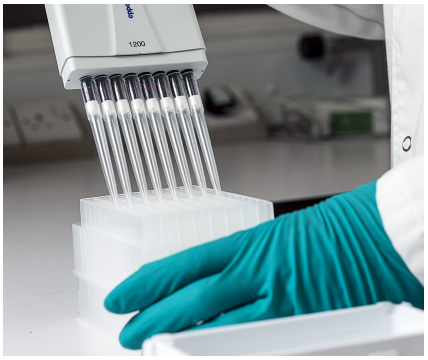
# Biological Sample Preparation using Solid Phase Extraction and Protein Crash Techniques in 96 Well Plates

## The Problem



Removing unwanted large biomolecules from ex-vivo samples such as blood, semen, serum, urine and spinal fluid in order to allow quantitative analysis of small molecules in these samples has been addressed in several ways. The prime interfering components that need to be removed are proteins. These macromolecules become very sticky and can cause damage to analytical instruments, especially chromatographs. The large size of the protein clogs up the small pores in a chromatography column.

Chromatography is the standard method of separation in Life Science research where quantitative analysis is required. It can be performed using Gas- or Liquid- chromatographs. Both are susceptible to damage by protein molecules. There are other naturally occurring substances which appear in serum that can also cause blockages in analytical columns. This group is known as phospholipids (PL), a derivative of fatty acids which as might be expected are also relatively "sticky".

### The Porvair Sciences Solution

Porvair sciences currently offer 2 types of 96 well filter plate for the removal of proteins from serum samples up to 2 ml in volume. The simplest and cost-effective method is the P3 (Protein Precipitation Plate), whilst the C18 SPE plate is more sophisticated. The choice of plate & method will be determined by the samples in use, the desired analyte and the level of sensitivity required.

Generally, samples processed on P3 plates are suitable for analysis on the wider bore columns of HPLC systems, whilst those prepared on a C18 SPE plate are suitable for GC analysis using capillary columns. The problems arise when these two systems are hyphenated to a Mass Spectrometer.

LC-MS is now a very common technique and very high resolution GC-MS can now be used to test for low levels of metabolites and drug-like compounds in serum or blood samples. However, at these levels the phospholipids become a bigger problem and a class of PLs known as lysophospholipids (LPL), which are not removed by the C18 silica, can cause anomalous effects in the GC-MS signals recorded.

The LPL causes a phenomenon known as Ion Suppression, in which the high background signal caused by the LPL masks the true signal generated by the analyte. It can both raise and lower the observed analyte signal and is thus difficult to correct for.

The Protein Crash technique used in the P3 plate does not remove either PL or LPL, but is very effective at removing proteins. It works by denaturing the protein using methanol or acetonitrile. The protein "crashes" out of solution and forms large sticky globules. These are trapped on an open-pore structure filter, beneath which is a smaller superhydrophobic filter which retains all liquids above it until a vacuum is applied to force the liquid containing the analytes of interest through the filter and into a collection plate below.

The traditional Solid Phase Extraction (SPE) method uses a "sandwich" of very fine silica, functionalized with long-chain hydrocarbons (18 carbon atoms) between two supporting frits. The long carbon chain is oleophilic and retards the passage of fatty acids and globular proteins. However, the smaller LPLs, which are highly polar due to an ionised phosphate group, pass straight through. The SPE plate needs extra conditioning and washing steps in addition to the loading step used in P3. It is therefore more time consuming and harder to automate.

In the experimental plate developed by PTL, an extra compound, Lanthanum Carbonate, is mixed with the c18 silica. Lanthanides bind strongly to phosphate groups and it is suggested that the LPL will bind to the lanthanide. However, without access to sensitive GC-MS equipment it is not possibly to quantify the efficacy of the lanthanide / phosphate removal system. It is also possible that other Lanthanide compounds could be used with better effect.

| Product Description |
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| **P3 Protein Precipitation Plate High Efficiency (packed individually, rigid shell)**<br>Product code: 240010 |
| **P3 Protein Precipitation Plate (packed individually in a rigid shell)**<br>Product code: 240100 |
| **P3 Protein Precipitation Plate (Bulk Packed in a bag of 5, no individual shells)**<br>Product code: 240200 |