Compatability of UHPLC with MS, are we more productive ?

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Introduction

Recent interest in LC-MS has revolved around the possibility of moving to smaller LC particles; the goal being increased efficiency and the advantages that this can provide as the inlet to MS. Although previous work[1] has shown that for short fast gradients small UHPLC particles offer little or no improvement in peak capacity when compared with well-packed 3um particle columns, they do offer improved sensitivity under these same conditions and greater sensitivity in LC translates to greater sensitivity in the MS. Where small UHPLC particles do show a significant efficiency increase over tradition columns is in longer columns and shallower gradients, so much so that the debate as to their usefulness has ceased.

Whilst MS can itself provide resolution, for critical applications and highly complex mixtures LC resolution is still necessary in order to reduce matrix effects, increase target identification and maintain sensitivity. In this poster we discuss the use of LC across the pH range, how to maximise resolution prior to MS detection and the ability to move to smaller particles as well as the implications that this has in analyte response and sensitivity in the MS detector.

Methodology

By looking at the Carr equation(figure 1) we can see that the three factors contributing to resolution are efficiency, retention and selectivity. The variable to be utilised in UHPLC is efficiency (N) but even this is a reasonably shallow slope in relation to what can be achieved by the selectivity (α) term.
How can we achieve this selectivity and still maintain compatibility with MS, how does the introduction of UHPLC affect our method development and use of LC-MS?
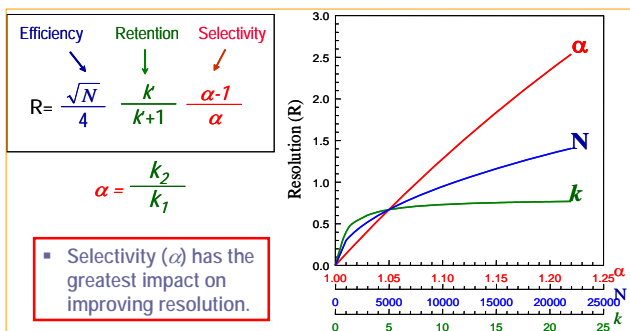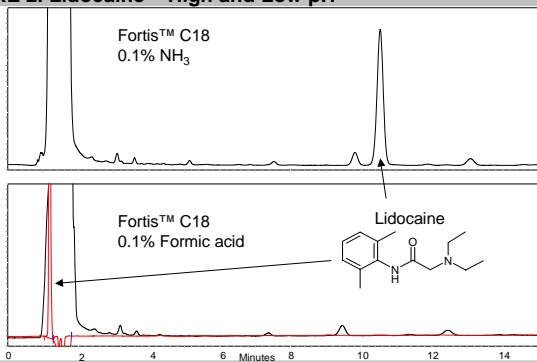
FIGURE 1. Contributions to Resolution

$$R = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha-1}{\alpha}$$

$$\alpha = \frac{k_2}{k_1}$$

- Selectivity (α) has the greatest impact on improving resolution.

FIGURE 2. Lidocaine – High and Low pH

Fortis™ C18 0.1% NH₃

Fortis™ C18 0.1% Formic acid

Lidocaine

Retention away from matrix through pH adjustment

One major contributor to selectivity is the use of pH, if we consider the separation of Lidocaine (Figure 2), a basic molecule. At low pH there is no retention due to its ionised form being polar, from a MS point of view this lack of retention will lead to problems from the matrix that the sample is in. If however we can gain sufficient LC retention then we are able to suppress any problem arising from matrix interference. The use of a much higher pH leads to the basic analyte existing in its neutral state and therefore retaining by hydrophobicity (Figure 3). The MS compromise here is that the source (ESI or APCI) will have to reform an ion in order to detect with high sensitivity. Another advantage of using high pH in this instance is that the molecule can now be eluted with higher organic solvent contribution which aids the mobile phase vaporisation and leads to more sensitivity.

Resolution of same m/z samples through selectivity

In Figure 4 we see the advantage of gaining good selectivity, we can move to a smaller 2.1um particle from a standard C18 3um column and gain efficiency, but in the case of the two positional isomers we have still not achieved baseline separation. Since the m/z is the same for these analytes then qualification and more importantly quantitation is made difficult with MS. Changing the selectivity with the use of a di-phenyl stationary phase gives us sufficient separation that we can now afford to reduce the column length and gain more speed
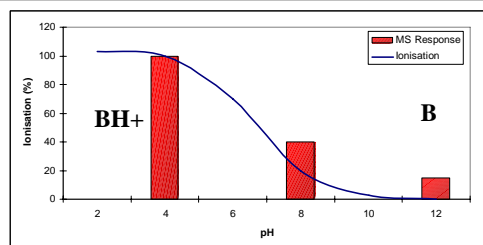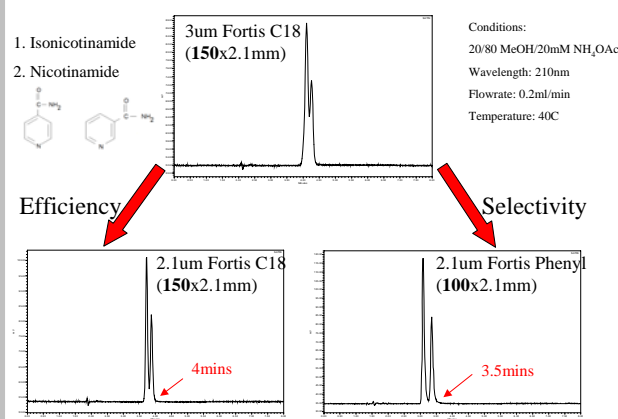
FIGURE 3. Ionisation and MS response vs pH

BH+   B

FIGURE 4. Positional Isomers - Resolution of Nicotinamides

1. Isonicotinamide
2. Nicotinamide

3um Fortis C18 (**150**x2.1mm)

Conditions:
20/80 MeOH/20mM NH₄OAc
Wavelength: 210nm
Flowrate: 0.2ml/min
Temperature: 40C

Efficiency   Selectivity

2.1um Fortis C18 (**150**x2.1mm)   4mins

2.1um Fortis Phenyl (**100**x2.1mm)   3.5mins

Improved response & resolution through column selection

If we look at a highly complex environmental sample, 135 transitions, analysed on two C18 columns we can see some more parameters for good separation and sensitivity, stronger retention on one column leading to better resolution. The other variable affecting peak height here is peak width, even on the 3um columns used here the sensitivity of sample is quite different

FIGURE 5. Resolution & Sensitivity Gains

Fortis™ C18   6.0e5

Atlantis™ dC18   4.8e5
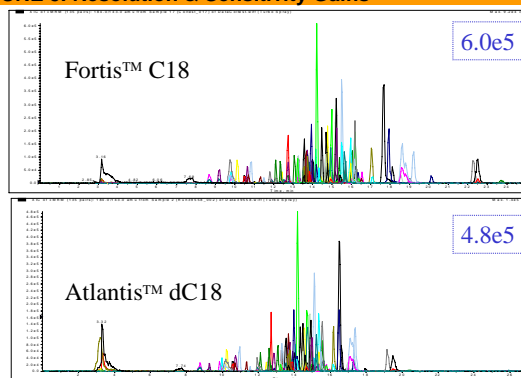
Discussion – Productivity ?

Are we more productive with UHPLC attached to MS and relying upon efficiency alone? We set out to ask this question: undoubtedly we can do method development in a shorter time if we can speed up our analysis. However in terms of throughput of samples serious consideration has to be given to the daily logistics of UHPLC use:

1. The ability to run twice the number of sample overnight due to reduced analysis times still leaves us with the issue of increased same prep and data analysis time.
2. We have an 'extreme pressure' system, will this give us more issues in repair and 'down-time'?
3. Will a chemist wait at the open access system due to 2x3min runs, whereas they went and did other work when it was 2x10min runs, more productive?
4. Do we need more/better sample clean up due to potentially easier blockages?
5. Is our method development robust and reproducible with UHPLC to HPLC to Prep?
6. Is our data collection rate fast enough for the sharper peaks now eluting?
7. Is our tubing set-up and length of tubing appropriate for the new low 'dead-volume' LC system?

Conclusion

UHPLC is definitely the next evolution in chromatography, the use of small particles offers us more efficiency which can be used to increase speed, resolution and sensitivity. However over reliance upon the term "efficiency" must be avoided, efficiency alone will not be sufficient for good chromatography - selectivity plays a vital role here.
We have shown that through the correct selection of particle size, pH and phase chemistry we are able to reduce analysis time and improve our MS sensitivity and target identification. Nothing should detract from good chromatography, no matter how good the MS. If LC is not optimum then the MS will be compromised as we have demonstrated.

References
1. K.Butchart, et al. Int. Labmate(2007) Vol. XXXII Issue V
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