OPTIMIZE TRAPPING GUIDE

A PRIMER FOR THE LC OR LC/MS TECHNIQUE OF OFF-LINE AND ON-LINE TRAPPING.

INTRODUCTION

Sample matrix components such as salts, detergents and contaminants present problems for mass spec analysis. Trapping is a chromatography technique that allows for the concentration or purification of a sample. A trap cartridge is a packed column bed loaded with a material to create desirable conditions for separating the target compound from the rest of the sample matrix. By selecting a packing material that has a strong affinity for the target compound, the analyte is retained in the trap while the rest of the sample matrix flows through. Alternately, a material can be selected which has no affinity for the target compound but that binds other unwanted matrix components.

Trap columns are uni-directional or bi-directional and are used either on-line or off-line for sample pre-concentration and clean-up. Trap column bed materials need not be similar to the primary LC column bed materials and can be selected based on sample clean-up needs. Desirable characteristics of a trap include low back pressure, bi-directional flow, robust bed, ability to regenerate the packed bed and low swept volume.

TRAP COLUMNS VS. GUARD COLUMNS

Choosing between traps and guard columns is determined by the application. A guard column is a short, disposable pre-column which removes particulates and contaminants that would otherwise shorten the life of the expensive LC column. A guard column protects the primary column. When selecting a guard column, a bonded phase similar to the primary column should be used.

Optimize Technologies offers several trapping options for LC applications:

- OPTI-TRAP™ - suitable for medium pressure manual and on-line applications (1,500 psi)
- OPTI-LYNX™ - a quarter-turn quick-connect system for HPLC applications (6,000 psi)
- EXP® for UHPLC applications up to 20,000+ psi

Trap columns are offered in a range of bed volumes from 0.12µL to 100µL and can be loaded with a variety of packing materials found in the Trap Column section (pages 29-38). For additional packing materials, please contact Optimize directly.

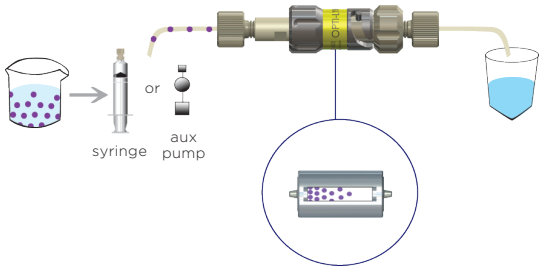
Trapping is a valuable technique for handling a variety of processes such as sample clean-up, purification, pre-concentration, desalting and detergent removal. In order to retain a target compound within a trap cartridge while flushing the sample matrix and any unwanted contaminants to waste, a packed bed with an affinity for the target compound is used. Alternatively, a packed bed with no affinity for the target compound may be used in order to keep the desired analyte unretained while having the undesired contaminant bound to the stationary phase.

In cases where samples are undesirably dilute, it is possible to increase the concentration of the target analyte in a sample either off-line or on-line. Using a trap cartridge allows the reduction of volume of a sample matrix while concentrating the analyte.

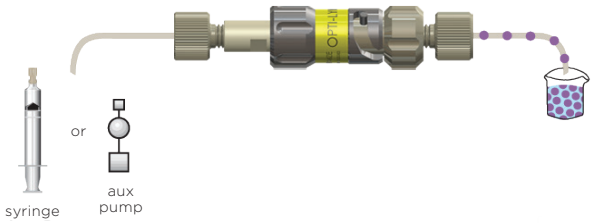
TRAP CARTRIDGES FOR SAMPLE PRE-CONCENTRATION

OFF-LINE SAMPLE PRE-CONCENTRATION

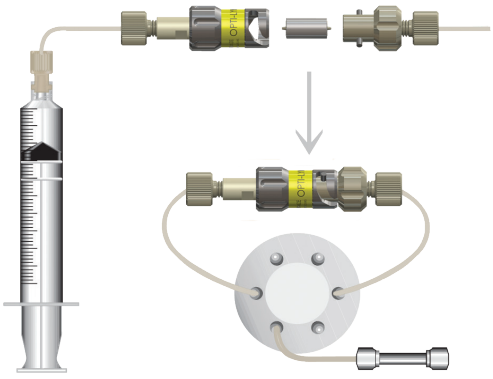
Using a syringe or a small pump along with a trap cartridge that has an affinity for the target analyte, a sample matrix is driven across the trap bed at a flow rate within the recommended range. A slower flow rate is generally considered better.



The sample matrix will be sent to waste while the target is analyte retained within the trap. The target analyte is now able to be eluted in a small volume of stronger solvent.



A quick rinse step prior to the elution step is advantageous if salts are present in the sample matrix. Elution takes place either by manual delivery of solvent or by installing the trap cartridge into a holder in-line upstream from an analytical column or within an injection loop.

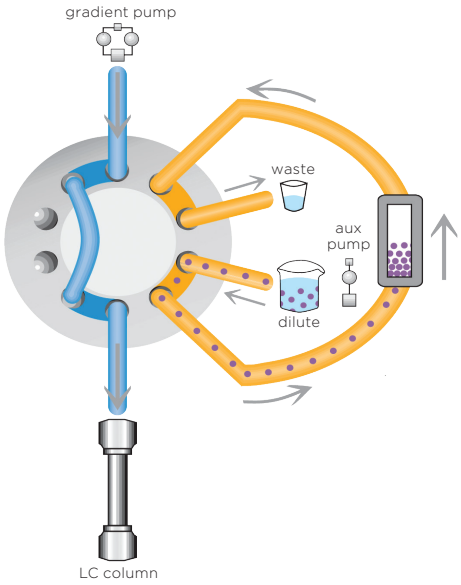


TRAP CARTRIDGES FOR SAMPLE PRE-CONCENTRATION

OFF-LINE SAMPLE PRE-CONCENTRATION

Pre-concentration can be automated by placing a trap cartridge in-line in the loop of an injection or switching valve. This setup allows two different sources to push solvents through the trap depending on the position of the valve.

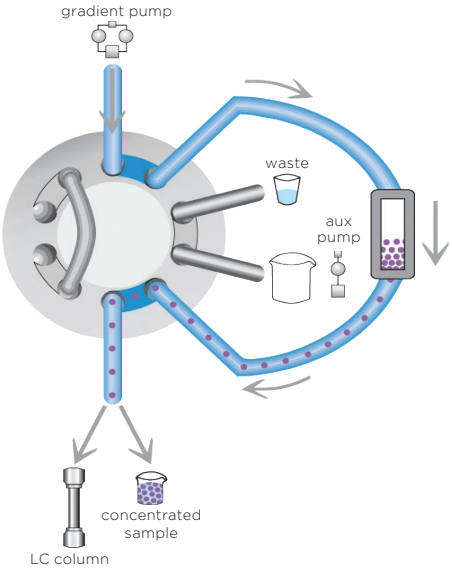
The sample solution is pushed through the trap bed by an auxiliary pump during the loading phase. After the sample matrix is flushed to waste, it may be beneficial to wash the trap bed with a salt-free solution, ensuring that any buffer salts are rinsed away.



Additional samples may be collected by repeating the sample-loading step with a flushing solvent in order to ensure that any additional sample remaining in the tubing makes it across the packing material during elution.

Using a small volume of suitably strong organic solvent, the concentrated sample can now be eluted.

Eluent can be sent directly to a mass spectrometer or to an analytical column for further separation. If the analyte contains a complex mixture of proteins and peptides, it may be desirable to follow the pre-concentration step with a two-dimensional LC configuration.



DETERGENT REMOVAL VIA TRAPPING

DETERGENT REMOVAL

Detergents may be present as a result of SDS PAGE analysis or as additions in order to help solubilize a sample. Prior to LC or LCMS analysis, these detergents must be removed.

Many off-line detergent removal methods are time consuming and may result in a significant loss of sample. An on-line trap provides a more convenient and efficient method for detergent removal.

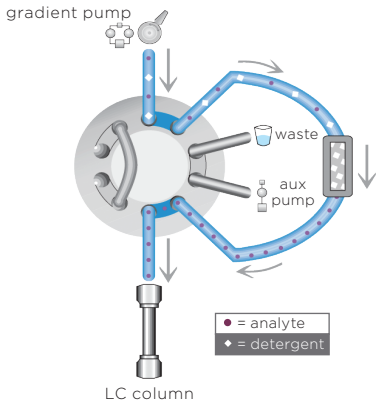
The type of detergent present in a protein sample affects the method of removal. Generally, there are three types of detergents that may be present in a sample: ionic, zwitterionic and non-ionic. Regardless of the method used, the idea is the same: trap the protein, wash detergent to waste and elute the protein or trap the detergent while allowing the protein to pass through.

SDS & IONIC DETERGENT REMOVAL

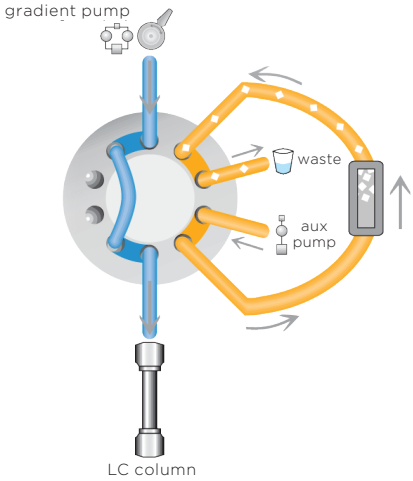
Ionic detergents such as SDS (sodium dodecyl sulfate) may be removed using a simple ion exchange trap. The packing material used in the trap must have an affinity for the type of charge on the polar head group of the detergent. Anionic detergents require the use of a strong anion exchange (SAX) phase such as a quaternary amine. Alternatively, cationic detergents require use of a strong cation exchange (SCX) phase such as benzenesulfonic acid.

SDS removal is accomplished by using a polymer-based anion exchanger. Mobile phase with a pH of 4.4 or less is used in order to provide conditions where the trap has a maximum affinity for SDS and minimal affinity for the protein sample. The low pH ensures protonated anionic side chains of a protein, reducing the chance of protein interaction with the packing material. Polymeric supports are a more resilient option at a low pH than silica-based anion exchangers. Selective binding of SDS to the trap should occur as the sample is pumped through the trap.

The protein should pass through unretained and may be sent for immediate analysis, or subjected to further on-line purification steps, such as concentration and desalting.



Detergent (SDS) binds to trap while protein/ peptide travels to LC column



The anion exchange trap will have a finite capacity for SDS and must be regenerated before that capacity is exceeded.

This can be accomplished with a mobile phase that has a high concentration of organic eluent and is strongly acidic. A pH below 2 and an organic content above 90% should be sufficient.

DETERGENT REMOVAL VIA TRAPPING

NON-IONIC DETERGENT (NID) REMOVAL

Non-ionic detergents have hydrophobic characteristics and no charge. Therefore, ion-exchange approaches cannot be used. In NID removal, it is best to temporarily adsorb the protein within a trap while detergent is flushed to waste.

A packed bed with affinity for the target protein and little to no affinity for non-ionic detergents is used for separation. This packed bed may consist of a single phase such as silica or polymeric SCX or even a mixture of phases such as SCX/SAX. The most beneficial chemistry for a particular protein sample may need to be determined empirically.

Generally, a sample is delivered to a trap using a mobile phase with a low percentage of organic modifier. The proteins should bind to the packing material while the NID passes through unretained. (fig. 1)

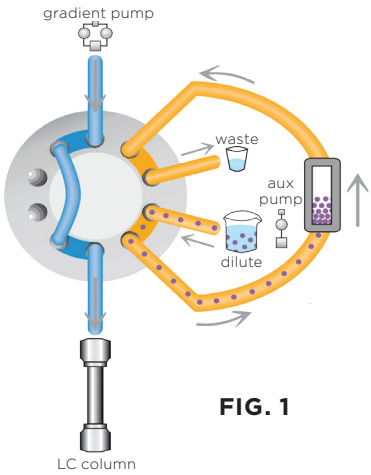


FIG. 1

If the isoelectric point (pI) of a protein is at or near the pH of a mobile phase, it may pass through the column unretained. If the pI of a protein is known, pH should be kept below pI for optimal interaction with an SCX trap, and either above or below for a mixed mode SCX/SAX trap. A salt solution of 0.5M concentration can be used to elute the protein after all of the detergent has passed through the trap. A reverse phase bed can be used to desalt the protein before sending it to an MS. In order to keep the desalting trap out of the flow stream during detergent removal, switching valves are required. (fig. 2)

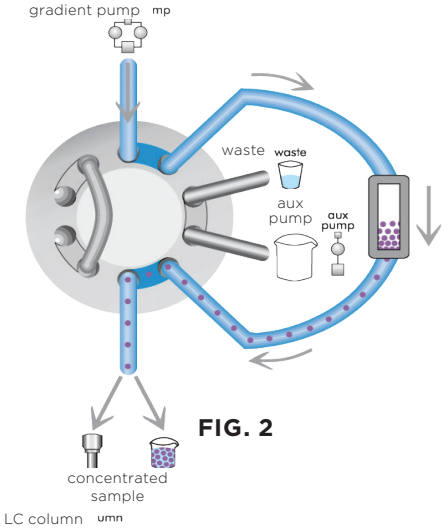


FIG. 2

Employing a “normal phase” trap is also a method for discarding of NID. The protein is loaded in high concentrations of organic solvent (80-95% acetonitrile) onto a highly polar stationary phase. The highly organic matrix is introduced in order to maximize the affinity of the sample for the polar stationary phase, and ensures near-complete binding of the sample and elimination of detergent. A gradient of decreasing organic or increasing salt concentration is then used to elute the protein.

TRAP SPECIFICS

SMALL MOLECULE CONCENTRATION & DESALTING TRAP

This trap contains a small pore, large particle, hydrophilic C18 silica (ODS-AQ) reversed-phase packing material and is designed to bind small molecules (0.1-10 kD). This includes many organic molecules such as pharmaceuticals, petrochemicals and natural products. Concentration of samples is possible with maximum efficiency. This trap removes salts (8M) and non-volatile buffers and is used at a pH range of 2-7.5.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “B solvent” (typically 90/10/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
2. Equilibrate the trap with 5-10 trap volumes of “A solvent” (typically 2/98/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
3. Add appropriate amount of acetonitrile and ion-pairing acid to sample to equal the composition of “A solvent.”
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.
5. Remove salts from trap and flush to waste by washing with approximately 5 trap volumes of “A solvent.”
6. Elute small molecules from trap. If performing on-line trapping, actuate the valve to the INJECT position and then run an increasing gradient of acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% acetonitrile or “B solvent.”
7. For full regeneration, flush the trap with several trap volumes of IPA.

TRAP SPECIFICS

PEPTIDE CONCENTRATION & DESALTING TRAP

Small biological molecules ranging from 0.5-50 kD can be bound and concentrated with a peptide concentration & desalting trap. This is done by using a medium pore, large particle, polymeric reversed-phase packing material with retention similar to a C8 phase. Operating at a pH range of 1-13, this trap removes salts (8M) and non-volatile buffers.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “B solvent” (typically 90/10/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
2. Equilibrate the trap with 5-10 trap volumes of “A solvent” (typically 2/98/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
3. Add appropriate amount of acetonitrile and ion-pairing acid to sample to equal the composition of “A solvent.”
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.
5. Remove salts from trap and flush to waste by washing with approximately 5 trap volumes of “A solvent.”
6. Elute peptides from trap. If performing on-line trapping, actuate the valve to the INJECT position and then run an increasing gradient of acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% acetonitrile or “B solvent.”
7. For full regeneration, flush the trap with 70:30 formic acid:IPA.

TRAP SPECIFICS

PROTEIN CONCENTRATION & DESALTING TRAP

When working with large biological molecules ranging from 5-500 kD, a protein concentration and desalting trap may be used for concentration or removal of salts (8M) and non-volatile buffers. The packed bed consists of a large pore, large particle, polymeric reversed-phase packing material with retention similar to a C4 phase. This functions at a pH range from 1-13.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “B solvent” (typically 90/10/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
2. Equilibrate the trap with 5-10 trap volumes of “A solvent” (typically 2/98/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
3. Add appropriate amount of acetonitrile and ion-pairing acid to sample to equal the composition of “A solvent.”
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.
5. Remove salts from the trap and flush to waste by washing the trap with approximately 5 trap volumes of “A solvent.”
6. Elute proteins from the trap. If performing on-line trapping, actuate the valve to the INJECT position and then run an increasing gradient of acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% acetonitrile or “B solvent.”
7. For full regeneration, flush the trap with 70:30 formic acid:IPA.

TRAP SPECIFICS

SDS REMOVAL TRAP

A large pore, large particle, polymeric strong anion exchange packing material is used for these traps. They are designed to bind anionic detergents such as sodium dodecyl sulfate (SDS) at low pH (2-4). This trap removes SDS at concentrations as high as 1%. If higher concentrations of SDS are present in a sample, the risk of forming micelles that trap analytes along with the SDS micelle complex. Such samples must be diluted below 1% first. The trap works at a pH range of 1-13.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “B solvent” (typically 90/10/0.005-0.1% acetonitrile/ H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
2. Equilibrate the trap with 5-10 trap volumes of “A solvent” (typically 2/98/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
3. Add an appropriate amount of acetonitrile and ion-pairing acid to sample to equal the composition of “A solvent.” Note: pH must be between 2-4.
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap. SDS will bind to the trap while proteins pass through.
5. Capture proteins as they pass through the SDS removal trap for further analysis. If performing on-line trapping, add 5-10 trap volumes of “A solvent” to allow concentration and desalting of proteins on the protein trap. If performing manual SDS removal, add 1-2 trap volumes of “A solvent” to allow all proteins to pass through SDS removal trap.
6. Actuate the valve to the INJECT position and elute proteins from concentration and desalting trap by running an increasing gradient of acetonitrile. While in the inject position, also clean the SDS trap and route retained SDS to waste by flushing with 5-10 trap volumes of 90% acetonitrile/ 0.1% HCl.
7. Fully regenerate trap by flushing with 90% acetonitrile/ 0.1% HCl.

TRAP SPECIFICS

NID (NON-IONIC DETERGENT) REMOVAL TRAP

Using a mixed bed of large pore, large particle, silica-based weak anion and weak cation exchange packing material, this trap is designed to bind charged proteins and/or peptides. This trap removes non-ionic detergents such as Triton X-100 and Tween-80 by allowing the uncharged detergents to pass through. The trap works at a pH range of 2-7.5.

Quick Reference

1. Clean the trap with 5-10 volumes of 10% acetonitrile/0.5M NaCl.
2. Equilibrate the trap with 5-10 trap volumes of 10% acetonitrile/10mM buffer, pH 7.0 (or some other pH not corresponding to the pI of proteins).
3. Add appropriate amount of acetonitrile and buffer solution to sample to allow sample to contain 10% acetonitrile buffered at pH 7.0 (or at some other pH not corresponding to the pI of proteins).
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap. NID will pass through the trap while proteins remain on the NID removal trap.
5. Release proteins from the NID removal trap using 1-2 trap volumes of 10% ACN/0.5M NaCl. If performing on-line trapping, then load 5-10 trap volumes of “A solvent” (typically 2/98/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid) to allow concentration and desalting of proteins on the protein trap. Note: some proteins require up to 5% ACN in “A solvent.”
6. Actuate the valve to the INJECT position and elute proteins from concentration and desalting trap by running an increasing gradient of acetonitrile.
7. Fully regenerate trap by flushing with 10% acetonitrile/0.5M NaCl.

TRAP SPECIFICS

SCX (STRONG CATION EXCHANGE) TRAP

A packed bed consisting of silica-based strong cation exchange material with medium pores and large particles is designed to bind small positively charged molecules from 0.5 to 50 kD. At a pH of 2.7-3.0, peptides will lose their negative charge and have a net positive charge. The trap is used in a pH range of 2.7 to 7.0. A pH of less than 2.7 will destroy the phase.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “high salt buffer, pH 3” of choice.
   Example: 5mM NaH2PO4, pH 3.0, with 25% acetonitrile and 0.25M KCl. Note: If using a peptide concentration and desalting trap in tandem with SCX trap for 2D analysis, a good buffer is 5/90/2.5/2.5/0.05% acetonitrile/H2O/30% ammonium hydroxide/formic acid/HFBA (“D buffer”).
2. Equilibrate the trap with 5-10 trap volumes of “low salt buffer.” Example: 5mM NaH2PO4, pH 3.0, with 25% acetonitrile. Note: If using a peptide concentration and desalting trap in tandem with SCX trap for 2D analysis, a good buffer is 5/95/0.1/0.005% acetonitrile/H2O/ formic acid/HFBA (“C buffer”).
3. Add an appropriate amount of acetonitrile and buffer to the sample to obtain pH 3.0 and 25% acetonitrile to match the “C buffer.”
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.
5. Release peptides from the trap using 1-2 trap volumes of “high salt buffer” or perform salt steps with increasing concentrations of salt. If performing on-line trapping, then load 5-10 trap volumes of “C buffer” to allow concentration and desalting of peptides on peptide trap.
6. Actuate the valve to the INJECT position and elute peptides from concentration and desalting trap by running an increasing gradient of acetonitrile.
7. For full regeneration, flush the trap with a "high salt buffer" of choice.

TRAP SPECIFICS

ISRP PROTEIN REMOVAL TRAP

This technique utilizes an Internal Surface Reversed-Phase trap, which contains a very small pore, large particle, silica-based internal surface, reversed-phase packing material. This trap is designed to bind small molecules (0.1-5 kD) onto C18 chains within the internal surface of the pores of the packing material. Protein removal from plasma, urine and serum samples is possible by excluding the proteins from the shielded hydrophobic phase. This allows them to pass through the interparticulate spaces. This works at a pH range of 2-7.5.

Quick Reference

1. Clean the trap with 5-10 trap volumes of “B solvent” (typically 90/10/0.005-0.1% acetonitrile/H2O/ion-pairing acid such as trifluoroacetic acid or heptafluorobutyric acid).
2. Equilibrate the trap with 5-10 trap volumes of equilibration buffer, pH 7.0. Example buffer: 5/95 acetonitrile/180mm ammonium acetate.
3. Add appropriate amount of acetonitrile and buffer to sample to equal the composition of the equilibration buffer.
4. Load sample onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.
5. Remove proteins and salts from trap and flush to waste by washing with approximately 5 trap volumes of equilibration buffer.
6. Elute small molecules from trap. If performing on-line trapping, actuate the valve to the INJECT position and then run an increasing gradient of acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% acetonitrile or “B solvent.”
7. Fully regenerate the trap by flushing it with IPA.