

Monodisperse Fully Porous Particle (MFPP) Use For Increasing Resolution in Liquid Chromatography



Dr Yvonne Walsh, Ken Butchart, Mark Woodruff • Fortis Technologies Ltd, 45 Coalbrookdale Road, Cheshire, CH64 3UG, UK.

Introduction

In this poster we discuss the use of a new range of stationary phase chemistries allied with monodisperse fully porous silica particles (MFPP).

One of the major challenges in LC and LC-MS is achieving full resolution of compounds especially when metabolites and/or isomeric species are involved. By combining the high efficiency of a monodisperse silica with new diverse stationary phase ligands, we have the potential to gain more selectivity and resolution between analytes.

Whilst C18 and C8 alkyl chain stationary phases are the most common choice for starting method development, they cannot achieve all separations with the required resolution sufficient to provide accurate qualitative results. The use of orthogonal stationary phases containing halogenated, aromatic or polar character allows differing mechanisms other than just hydrophobicity to be employed.

We discuss the use of several mixed stationary phases which allows more mechanisms of interaction to be used in the separation process to obtain more resolution.

Unique L1 Column Chemistries

In the resolution equation, selectivity (α) is by far the strongest contributor to resolution so having a range of chemistries that provide a broad range of selectivity is crucial. With C18 (USP L1) phases being the most widely used as a starting point in method development it is therefore useful to provide extra selectivity options within this column classification.

FIGURE 1. Unique L1 Classification C18 Phases

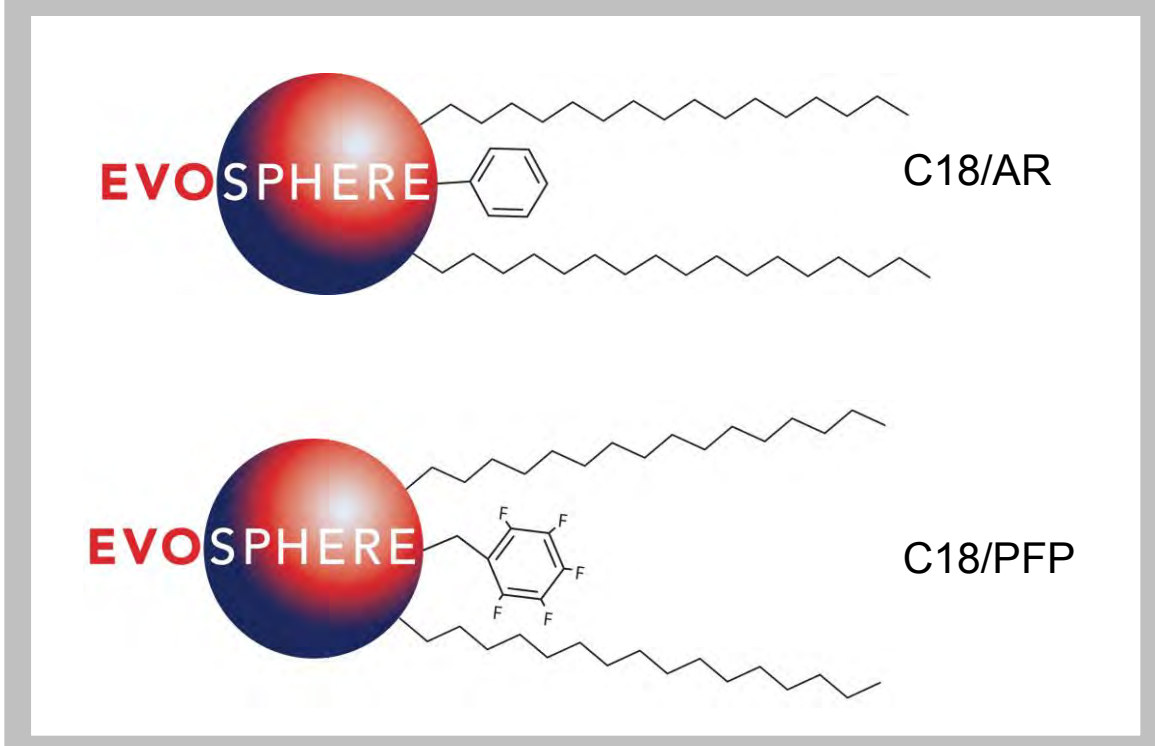
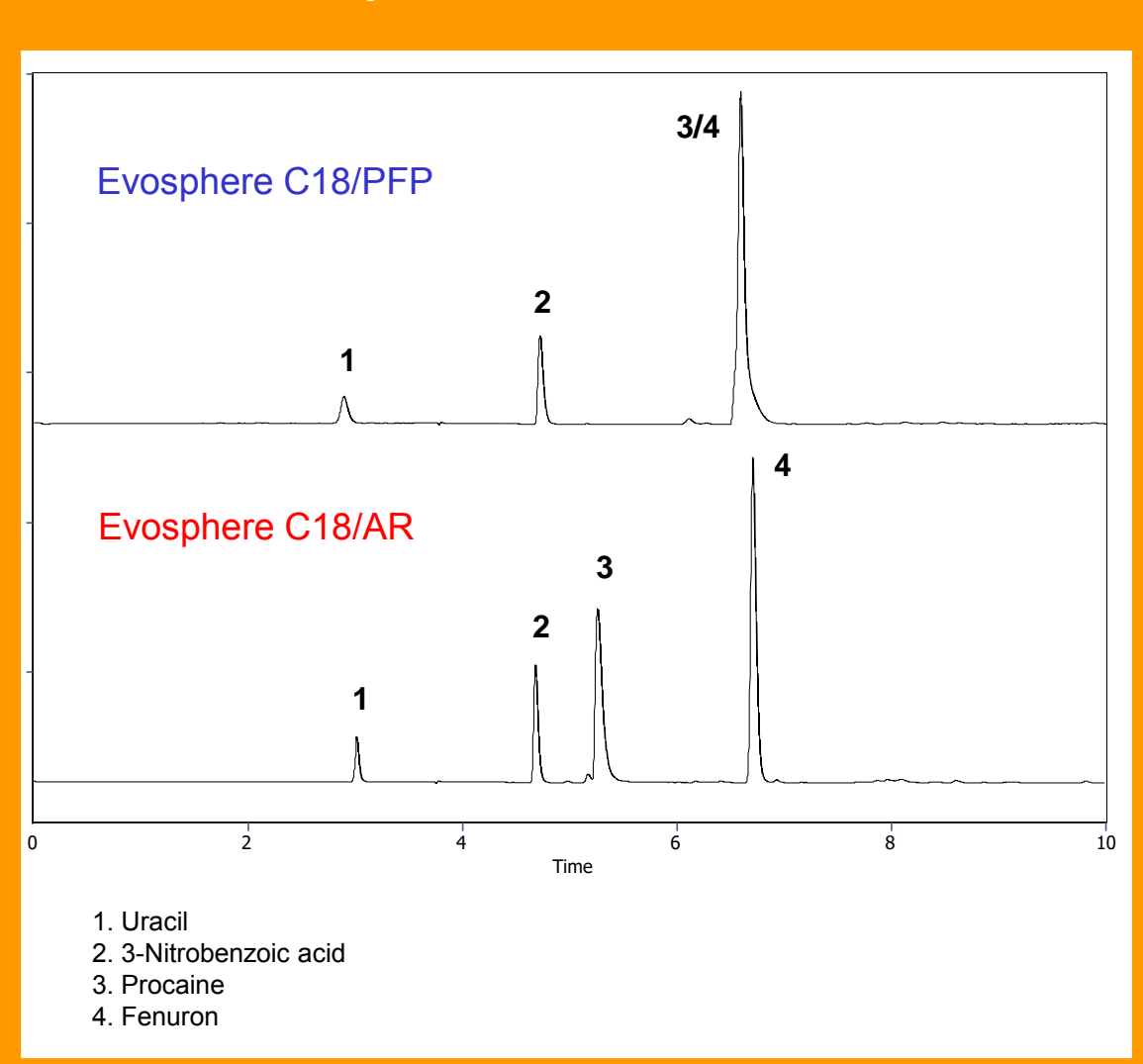


Figure 2 shows how two predominantly C18 phases, both in the USP L1 category, can offer orthogonal selectivity for acids, bases and neutral compounds. This makes these phases highly complementary to each other when developing a new method from scratch. The inclusion of a second ligand in these phases imparts extra mechanisms of interaction such as steric, pi-pi and ion charge.

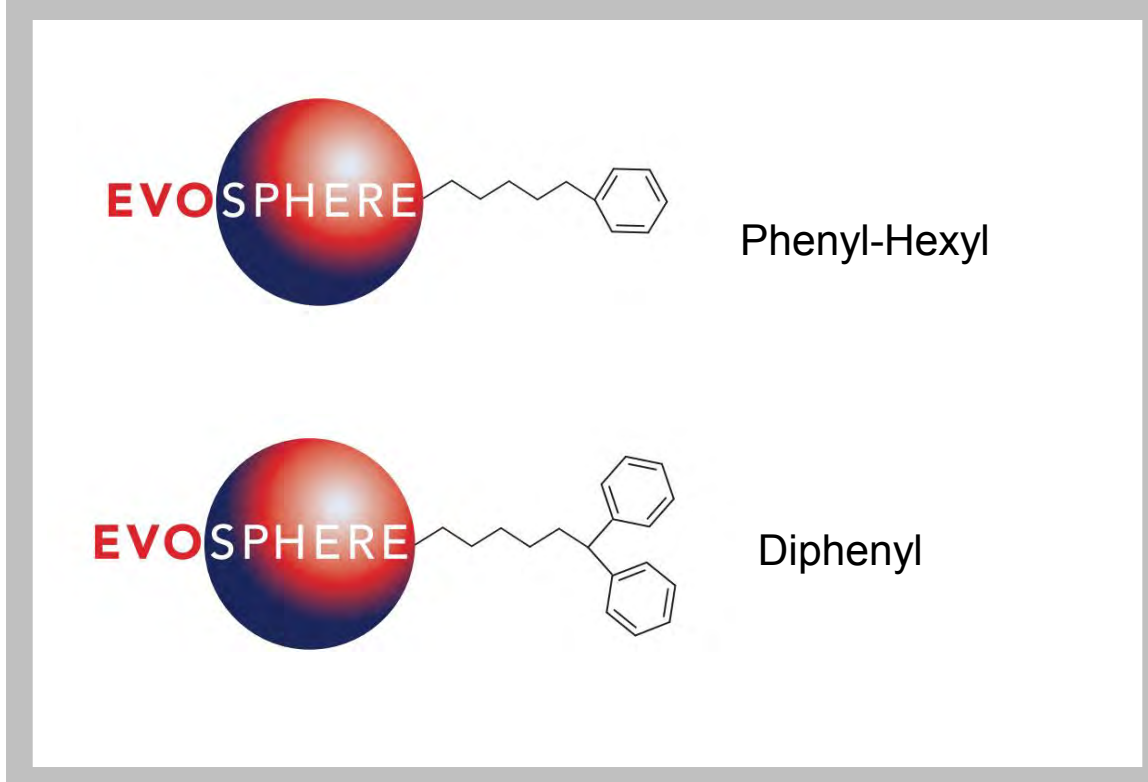
FIGURE 2 Selectivity between C18's



Unique Phenyl Chemistries

Multiple aromatic based stationary phase choices have been designed on these MFPP's so that increased modes of interaction can be applied to the resolution problem.

FIGURE 3. Aromatic based stationary phases



Although the Phenyl phases shown in figure 3 appear similar the addition of the branched structure in the Diphenyl phase chemistry leads to significant selectivity changes due to the extra steric interaction that occurs around the branched structure. With multiple interactions taking place difficult to resolve compounds, such as positional isomers and metabolites, are no longer the problem they once were.

FIGURE 4. Separation of Explosives

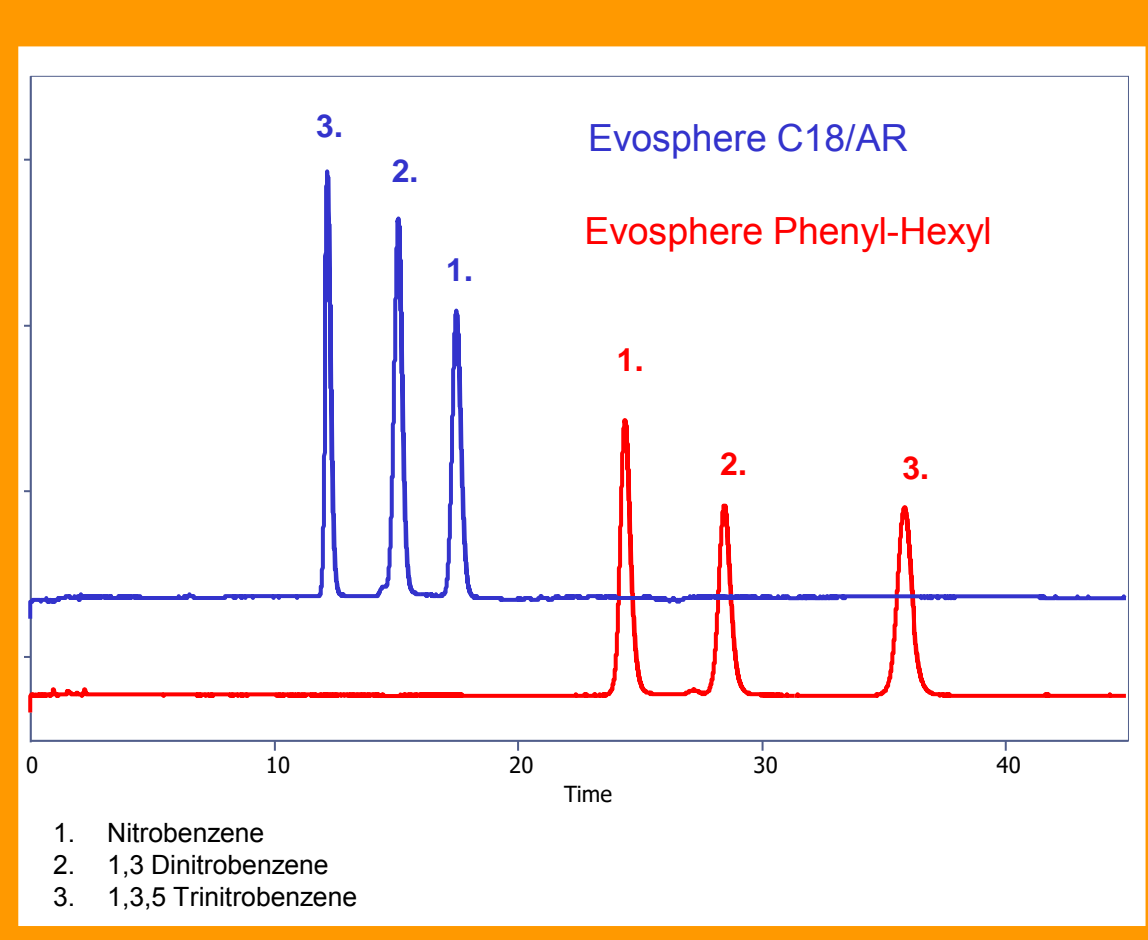


Figure 4 shows how a selection of positional changes on explosives can be separated on two contrasting stationary phases. Both phases are based on monodisperse fully porous particles (MFPP) so provide high efficiency and sensitivity. However it is the choice of stationary phase chemistry which offers the most in terms of resolution and selectivity. The Evosphere C18/AR giving a different elution order to that of a phenyl-hexyl bonded phase.

FIGURE 5. Separation of Analgesic Drugs

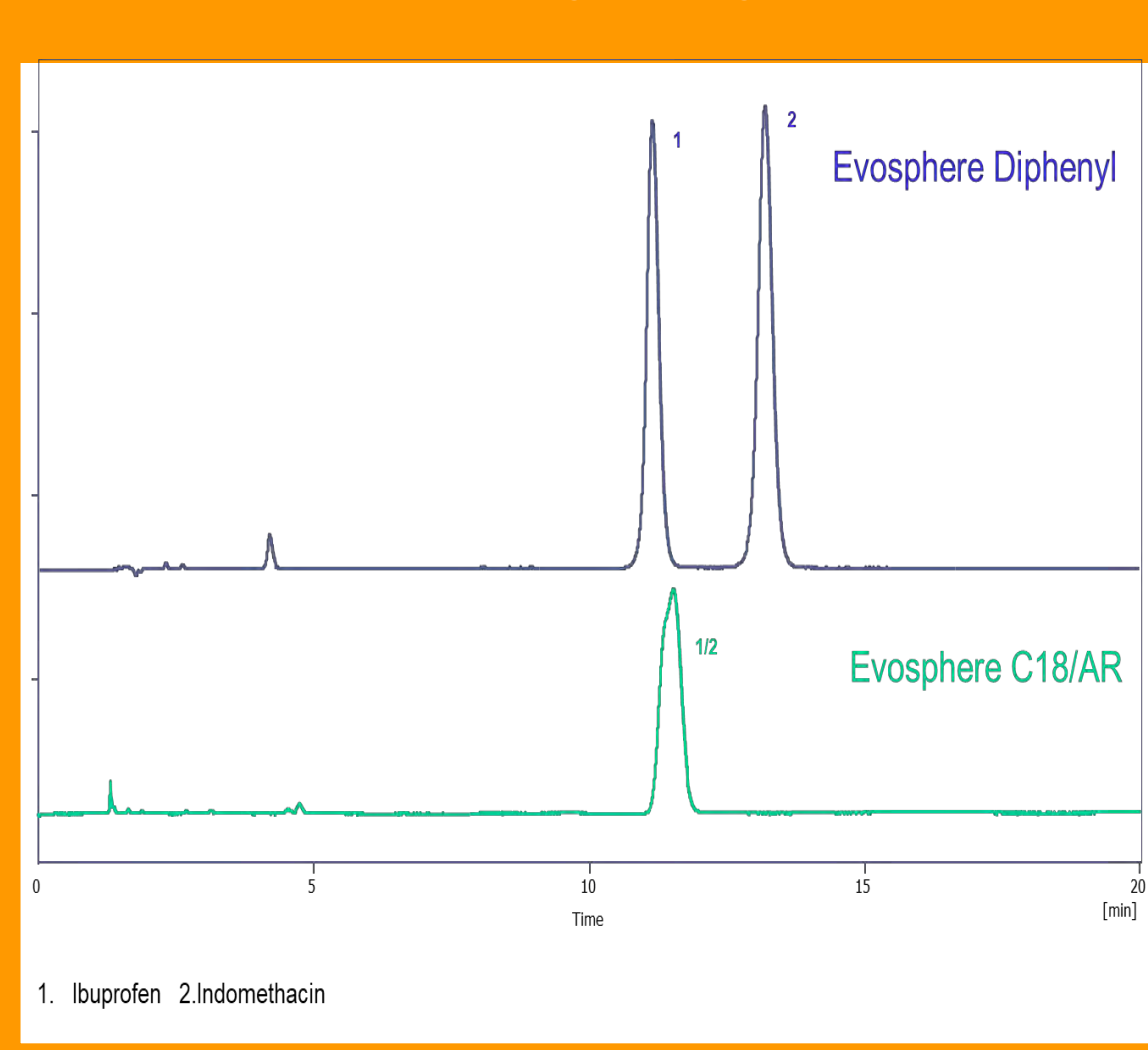


Figure 5 shows how the extra selectivity of the Diphenyl stationary phase offers enhanced resolution compared to an alternative hydrophobic/aromatic containing stationary phase.

Options for Polar and Basic Compounds

Choice of stationary phase for more polar analytes is often more complicated by the fact that many analytes require the use of high water content mobile phases for solubility. This can lead to 'phase collapse' on a standard C18 stationary phase.

If polarity can be designed into the bonding process either by addition of a polar-embedded functionality or as polar endcapping then hydrophilic analytes can be retained and therefore resolved from each other.

FIGURE 6. Polar Capped and Polar Embedded Phases

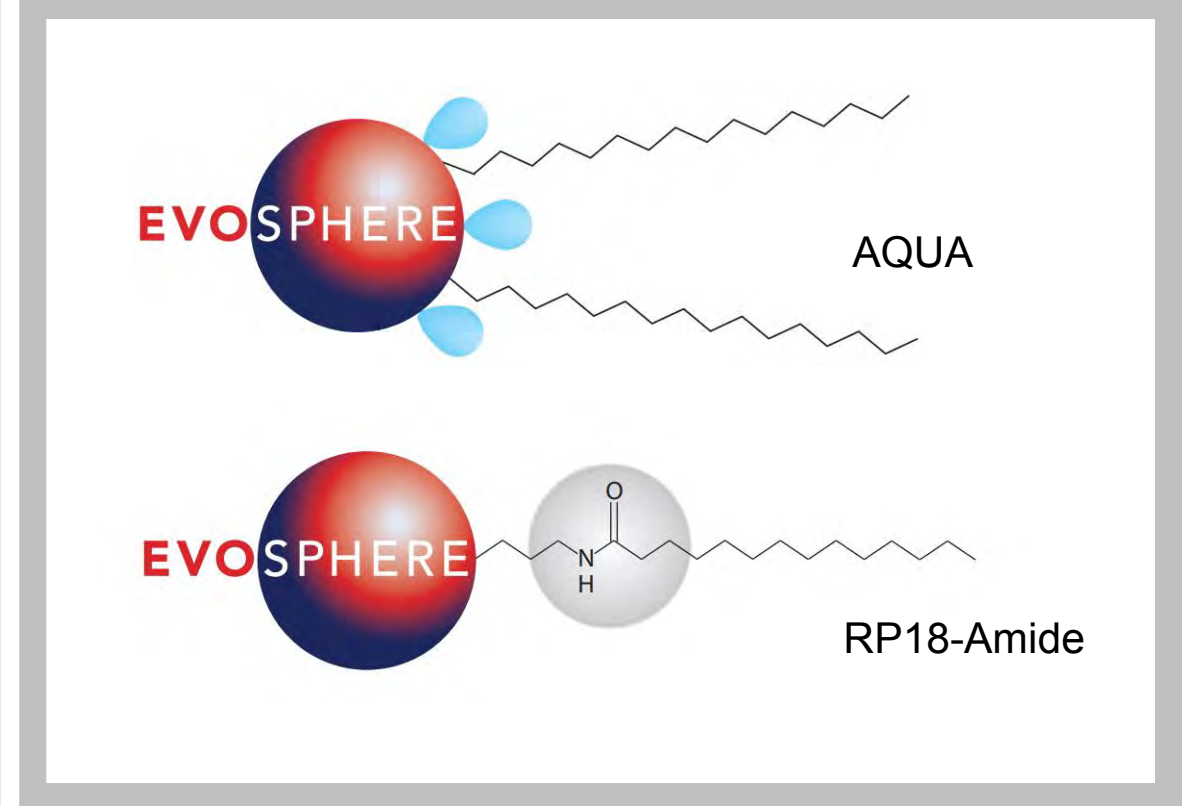


Figure 6 shows the new polar end-capped and polar embedded stationary phases with their successful application shown in figures 7 and 8.

FIGURE 7. Separation of polar compounds

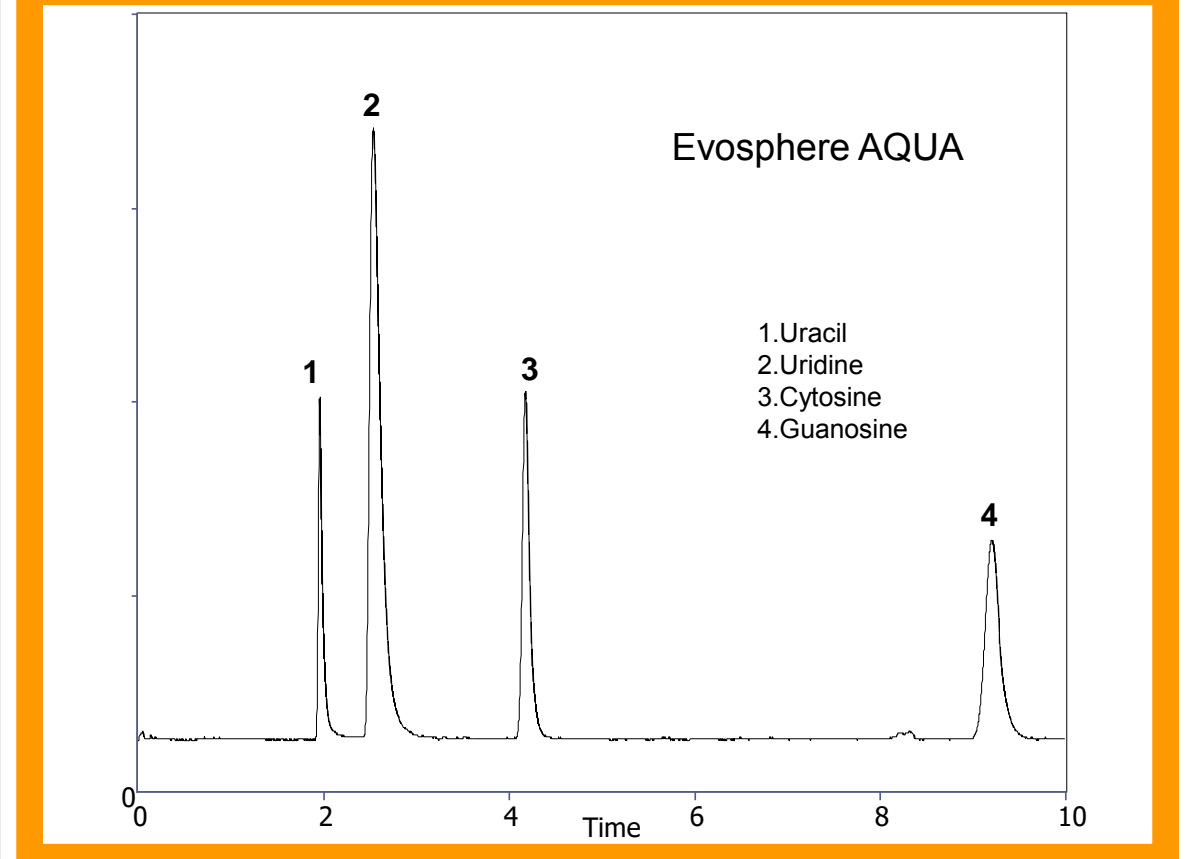
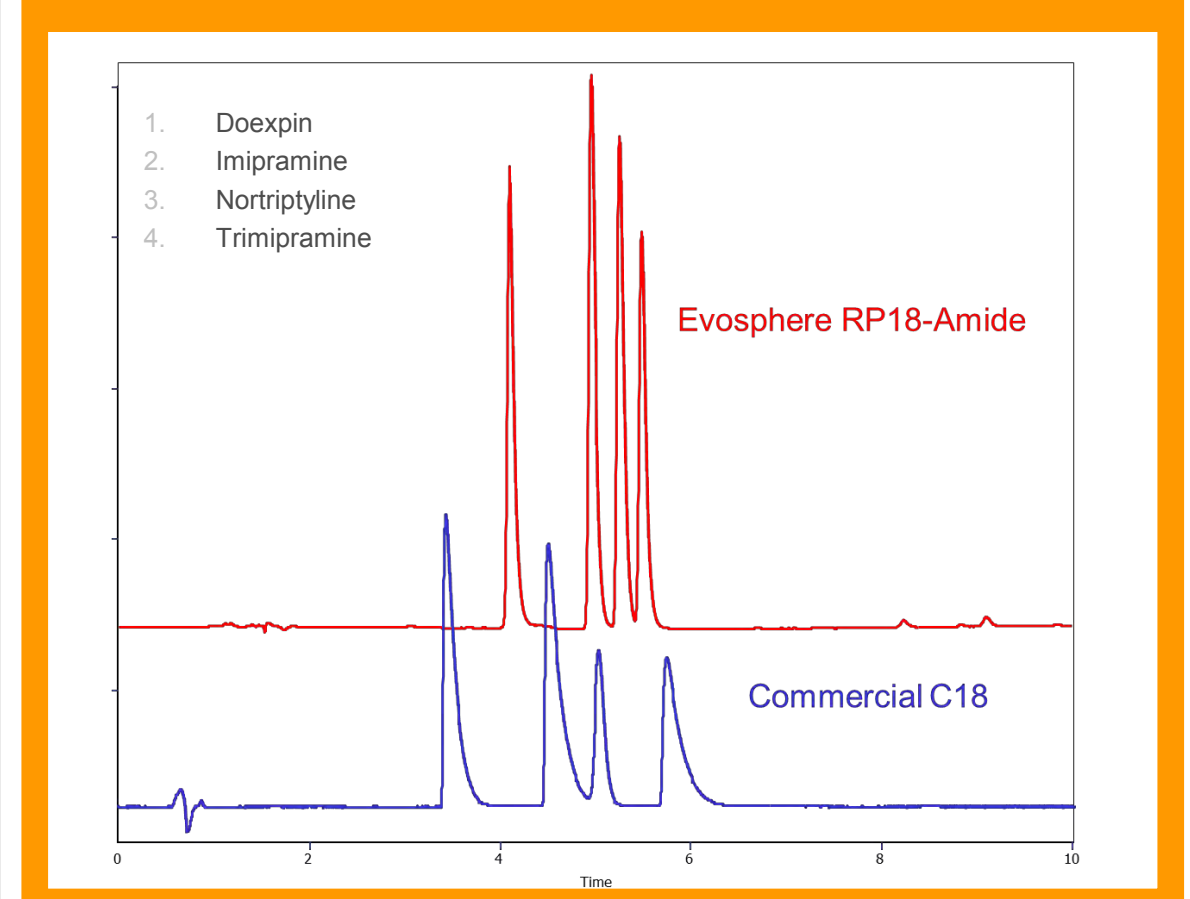


Figure 8 shows the performance benefit of the new RP18-Amide phase versus a conventional C18 product.

FIGURE 8. Tricyclic Antidepressants Separation



Conclusion

In this poster we have discussed the use of a new Monodisperse fully porous silica particle which increases efficiency over traditional silica columns. We then show applications highlighting some novel new selectivity options which can vastly improve the analyst's ability to separate complex sample mixtures.

This ability to gain extended resolution in turn leads to more robust and reproducible methods being developed.

The Evolution of HPLC Columns...



* Evosphere® is a registered trademarks of Fortis Technologies Ltd.
* Fortis Technologies recognises the trademarks of all other manufacturers.



A CALIBRE SCIENTIFIC COMPANY