • Increase elution solvent volume • Optimize mass transfer • Optimize nature of elution solvent	• FRACTIONATE, allows for the collection of each SPE step into its own collection vessel for further testing • LOAD & COLLECT • Collect multiple fractions • Collect multiple fractions, decrease liquid dispense flow, increase air/gas • Collect with varying eluent solvents

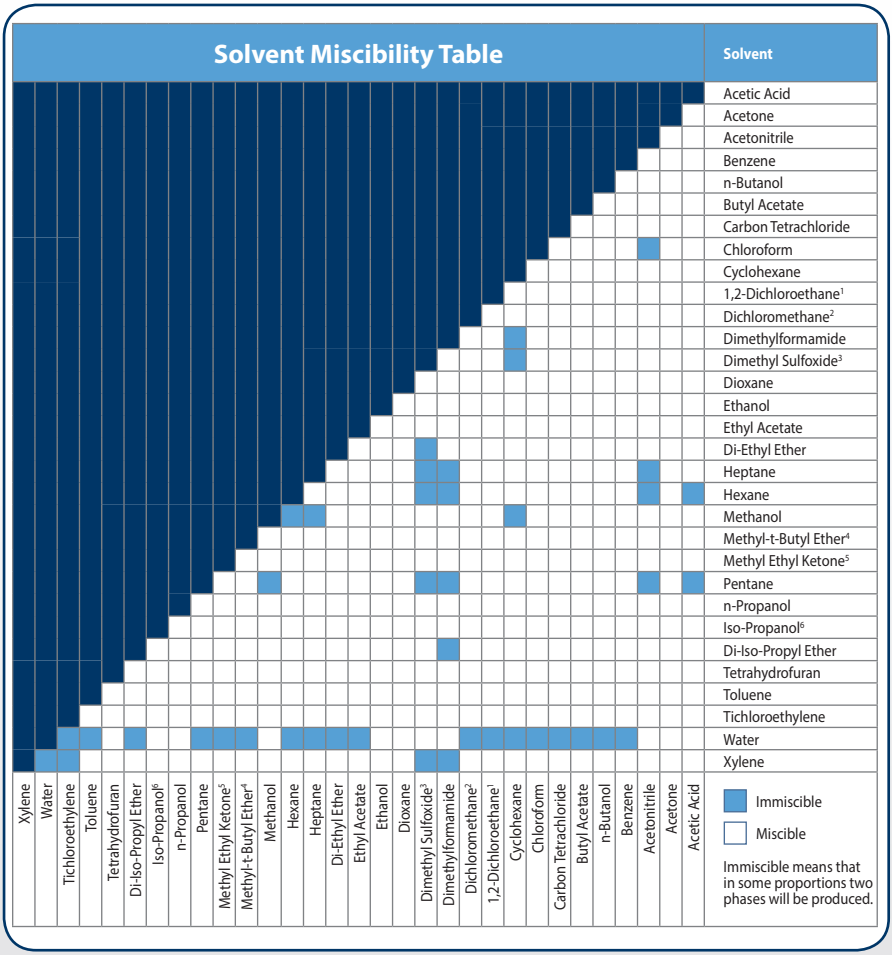
Problem	Possible Cause	Corrective Action	Gilson TRILUTION® LH SPE Task
Interference	• Poor washing efficiency	• Optimize mass transfer • Increase solvent volume • Optimize washing solvent	• WASH, increase air/gas time • WASH, increase volume • Collect multiple washes
Carry-over	• Inadequate probe rinsing	• Rinse after each liquid handling step • Optimize the rinsing step	• RINSE PROBES • RINSE PROBES increase flow rate and volume
	• Inadequate rinsing solvent	• Add extra-rinsing solvents • Select a different rinsing solvent	• RINSE PROBES
Low Throughput	• Time consuming tasks	• Optimize protocol	• Decrease solvent volume
	• Unnecessary steps	• Optimize arm and pump movements	• Increase flow rates rinsing and washing steps
	• Improper methodology	• Optimize protocol • Eliminate evaporation step • Reconsider the extraction mechanism and the sorbent	• Decrease rinsing and washing steps
LOQ not reached	• Eluent too dilute	• Minimize the evaporation step • Concentrate sample by evaporating to a smaller volume • Trace enrichment • Use of disk column	• Collect with various volumes of eluant • EVAPORATE • ELUTE and concentrate eluant volume • ELUTE
Poor Reproducibility	• Improper conditioning	• Refer to the SPE manufacturer's recommendations	• CONDITION
	• Incomplete aspiration of sample	• Reduce aspiration flow rate	• LOAD, decrease rate of aspiration
	• Improper mixing of the eluent before injection	• Dilute sample • Mix the eluent before injection • Optimize the mixing	• DILUTION • MIX, increase number of cycles, change mixing height

Appendix B: Solvent & Sorbent Polarity Chart

Relative Polarity	Compound Formula	Chemical Group	Representative Solvents	Eluting Strength (ε_0)
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
	$R\text{-}NR_2$	Amines	Pyridine	0.55
			Triethylamine	0.73
	R-OH	Alcohols	Isopropanol	0.63
			Ethanol	0.70
			Methanol	0.73
	$R\text{-}CO\text{-}NR_2$	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	NH_2/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 Sulfonic acid
	Yes	100	
6.6	Yes	100	
	Yes	100	SAX/Quaternary Amine
6.2	Yes	100	
10.2	Yes	100	

Appendix C: 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-Dichloroethane[1]	3.5	1.444	225	84	0.79	0.81
Dichloromethane[2]	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide[3]	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 Ether[4]	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone[5]	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-Propanol[6]	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

Appendix D: Temperature Conversion Formulas

Temperature Conversion Formulas	
˚C = ˚F – 32 ÷ 1.8	˚C = degrees Celsius ˚F = degrees Fahrenheit ˚K = degrees Kelvin ˚R = degrees Rankine
˚C = ˚K – 273.15	
˚F = 1.8 x ˚C + 32	
˚F = ˚R – 459.67	
˚K = ˚C + 273.15	
˚R = ˚F + 459.67	

**Info edited from: http://www.sengpielaudio.com/ConvPress.htm*

Appendix E: Pressure Conversion Chart

*Unit of Measure	Equivalent Measure
Pounds per square inch (psi, PSI. lk/in^2, lb/sq in)	Normal atmospheric pressure is 14.7 psi
Atmosphere (atm)	Normal atmospheric pressure = 1 atmosphere (atm) = 14.6956 psi = 760 torr
Bar (bar)	The bar is very similar to 1 atmosphere unit. 1 bar = 750.062 torr = 0.9869 atm = 100,000 Pa
Pascal (Pa)	1 pascal is equal to the force of 1 Newton per square meter. 1 Newton is the force required to accelerate 1 kg • m/s^2 1 pascal = 10 dyne/cm 2 = 0.01 mbar 1 atm = 101,325 Pascals = 760 mm Hg = 760 toor = 14.7 psi
Kilopascal (kPa)	1kilopascal = 1,000 Pa 101.325 kPa = 1 atm = 760 torr 100 kPa = 1 bar = 750 torr
Megapascal (MPa)	1 megapascal = 1,000 kPa = 1,000,000 Pa

**Info edited from: http://www.sengpielaudio.com/ConvPress.htm*

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