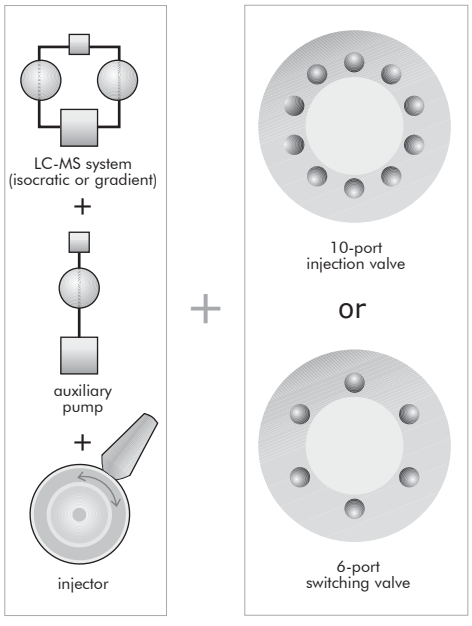
APPLICATION NOTE #101

trapping: a general guide

Trap Cartridges are available in a variety of configurations, bed sizes and chemistries.

Clean up & enrich your protein, peptide and small molecule samples on-line with OPTI-LYNX quick-connect trap cartridges.



Trap cartridges are a convenient means of handling a variety of sample clean-up and enrichment processes. Applications include sample clean-up, purification, pre-concentration, desalting and detergent removal, and more. This application note discusses general hardware requirements and other considerations necessary for setting up a basic trapping rig.

THE TRAPPING TECHNIQUE

Trapping is a chromatographic technique, but it has more in common with SPE than with LC. Trapping applications are usually devised such that the target compound (which can be either a desired analyte or an undesired contaminant) is usually in one of two retention states - either completely bound to the stationary phase within a trap cartridge, or completely unbound and free to elute. The term "trap cartridge" typically refers to a packed bed with suitable capacity to completely retain a given amount of target analyte (or contaminant) within a sample.

Simply put, the goal is usually to create conditions where either 1) analytes stick to the trap while the sample matrix and any contaminants within it are washed through to waste, or 2) contaminants stick to the trap, while the sample is unretained and is carried straight to the LC column or MS. Various refinements of this technique allow a number of common tasks to be handled on-line in more convenient fashion.

Here are just a couple of application examples:

matrix/contaminant removal...

Often, sample matrix components such as salts and detergents, or even the sample solvent itself, are not compatible with MS analysis. With the right combination of stationary phase and solvent conditions, target analytes can be trapped and these undesirable components flushed to waste before the sample is analyzed via LCMS.

sample preconcentration...

An excessively dilute sample can be concentrated prior to analysis by running the sample through a trap cartridge with affinity for the target analyte. After sufficient analyte has been trapped by the cartridge, the concentrated analyte can be eluted from the trap using a small volume of eluting solvent.

REQUIRED EQUIPMENT

For fully automated sample trapping capability, a remotely actuated 10 port injection valve or six port switching valve is required. But you could easily perform many trapping applications in simpler fashion using manual/off-line sample loading and flushing. Our current concern is with set-up of a semi-automated system, so we will rely on dedicated valves to control solvent switching. We'll need…

- Existing LC-MS system, isocratic or gradient
- 10-port injection valve (or 6-port switching valve)
- Auxiliary pump and injector or autosampler for loading of sample onto trap

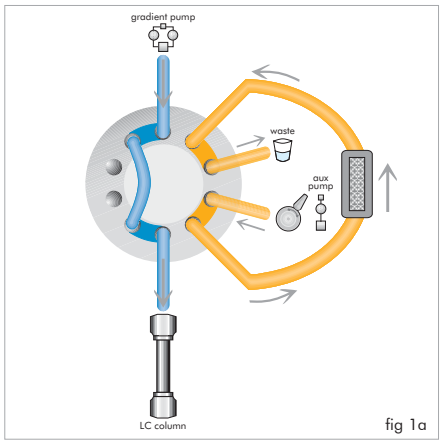


fig 1a

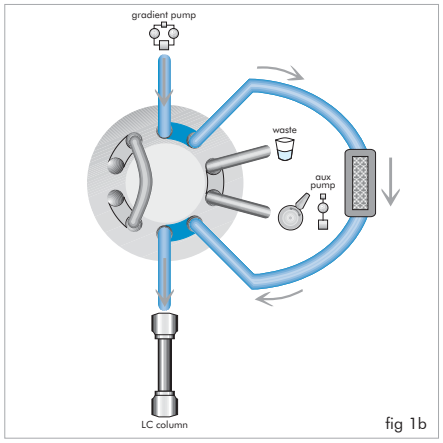


fig 1b

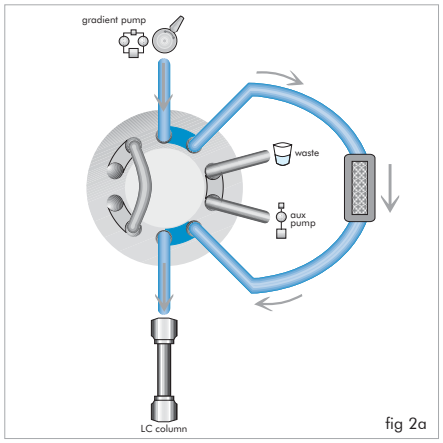


fig 2a

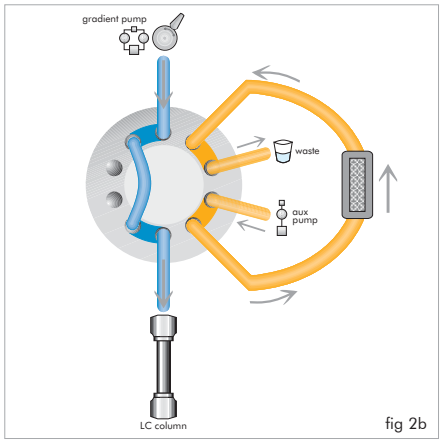


fig 2b

CASE #1: TRAP SAMPLE, FLUSH MATRIX

Figures 1a & 1b show an ideal connection scheme for facilitating off-line sample trapping and flushing, followed by direct elution onto the LC column.

LOADING THE TRAP

In fig 1a, the 10-port valve is in position for trap loading. The flow from the gradient LC pump is shorted directly to the LC column, allowing column equilibration or flushing to occur while the trap is being loaded. The trap cartridge is on it's own liquid "circuit", and is isolated from the high-pressure LC solvent path. Solvents delivered by the auxiliary pump pass through the trap and go directly to waste. An injection valve or autosampler placed between the auxiliary pump and trap allows for convenient introduction of sample to the trap cartridge.

Trap cartridge chemistry and carrier solvent are selected such that the trap has good selectivity for the analytes in the sample, but little affinity for ionic salts and other contaminants.

Once the sample is loaded and the matrix flushed to waste, elution onto the LC column or MS can begin. However, it may be desirable to wash with one or more additional solvents, depending on the nature of potential contaminants.

ELUTING THE SAMPLE

The target analyte is now adsorbed onto the stationary phase of the trap cartridge, and other components of the sample matrix have been flushed to waste. If loading conditions were optimal, the sample will also be located at the top of the trap bed. The simplest and most effective way to get the analyte off the trap and onto the LC column is to use the LC solvent flow to elute it. And so the 10-port valve is switched to the elution position (fig1b). Note that the valve has been plumbed so that LC solvent flows through the trap cartridge in reverse (i.e. in the opposite direction to that in which the cartridge was loaded). This ensures the fastest possible elution of solvent from the trap, and minimizes dilution and dispersion of the analyte.

CASE #2: PASS ANALYTE, TRAP CONTAMINANT

If a sample contains just one or two contaminants that must be removed prior to LCMS analysis, packing materials with affinity for only the contaminants can be used to separate the target analyte from undesirable matrix components. Figures 2a & 2b illustrate the fluid connections required.

INJECTING THE SAMPLE

Sample injection takes place with the trap cartridge in-line between the injection valve and LC column (fig 2a). Once the injection is made, sample and matrix flow through the trap. The target analyte is by design unretained by the trap cartridge, but target contaminants will be bound in the trap. The analyte exits the trap and proceeds to the LC column for separation.

REGENERATING THE TRAP

Since the trap cartridge has a finite capacity for adsorption of contaminants, the cartridge must either be replaced or regenerated before that capacity is exceeded. Otherwise, contaminants will get through the trap and onto the LC column during a run. If solvent conditions can be created that will cause near-complete elution of the contaminants from the trap, it can be regenerated every few runs and kept in use. Fig 2b shows the trap cartridge switched out of the flow path. The auxiliary pump is used to send regeneration solvent through the trap to waste, cleaning it of contaminants.