Monodisperse HPLC Particles

An aid to Method Design with Evosphere particles

The Evolution of HPLC Columns

EVOSPHERE

Method Development - Keeping it simple

Many United States Pharmacopeia (USP) methods were developed at a time when HPLC column technology was still at an early stage, with limited stationary phase options available and low performing Type A silica as the underlying support.

The high levels of impurities present in old Type A silicas mean that many methods had to make use of Ion-Pair (IP) reagents and inorganic buffer salts to achieve retention and suitable peak shape. However use of such additives is now considered undesirable due to incompatibility with modern detection methods such as Mass spectroscopy (MS).

The best practise for method development is to make use of modern ultrapure Type B silica's in combination with the ever increasing selection of stationary phase chemistries designed to give optimal resolution and peak shape. By doing so we can avoid developing troublesome methods that struggle with reproducibility and incompatible with MS.

Modern Solutions - Evosphere High efficiency columns

Evosphere® columns contain an ultrapure Type B monodisperse porous silica support with a uniform smooth surface, the high degree of monodispersity generates very high efficiency HPLC performance due to the reduced flow path dispersion (Eddy diffusion) within the column. The broad range of Evosphere stationary phase selectivity's available provide the capability to resolve more compounds in less time, whilst at the same time achieving greater sensitivity. They also provide the capability to remove the need for IP reagents and inorganic buffers from the mobile phase due to the increased interaction mechanisms present.

Monodisperse Evosphere particles are available in 1.7µm, 3µm and 5µm particle sizes.

Do's and Dont's

DO

- **Use MS friendly organic buffers, such as formic acid and ammonium acetate**
- **Make use of stationary phase selectivity - use more polar stationary phases for polar analytes**
- **Use stationary phase secondary interactions - dipole and steric interactions for isomers/ metabolites and related species**
- **Avoid complex mobile phases - these lead to less robust methods**
- **Use the correct buffer for the pH required - see our handy table below**

DONT

- **Do not use phosphate or perchlorate buffers**
- **Do not use TEA or TFA in mobile phase**

	pKa	Useable pH range	MS compatible
Formic acid	3.8	2.8 - 4.8	Yes
Acetic acid	4.8	3.8 - 5.8	Yes
Ammonium formate	3.8 & 9.2	2.8 - 4.8 & 8.2 - 10.2	Yes
Ammonium acetate	4.8 & 9.2	3.8 - 5.8 & 8.2 - 10.2	Yes
Ammonium bicarbonate	9.2	8.3 - 10.2	Yes

Leverage Modes of Selectivity

Evosphere columns have been designed to not only be a high efficiency particle but also with 8 novel stationary phases all designed to be orthogonal.

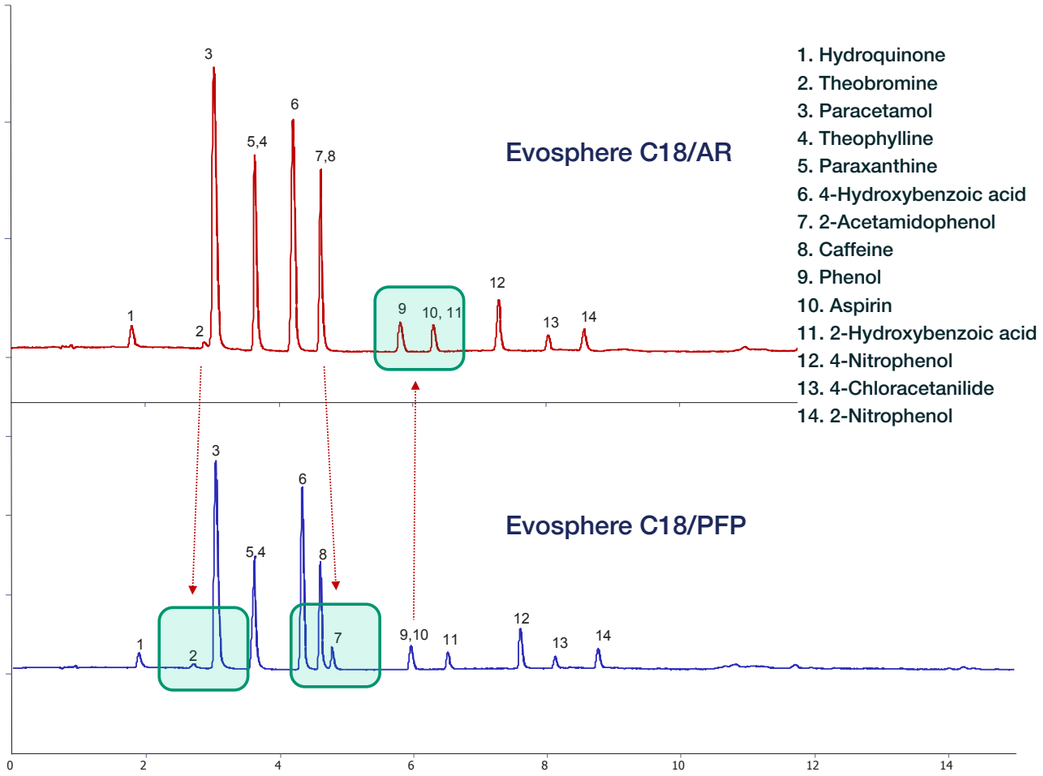
When developing a new method in chromatography having a diverse range of selectivities allows a choice to be made dependant upon initial knowledge of the compound types and classes: choose phases based on similarity i.e. Evosphere C18/AR and C18/PFP both having a high hydrophobicity, but subtle changes in steric arrangement and dipole charge. Alternatively choose stationary phases that are as orthogonal as possible from each other, allowing for the best probability of a generic gradient screen to ascertain the best starting column, The chosen column can then be taken forward for further optimisation.

Question 1: How do I leverage multiple modes of interaction in order to maximise resolution

Answer 1 : Screen the unknown or known analyte mixes across different stationary phase columns in a systematic way to determine which phase is the most beneficial for future method optimization.

Question 2: How do I maximise the selectivity space in order to develop the most robust method?

Answer 2: Compare Acetonitrile and Methanol mobile phases to explore multiple modes of interaction in addition to comparing different bonded phase columns. (see page 5&6)



Evosphere C18/AR and Evosphere C18/PFP are both USP L1 columns, however they are designed to enhance separation capabilities by offering different mechanisms of interaction to each other, leading to orthogonal separations.

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'.

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.

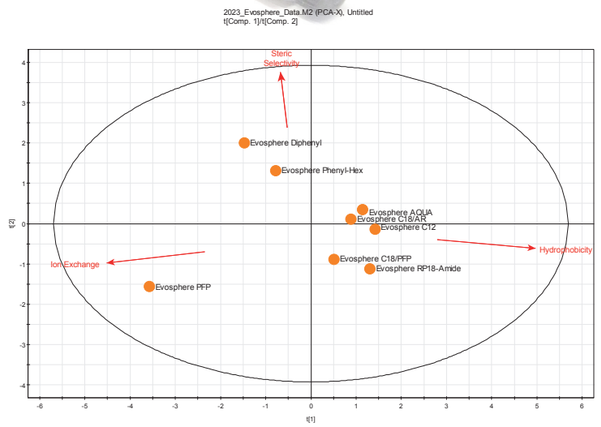


Figure 1. Principle Component Analysis

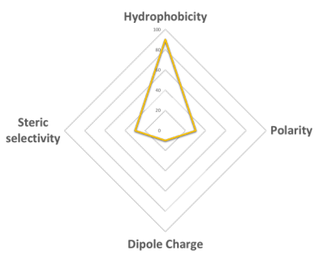


Figure 2. Evosphere C12

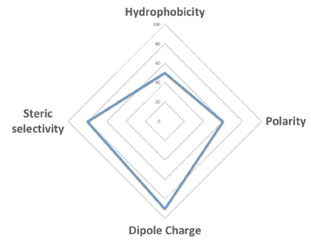


Figure 3. Evosphere Diphenyl

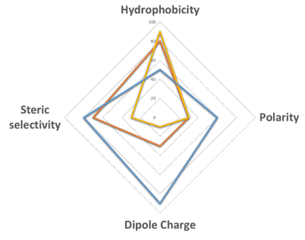


Figure 4. Method Development screening: Evosphere C12, Diphenyl and C18/PFP

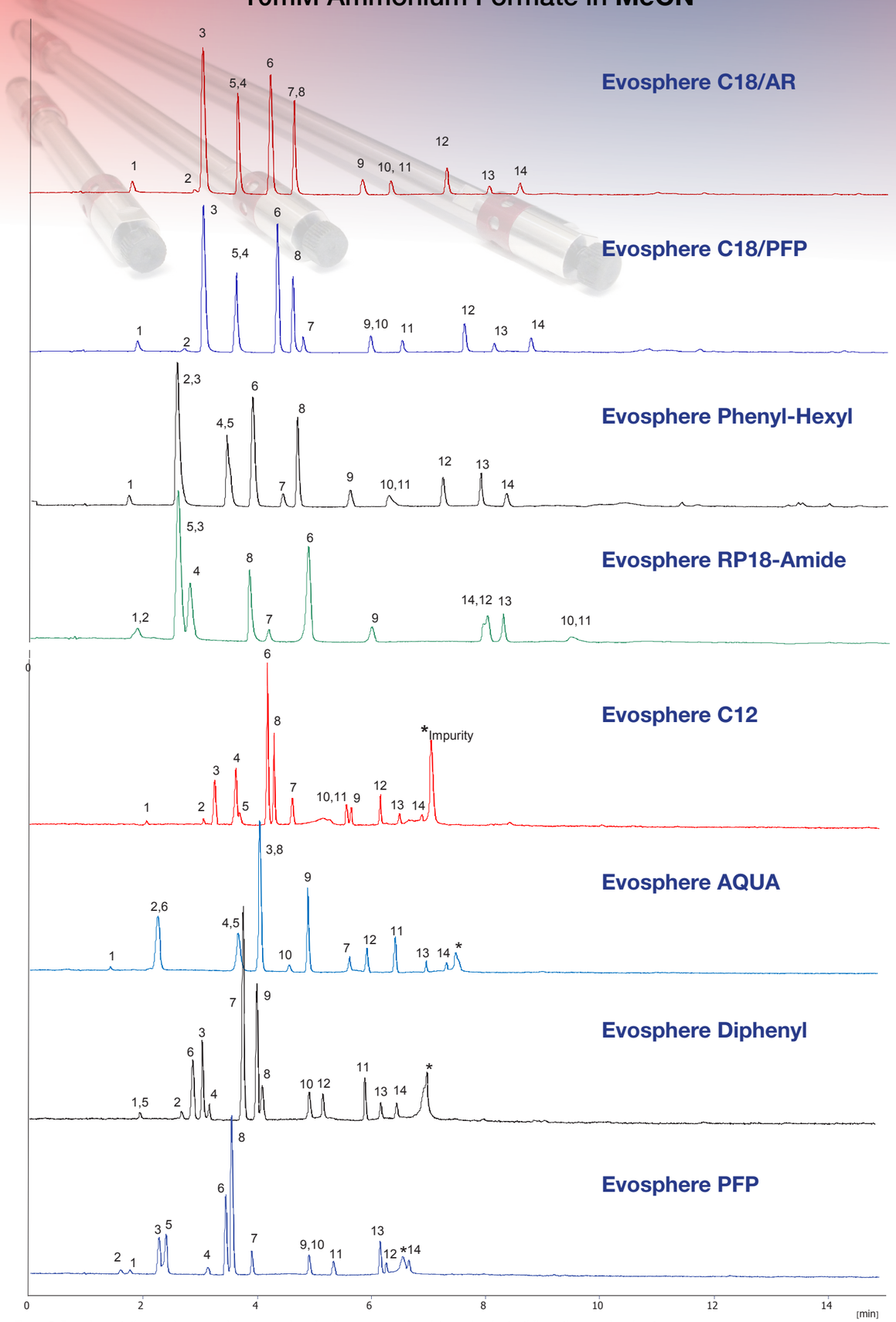


Figure 5. Evosphere stationary phases used as a screening of a diverse range of analytes, using Acetontirile as the organic solvent

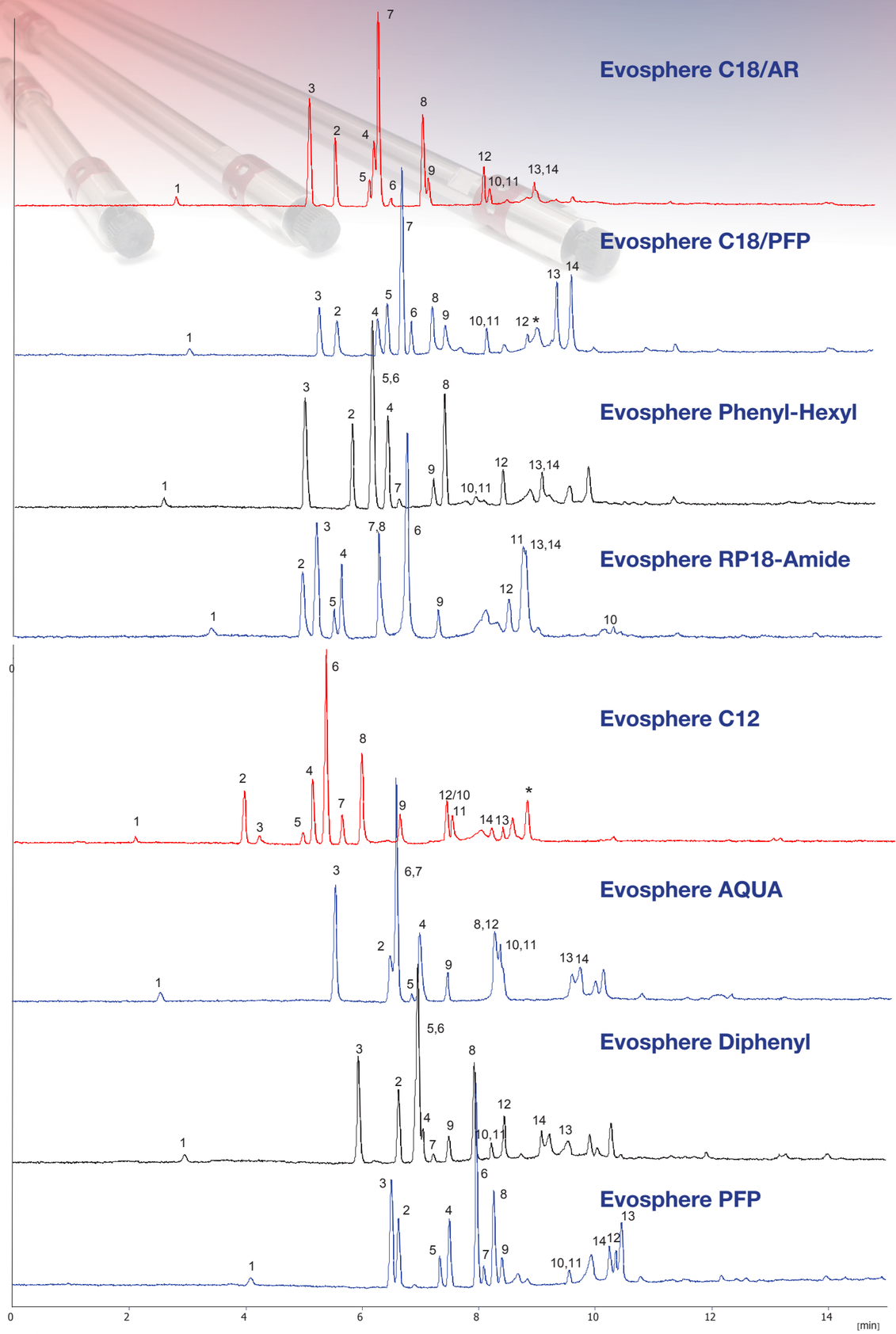


Figure 6. Evosphere stationary phases used as a screening of a diverse range of analytes, using Methanol as the organic solvent

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.

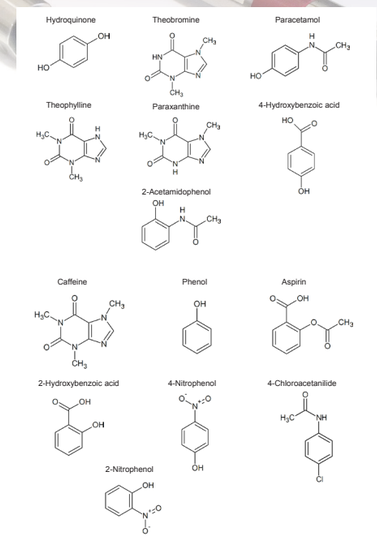
Experimental Conditions

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: 40ºC
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 or neutral. Monodisperse fully porous particles ensure that maximum efficiency can be achieved even without the need to move to a UHPLC system.

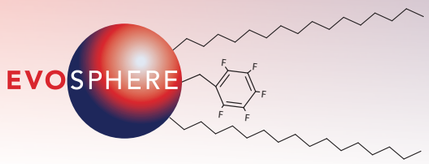
Column screening using some or all of the phases and alternate solvents can also be used to initially screen complex mixtures. Once these initial screens have taken place with the compounds of interest then the analyst can determine which phase and mobile phase combination to optimise further with.

If you wish to discuss this further then you can contact our technical support team on technicalsupport@fortis-technologies.com in order to request assistance on method development. Or our online web database has many compounds previously run on Evosphere columns as a starting point

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

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

Stationary Phase Choice

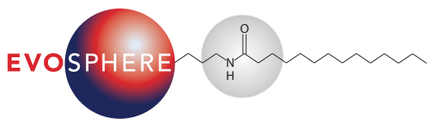


- **Evosphere C18/PFP**
- **Orthogonal Selectivity**
- **Method development starting point**

Evosphere C18/PFP is designed to provide characteristics which will enhance method development. It provides the ability to obtain sharp peak shapes whilst retaining and separating a wide variety of compounds both hydrophobic and hydrophilic.

- **Evosphere C18/AR**
- **Orthogonal Selectivity**
- **Method development starting point**

Evosphere C18/AR is designed to provide increased resolution between compounds, having a combination of hydrophobicity and aromatic selectivity will lead to enhanced resolution. USP L1 column.



- **Evosphere RP18-Amide**
- **Orthogonal Selectivity**
- **Excellent method development option**

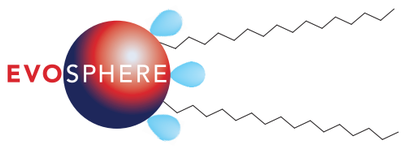
Evosphere RP18-Amide is designed to provide polar characteristics which will enhance resolution in method development. It provides orthogonal selectivity to alkyl chain phases due to its polar-embedded group. Sharp peak shapes, extra selectivity and increased retention can all be obtained.

- **Evosphere Diphenyl**
- **Separate positional isomers**
- **Stable ligand, No “MS” bleed**

Evosphere Diphenyl is designed to provide pi-pi, steric and hydrophobic characteristics which will enhance selectivity and the ability to develop methods. Particularly suited to positional isomers and other closely related species such as metabolites.

- **Evosphere Phenyl-Hexyl**
- **Separate metabolites**
- **Excellent resolution**

Evosphere Phenyl-Hexyl is designed to provide characteristics which will enhance selectivity. It provides alternate selectivity to a pure hydrophobic stationary phase whilst still maintaining the key attributes of robustness and reproducibility.



- **Evosphere AQUA**
- **Separate polar analytes**
- **Excellent stability**

Evosphere AQUA is designed to provide characteristics which will enhance retention of highly polar analytes. Reproducible surface characteristics provide robust separations. Combination of hydrophobic and hydrophilic nature.

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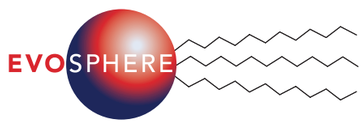
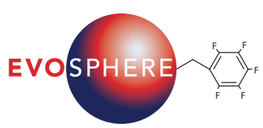


- **Evosphere C12**
- **Ultra High Efficiency**
- **Method development starting point**

Evosphere C12 is designed to provide characteristics which will enhance method development. The dense C12 ligand provides the ability to obtain sharp peak shapes whilst retaining and separating a wide variety of acid, base and neutral compounds with excellent robustness.



- **Evosphere PFP**
- **Orthogonal Selectivity**
- **Combined with Ultra High Efficiency particles**

Evosphere PFP (PentaFluoroPhenyl) is designed to provide characteristics which will enhance selectivity. It provides alternate selectivity to a hydrophobic stationary phase whilst still maintaining the key attributes of robustness and reproducibility.

- **Evosphere HILIC**
- **High polarity compounds**
- **Combined with Ultra High Efficiency particles**

Evosphere HILIC is designed to provide characteristics which will enhance retention of polar compounds. Hydrophilic interaction chromatography uses partition, ion-exchange and hydrogen bonding to retain high polarity analytes.

Evosphere Monodisperse Particles

	Particle Size	Surface Area	Pore Size	% C	pH range	USP
Evosphere C12	1.7µm 3µm 5µm	350m²/g	100Å	17	1-11	L87
Evosphere C18/AR	1.7µm 3µm 5µm	350m²/g	100Å	17	2-9	L1
Evosphere C18/PFP	1.7µm 3µm 5µm	350m²/g	100Å	17	2-9	L1
Evosphere RP18-Amide	1.7µm 3µm 5µm	350m²/g	100Å	20	2-9	L60
Evosphere Phenyl-Hexyl	1.7µm 3µm 5µm	350m²/g	100Å	14	2-9	L11
Evosphere Diphenyl	1.7µm 3µm 5µm	350m²/g	100Å	15	2-9	L11
Evosphere PFP	1.7µm 3µm 5µm	350m²/g	100Å	13	2-9	L43
Evosphere AQUA	1.7µm 3µm 5µm	350m²/g	100Å	18	2-9	L96
Evosphere HILIC	1.7µm 3µm 5µm	350m²/g	100Å	n/a	2-7	L3

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