Chromatography Solutions

Technical note #SP002

J.T.Baker® BAKERBOND® Protein Precipitation (PPP) and Supported Liquid Extraction (SLE) Plates for Efficient LC-MS Sample Preparation

INTRODUCTION

Liquid chromatography coupled with mass spectrometry (LC-MS) is a highly specific, sensitive and rapid technique that can be used for the determination of low-level target analytes in complex biological matrices and is routinely applied for many bioanalytical analyses. Using this approach, analytes are separated by LC, prior to detection by MS. The MS can be used to provide highly sensitive and selective identification and quantification of target analytes.

Biological matrices, such as plasma, serum and urine, are usually highly complex, comprising of high concentrations of endogenous components, alongside often low relative concentrations of the target analyte(s). Injection of neat biological fluids onto the LC-MS system is typically not practical, as this would result in a plethora of issues including, but not limited to:

- Particulate build-up on the column, resulting in increased back pressure
- Back pressure build-up/blockage due to matrix components precipitated in the mobile phase
- Contamination of the column, resulting in poor peak shape and retention time shifts
- Contamination of the LC-MS instrumentation, causing sensitivity and carryover issues

These issues are highly undesirable, particularly in high throughput scenarios, as they result in significant reduction in chromatographic performance and column lifetime, more frequent instrument down-time and increased instrument maintenance and cleaning requirements.

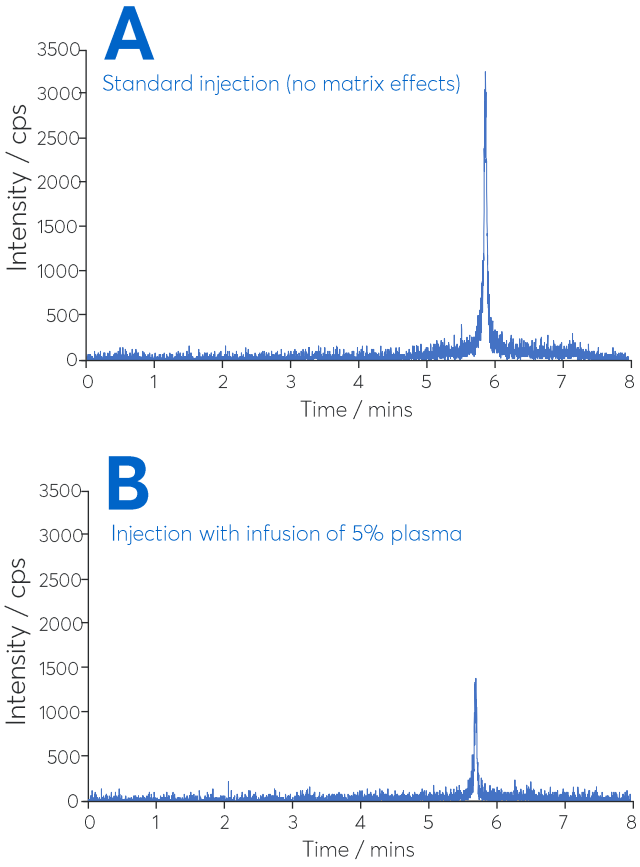
An additional and critical consideration is the effect that matrix components can have on the analytical data obtained from the assay. Despite chromatographic separation of the target analytes, the complexity of biological matrices and typically low relative abundance of target analytes, means that matrix components can interfere with analyte response, preventing accurate

Quantification.[1] If, as is likely in complex samples, more than one compound is eluted simultaneously from the LC column, then there is potential for interference. This ultimately can result in either a decrease (suppression) or increase (enhancement) in signal response for the target analyte. The matrix can therefore have a significant impact on sensitivity, and the accuracy of analyte quantification.

An example of the potential impact of interfering matrix components is demonstrated in Figure 1. In Figure 1A, a standard solution of estrone was injected and analysed on a reversed-phase gradient using an ACE® Excel® SuperC18 column and the 269.1 → 145.0 MRM transition monitored. The experiment was then repeated with infusion of a 5% solution of protein precipitated plasma directly into the MS source. The presence of plasma components has a significant impact on the response obtained. A 48 % reduction in MS detector response was obtained. This loss in sensitivity is a result of the effect on estrone ionisation by matrix components.

STRATEGIES TO REMOVE MATRIX COMPONENTS

It is clear that matrix components which may interfere with the analysis, should preferably be removed prior to analysis. The purpose of sample preparation is to remove these interferences in an efficient, reliable and reproducible manner. J.T.Baker® BAKERBOND® provide several approaches for sample preparation and removal of matrix components from biological samples, including protein precipitation (PPP), supported liquid extraction (SLE) and solid phase extraction (SPE).[2] This technical note focusses on the application of PPP and SLE for sample clean-up. These approaches utilise a 96 well plate format (Figure 2) to allow for simplified, high-throughput application in analytical workflows.



Column:	Avantor® ACE® Excel SuperC18
Particle Size:	2 µm
Dimensions:	100 x 2.1 mm
Mobile Phase:	A: 20 mM ammonium acetate (aq) B: 20 mM ammonium acetate in MeOH/H_2O (90:10)

Gradient:

Time (mins)	% B
0	2
0.1	60
2.1	60
2.2	98

Flow Rate:	0.4 mL/min
Injection:	1.0 µL
Temperature:	50 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system
Ionisation mode:	ESI, negative mode
Source temp:	350 °C
Curtain gas:	38 psig
Source voltage:	-3500 V
Ion source gas 1:	40 psig
Ion source gas 2:	70 psig

Figure 1: Demonstration of the influence of matrix effects on the analysis of estrone (MRM 269.1→ 145.0) by LC-MS. A: injection of a 100 ng/mL estrone standard. B: Injection of a 100 ng/mL estrone standard with infusion of a 5 % rat plasma solution into the MS source at a flow rate of 7 µL/min.

Figure 2: J.T.Baker® BAKERBOND® 96 well plate.

PROTEIN PRECIPITATION PLATES

Biological samples containing proteinaceous matrix components, that may interfere with downstream analytical methods, require clean-up prior to analysis to remove these substances. Additionally, such components can precipitate when introduced into the LC mobile phase, leading to column or system blockages. Isolation of high quality, protein-free samples is therefore a vital prerequisite for successful LC-MS analysis. J.T.Baker® BAKERBOND® 96 well Protein Precipitation Plates use a crash method to provide a simple and effective solution to endogenous protein removal. Proteins in the sample are precipitated using an organic solvent and removed by filtration through a hydrophobic frit.

The 96 well format allows for high-throughput and reproducible protein removal. Precipitation of proteins is achieved by the addition of 3 volumes of acetonitrile, followed by gentle mixing and incubation for a short period, typically up to three minutes.[3] The sample is then recovered by application of a vacuum or centrifugation. The novel hydrophobic frit is designed to retain the sample without it draining away under high organic conditions, whilst the low-binding characteristics ensure maximum analyte recovery. These attributes and ease of automation make J.T.Baker® BAKERBOND® Protein Precipitation Plates an ideal choice for high-throughput protein precipitation

SUPPORTED LIQUID EXTRACTION

Traditional liquid-liquid extraction has been used for centuries to enrich or purify solutes, based on differential solubility in aqueous and organic solvents. The dissolved analyte solution is combined with an appropriate immiscible aqueous or organic solvent and shaken. The analyte preferentially partitions into one of the phases, whilst other sample components ideally partition into the

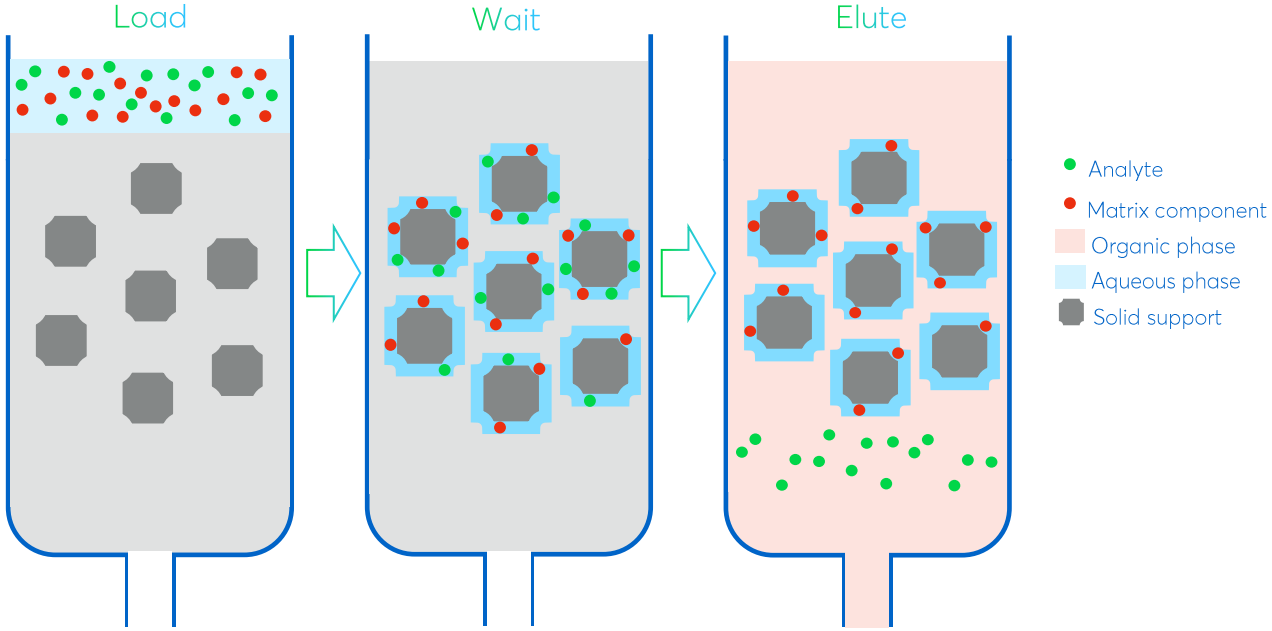


Figure 3: Schematic representation of extraction of an aqueous analyte using the SLE approach.

opposite phase. The phase containing the analyte is then removed, the extraction repeated and the sample concentrated by evaporation prior to analysis. Although effective, this process is typically lengthy, requires considerable volumes of solvent and is more suited to extraction of large volume samples. Smaller scale versions of liquid extraction have been commercialised, however the process is highly dependent on the mixing of two immiscible liquids, which can be challenging when dealing with very small volumes.

SLE can overcome these issues and can be performed on a smaller scale, is compatible with biological samples and can be applied using a high degree of automation. The technique is widely applied for the clean-up of complex matrices in a diverse range of analytical fields, including environmental, clinical, forensics and food and beverage. In SLE, the aqueous solvent, which is pH adjusted to ensure the analyte is non-ionised, is supported as a very thin layer on an inert support (Figure 3), typically diatomaceous earth (a siliceous material composed of the fossilised exoskeletons of diatoms), whilst the immiscible organic solvent percolates over the support to facilitate analyte transfer.[4] The high surface area of the support, combined with the thin aqueous film, makes the transfer significantly more efficient compared to traditional liquid-liquid extraction, with dramatic reduction in solvent requirements and greater reproducibility and recoveries. Additionally, sample processing is more efficient as laborious mixing steps are eliminated, emulsion formation is avoided and the process is readily automated for high-throughput analysis. Targeted analyte extraction can also be achieved through judicious selection and optimisation of the aqueous phase. J.T.Baker® BAKERBOND® SLE plates incorporate a diatomaceous earth packing material into a high-throughput 96 well plate format for quick, reproducible sample processing and excellent analyte recovery from biological matrices. This SLE solution is also available with two sorbent bed weights and a 3 mL cartridge format for larger volume samples.

CONCLUSION

The use of appropriate sample preparation techniques, prior to the analysis of biological samples, is highly beneficial. Their use typically results in improvements to method reproducibility, sensitivity and quantification accuracy, along with reduced LC-MS system maintenance requirements and down-time. PPP and SLE are effective and widely utilised techniques for the removal of endogenous matrix components from complex matrices. In addition, regulatory considerations such as requirements for analyte resolution from interfering peaks that can potentially invalidate a method, variability in sample interference between samples and an understanding of sample matrix effects as part of bioanalytical validation support the use of sample preparation protocols.

J.T.Baker® BAKERBOND® PPP and SLE plates provide a high performance, reproducible and fully automatable solution for the quick and efficient removal of proteins using PPP and selective removal of matrix components with SLE prior to LC-MS analysis. The removal of interfering matrix components improves the reproducibility and sensitivity of bioanalytical assays, whilst also extending the lifetime of the LC column and reducing LC-MS maintenance and instrument down-time.

REFERENCES

1. Annesley, T. M. *Clinical Chemistry* **49** (2003) 1041–1044
2. J.T.Baker® BAKERBOND® spe 96-well plates
3. J.T.Baker® BAKERBOND® PPP protocol
4. J.T.Baker® BAKERBOND® SLE protocol

Protein Precipitation 96-well plates

Description	Sorbent weight	Pack size	Part Number
BAKERBOND® Protein precipitation plate	200 mg	1	8181-96
BAKERBOND® Protein precipitation plate	200 mg	5	8181-596

SLE 96-well plates & cartridges

Description	Sorbent weight	Pack size	Part Number
BAKERBOND® SLE 96-well plate	200 mg	1	8182-96
BAKERBOND® SLE 96-well plate	400 mg	1	8183-96
BAKERBOND® SLE 3 mL cartridge	200 mg	50	8182-02
BAKERBOND® SLE 3 mL cartridge	400 mg	50	8183-04

Accessories

Description	Part Number
Vacuum manifold for multi-well plates	8183-VM
Mid gasket for vacuum manifold 8183-VM	8190-RG
Top gasket for vacuum manifold 8183-VM	8185-RG
Space insert for vacuum manifold	8186-SI
Disposable Reservoir Tray 25pk	8188-RT