Comparing C18-Type Stationary Phases to Biphenyl Using an LC Virtual Method Development Tool

John Gallant, Melinda Urich Restek Corporation, Bellefonte, PA, USA

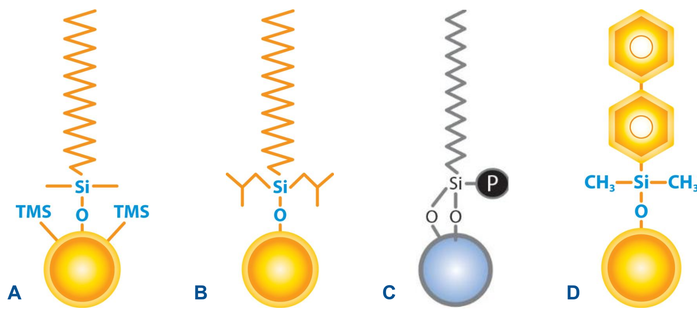
Introduction

The selection of an appropriate column is a critical component in the development of a successful LC-MS/MS method. Column selectivity is the single most important factor on the resolution of critical pairs. The complexity of multi-class panels can make separation and quantitation challenging, but by leveraging selectivity, difficult separations can be achieved. Understanding column chemistry is essential for developing high performing and robust LC-MS/MS methods. In this work, a virtual method development tool (*EZ*LC, Restek Corporation) was used to compare the selectivity of the Biphenyl phase to several C18-type phases.

Background

To compare the selectivity of the Biphenyl stationary phase to C18-type column chemistries, a diverse range of pesticide classes were chosen to evaluate. Using the pesticides library in the online modeller, compounds were selected from the following classes: insecticides, fungicides, herbicides, acaricides and growth regulators.

Four column chemistries were selected for analysis:



A – Raptor C18: Traditional TMS endcapped C18 phase bonded to a superficially porous particle.

B – Raptor ARC-18: C18-type phase with diisobutyl sidechains bonded to a superficially porous particle.

C – Ultra Aqueous C18: C18-type phase with a polar group modification bonded to a fully porous particle.

D – Raptor Biphenyl: Biphenyl ligand bonded to a superficially porous particle.

Method

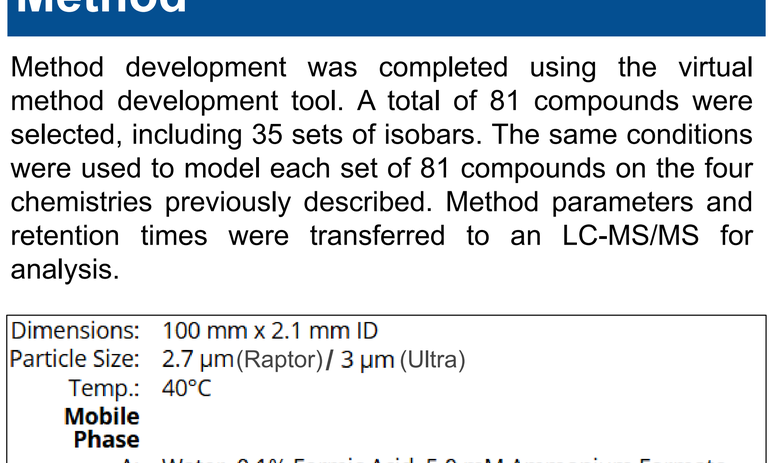
Method development was completed using the virtual method development tool. A total of 81 compounds were selected, including 35 sets of isobars. The same conditions were used to model each set of 81 compounds on the four chemistries previously described. Method parameters and retention times were transferred to an LC-MS/MS for analysis.

Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 µm (Raptor) / 3 µm (Ultra)
Temp.:	40°C
Mobile Phase	
A:	Water, 0.1% Formic Acid, 5.0 mM Ammonium Formate
B:	Methanol, 0.1% Formic Acid, 5.0 mM Ammonium Formate

Time (min)	Flow (mL/min)	%A	%B
0	0.6	85	15
7.5	0.6	25	75
12	0.6	0	100
12.01	0.6	85	15
14	0.6	85	15

Experimental Chromatogram

Figure 1: Experimental Chromatogram of 81 Compounds on Raptor Biphenyl



Modelled Chromatogram

Figure 2: Modelled Chromatogram of 81 Compounds on Raptor Biphenyl

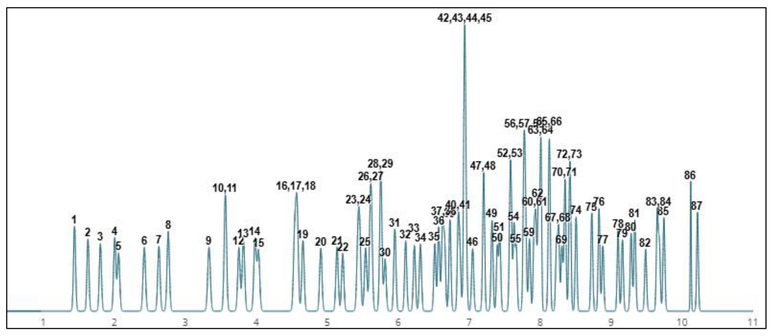


Table 1: Retention Time Comparison of modelled Data vs. Instrument Data.

Peak	Analyte	Mod t_R (min)	Exp t_R (min)	Diff (sec)	Peak	Analyte	Mod t_R (min)	Exp t_R (min)	Diff (sec)
1	Omethoate	1.44	1.45	**0.6**	45	Promecarb	6.96	6.95	**0.6**
2	Formetanate HCL	1.63	1.59	**2.4**	46	Terbutryn	7.05	7.05	**0.0**
3	Aldicarb sulfoxide	1.81	1.82	**0.6**	47	Cyproconazole isomer 2	7.20	7.18	**1.2**
4	Butoxycarboxim	2.01	2.02	**0.6**	48	Kresoxim-methyl	7.21	7.21	**0.0**
5	Flonicamid	2.06	2.08	**1.2**	49	Cyproconazole isomer 1	7.32	7.33	**0.6**
6	Pymetrozine	2.43	2.27	**9.6**	50	Fenhexamid	7.40	7.45	**3.0**
7	Monocrotophos	2.63	2.62	**0.6**	51	Triadimefon	7.43	7.46	**1.8**
8	Oxamyl	2.76	2.79	**1.8**	52	Diclobutrazol	7.58	7.56	**1.2**
9	Thiabendazole	3.34	3.20	**8.4**	53	Triticonazole	7.59	7.59	**0.0**
10	Thiamethoxam	3.57	3.55	**1.2**	54	Clethodim isomer 1	7.63	7.64	**0.6**
11	Dicrotophos	3.57	3.57	**0.0**	55	Boscalid	7.66	7.69	**1.8**
12	3-Hydroxycarbofuran	3.76	3.76	**0.0**	56	Furalaxyl	7.77	7.84	**4.2**
13	Dimethoate	3.82	3.84	**1.2**	57	Hexaconazole	7.78	7.79	**0.6**
14	Mevinphos isomer 1	3.99	4.00	**0.6**	58	Tebuconazole	7.80	7.81	**0.6**
15	Ethirimol	4.03	3.98	**3.0**	59	Mepanipyrim	7.85	7.84	**0.6**
16	Aldicarb	4.54	4.57	**1.8**	60	Flufenacet	7.91	7.92	**0.6**
17	Butocarboxim	4.57	4.59	**1.2**	61	Diniconazole	7.94	7.95	**0.6**
18	Mevinphos isomer 2	4.58	4.57	**0.6**	62	Tebufenozide	7.97	8.02	**3.0**
19	Carbetamide	4.66	4.65	**0.6**	63	Bifenazate	8.00	8.04	**2.4**
20	Thidiazuron	4.91	4.87	**2.4**	64	Etaconazole isomer 2	8.01	8.08	**4.2**
21	Acetamiprid	5.14	5.09	**3.0**	65	Etaconazole isomer 1	8.12	8.15	**1.8**
22	Fluometuron	5.22	5.19	**1.8**	66	Mandipropamid	8.13	8.14	**0.6**
23	Metribuzin	5.43	5.42	**0.6**	67	Dimethomorph isomer 1	8.23	8.25	**1.2**
24	Propoxur	5.46	5.45	**0.6**	68	Fenoxycarb	8.26	8.30	**2.4**
25	Simetryn	5.54	5.52	**1.2**	69	Fluquinconazole	8.31	8.33	**1.2**
26	Bendiocarb	5.61	5.60	**0.6**	70	Dimethomorph isomer 2	8.34	8.40	**3.6**
27	Monolinuron	5.62	5.59	**1.8**	71	Ipconazole isomer 1	8.36	8.38	**1.2**
28	Chlortoluron	5.76	5.72	**2.4**	72	Carfentrazone ethyl	8.41	8.47	**3.6**
29	Pyracarbolid	5.76	5.74	**1.2**	73	Ipconazole isomer 2	8.43	8.47	**2.4**
30	Carbofuran	5.82	5.82	**0.0**	74	Picoxystrobin	8.51	8.56	**3.0**
31	Carbaryl	5.95	5.94	**0.6**	75	Benalaxyl	8.73	8.81	**4.8**
32	Isoproturon	6.11	6.09	**1.2**	76	Tebufenpyrad	8.83	8.89	**3.6**
33	Thiophanate-methyl	6.23	6.22	**0.6**	77	Prochloraz	8.88	8.96	**4.8**
34	Cycluron	6.31	6.32	**0.6**	78	Trifloxystrobin	9.10	9.17	**4.2**
35	Flutriafol	6.52	6.49	**1.8**	79	Clethodim isomer 2	9.16	9.28	**7.2**
36	Fenobucarb	6.57	6.54	**1.8**	80	Pyraclostrobin	9.28	9.38	**6.0**
37	Methabenzthiazuron	6.63	6.60	**1.8**	81	Benzoximate	9.33	9.44	**6.6**
38	Siduron	6.66	6.66	**0.0**	82	Quinoxyfen	9.49	9.53	**2.4**
39	Isocarbophos	6.73	6.73	**0.0**	83	Etoxazole	9.65	9.74	**5.4**
40	Desmedipham	6.85	6.80	**3.0**	84	Propargite	9.68	9.82	**8.4**
41	Prometryne	6.87	6.88	**0.6**	85	Amitraz	9.74	9.88	**8.4**
42	Halofenozide	6.93	6.92	**0.6**	86	Hexythiazox	10.12	9.94	**10.8**
43	Phenmedipham	6.93	6.86	**4.2**	87	Fenazaquin	10.22	10.14	**4.8**
44	Paclobutrazol	6.94	6.91	**1.8**					

Comparing Column Chemistries

Column selectivity can easily be demonstrated by using the virtual tool. To demonstrate this, three unique examples were chosen, all using the same conditions listed in Table 1, and a subset of compounds listed in Table 2.

Example 1: Aromatic Retention Characteristics

Four analytes were selected from the previous list. Using the same conditions listed in Table 1, compounds were modelled on all four phases, and the retention time data was compared.

Figure 3: Analytes Containing a Phenyl Ring

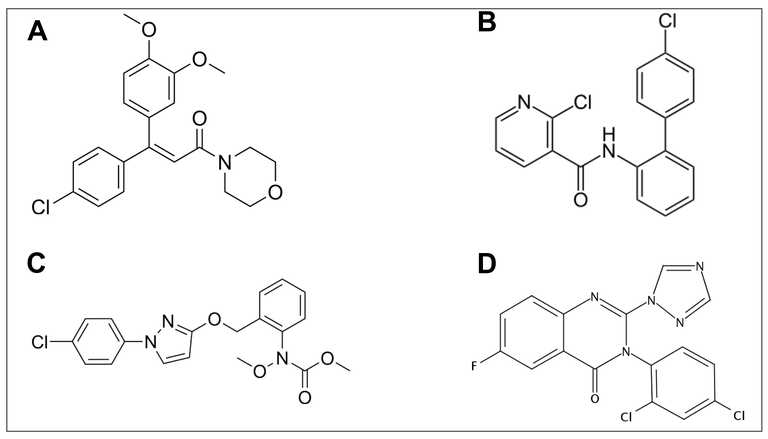


Table 2: Retention Time Comparison of Analytes Containing a Phenyl Ring

Structure Label	Column Chemistry / Analyte Name	C18 t_R (min)	ARC-18 t_R (min)	Aqueous C18 t_R (min)	Biphenyl t_R (min)
A	Boscalid	6.01	6.49	6.74	7.66
B	Dimethomorph Isomer I	5.93	6.52	6.99	8.23
	Dimethomorph Isomer II	6.20	6.71	7.09	8.34
C	Pyraclostrobin	7.39	7.81	7.89	9.28
D	Fluquinconazole	6.43	6.91	7.13	8.31

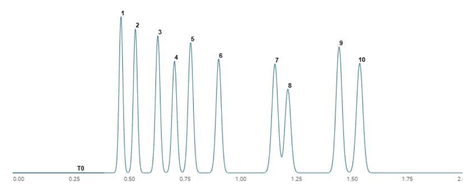
Comparing Column Chemistries

Example 2: Early Eluters

For each column chemistry, the 10 most polar compounds in the panel were plotted to assess retention characteristics for the early eluters.

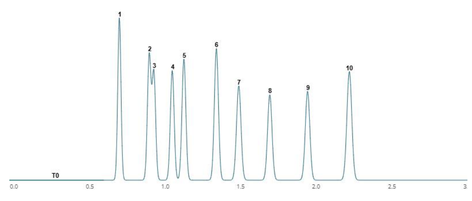
Raptor C18

Peak	Analyte	t_R (min)
1	Formetanate HCL	0.46
2	Pymetrozine	0.53
3	Omethoate	0.63
4	Aldicarb sulfoxide	0.70
5	Butoxycarboxim	0.77
6	Oxamyl	0.90
7	Flonicamid	1.15
8	Thiamethoxam	1.21
9	Monocrotophos	1.44
10	Thiabendazole	1.53



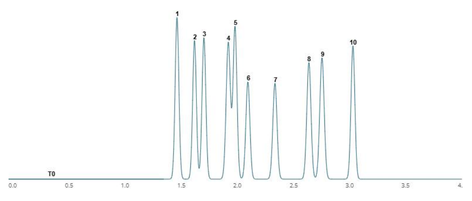
Raptor ARC-18

Peak	Analyte	t_R (min)
1	Formetanate HCL	0.70
2	Omethoate	0.89
3	Pymetrozine	0.92
4	Aldicarb sulfoxide	1.05
5	Butoxycarboxim	1.12
6	Oxamyl	1.34
7	Flonicamid	1.49
8	Thiamethoxam	1.69
9	Monocrotophos	1.94
10	Thiabendazole	2.22



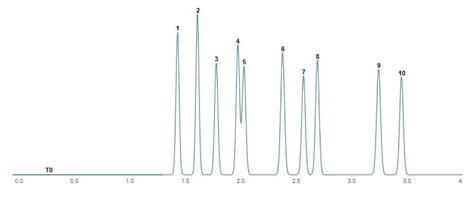
Ultra Aqueous C18

Peak	Analyte	t_R (min)
1	Omethoate	1.46
2	Formetanate HCL	1.62
3	Butoxycarboxim	1.70
4	Flonicamid	1.92
5	Aldicarb sulfoxide	1.98
6	Oxamyl	2.09
7	Pymetrozine	2.33
8	Monocrotophos	2.64
9	Thiamethoxam	2.75
10	3-Hydroxycarbofuran	3.03



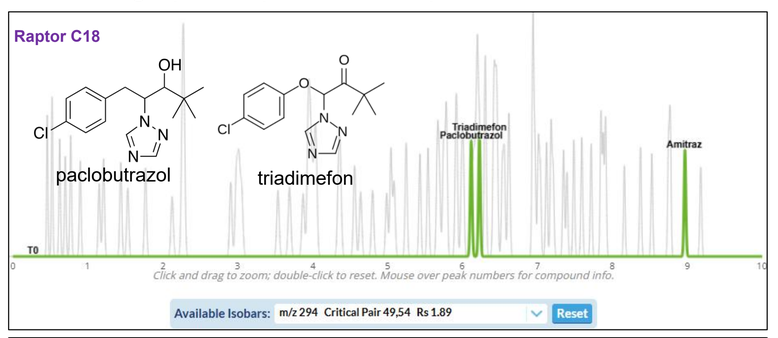
Raptor Biphenyl

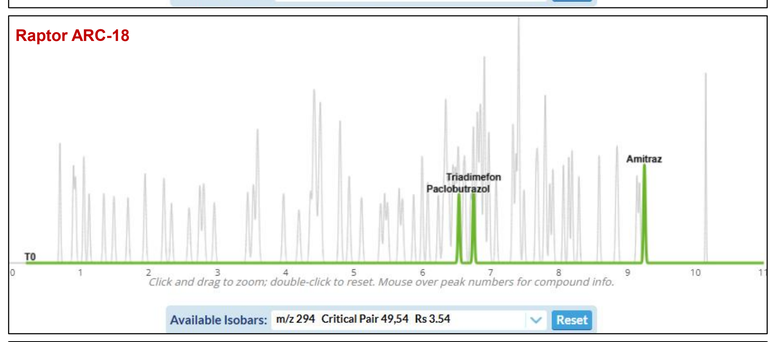
Peak	Analyte	t_R (min)
1	Omethoate	1.44
2	Formetanate HCL	1.63
3	Aldicarb sulfoxide	1.81
4	Butoxycarboxim	2.01
5	Flonicamid	2.06
6	Pymetrozine	2.43
7	Monocrotophos	2.63
8	Oxamyl	2.76
9	Thiabendazole	3.34
10	Thiamethoxam	3.57

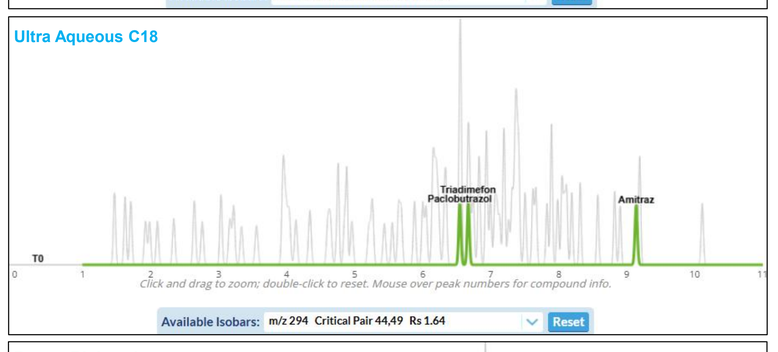


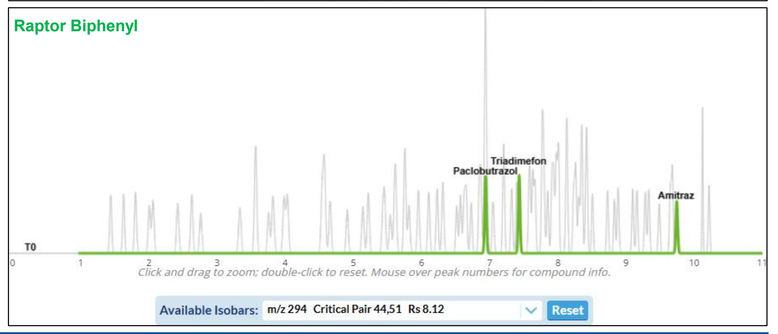
Example 3: Isobaric Resolution Capabilities

For each column chemistry, the separation of an isobaric pair, paclobutrazol and triadimefon, requiring chromatographic separation was plotted.









Discussion and Conclusions

The accuracy of the virtual method development tool was demonstrated by comparing the experimental chromatogram (Figure 1) to the modelled chromatogram (Figure 2). Of the 81 compounds in the panel, no compound had more than a 10.8 second difference in retention compared to the modelled result (Table 1). With the accuracy of the modeller verified, the selectivity of various C18-type stationary phases were compared with the Biphenyl data. Analytes containing a phenyl ring (Figure 3) were significantly more retained on the Biphenyl phase compared to C18-type phases (Table 2). This is due to the strong π-π interactions of the Biphenyl phase which are enhanced through the use of methanol in mobile phase B. Unlike acetonitrile, methanol does not contain π electrons and can be used to strengthen π-π driven separations. Example 2 demonstrates the effectiveness of the Biphenyl phase for early eluting compounds which show poor retention on Raptor C18 and Raptor ARC-18. Only Ultra Aqueous C18 shows comparable retention times to Raptor Biphenyl, however this is likely due Ultra being a fully porous particle while Raptor is a superficially porous particle, indicating higher retention factors for Raptor Biphenyl. Example 3, the separation of an isobaric pair, further demonstrates the advantage of Biphenyl's π-π retention mechanism for critical separations. The unique selectivity of the Biphenyl phase can be leveraged to achieve difficult separations not possible on C18-type phases.