Unique Di-phenyl Functionality and Selectivity in LC and LC-MS

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

Introduction

In Chromatography selectivity is often hard to achieve in a simple manner, complex samples often require a complex mobile phase, which is not seen as productive, reproducible or easily transferable between facilities.

In this poster we discuss the use of a novel di-phenyl bonded phase chemistry which allows the use of simple mobile phase systems even for complex sample types, such as positional isomers, loss/gain of functional groups and closely related metabolites.

We discuss the use of this unique stationary phase in terms of its physical and chemical characteristics and stability, particularly in MS where the unique bonding function allows for a stable baseline to be achieved. The more stable the baseline with less chemical interference the better the subsequent sensitivity and resolution achievable.
We compare to a traditional C18 chemistry to outline the extra selectivity. We also look at the correct choice of organic modifier in the mobile phase.

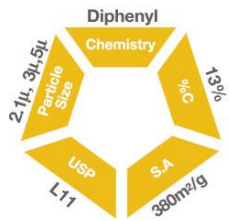
Advantages of Di-Phenyl

- **Unique Selectivity**
  - Three Interaction mechanisms
    - Hydrophobicity
    - Steric Selectivity
    - pi-pi
- **Separate Positional Isomers**
- **No "MS bleed"**
- **Enhanced Polar Retention**

Stationary Phase Characteristics

Fortis™ Phenyl

- Silica Template, Monofunctional phenyl bonding
- Unique di-phenyl chemistry
- Sharp Peak Shapes
- Highly Selective



Unique Structure

FIGURE 1. Di-phenyl structure

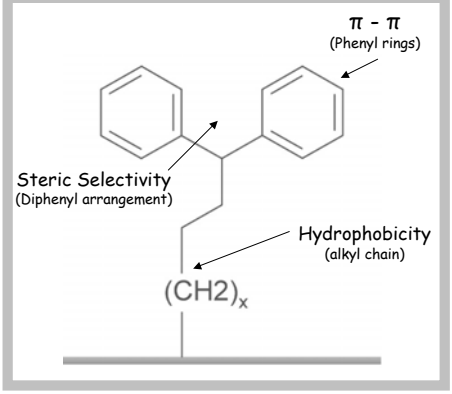


Figure 1. Shows the unique structure of the di-phenyl, showing how the three controlled mechanisms are applied to the chromatographic separation. The di-phenyl structure combined with extensive endcapping also provides significant coverage, ensuring peak shapes remain unaffected.

The alkyl chain linker has multiple functions:

1. providing hydrophobicity since it is rare that no hydrophobicity is needed.

2. by moving the phenyl rings away from the silica surface the phase becomes stable even under gradient conditions.

FIGURE 2. Fortis C18 vs Fortis di-phenyl

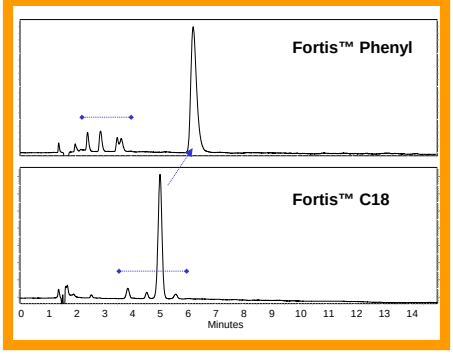


Figure 2. highlights how selectivity changes from a traditional C18 column to the new di-phenyl. The parent compound has retained longer on the di-phenyl due to its chemical nature, however all of the related species have now eluted away from the parent leading to better resolution, accuracy of qualitation and improved fractionation.

This type of selectivity change may not be typical of all samples however it highlights the orthogonal selectivity that can be achieved with the choice of a radically different stationary phase from alkyl chain that most people start with.

When looking for method development stationary phases, the more diverse the stationary phases the more chance of quickly finding a solution to the separation problem. Most people start with C18 or C8 and then move to a polar endcapped phase, however this is not orthogonal selectivity, since most polar endcapped phases start life as a C18 chemistry.

FIGURE 3. Separation of positional Isomers

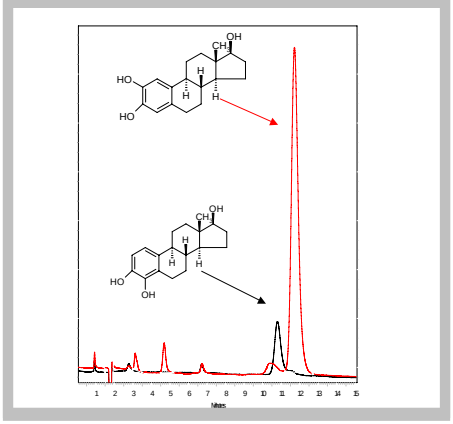


FIGURE 4. Comparison of basic positional Isomers

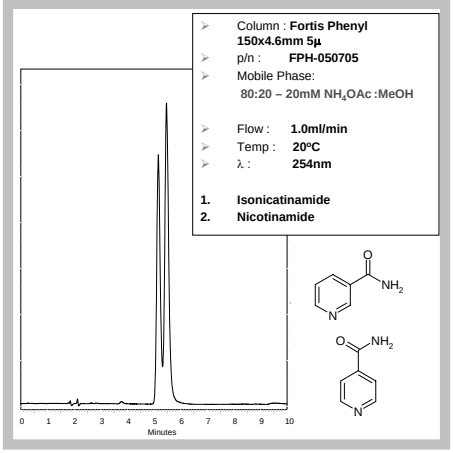


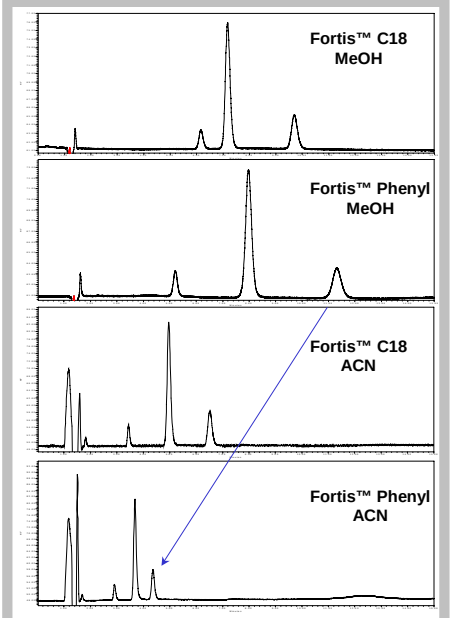
Figure 3 and 4 show how closely related species can be easily and quickly developed using the orthogonal nature of the di-phenyl vs a alkyl chain column. Mobile phases are simple; Water:Methanol for the steroids in Fig 3 and 20mM NH_4OAc:MeOH for the Nicotinamides in Fig 4. This allows the analyst to concentrate on other aspects if complex mobile phase production is not involved.

Mobile Phase Choice

Choice of mobile phase can be very important in running a phenyl column. Whilst many people have standardised upon ACN as the organic modifier of choice, MeOH is a better choice in order to let the π-π interactions occur on the phenyl rings. Using ACN can not only suppress retention but also selectivity.

It can be seen how maximum retention and resolution is obtained on Fortis Phenyl in MeOH mobile phase. Once the organic modifier is substituted for ACN not only is resolution reduced but also a large amount of retention is lost in relation to that lost on a C18.

FIGURE 5. Mobile Phase choice

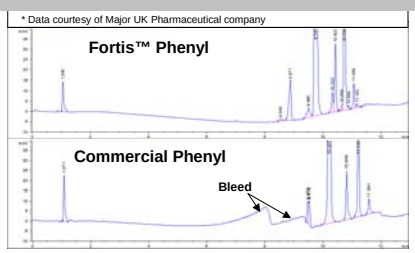


"MS-Bleed"

One of the issues with Phenyl functional stationary phases in the past has been the issue of "Phase Bleed". Due to the fact that the phenyl ring contains a chromatophore UV baselines could be seriously affected if the bonded phenyl ligand was not stable.

Due to the alkyl chain ligand in the Fortis Phenyl bonding, stability is no longer an issue

FIGURE 6. Phase Bleed



Conclusion

In this poster we have discussed the use of a new di-phenyl stationary phase. We have shown how this provides highly orthogonal selectivity to C18, most people's starting point. We have shown how it can be used to provide simple changes in selectivity with minimal changes to mobile phase systems and how this simplifies method development. Even positional isomers can be separated which makes the Fortis Phenyl perfect for metabolite profiling.