Evosphere Method Development Guide

Using Monodisperse Particles and Selectivity

Introduction

Monodisperse Fully Porous Particles (MFPP) combined with a new range of novel phase chemistries add a new dimension to the resolution and selectivity that can be achieved in HPLC and UHPLC. How does MFPP work and how do you pick the correct selectivity and optimise for a particular compound of interest ?

MFPP

Monodisperse particles lower band broadening by improving the homogeneity of the packing bed of the column, reducing the van-deemter curve, leading to increased efficiency and selectivity . More in-depth info can be found in the Evosphere® brochure[1] or in the technical white paper on LC-GC website[2] outlining how this works and the theories behind it such as the lower C-term in the van-deemter curve.

Phase selectivity

Evosphere now comes with 8 novel selectivities which can be used to gain increased resolution between compounds.

Principle Component Analysis (PCA) or spider diagrams can be utilised to help assist in understanding how the mechanisms operate across the various phases, and therefore how you can make an informed decision based upon the chemical entity to be retained and resolved from any metabolites and impurites also present. The new phases chemistries are:

- Evosphere C18/PFP
- Evosphere C18/AR
- Evosphere RP18-Amide
- Evosphere Diphenyl
- Evosphere Phenyl-Hexyl
- Evosphere AQUA
- Evosphere C12
- Evosphere PFP

The phases can be characterised by Hydrophobicity, dipole charge, polarity and steric selectivity and can be chosen as complementary or orthogonal in order to build a 'method development tool kit'.

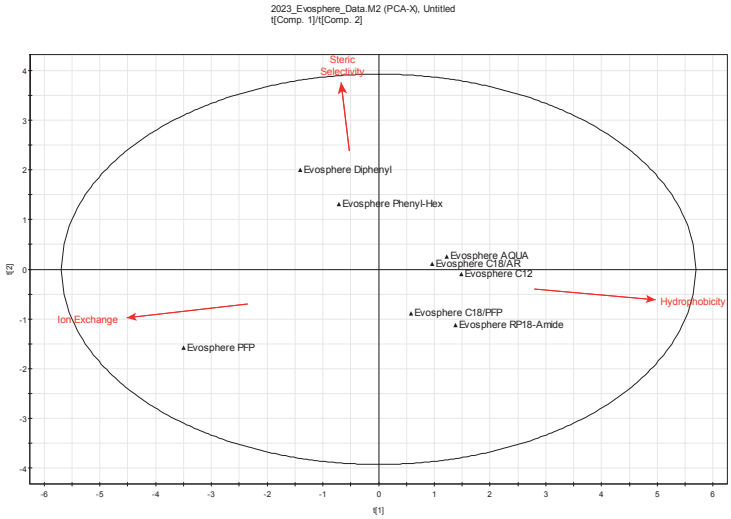


Figure 1. Principle Component Analysis

Either a stationary phase with the individual desired characteristic, such as hydrophobicity, can be chosen or a combination of phases with orthogonal characteristics can be combined to offer diversity. An example is shown in Figure 4 where a hydrophobic phase Evosphere C12, a dipole focused stationary phase, Evosphere Diphenyl and finally Evosphere C18/PFP are combined to potentially provide a diverse combination of characteristics.

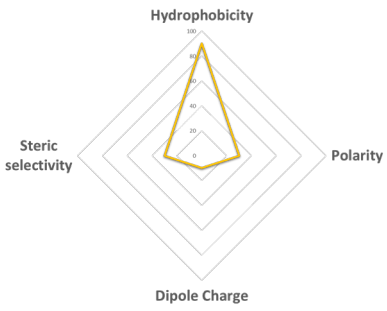


Figure 2. Evosphere C12

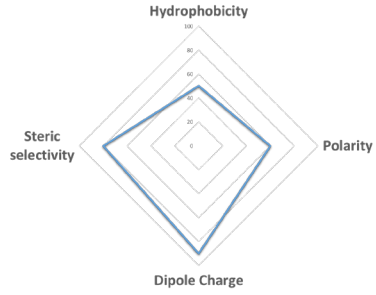


Figure 3. Evosphere Diphenyl

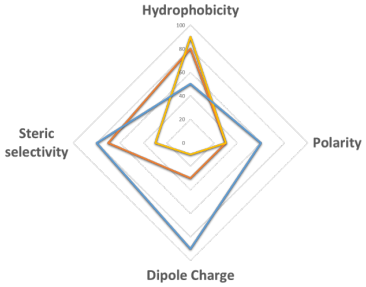


Figure 4. Method Development screening: Evosphere C12, Diphenyl and C18/PFP

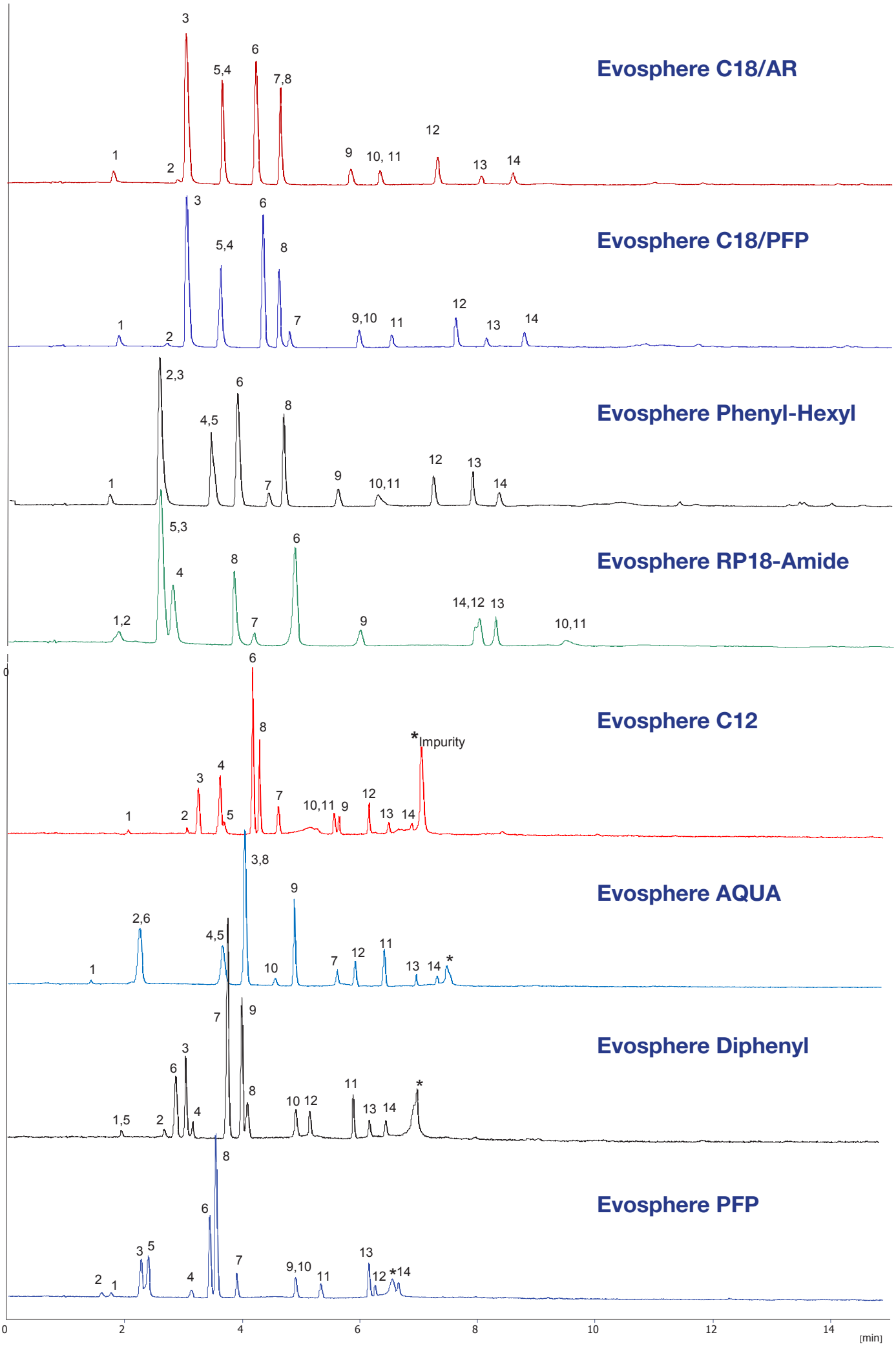


Figure 5. Evosphere stationary phases used as a screening of a diverse range of analytes, using Acetontirile as the organic solvent

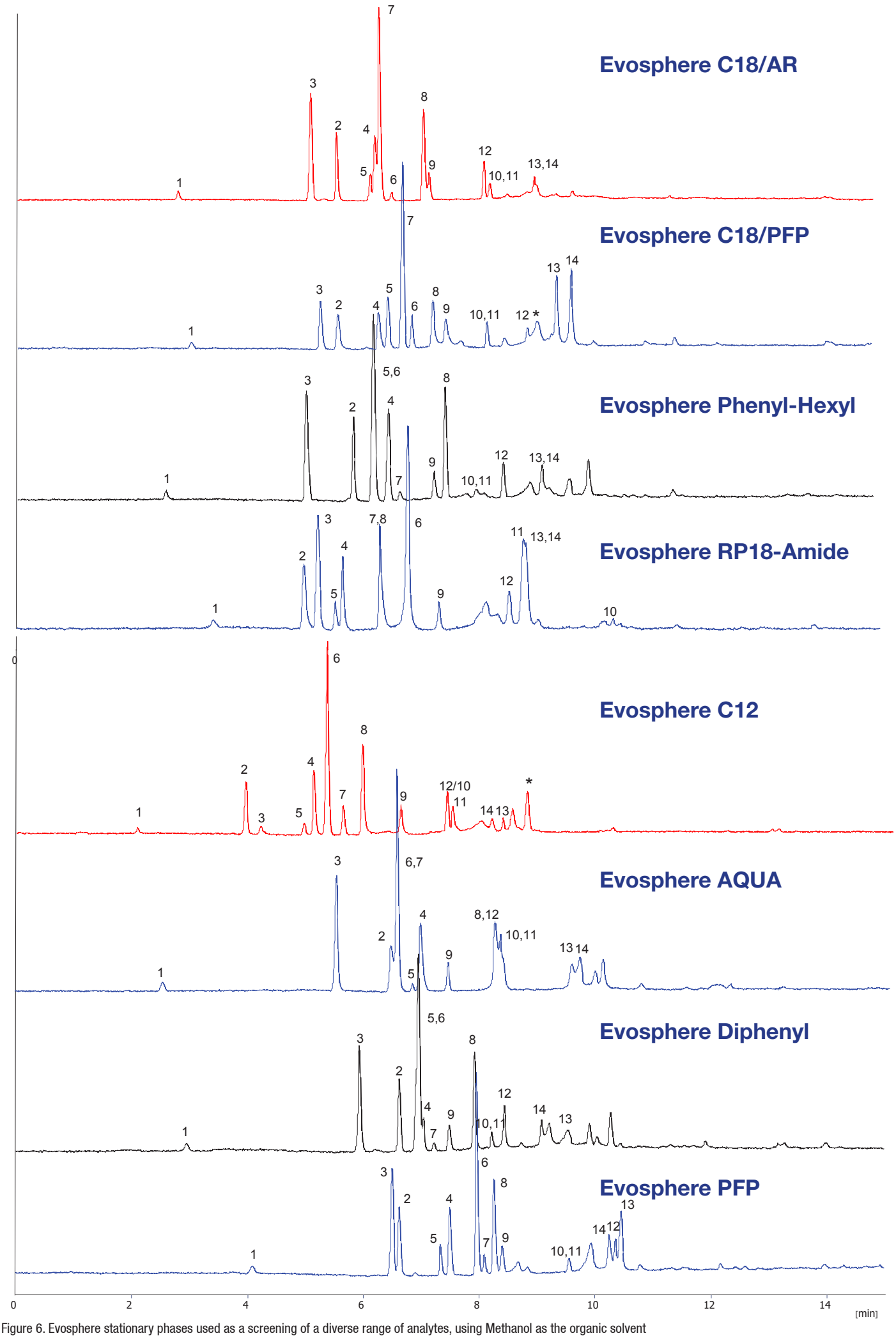


Figure 6. Evosphere stationary phases used as a screening of a diverse range of analytes, using Methanol as the organic solvent

Figure 5 and 6 show an example of how the phases can be quickly screened for a wide range of analytes, acidic, basic and neutral, in order to determine which stationary phase is going to be best for further optimisation.

Experimental Conditions

All Columns: 3µm 100x2.1mm

Mobile phase

A: 10mM Ammonium Formate pH 3.0

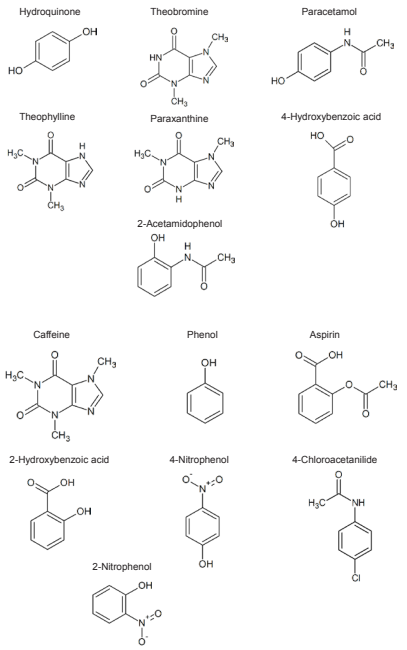
B1: 90:10 10mM Ammonium Formate pH 3.0 in Acetonitrile:Water 90:10

B2: 90:10 10mM Ammonium Formate pH 3.0 in Acetonitrile:Water 90:10

Flow Rate: 0.4ml/min

Temp: 40°C

Detection: 254nm



1. Hydroquinone
2. Theobromine
3. Paracetamol
4. Theophylline
5. Paraxanthine
6. 4-Hydroxybenzoic acid
7. 2-Acetamidophenol
8. Caffeine
9. Phenol
10. Aspirin
11. 2-Hydroxybenzoic acid
12. 4-Nitrophenol
13. 4-Chloracetanilide
14. 2-Nitrophenol

Conclusion

In this application guide we have shown how the new Evosphere HPLC columns can be used to improve separations and resolution between critical compounds. Understanding the differences between stationary phases is the first step in developing a new HPLC method. There are several new and unique orthogonal stationary phases available to aid in separation challenges. The correct choice of stationary phase can be aided by assessing principle component analysis or spider charts and an understanding of the fundamental chemistry of the compounds nature, acidic, basic neutral. Monodisperse fully porous particles ensure that maximum efficiency can be achieved even without the need to move to a UHPLC system.

Column screening using some or all of the phases and alternate solvents can also be used to initially screen complex mixtures. Once these initial screens have taken place with the compounds of interest then the analyst can determine which phase and mobile phase combination to optimise further with.

1. https://www.fortis-technologies.com/monodisperse-hplc/

2. https://chromtographyonline.com/view/the-effect-of-particle-mono-dispersity-in-hplc-column-performance

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