Enhanced Retention and Selectivity of Unsaturated Compounds Using π-π Interactions

Richard J. Lake*, Rebecca Wittrig Ph.D. & C. Vernon Bartlett

Restek Corporation, 110 Benner Circle, Bellefonte PA 16823, 1-800-356-1688

Abstract

Phenyl stationary phases are commonly used for analyses of neutral or aromatic compounds in reversed phase applications, in which they exhibit distinct selectivity differences, relative to butyl (C4) through octadecyl (C18 / ODS) alkyl chain stationary phases. However, a downside to phenyl phases is that they typically show only moderate retention, compared to ODS phases. In our laboratories, advances in phenyl bonding chemistry have increased overall retention while enhancing aromatic character. One such advance is the development of a biphenyl stationary phase. A biphenyl phase offers a more concentrated phenyl arrangement and a sterically favorable positioning of the phenyl groups, by presenting a surface chemistry that consists of two phenyl groups bonded end-to-end. The scope of these analyses was to determine if a biphenyl arrangement will show significantly different selectivity for unsaturated compounds, or compounds containing unsaturated functional groups, while maintaining a high retention capacity, similar to that of an ODS phase – a characteristic unlike conventional phenyl phases.

The Effect of Selectivity on Resolution

The effect and relative importance of selectivity, efficiency, and capacity on resolution are illustrated by the resolution equation. Mathematically, the selectivity term affects resolution to the greatest degree, showing that resolution is largely a function of selectivity. Selectivity, in turn, is controlled mainly by two factors - analyte interactions with the stationary phase and analyte interactions with the mobile phase. By choosing a stationary phase that, as closely as possible, produces optimum selectivity, less emphasis is placed on the mobile phase to provide selectivity, and complex samples can be more easily separated without the use of modifiers or complex mobile phases.

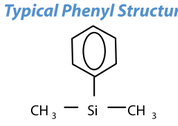
$$R = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\sqrt{N}\right)\left(\frac{k'}{1 + k'}\right)$$

Selectivity Efficiency Capacity

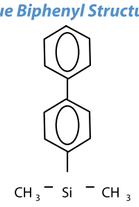
Chemical Composition of Phenyl Phases

A typical phenyl stationary phase consists of a single phenyl group bonded to a silica surface. A biphenyl phase offers a more concentrated phenyl arrangement, and a sterically favorable positioning of the phenyl groups, by presenting a surface chemistry that consists of two phenyl groups bonded end-to-end.

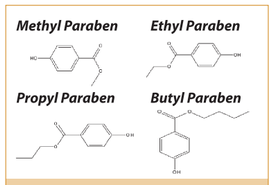
Typical Phenyl Structure



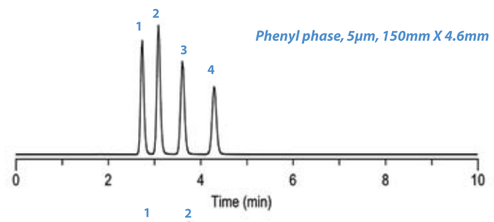
Unique Biphenyl Structure

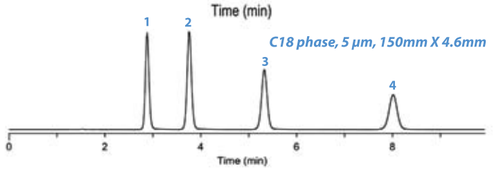


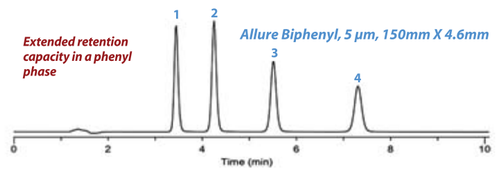
Analysis of Parabens



Instrument Conditions
Instrument: Waters 1525 Binary HPLC Pump and a Waters 2487 UV detector
Mobile Phase: 50:50 0.1% Acetic acid in Water:Acetonitrile
Flow rate: 1.0 ml / min, Isocratic
UV detection: 254 nm
Temp: Ambient







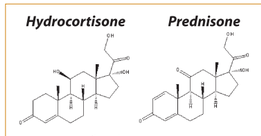
An analysis of parabens illustrates the superior retention capacity of the biphenyl phase. Each paraben in the test group consists of an aromatic ring with a carboxylic functional group through which a methyl, ethyl, propyl, or butyl chain is attached. The length of the attached alkyl chain is the only structural difference among the analytes. The retention capacity of the biphenyl phase for the parabens is similar to that of an ODS phase, and is much greater than that of a phenyl phase. Note that selectivity among the three columns shows no distinct differences, as the aromatic backbone is unchanged.

Summary of Retention and Capacity Factors (k') for the Analysis of Parabens

Peak #	Compound	Capacity Factor (k')		
		Phenyl	*C18*	*Biphenyl*
1	Methyl Paraben	0.88	1.45	1.91
2	Ethyl Paraben	0.97	2.20	2.58
3	Propyl Paraben	1.48	3.53	3.64
4	Butyl Paraben	1.95	5.81	5.15

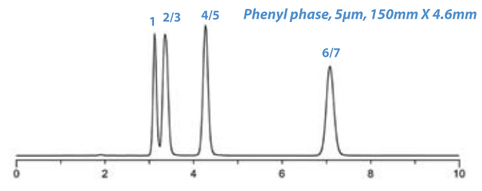
Analysis of Corticosteroids

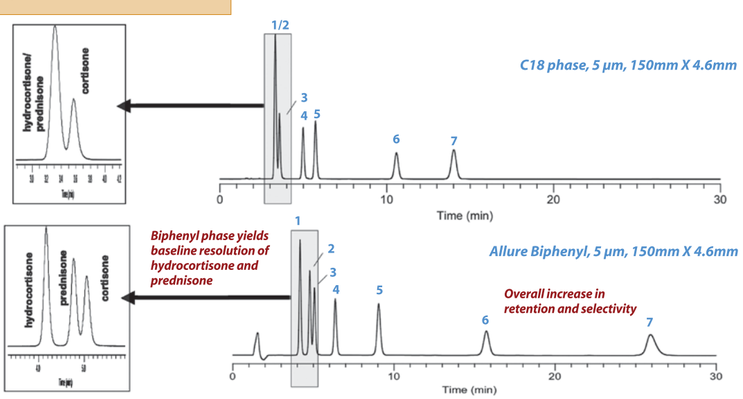
Next, compounds that differ in the number and positions of double bonds then were investigated to determine selectivity differences, if any, among typical stationary phases. Steroids are an excellent model for studying the effects of differences in hydrocarbon ring saturation on chromatographic behavior, in that all members are based on a common conjugated ring system, with differences in double bond positions and ring constituents producing their chemical diversity. We first assayed a group of seven corticosteroids, using biphenyl, phenyl, and ODS stationary phases, to ascertain if any phase shows enhanced selectivity. The biphenyl phase demonstrated better selectivity for steroids. Relating the structures of hydrocortisone, cortisone, and prednisone to the chromatographic data provided an explanation of these results. Structurally, hydrocortisone and prednisone contain the identical position 17 functional group, whereas cortisone contains a similar functional group with different orientation. The ODS stationary phase provided baseline resolution of cortisone, but allowed coelution of hydrocortisone and prednisone. Hence, the ODS phase appears to show selectivity based on differences in functional group orientation, but not on subtle differences in ring structure. In contrast, the biphenyl phase was capable of differentiating hydrocortisone and prednisone, which differ in double bonding between positions 1 and 2 of the ring structure. These results indicate that the biphenyl phase, through π-π interactions, can resolve compounds with differences in hydrocarbon ring carbon-to-carbon bond structure.



Instrument Conditions
Instrument: Waters 1525 Binary HPLC Pump and a Waters 2487 UV detector
Mobile Phase: 60:40 Water:Acetonitrile
Flow rate: 1.0 ml / min, Isocratic
UV detection: 254 nm
Temp: Ambient

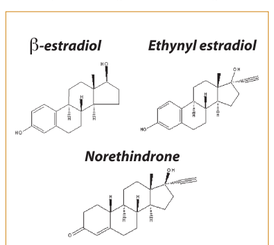
	Peak	Retention Time (min.) phenyl	C18	biphenyl
1.	hydrocortisone	3.14	3.33	4.19
2.	prednisone	3.32	3.33	4.79
3.	cortisone	3.32	3.59	5.08
4.	dexamethasone	4.28	5.00	6.37
5.	corticosterone	4.28	5.74	9.01
6.	cortisone acetate	7.09	10.59	15.75
7.	desoxycorticosterone	7.09	14.03	25.94



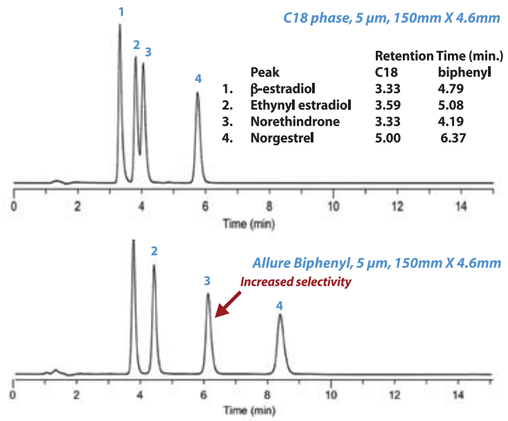


Analysis of Contraceptive Hormones

The chemical structures of β-estradiol, ethynyl estradiol, and norethindrone (Figure 10) show differences and similarities in functional group and ring structures comparable to those among the corticosteroids. As expected, the ODS phase completely resolved β-estradiol and ethynyl estradiol, which differ in the position 17 ethynyl functional group, but could not resolve ethynyl estradiol and norethindrone, which have identical position 17 functional groups, but small differences in hydrocarbon ring saturation. Overall, retention and selectivity of the biphenyl phase again were markedly better, relative to the ODS phase, as the biphenyl phase provided complete resolution of all compounds. Most significantly, the biphenyl phase showed greater capability for resolving differences in ring structures, as noted by the greater resolution between ethynyl estradiol and norethindrone.



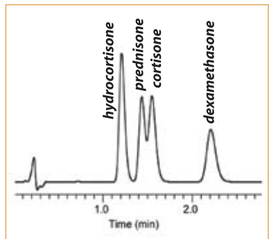
Instrument Conditions
Instrument: Waters 1525 Binary HPLC Pump and a Waters 2487 UV detector
Mobile Phase: 60:40 Water:Acetonitrile
Flow rate: 1.0 ml / min, Isocratic
UV detection: 220 nm
Temp: Ambient



	Peak	Retention Time (min.) C18	biphenyl
1.	β-estradiol	3.33	4.79
2.	Ethynyl estradiol	3.59	5.08
3.	Norethindrone	3.33	4.19
4.	Norgestrel	5.00	6.37

Fast HPLC Using a Conventional System

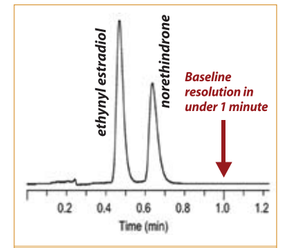
Corticosteroids Mix
Allure Biphenyl, 3 µm, 30mm X 4.6mm



Mobile Phase: 60:40 Water:Acetonitrile
Flow rate: 1.0 ml / min, Isocratic
UV detection: 254 nm
Temp: Ambient

Resolution is effected to varying degrees by selectivity (α), capacity (k') and efficiency. α & k' control resolution to the greatest degree, and they are controlled mostly by analyte interaction with the stationary and mobile phases. Efficiency, on the other hand, is mainly controlled by dimensional factors, like particle size, column length and column ID, and effects resolution to a lesser degree. Thus, because a biphenyl stationary phase provides improved selectivity and retention, a fast HPLC application is possible without changing column ID and particle size.

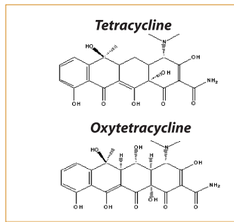
Common Contraceptive Mix
Allure Biphenyl, 3 µm, 30mm X 4.6mm



Mobile Phase: 65:35 Acetonitrile:Water
Flow rate: 1.5 ml / min, Isocratic
UV detection: 220 nm
Temp: Ambient

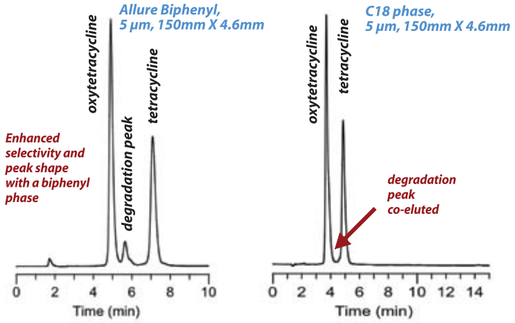
Analysis of Tetracyclines

The analysis of tetracyclines was performed to further compare α and k' of the biphenyl and ODS phases. The selectivity of the stationary phases was evaluated by determining the resolution and selectivity (α) between tetracycline and oxytetracycline. These two compounds are a good indication of selectivity because they are both comprised of a four ring hydrocarbon system and have similar chemical structures. The biphenyl phase produced the best results based upon this criterion, demonstrating that the biphenyl stationary phase exhibits pi-pi bonding with the ring structures of the tetracyclines. This mechanism showed an increase in retention capacity when compared to a mechanism based upon hydrophobicity, as in the alkyl chain phase of an ODS phase.



Instrument Conditions
Instrument: Waters 1525 Binary HPLC Pump and a Waters 2487 UV detector
Mobile Phase: 80:20 20mM Ammonium Phosphate in Water (pH=2.5)
Flow rate: 1.0 ml / min, Isocratic
UV detection: 254 nm
Temp: Ambient

A system suitability test was also performed to evaluate the capacity factors and USP tailing factors were used to further evaluate stationary phases. Again, the biphenyl column demonstrated the greatest selectivity (alpha) and the greatest peak shape (USP tailing) under simple, isocratic conditions.



Selectivity Between Oxytetracycline and Tetracycline

Stationary Phase	Resolution (R)	Selectivity Factor (_)
Biphenyl	5.28	1.61
Pentafluorophenyl Propyl	4.49	1.59
Octadecylsilane (C18)	3.31	1.50
Cyanopropyl	NA	1.34
Octylsilane (C8)	NA	0.47
Pentafluorophenyl	NA	NA

System Suitability of Tetracycline

Stationary Phase	Mean Capacity Factor (k')	Mean USP Tailing Factor
Biphenyl	5.678	1.06
Pentafluorophenyl Propyl	8.115	1.28
Octadecylsilane	3.734	1.21

Conclusion

These analyses demonstrated that a biphenyl stationary phase, through π-π interactions, offers a unique alternative when analyzing compounds with saturation differences in the hydrocarbon ring structure, and increased retention and efficiency, relative to traditional phenyl phases, when π-bonding is absent. A biphenyl phase, with the aromatic rings bonded end-to-end, offers characteristics of both phenyl and alkyl phases. By combining these two chemistries, unique and enhanced chromatographic performance can be achieved.