

Application Note

Introduction

Metabolites cause all sorts of problems in chromatography separations. Due to the small chemical changes that they normally exhibit from the parent compound it is more difficult to achieve successful separation of metabolites. If successful separation can be achieved of these critical compounds then accuracy can be maintained, better qualification and quantification results will ensue.

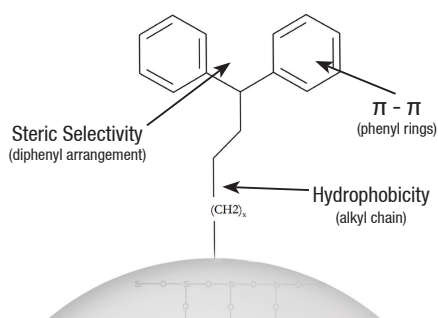
Whilst MS has become the pre-eminent technology for the detection of analyte species,

“Even MS struggles to differentiate certain analyte species from each other. The need for good chromatography is paramount”

even MS struggles to differentiate certain analyte species from each other, compounds such as positional isomers have the same mass to charge ratio and therefore provide the same response in MS detection.

Unique Selectivity

Using a unique stationary phase chemistry in chromatography allows us to separate metabolites which are not achievable in RP chromatography with a C18 chemistry. By providing three mechanisms of interaction the Fortis™ Diphenyl stationary phase allows the analyst to separate closely related species without having to resort to

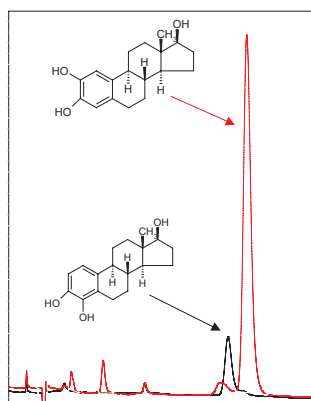


Metabolite profiling

complex method development strategies.

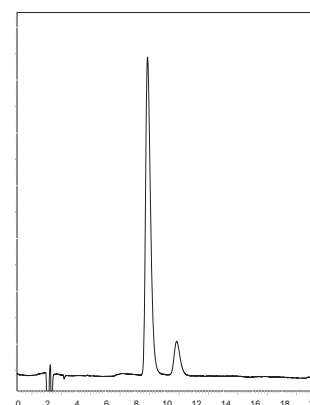
Experimental

Positional isomers prove troublesome to chromatographers due to the identical nature of



Column: Fortis Diphenyl 150x4.6mm 5μ
Mobile Phase: 40:60 Water : MeOH **Flow:** 1.0ml/min
Temp: 20°C **Wavelength:** 210nm

tabolite change. Resolving the analytes by this hydroxyl group is again traditionally difficult in RP C18 stationary phase.

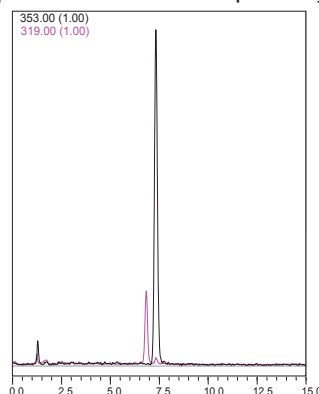


Quinidine and Dihydroquinidine

Column: Fortis Diphenyl 150x4.6mm 5μ
Mobile Phase: 70:30 Water : MeOH + 0.1% formic acid
Flow: 1.0ml/min **Temp:** 25°C **Wavelength:** 235nm

the functional groups present, and in MS the isomers provide the same mass to charge ratio.

In the example below the separation of two metabolites differing only by the presence of a chlorine atom is highlighted, the Diphenyl stationary phase resolves the two compounds by its steric mechanism even with a simple ACN water mobile phase system.



PK11195 and dechlorinated PK11195

Column: Fortis Diphenyl 150x4.6mm 5μ
Mobile Phase: 60:40 ACN : Water **Flow:** 1.0ml/min
Temp: 20°C **MS Detection**

Conclusion

Fortis™ Diphenyl allows us to separate positional isomers, whether acidic, basic or neutral. It will also provide success in the differentiation of small changes that often occur between metabolites such as a change/addition/loss in a functional group atom present. The Fortis Diphenyl simplifies method development of these molecular groups helping keep mobile phase choice to a minimum.

Quinidine and its hydroxylated analogue provide an example of a common me-