

Agilent Zorbax RP-HPLC Bonded Phases

Several types of bonded phases are required in order to achieve optimum resolution for a wide range of compounds, whilst providing maximum column lifetime. Zorbax® SB is recommended for low pH use. Zorbax Eclipse XDB and Bonus-RP are optimum for mid pH range analyses. Zorbax Extend-C18 is designed for high pH applications.

Figure 1. pH range for optimum column life

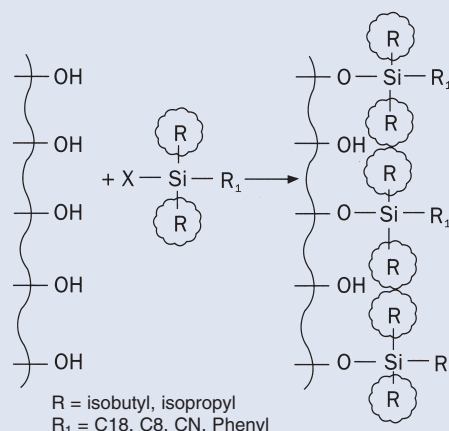
Recommended Column	pH Range for Optimal Column Life												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ZORBAX StableBond													
ZORBAX Eclipse XDB													
ZORBAX Bonus-RP													
ZORBAX Extend-C18													

ZORBAX Rx and SB

Zorbax Rx phases utilise a new generation highly purified base silica. Consequently the Zorbax Rx-C8 and Rx-C18 materials are well suited for the analysis of basic molecules. In the Zorbax Rx-C18 material a dimethyloctadecylsilyl reagent is bonded to the Zorbax Rx silica surface. Zorbax Rx-C8 and SB-C8 materials are identical. They are manufactured using a sterically protected octylsilane.

Zorbax StableBond phases are designed for separating acidic, neutral and basic molecules at low pH. The Zorbax SB - C18, SB-CN and SB-Ph materials are also manufactured by bonding unique monofunctional silanes to Zorbax Rx silica (Figure 2). Bulky side-chain groups associated with the silanes sterically protect the siloxane bond from hydrolytic attack at low pH.

Figure 2. Reaction of monofunctional sterically protected silane with hydroxylated silica



Zorbax StableBond packings are engineered to be safely used under low pH conditions. For this reason, endcapping is not used on any Zorbax SB stationary phases so that premature column failure due to rapid stripping of the unstable endcapping silanes e.g. TMS, can be avoided.

Zorbax Rx and SB Phases

Material	Bonded Phase Ligand	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Optimum pH
Zorbax Rx-C8	Diisopropyl octyl	3.5, 5, 7	80	180	5.5	1-6
Zorbax Rx-C18	Dimethyl octadecyl	3.5, 5, 7	80	180	12	1-6
SB-C3	Diisopropyl propyl	5	80	180	4	1-6
SB-C8	Diisopropyl octyl	3.5, 5, 7	80	180	5.5	1-6
SB-C18	Diisobutyl octadecyl	3.5, 5, 7	80	180	10	1-6
SB-CN	Diisopropyl cyanopropyl	3.5, 5, 7	80	180	4	1-6
SB-Phenyl	Diisopropyl phenyl	3.5, 5, 7	80	180	5.5	1-6
SB-Aq ¹	Proprietary	5	80	180	Proprietary	1-6

¹ See p.228 for further information

Zorbax SB Phase Features

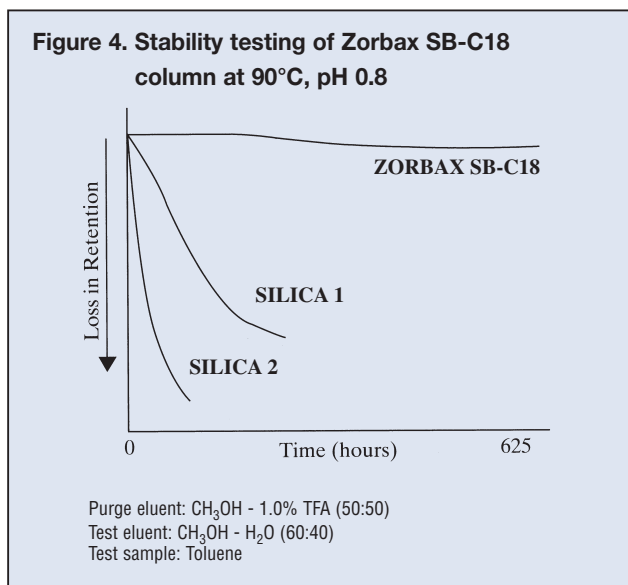
- Stability at low pH

Cyano- and phenyl-bonded phases offer alternative reversed-phase selectivities to that achievable with C18-bonded phases. However cyano- and phenyl-bonded phases are usually avoided by chromatographers because of their reputation for poor stability and short column lifetimes.

Through the use of sterically protected silanes, Zorbax SB-C8, CN, and even phenyl bonded phases can be made that have stability and lifetime comparable to C18 bonded phases (Figure 3). This patented technology is responsible for the long column lifetimes exhibited by the Zorbax 'StableBond' HPLC columns.

- Extended Temperature Use

For Zorbax SB-C18 an octadecyldiisopropyl silane is bonded to the Zorbax silica. The enhanced stability of the SB-C18 column is shown in a study at 90°C using a low pH mobile phase comparing a number of commercial columns (Figure 4).

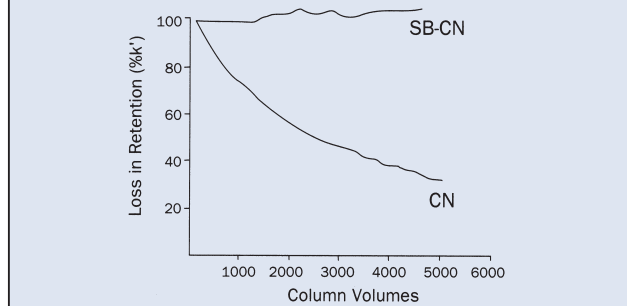


- Rapid Resolution™ Columns

Specially engineered Zorbax SB-C8 and SB-C18 Rapid Resolution™ columns can reduce analysis time by more than 60% and eliminate as much as 50% of the solvent waste compared to conventional HPLC columns. These columns provide savings in time and solvent waste without the disadvantages of low resolution and frequent column plugging that is common with other columns packed with 3 micron material.

FOR WIDE PORE ZORBAX SB 300Å PHASES REFER TO P. 232

Figure 3. Loss in bonded phase (retention) for standard CN and SB-CN bonded columns



- High Efficiency 3.5µm Particles

Zorbax SB packing material of 3.5µm particle size generates more plates per metre and can be operated at higher linear velocity (flow rate) than the 5µm packing material that is typically used in standard high resolution columns. This means that short Zorbax SB-C8 and SB-C18 Rapid Resolution columns can be used to significantly reduce analysis time without sacrificing resolution.

- Minimum Particle Size Distribution

Typical '3 micron' columns use 0.5µm porosity inlet frits. The broad distribution of packing particle sizes prevent the use of larger porosity frits. These frits plug with particulate material much more readily than the 2µm frits that are typically used on columns packed with 5µm material. For this reason '3 micron' columns have often been inconvenient to use because of the frequent need to replace frits.

Zorbax SB 3.5µm material is precisely sized with an average particle diameter of 3.5µm and a very narrow size distribution. This approach eliminates the need to use 0.5µm frits on the column inlet. In fact, these columns use the identical 2µm frits used with 5µm packing so they are no more likely to plug and just as easy and convenient to use. In addition, columns packed with the Zorbax SB 3.5µm material are designed to operate at pressures compatible with most HPLC systems.

- Narrow Bore Columns

Selected narrow bore Zorbax columns are available in two diameters, 3.0 mm (medium bore) and 2.1 mm (narrow bore). These columns are designed to minimise solvent usage whilst still providing highly efficient separations. Those readily available are shown in the range list. It may be possible to supply further columns not listed.

Zorbax Eclipse™ XDB

- Four bonded phases for different selectivity
- Good peak shape for acids, bases and neutrals
- Wide pH range 2 - 9

Zorbax Eclipse™ XDB columns feature eXtra Dense Bonding and double endcapping through a proprietary process to cover as many active silanols as possible. This dense bonding minimises unwanted interactions between analytes and surface silanols of the silica particle. These particles resist dissolution at mid-pH for increased column stability.

Four bonded phase choices are available for method development optimisation. Zorbax Eclipse XDB columns can be used over a wide pH range of 2 - 9 (best at 3 - 8). They are available in 1.8, 3.5, 5 and 7µm particle sizes for high speed, high resolution, analytical and preparative scale separations. For 1.8µm Zorbax Eclipse XDB columns, see also page 265.

Zorbax Eclipse XDB Phases

Phase	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Endcapped	pH Range*	Temperature Limit (°C)
C8	80	180	7.6	Double	Max. 2-9 Recomm. 3-8	60
C18	80	180	10	Double	Max. 2-9 Recomm. 3-8	60
Phenyl	80	180	7.2	Double	Max. 2-9 Recomm. 3-8	60
CN	80	180	4.3	Double	Max. 2-9 Recomm. 3-8	60

* The solubility of silica in the eluent determines column lifetime at high pH. Column lifetime at pH >7 is expected to decrease with increasing temperature and increasing water content in the eluent. Below pH 3, lifetime for Eclipse XDB columns will also decrease with increasing temperature.

Ordering Information (see p.264 for microbore and capillary columns and p.266 for RRHT 1.8µm columns)

Description	Particle Size (µm)	Dimensions (mm)	Eclipse XDB-C18 Catalogue No.	Eclipse XDB-C8 Catalogue No.	Eclipse XDB-Ph Catalogue No.	Eclipse XDB-CN Catalogue No.	Price
Narrow Bore LC-MS	5	50 x 2.1	960967-902	960967-906	960967-912	960967-905	£196
Narrow Bore Rapid Resolution	3.5	100 x 2.1	961753-902	961753-906	-	961753-905	£310
Narrow Bore	5	150 x 2.1	993700-902	993700-906	993700-912	993700-905	£310
Solvent Saver Plus	3.5	150 x 3.0	963954-302	963954-306	963954-312	963954-305	£345
Solvent Saver	5	150 x 3.0	993967-302	993967-306	993967-312	993967-305	£310
Solvent Saver	5	250 x 3.0	990967-302	990967-306	990967-312	990967-305	£345
Rapid Resolution	3.5	50 x 4.6	935967-902	935967-906	935967-912	-	£219
Rapid Resolution	3.5	75 x 4.6	966967-902	966967-906	966967-912	966967-905	£268
Rapid Resolution	3.5	100 x 4.6	961967-902	961967-906	-	961967-905	£310
Rapid Resolution	3.5	150 x 4.6	963967-902	963967-906	963967-912	963967-905	£345
Analytical	5	150 x 4.6	993967-902	993967-906	993967-912	993967-905	£310
Analytical	5	250 x 4.6	990967-902	990967-906	990967-912	990967-905	£345
Semi-Preparative	5	250 x 9.4	990967-202	990967-206	-	-	£813
Guard Cartridges							
Narrow Bore ¹ (4/pk)	5	12.5 x 2.1	821125-926 ²		-	821125-935	£113
Analytical ¹ (4/pk)	5	12.5 x 4.6	820950-925	820950-926	820950-927	820950-935	£113
Rapid Resolution Cartridges (Requires hardware kit 820555-901, £133)							
Narrow Bore	3.5	15 x 2.1	975700-902	975700-906	-	-	£123
Narrow Bore (3/pk)	3.5	15 x 2.1	975700-932	975700-936	-	-	£304
Narrow Bore	3.5	30 x 2.1	973700-902	973700-906	-	-	£152
Narrow Bore (3/pk)	3.5	30 x 2.1	973700-932	973700-936	-	-	£382
Analytical	3.5	15 x 4.6	931975-902	931975-906	-	-	£123
Analytical (3/pk)	3.5	15 x 4.6	931975-932	931975-936	-	-	£304
Analytical	3.5	30 x 4.6	933975-902	933975-906	-	-	£152
Analytical (3/pk)	3.5	30 x 4.6	933975-932	933975-936	-	-	£382
High Resolution and Efficiency HP-Merck Cartridge (Requires reusable hardware kit 5021-1845, £173)							
Analytical	3.5	75 x 4.6	7995118-344	7995108-344	-	-	£258
Analytical	5	150 x 4.6	7995118-595	7995108-595	-	-	£265
Analytical	5	250 x 4.6	7995118-585	7995108-585	-	-	£311

¹ Guard cartridges require a reusable hardware kit 820888-901 (£114)

² Packed with Eclipse XDB-C8

Ordering Information

Zorbax Rx

Description	Particle Size (µm)	Dimensions (mm)	Rx-Sil Catalogue No.	Rx-C18 Catalogue No.	Rx-C8 Catalogue No.	Price
Narrow Bore	5	150 x 2.1	-	883700-902	883700-906	£311
Solvent Saver	5	150 x 3.0	-	883967-302	883975-306	£311
Solvent Saver	5	250 x 3.0	-	880967-302	880975-306	£346
Rapid Resolution	3.5	75 x 4.6	-	866967-902	866953-906	£269
Rapid Resolution	3.5	150 x 4.6	-	863967-902	863953-906	£346
Analytical	5	150 x 4.6	883975-901	883967-902	883967-901	£311
Analytical	5	250 x 4.6	880975-901	880967-902	880967-901	£346
Semi-Preparative	5	250 x 9.4	880975-201	880967-202	880967-201	£813
Reliance Cartridges						
Analytical ¹	5	80 x 4.0	-	820967-902	820967-901	£131
Narrow Bore Guard Cartridge ² (4/pk)	5	12.5 x 2.1	821125-919	-	-	£113
Guard Cartridge ² (4/pk)	5	12.5 x 4.6	-	821125-926 ⁴	-	£113
Preparative Guard Cartridge ³ (2/pk)	7	15 x 9.4	820950-919	820950-914	820950-913	£113
Preparative Guard Cartridge ³ (2/pk)	7	15 x 9.4	820675-119	820675-115 ⁵		£120

Zorbax SB (80Å pore size) (see p.262 for 300Å, p.264 for microbore and capillary and p.265 for RRHT 1.8µm columns)

Description	Particle Size (µm)	Dimensions (mm)	SB-C18 Catalogue No.	SB-C8 Catalogue No.	SB-CN Catalogue No.	SB-C3 Catalogue No.	SB-Phenyl Catalogue No.	SB-Aq Catalogue No.	Price
Narrow Bore LC-MS	5	50 x 2.1	860975-902	860975-906	860975-905	860975-909	860975-912	860975-914	£205
Narrow Bore Rapid Resolution	3.5	100 x 2.1	861753-902	861753-906	861753-905	-	861753-912	861753-914	£311
Narrow Bore	5	150 x 2.1	883700-922	-	883700-905	883700-909	883700-912	-	£311
Solvent Saver Plus	3.5	150 x 3.0	863954-302	863954-306	863954-305	-	863954-312	863954-314	£346
Solvent Saver	5	150 x 3.0	883975-302	883975-306	883975-305	883975-309	883975-312	883975-314	£310
Solvent Saver	5	250 x 3.0	880975-302	880975-306	880975-305	880975-309	880975-312	880975-314	£346
Rapid Resolution	3.5	50 x 4.6	835975-902	835975-906	835975-905	-	835975-912	835975-914	£219
Rapid Resolution	3.5	75 x 4.6	866953-902	866953-906	866953-905	-	866953-912	866953-914	£269
Rapid Resolution	3.5	100 x 4.6	861953-902	861953-906	861953-905	-	861953-912	861953-914	£311
Rapid Resolution	3.5	150 x 4.6	863953-902	863953-906	863953-905	-	863953-912	863953-914	£346
Analytical	5	150 x 4.6	883975-902	883975-906	883975-905	883975-909	883975-912	883975-914	£311
Analytical	5	250 x 4.6	880975-902	880975-906	880975-905	880975-909	880975-912	880975-914	£346
Semi-Preparative	5	250 x 9.4	880975-202	880967-201	880975-205	880975-209	880975-212	-	£813
Rapid Resolution Cartridges (Requires reusable hardware kit 820555-901, £133)									
Narrow Bore	3.5	15 x 2.1	875700-902	875700-906	-	-	-	-	£123
Narrow Bore 3/pk	3.5	15 x 2.1	875700-932	875700-936	-	-	-	-	£304
Narrow Bore	3.5	30 x 2.1	873700-902	873700-906	-	-	-	-	£152
Narrow Bore 3/pk	3.5	30 x 2.1	873700-932	873700-936	-	-	-	-	£382
Analytical	3.5	15 x 4.6	831975-902	831975-906	-	-	-	-	£123
Analytical 3/pk	3.5	15 x 4.6	831975-932	831975-936	-	-	-	-	£304
Analytical	3.5	30 x 4.6	833975-902	833975-906	-	-	-	-	£152
Analytical 3/pk	3.5	30 x 4.6	833975-932	833975-936	-	-	-	-	£382
High Resolution and Efficiency HP/Merck Cartridges (Requires reusable hardware kit 5021-1845, £173)									
Analytical	3.5	75 x 4.6	7995218-344	7995208-344	-	-	-	-	£258
Analytical	5	150 x 4.6	7995218-595	7995208-595	-	-	-	-	£265
Analytical	5	250 x 4.6	7995218-585	7995208-585	-	-	-	-	£311
Reliance Cartridges									
Analytical ¹	5	80 x 4.0	820975-902	820975-906	820975-905	-	820975-912	-	£131
Narrow Bore Guard Cartridge ² (4/pk)	5	12.5 x 2.1	821125-926 ⁴		821125-924 ⁶		821125-926 ⁴	821125-933	£113
Guard Cart ² (4/pk)	5	12.5 x 4.6	820950-920	820950-915	820950-916	820950-922	820950-917	820950-933	£113
Preparative Guard Cartridge ³ (2/pk)	7	15 x 9.4	820675-115 ⁵		820675-124 ⁷		820675-115 ⁵	-	£120

¹ Reliance cartridges require a reusable hardware kit 820678-901 (£82)

² Guard cartridges require a reusable hardware kit 820888-901 (£114)

³ Preparative guard cartridges require a reusable hardware kit 840140-901 (£138)

⁴ Packed with Zorbax Eclipse XDB-C8

⁵ Packed with Zorbax SB-C8

⁶ Packed with Zorbax 300SB-C3, 5µm

⁷ Packed with Zorbax 300SB-C3, 7µm

Zorbax® Bonus-RP

- Unique reversed-phase selectivity
- Novel bonding technology with embedded polar group and steric protection
- Excellent peak shape for basic compounds at low and mid pH
- Usable in 100% aqueous eluents

Zorbax® Bonus-RP has a polar amide group embedded in a long alkyl chain (see Figure 7). This novel bonding reduces interactions between basic compounds and the silica support, improving peak shape for the most difficult basic compounds. Peak shape and column lifetime are further improved by triple endcapping. In addition, diisopropyl side groups provide steric protection against acid hydrolysis for good lifetime at low pH. Zorbax Bonus-RP columns provide alternative selectivity to C18 and C8 bonded phases.

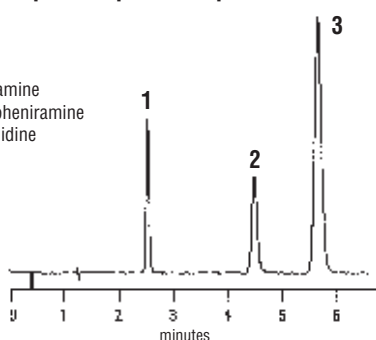
Zorbax Bonus-RP Phase

Phase Type	Polar-embedded alkyl
Particle Size (µm)	3.5, 5
Pore Size (Å)	80
Surface Area (m ² /g)	180
Carbon Load (%)	9.5
Endcapped	Triple
pH Range	2 - 9
Temperature Limits (°C)*	60

* Temperature limits are 60°C up to pH 8, 40°C from pH 8-9

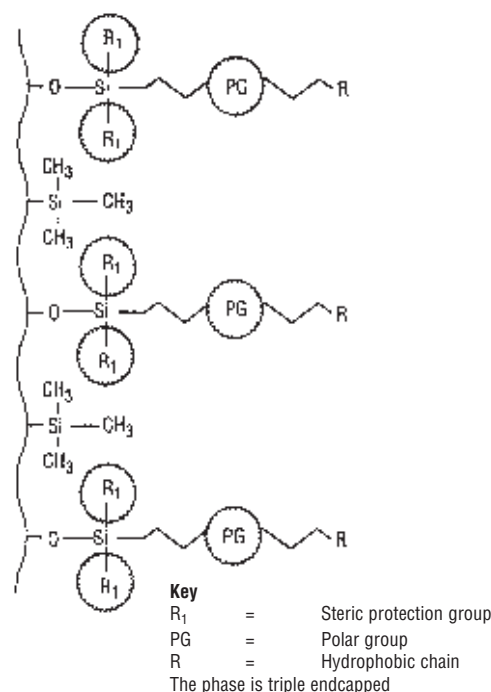
Figure 8. Improved peak shape for basic drugs

1. Doxylamine
2. Chlorpheniramine
3. Triprolidine



Column: Zorbax Bonus-RP (150 x 4.6mm) Flow rate: 1.5ml/min
Eluent: 25mM NH₄Ac, pH 5.5 - CH₃CN (80:20) Temperature: 40°C

Figure 7. Zorbax Bonus-RP structure



Ordering Information

Description	Particle Size (µm)	Dimensions ² (mm)	Catalogue No.	Price
Narrow Bore for LC-MS, Combinatorial chemistry	3.5	50 x 2.1	861700-901	£219
	5	50 x 2.1	861971-901	£205
Narrow Bore Rapid Resolution	3.5	100 x 2.1	861768-901	£311
Narrow Bore	5	150 x 2.1	883725-901	£311
Solvent Saver Plus	3.5	100 x 3.0	864668-301	£311
Solvent Saver Plus	3.5	150 x 3.0	863668-301	£346
Solvent Saver	5	150 x 3.0	883668-301	£310
Solvent Saver	5	250 x 3.0	880668-301	£346
Rapid Resolution	3.5	75 x 4.6	866668-901	£269
Rapid Resolution	3.5	100 x 4.6	864668-901	£311
Analytical	3.5	150 x 4.6	863668-901	£346
Analytical	5	150 x 4.6	883668-901	£311
Analytical	5	250 x 4.6	880668-901	£346
Guard Cartridges				
Narrow Bore ¹ (4/pk)	5	12.5 x 2.1	821125-928	£113
Analytical ¹ (4/pk)	5	12.5 x 4.6	820950-928	£113

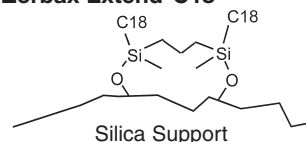
¹ Guard cartridges require a reusable hardware kit 820888-901 (£114)

² Microbore (1mm i.d.) columns listed on p.264

Zorbax® Extend-C18 and 300Extend-C18

Zorbax® Extend-C18 and 300Extend-C18 are novel phases, based on the Zorbax Rx-Sil particle. Zorbax Extend-C18 is designed for high pH applications. The patented chemistry of Extend-C18 incorporates a novel C18-C18 bidentate structure (Figure 9), and the double endcapping procedure enhances its stability at high pH.

Figure 9. The unique bidentate structure of Zorbax Extend-C18



Zorbax Extend-C18 Phases

Zorbax Phase	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Temperature Limits (°C)	pH Range
Extend-C18	80	180	12.5	60°C at pH <8, 40°C at pH >8	2.0 - 11.5
300Extend-C18	300	45	4		

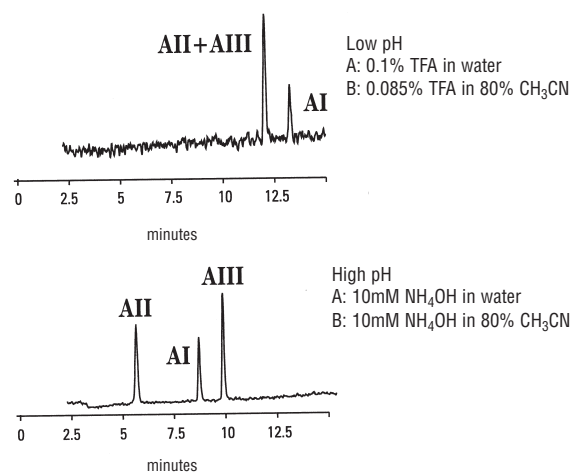
The Extend-C18 column can be used for the analysis of smaller molecules (MW<4000), and the 300Extend-C18 column is suitable for the efficient separation of large proteins and polypeptides.

The unique high pH stability and excellent resolution of Zorbax Extend-C18 extends column utility to a variety of applications:

- Using ammonium hydroxide as eluent with LC-MS of peptides or small molecules (Extend-C18) and proteins and polypeptides (300Extend-C18) for excellent signal/noise performance and alternative selectivity
- Separating basic compounds above their pK_a in free base (uncharged) form giving added retention and/or alternative selectivity
- General separation of basic, acidic and neutral compounds at high pH often providing different selectivity
- Rapid separations using 3.5µm and 1.8µm columns

Figure 10 shows the increased selectivity and LC-MS sensitivity achieved for angiotensins I, II and III at pH 10 (NH₄OH eluent) compared with the low pH assay (TFA).

Figure 10. Improved resolution of angiotensins at high pH



Column: Zorbax Extend-C18 (150 x 2.1 mm)
Flow rate: 0.2ml/min
Gradient: 15-50% B in 15mins
Temperature: 35°C
Sample: Angiotensins I, II and III (50pmol each)
LC-MS: Positive ion ESI

Ordering Information (see p.264 for microbore and capillary columns and p.265 for RRHT 1.8µm columns)

Description	Particle Size (µm)	Dimensions ² (mm)	Extend-C18 Catalogue No.	300Extend-C18 Catalogue No.	Price
Rapid Resolution	3.5	50 x 2.1	735700-902	765750-902	£241
Narrow Bore Rapid Resolution	5	50 x 2.1	760450-902	-	£226
Narrow Bore Rapid Resolution	3.5	100 x 2.1	761753-902	761775-902	£343
Narrow Bore Rapid Resolution	3.5	150 x 2.1	-	763750-902	£382
Narrow Bore	5	150 x 2.1	773700-902	-	£342
Solvent Saver Plus	3.5	100 x 3.0	764953-302	-	£342
Solvent Saver Plus	3.5	150 x 3.0	763954-302	-	£382
Solvent Saver	5	150 x 3.0	773450-302	-	£341
Solvent Saver	5	250 x 3.0	770450-302	-	£380
Rapid Resolution	3.5	50 x 4.6	735953-902	765973-902	£241
Analytical	5	50 x 4.6	746450-902	-	£226
Rapid Resolution	3.5	75 x 4.6	766953-902	-	£297
Rapid Resolution	3.5	100 x 4.6	764953-902	761973-902	£343
Rapid Resolution	3.5	150 x 4.6	763953-902	763973-902	£382
Analytical	5	150 x 4.6	773450-902	773995-902	£342
Analytical	5	250 x 4.6	770450-902	770995-902	£381
Guard Cartridges					
Narrow Bore ¹ (4/pk)	5	12.5 x 2.1	821125-930	821125-932	£124
Analytical ¹ (4/pk)	5	12.5 x 4.6	820950-930	820950-932	£124

¹ Guard cartridges require a reusable hardware kit 820888-901 (£114)

² Microbore (1mm i.d.) columns listed on p.264