MULTIDIMENSIONAL APPROACH FOR THE SEPARATION OF COMPLEX BIOLOGICAL SAMPLES

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INTRODUCTION

The field of proteomics and medical analysis has propelled the demand for high resolution separation. The traditional benchmark for a high resolution technique is 2D electrophoresis, which has the disadvantage of the analytes being embedded in a resin and extensive post separation treatment is required if further analysis is needed. To improve the resolution in liquid chromatography the multidimensional approach, which was so successful in electrophoresis is adopted and modified for LC. The general approach is to utilize two different selectivities (charge and hydrophobicity or size and hydrophobicity) to subdivide a complex sample and analyse the fractions separately. Most commonly the low resolution separation forms the first dimension, while the high resolution RP separation is the second dimension. There are a large number of possible setups for multidimensional LC with various degrees of complexity.

THE PROTECOL™ APPROACH TO 2D-LC

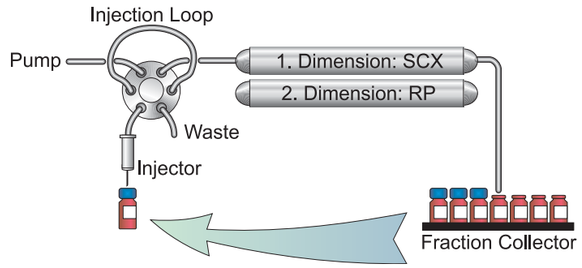
All components of the ProteCol system are precision engineered to minimize void volumes. All tubing used is made of PEEKsil™ (PEEK™ coated fused silica) combining the advantages of fused silica with the ruggedness of PEEK tubing. The capillary ends are precisely square cut and polished to allow true zero volume connections. All packing materials are chosen to give optimum performance in multi-dimensional LC for both peptides in proteomics and small molecules in drug discovery.

POSSIBLE SETUPS FOR TWO DIMENSIONAL LC:

Off-line 2D-LC:
In the first step the whole sample is separated on the strong cation exchange column and fractions are collected. Those fractions are then rerun using a reversed phase column.

Advantages:
- LC equipment does not need to be modified
- The first dimension can be a high resolution separation with a large number of fractions

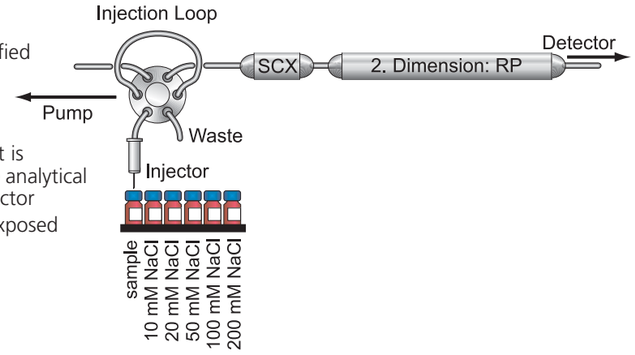
Disadvantages:
- Very time consuming, slow process
- Can not be automated



In-line 2D-LC:
A small SCX precolumn is attached to a analytical reversed phase column. The sample is loaded onto the SCX column and stepwise eluted by injecting small amounts of salt solutions of increasing ionic strength. After loading and after each elution step a standard RP gradient is run to perform the second dimension separation.

Advantages:
- LC equipment does not need to be modified
- The process can be automated

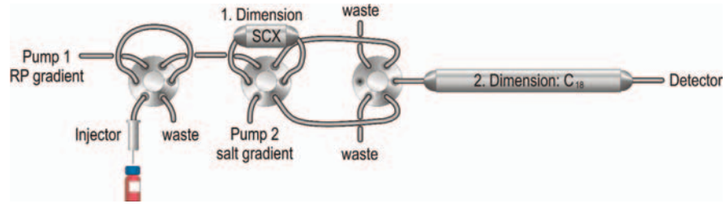
Disadvantages:
- Small amounts of salt is pumped through the analytical column into the detector
- The SCX column is exposed to organic solvents



2D-LC using off-line trap column:
A trap column packed with strong cation exchange packing and placed into a injection valve. After eluting of the trap column the trap column is taken off-line for the reversed phase analysis of the fraction.

Advantages:
- The ion exchange column is used off-line. It is therefore not exposed to organic solvents
- Having the first dimension column off-line reduces void volumes during the second dimension separation, thus improving the chromatographic performance
- Since the SCX- and the RP part of the system are separated they can be regenerated independently

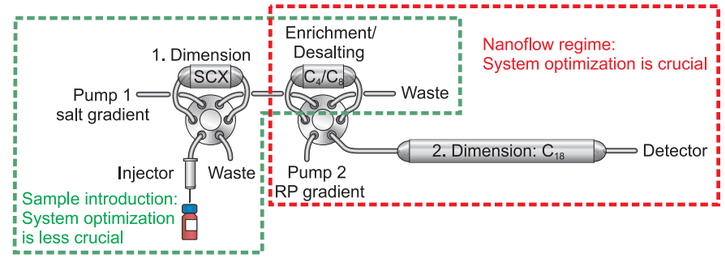
Disadvantages:
- Small amounts of salt is pumped through the analytical column into the detector
- Depending on the equipment used the injector part might have to be reconfigured / modified



Multidimensional LC using trap and desalting column:
The above configuration can be further sophisticated by eluting from the SCX column onto a C4 or C8 desalting column.

Advantages:
- Only allows MS compatible solution to the detector

Disadvantages:
- Some sample components which are not retained by the desalting column might go to waste and will not be analysed
- Need to introduce a second 6-port valve for the desalting column



POSSIBLE SETUPS FOR TWO DIMENSIONAL LC:

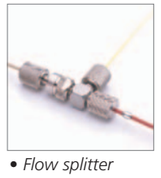

• *ProteCol™ – precolumn / guard column*


• *ProteCol™ – trap / desalting column*

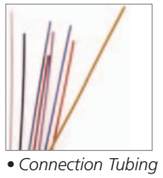

• *ProteCol – analytical column*


• *In-line filters*


• *Zero-volume unions*


• *Flow splitter*


• *ProteCol flowmeter*


• *Connection Tubing*

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