ZORBAX Solvent Saver Columns: Cost Savings and LC-MS Compatibility

- **Solvent and waste disposal 60% less than for 4.6 mm I.D. columns**
- **The same resolution and selectivity as larger-diameter columns**

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With the wide variety of column diameters and lengths available, the usefulness and substantial benefits of Agilent's 3.0 mm I.D. ZORBAX Solvent Saver columns can sometimes be overlooked. Many researchers use 4.6 mm I.D. columns exclusively, and may be hesitant to switch to smaller-diameter columns. However, Agilent's 3.0 mm I.D. Solvent Saver columns can provide significant advantages (see Table 1), and still deliver the same resolution and selectivity as the larger-diameter columns (shown in Figure 1).

Table 1. Advantages of Agilent ZORBAX Solvent Saver and Solvent Saver Plus Columns

- 60% reduction in mobile phase usage and waste generation
- 2- to 3-fold signal-to-noise (S/N) ratio improvement
- Optimum LC/MS performance without changing columns for common interfaces (ESI, APCI, APPI)
- In most cases, no need for time-consuming and costly system modification or replumbing

Cost Savings, Signal-to-Noise Improvements, and Optimum Linear Velocity

Solvent Saver columns can provide significant solvent savings and lower waste disposal costs because the flow rate needed for 3.0 mm columns is

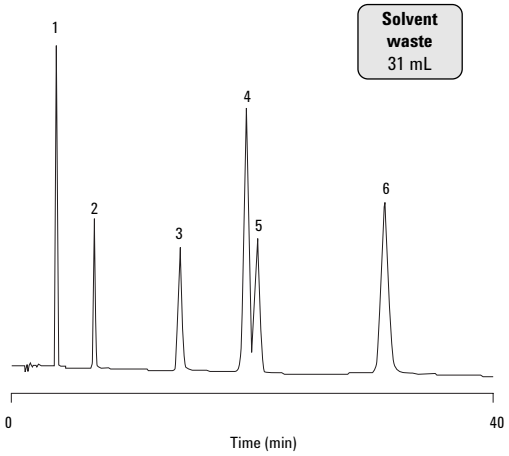
Figure 1. Separation of Antibacterials on 4.6 and 3.0 mm I.D. Columns

Mobile Phase: 80% A/20% B
A = 0.2 M Na_2HPO_4, 0.1 M citric acid (adjust to pH 2.6)
B = Acetonitrile
Column Temperature: Ambient
Sample: Antibacterials

Peak Identification
1. Sulfamerazine
2. Furazolidone
3. Oxolinic acid
4. Sulfadimethoxine
5. Sulfaquinoxaline
6. Nalidixic acid

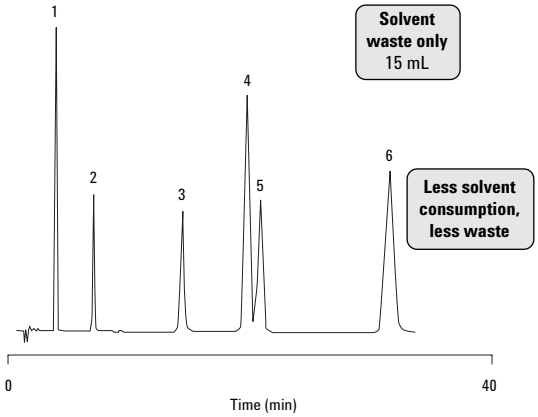
Standard Column

Column: **ZORBAX SB-C18 4.6 x 150 mm, 5 µm**
Part Number: **883975-902**
Flow Rate: 1.0 mL/min
Injection Volume: 3 µL
Detector Cell Volume: 8 µL



Solvent Saver Column

Column: **ZORBAX SB-C18 Solvent Saver 3.0 x 150 mm, 5 µm**
Part Number: **883975-302**
Flow Rate: 0.5 mL/min
Injection Volume: 2 µL
Detector Cell Volume: 8 µL



40% of the flow rate for 4.6 mm I.D. columns. Such savings can add up quickly, as these are two of the more expensive items in many laboratory budgets.

Another positive feature of Solvent Saver columns is the potential two- to three-fold signal-to-noise (S/N) improvement for the same sample mass injected. Researchers, in particular, can use this capability to their advantage when they need a small boost in the S/N ratio, especially for some environmental and pharmaceutical applications.

The most important reason why people choose Solvent Saver columns is that they are ideal for a variety of LC-MS applications and can be used effectively with all of the common MS interfaces. The flow rate ranges used for electrospray (1 µL/min. to 0.5 mL/min.) and for APCI and APPI (0.2 to 2 mL/min.) overlap in the range where Solvent Saver columns deliver their optimum efficiency (0.2 to 0.8 mL/min.). This is a big advantage and a nice convenience, because one can easily switch between interfaces without changing columns, instrument tubing, or detector flow cells.

Most Solvent Saver geometries can be used effectively with the 0.17 mm I.D. tubing and flow cell that come standard with Agilent 1100 liquid chromatography instruments and detectors, because their column volumes and associated analyte peak volumes are sufficiently large relative to normal extra-column volumes. The 100 mm and shorter Solvent Saver Plus (3.5 µm) columns produce smaller peak volumes so that they will deliver their optimal efficiency using 0.12 mm I.D. tubing and the semi-micro flow cell (4.5 µL volume).

Adapting Methods for Isocratic Separations

To use a 3.0 mm column in place of a 4.6 mm column of the same length, you must reduce the flow rate by a factor of $(3.0/4.6)^2$ or 0.42. For example, to adapt an isocratic separation at 1.0 mL/min. on a 4.6 x 150 mm, 5 µm particle size column to a 3.0 x 150 mm column, you decrease the flow rate to 1 x $(3.0/4.6)^2$ = 0.42 mL/min. Since column length and particle size stay the same, the column efficiency (plate count), retention time, and resolution remain the same.

Depending on the sample solvent composition used in the method, it may be possible to keep the same injection volume, thereby producing more than a two-fold peak height increase $(4.6/3.0)^2$. If, for your method, the percent of organic modifier in the sample solvent is high relative to that of the mobile phase, it may be necessary to decrease the injection volume for the 3.0 mm I.D. column by the same factor of 0.42. For example, to convert a 10 µL injection of sample in 100% organic modifier for a 4.6 x 250 mm column, you may have to reduce the injection volume to 4 µL or lower for a 3.0 x 250 mm column. Alternatively, the sample solvent can be diluted five- to ten-fold with water or buffer, and then a 20 µL or larger injection can be made, thus producing the same or an improved signal-to-noise ratio.

Adapting Methods for Gradient Separations

For gradient separations, the first adjustment is the same as that for isocratic separations—decrease

Table 2. Adapting Gradient Separations to 3.0 mm I.D. Columns[1]

	Agilent 1100 LC Binary with Mixer[2]	Agilent 1100 LC Binary with Mixer[2]	Agilent 1100 LC Binary (No Mixer)[3]	Agilent 1100 LC Quaternary[4]
Column	4.6 x 150 mm 5-µm Analytical	3.0 x 150 mm 5-µm Solvent Saver	3.0 x 150 mm 5-µm Solvent Saver	3.0 x 150 mm 5-µm Solvent Saver
Column Volume V_m (mL)	1.5	0.64	0.64	0.64
System Dwell Volume (µL)	900	900	480	1100
Gradient Time (min.)	30	30	30	30
Injection Volume (µL)	10	4	4	4
Flow Rate (mL/min.)	1.0	0.42	0.42	0.42
Gradient Program Time (min.)	Time 0 %B 5 Time 30 %B 95	Time 0 %B 5 Time 30 %B 95	Time 0 %B 5 Time 30 %B 95	Time 0 %B 5 Time 30 %B 95
System Re-equilibration Time	0.90 mL/1 mL/min or 0.9 min	0.90 mL/0.42 mL/min or 2.1 min.	0.48 mL/0.42 mL/min or 1.1 min.	1.1 mL/0.42 mL/min or 2.6 min.
Column Equilibration Time (10 column volumes)	15 min.	(0.64 mL x 10)/0.42 mL/min or 15 min.	(0.64 mL x 10)/0.42 mL/min or 15 min.	(0.64 mL x 10)/0.42 mL/min or 15 min.
Total Post Time	16 min.	17 min.	16 min.	17.6 min.
Dwell Volume Adjustment	None	900 µL x 0.64 mL/1.5 mL or 382 µL	480 µL x 0.64 mL/1.5 mL or 204 µL	1100 µL x 0.64 mL/1.5 mL or 469 µL
Injection Delay	None	(0.900 mL - 0.382 mL)/ 0.42 mL/min or 1.22 min.	(0.480 mL - 0.204 mL)/ 0.42 mL/min or 0.65 min.	(1.1 mL - 0.47 mL)/ 0.42 mL/min or 1.48 min.
Injector Program	None	DRAW 4 µL Remote StartPulse Wait 1.22 min. Inject	DRAW 4 µL Remote StartPulse Wait 0.65 min. Inject	DRAW 4 µL Remote StartPulse Wait 1.48 min. Inject

[1] Assumes 0.17 mm I.D. tubing throughout

[2] At maximum pressure, dwell volume is 900 µL; at minimum pressure, dwell volume is 600 µL

[3] At maximum pressure, dwell volume is 480 µL; at minimum pressure, dwell volume is 180 µL.

[4] At maximum pressure, dwell volume is 1100 µL; at minimum pressure, dwell volume is 800 µL.

the flow rate to F x $(3.0/4.6)^2$ as described above. The second critical adjustment is to decrease the effective LC system dwell volume by the ratio of the column volumes ($V_{3\,mm}/V_{4.6\,mm}$). (Column volume is approximately 0.1 mL/cm for a 4.6 mm I.D. column, and 0.042 mL/cm for a 3.0 mm column.) To illustrate this, for an Agilent 1100 LC binary system with 0.17 mm I.D. standard tubing, the dwell volume (with mixer) ranges from approximately 600 to 900 µL, depending on flow rate and backpressure. Therefore, for the maximum 900 µL dwell volume, the corrected dwell volume must be decreased to 382 µL ([0.64 mL/ 1.5 mL] x 900 µL). This can be accomplished using an injection delay with an injector program (see Table 2). Similar examples are shown for the 1100 LC binary system (without mixer) and for the 1100 LC quaternary system. If other instruments, tubing lengths, or inner diameters are used, adjustments can be made by measuring and correcting for the system dwell volume.

New Phases

New phases have been added to the ZORBAX Solvent Saver and Solvent Saver Plus family, including SB-C3, SB-Aq, Bonus-RP, Extend-C18, and Rx-C18.

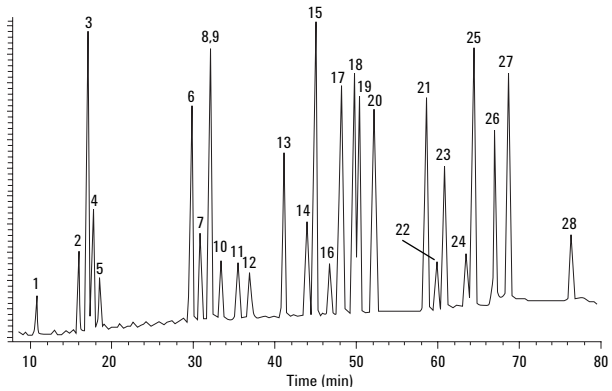
Figure 2. Analysis of Pesticides in Drinking Water

This example analysis of 28 pesticides requires a 70-minute gradient and a total analysis time of 80 minutes. If a standard 4.6 mm I.D. column were used, almost 66 mL of mobile phase would be consumed during the analysis, plus an additional 25 mL for equilibration of the column at initial gradient conditions. **The ZORBAX Solvent Saver column allows you to accomplish this separation with less than 28 mL of mobile phase plus another 10 mL for equilibration—a 58% savings in mobile phase.**

Column:	**ZORBAX SB-C18 Solvent Saver 3.0 x 250 mm, 5 µm**
Part Number:	**880975-302**
Mobile Phase:	A = 95:5 (v/v) 2 mM Sodium Acetate, pH 6.5/Acetonitrile (ACN) B = 100% ACN
Gradient Program:	10% B for 2 min. 10% to 45%B in 70 min.
Injection Volume:	25 µL
Flow Rate:	0.35 mL/min.
Column Temperature:	40°C
Detection:	245 nm

Peak Identification

1. Desisopropylatrazine
2. Metamitron
3. Fenuron
4. Chloridazon
5. Desethylatrazine
6. Metoxuron
7. Carbetamid
8. Bromacil
9. Hexazinone
10. Simazine
11. Metribuzin
12. Desethylterbutylazine
13. Carbutilat
14. Methabenzthiazuron
15. Chlortoluron
16. Atrazine
17. Monolinuron
18. Diuron
19. Isoproturon
20. Metobromuron
21. Metazachlor
22. Buturon
23. Propazine
24. Dimefuron
25. Terbuthylazine
26. Linuron
27. Chlorbromuron
28. Chloroxuron



ORDER GUIDE

ZORBAX StableBond 3.0 mm I.D. Solvent Saver and Solvent Saver Plus Columns[1]

Length (mm)	Particle Size (µm)	Part Number SB-C18	SB-C8[2]	SB-CN	SB-C3	SB-Phenyl	SB-Aq	300SB-C18	300SB-C8	300SB-C3
250	5	880975-302	880975-306	880975-305	880975-309	880975-312	880975-314			
150	5	883975-302	883975-306	883975-305	883975-309	883975-312	883975-314			
150	3.5	863954-302	863954-306	863954-305	863954-309	863954-312	863954-314	863974-302	863974-306	863974-309
100	3.5	861954-302	861954-306	861954-305	861954-309	861954-312	861954-314		861973-306	

Additional ZORBAX 3.0 mm I.D. Solvent Saver and Solvent Saver Plus Columns

Length (mm)	Particle Size (µm)	Part Number XDB-C18	XDB-C8	XDB-Phenyl	Bonus-RP	Extend-C18	Rx-C18	Rx-C8
250	5	990967-302	990967-306	990967-312	880668-301	770450-302	880967-302	880975-306
150	5	993967-302	993967-306	993967-312	883668-301	773450-302	883967-302	883975-306
150	3.5	963954-302	963954-306	963954-312	863668-301	763954-302	863967-302	863954-306
100	3.5	961967-302	961967-306	961967-312	864668-301	764953-302	861967-302	861954-306
75	3.5	966954-302						
50	3.5					735954-302		

[1] Configurations not shown available by custom column request.

[2] ZORBAX SB-C8 and Rx-C8 phases are identical.

To learn more about Agilent ZORBAX Solvent Saver columns, please visit www.agilent.com/chem and look under "Products and Services," then "Columns and Accessories."