Effect of Matrix loading on Signal Response in Bioanalysis

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Introduction

In recent years there has been more emphasis placed on speed in bioanalysis . Utilising ultra high pressure liquid chromatography (UHPLC) has become more widespread, analysts can now routinely utilise smaller particles to increase efficiency of the separation, leading to increased sensitivity, speed and/or resolution. Another option that has recently been adopted is the use of “fused-core” or “core-shell” particles, which have the potential benefit of efficiency without the elevated back-pressure encountered with UHPLC particle columns (and therefore precluding the need for specialist UPLC pumping systems).

In this poster we discuss the various options available for high throughput bioanalysis and assess some of the implications of these technologies in comparison to traditional 3mm particle HPLC configurations. Spiked blood samples are analysed, at typical concentrations from pharmokinetic studies.

We consider fundamental issues such as peak capacity, peak width, surface area and matrix effects and how they may compromise the resolution that can be achieved.

Background

There are multiple implications of matrix effects with biological samples including,
- the speed of analysis
-column overload occurs from both the matrix and the analyte.
-broader peak widths
-compromised speed in terms of the theoretical maximum.
-peak width of the analytes affects peak capacity.

How fast can we actually go, whilst still retaining accuracy of analysis?

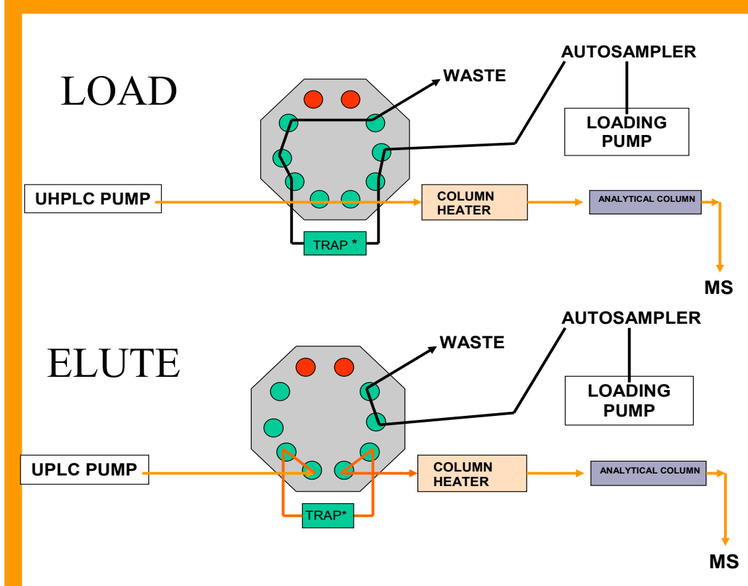
Another variable often used in increasing speed is the use of elevated temperatures, the compromises with increasing speed this way are discussed such as thermally labile compounds and limitations in peak capacity etc.

Applications highlighting all of these effects are discussed in a bioanalytical context - comparing peak shapes to peak capacity and realistically achievable analysis times when applied to samples from pharmacokinetic studies. We discuss the use of speed in realistic terms of peak capacity, peak width and sample loading.

Methodology

The current set up we are using is a dual pump, trap wash and elute system, the diagrams below show the trap plumbing used (the same plumbing can be set up on a standard 6 port valve).

FIGURE 1. Chromatographic set-up



For the loading pump we are using MF5 (spiked with 0.1% TFA) as MP A, and 40/30/30 MeOH/IPA/Acetone with 1% acetic acid as MPB (used as a strong wash across the injector and trap between injections)

For the eluting pump we have MF5, MF4 as mobilephase due to the methanol based MP giving better response than the original 0.1% formic /Acetonitrile we started with.

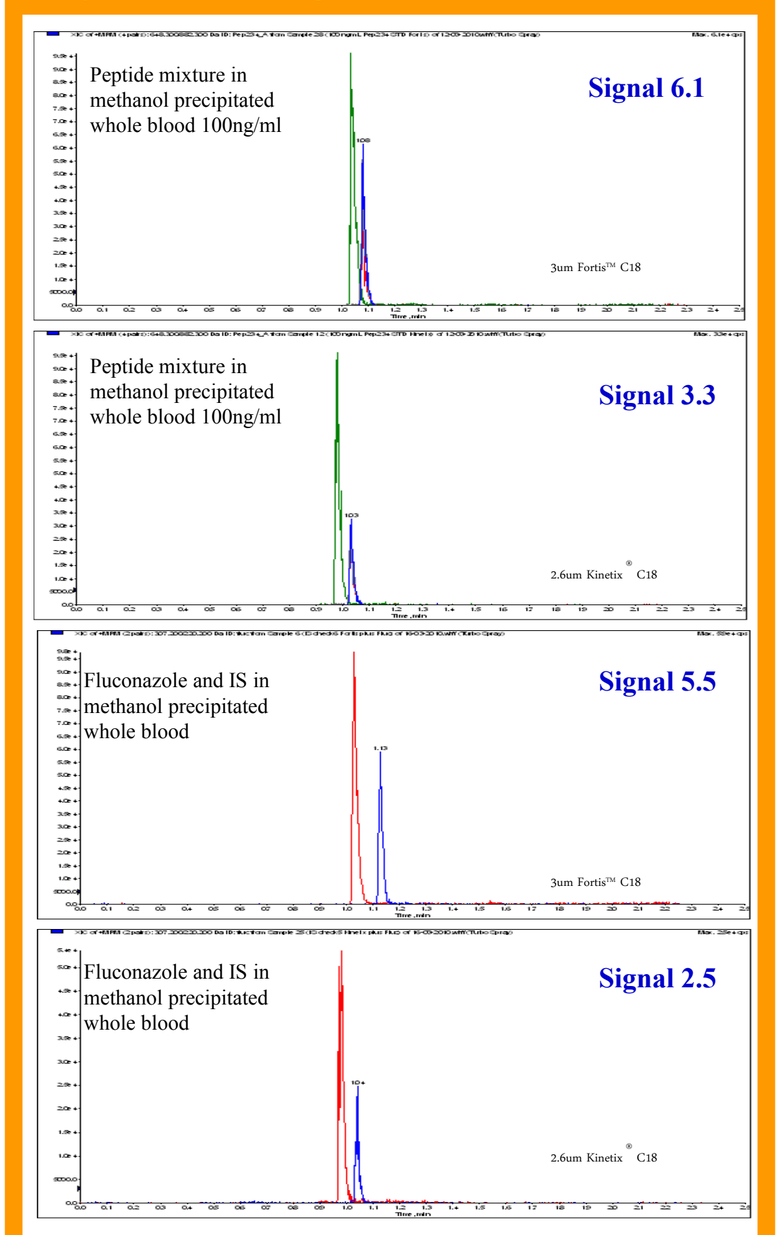
The initial load onto the trap cartridge is held for 30 seconds (0.5 mL of MPA) then the trap switches in line with the eluting pump at 0.5 mins, the eluting gradient runs through the trap to analytical column, then at 1.5 minutes the trap is taken back out of line to wash with the strong solvent (loading MPB) whilst the eluting column finished the chromatography. The trap cartridge is a Halo™ C18 4.6 x 5 mm cartridge. Chromatography is performed on a 3 x 50mm analytical column.

50 µL of blood “crashed” with 500 µL of methanol: 150 µL of supernatant transferred to assay plate and 150 µL of 0.1% TFA in water added, samples mixed and 200 µL injected.

Results

Chromatograms in figure 2 show varying retention times and signal response for sample components. These variances arise from differences in the surface area values of the column phases.

FIGURE 2. Test compounds from whole blood extracts – comparison with fused core and conventional phase, comparison of sensitivity and retention



Due to the nature and construction of the core shell materials they have a limited surface area for sample interaction ~200m^2g^{-1}, whereas more traditional hplc phases can provide surface area values > 350m^2g^{-1}.

This lack of surface area and hence sample loadability means that overload of the sample matrix results in lower sample peak heights/sensitivity once the matrix response is subtracted.

This drawback is also evident in most sub 2um UHPLC phases(see figure 3), however the 1.7um Fortis C18 provides a high surface area which results in increased retention (see figure 4) and improved sample loading (see figue 5).

FIGURE 3. Phase Surface Area

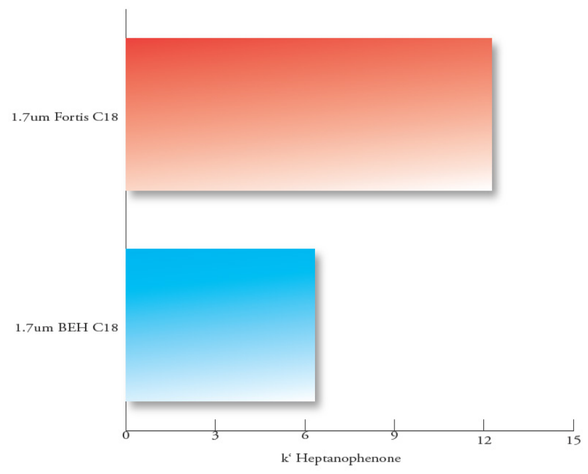
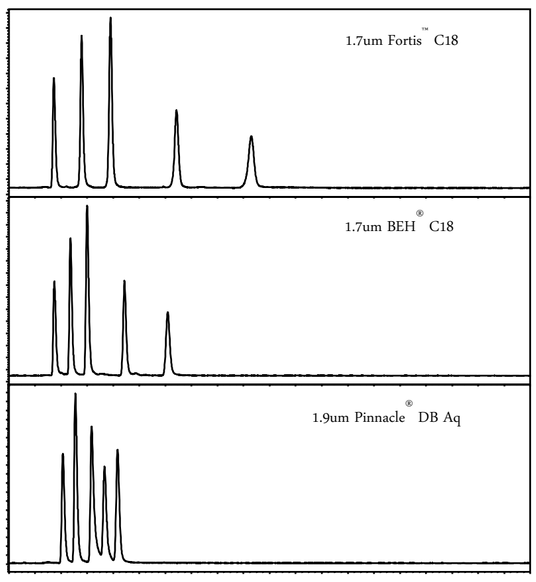
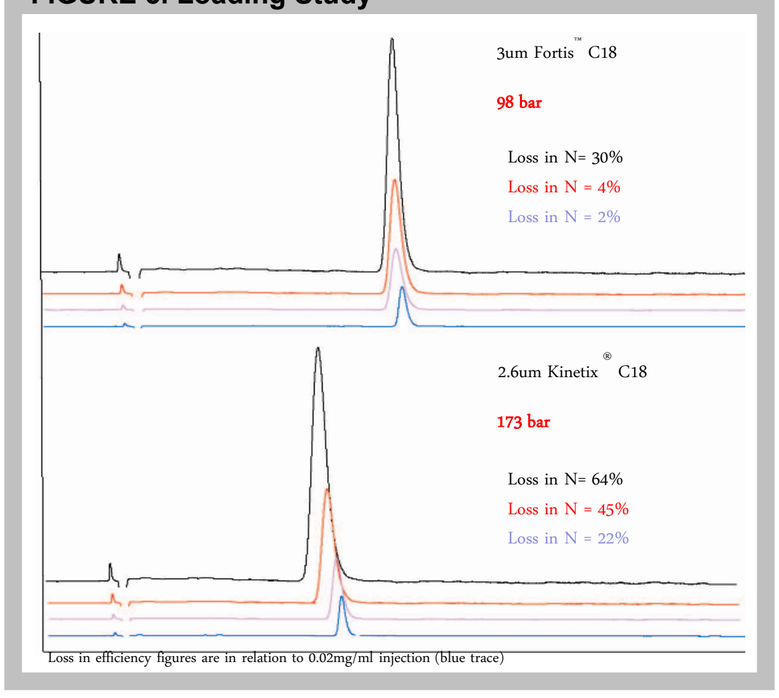


FIGURE 4. Retention on sub 2um UHPLC columns



Column: 50x2.1mm C18 1.7um
Flow: 0.4ml/min Temperature: 25°C Wavelength: 254nm

FIGURE 5. Loading Study



Loss in efficiency figures are in relation to 0.02mg/ml injection (blue trace)

This high surface area(S.A.) can then be utilised in a number of ways. Firstly increased retention and therefore more resolving power or secondly reduction of column length to reduce system backpressure (see figures 6+7).

FIGURE 6. Using S.A. to Reduce column length

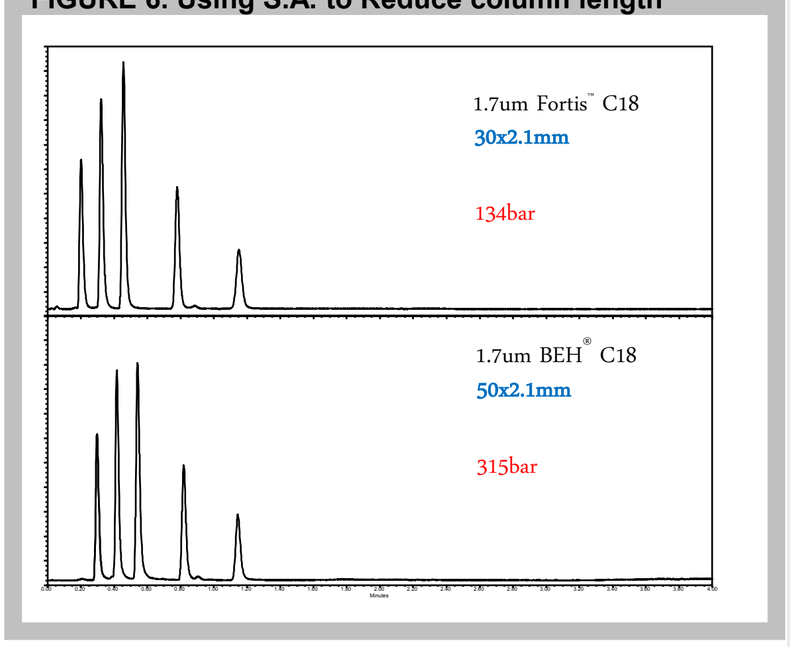
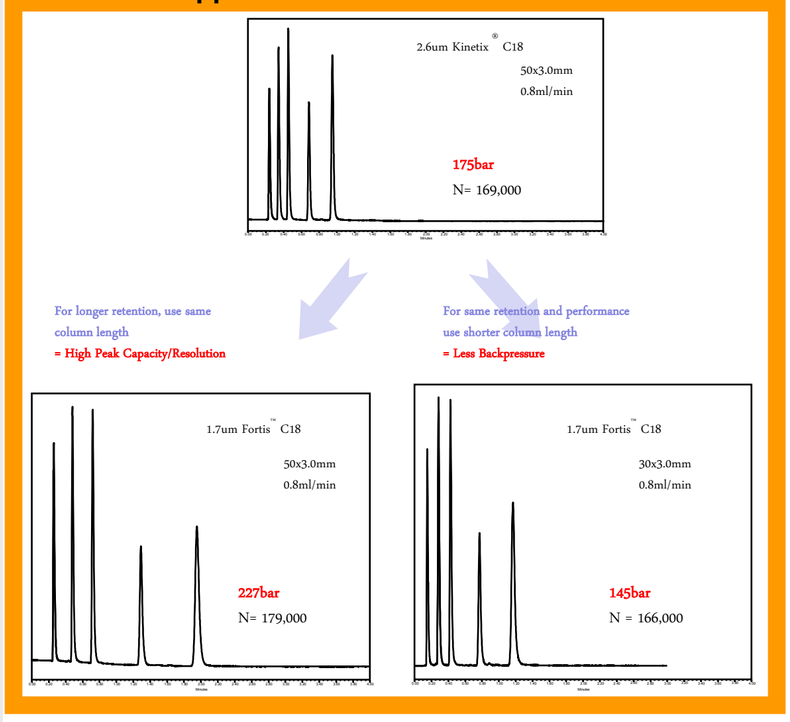


FIGURE 7. Application of Surface Area



Discussion

Fused-core and <2µ uHPLC HPLC columns have advantages of speed and sensitivity of analysis vs. traditional HPLC. However, there are disadvantages to these column types for bioanalysis:

- **Susceptible to blockages.**
- **Higher operating back pressures.**
- **Limited to smaller sample volumes.**
- **Requires cleaner extracted samples (time consuming sample preparation).**

‘Not applicable to analysis of ‘crude’ discovery samples’

The data we have so far eludes to better sensitivity, less ion suppression and lower back pressures when using fully porous 3um particle phases, this equals better quality data and reliability.

Further work will now be carried out using fully porous particles including the new higher surface area sub 2um particles now available.

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