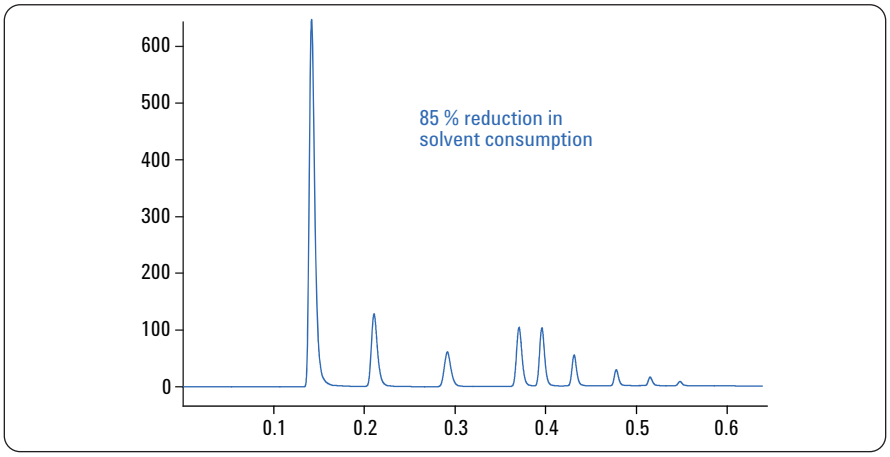
Reducing HPLC Solvent Consumption

Combating the worldwide shortage of acetonitrile by deploying short LC columns with small internal diameters and particles

Technical Note



Introduction

Towards the end of 2008 the deterioration of the global economy led to a worldwide shortage of acetonitrile – a solvent used extensively as a mobile phase component in HPLC analysis. In January 2009 the price of acetonitrile was recorded at about 100 US$ per liter with disposal costs about double this price. The immediate challenge for analytical laboratories is to reduce their consumption of this HPLC solvent as much as possible. The degree to which each laboratory is able to reduce acetonitrile consumption depends on the individual operating environment. Regulated laboratories that work with validated methods have less flexibility to change these methods and thereby have less opportunity to reduce solvent consumption. Different strategies must be followed than in non-regulated environments, in which the opportunity to reduce solvent consumption is must greater.

Agilent Technologies

Overview

The Agilent portfolio of LC equipment, including the 1120 Compact LC, 1200 Series HPLC and 1200 Series RRLC (Rapid Resolution LC), offers a wide range of possibilities to reduce solvent consumption.

- Using columns with smaller inside diameters, but of the same length, can reduce solvent consumption and costs by 50 to 60 %
- Using columns of shorter lengths and small particle sizes, for instrumentation able to support backpressures up to 400 bar, can reduce solvent consumption and costs by up to 70 %
- Using short columns with sub-2-micron particles, for fast or ultra-fast runs on instrumentation able to support backpressures greater than 400 bar, can reduce solvent consumption and costs by up to 85%

In regulated environments where laboratories use validated methods, there is often not much flexibility for the laboratory to make significant changes to the methods.Nevertheless, a strategy to reduce solvent consumption can be developed when small changes such as internal column diameter or particle size are allowed. When larger changes are allowed significant reductions can be achieved. Table 1 summarizes the extent to which the European and US Pharmacopeias allow variations in dimensions and particle sizes for LC columns.[1,2]

Dimension	USP	EP
Length	± 70 %	± 70 %
Internal diameter	± 25 %	± 25 %
Particle size	50 % smaller, not larger	50 % smaller, not larger

Table 1
LC column dimension and particle size variations allowed by EP and USP.

A variety of different approaches can be adopted whereby the most promising strategy is either to reduce column length and particle size, or to reduce the internal diameter of the column.

- Reduce internal column diameter from 4.6 to 2.1 mm. Leave particle size unchanged or reduce by half. For this approach the LC system must have low delay and dispersion volumes to support the use of 2.1 mm id columns.

- Reduce internal column diameter from 4.6 to 3.0 mm. Leave particle size unchanged or reduce by half. Typically, conventional LC equipment can be used for this approach.

- Use short, 4.6 mm id columns packed with 1.8 µm particles at high flow rates to keep run and equilibration times as short as possible. Here, special LC equipment is required, which supports backpressures in excess of 400 bar.

- Use short, 2.1 mm id columns packed with 1.8 µm particles at conventional or high flow rates. For this approach the LC system must have low delay and dispersion volumes to support the use of 2.1 mm id columns. If high flow rates are used, the LC instrumentation must allow backpressures greater than 400 bar.

- Use short, 3 mm id columns with 3.5 µm particles, which can also reduce solvent consumption significantly compared to 4.6 mm id columns packed with 5 µm particles. 50 mm x 4.6 mm id columns with 1.8 µm particles can also be used when flow rates are applied that do not exceed the 400 bar limit. An advantage of this approach is that conventional LC equipment can be used.

The following examples demonstrate the effectiveness of each of these approaches in terms of reduction in solvent consumption. Agilent ZORBAX columns were used throughout the investigation. Different Agilent LC systems were deployed for each strategy. The exact system configuration is given with the chromatographic results. A good tool to change methods is the "Agilent Method Translator and Cost Savings Caluculator", which is provided for free on a CD[3].

Chromatographic conditions

Sample:	Phenone mix, 100 ng/µL each, dissolved in water/acetonitrile (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Columns:	ZORBAX RRHT SB C18, 100 x 2.1 mm, 1.8 µm for 600 bar operation, ZORBAX RRHT SB C18, 100 x 4.6 mm, 1.8 µm for 600 bar operation
Solvents:	A: Water, B: Acetonitrile
Gradient:	0 min, 35 %B; 5 min, 95 %B, hold for 1 min
Stop time:	8 min
Post time:	5 min
Flow rates:	0.6 mL/min for 2.1 mm id column 1.5 mL/min for 4.6 mm id column
Injection vol.:	1 µL, 10 second needle wash
Column temp.:	50 °C
Detection:	Signal wavelength 245/10 nm, reference 360/80 nm 20 Hz data acquisition, peak width > 0.01 min, slit 8 nm, standard flow cell with 10 mm path length and 13 µL volume for 4.6 x 50 mm column micro flow cell 3 mm path length, 2 µL volume for 2.1 x 50 mm column

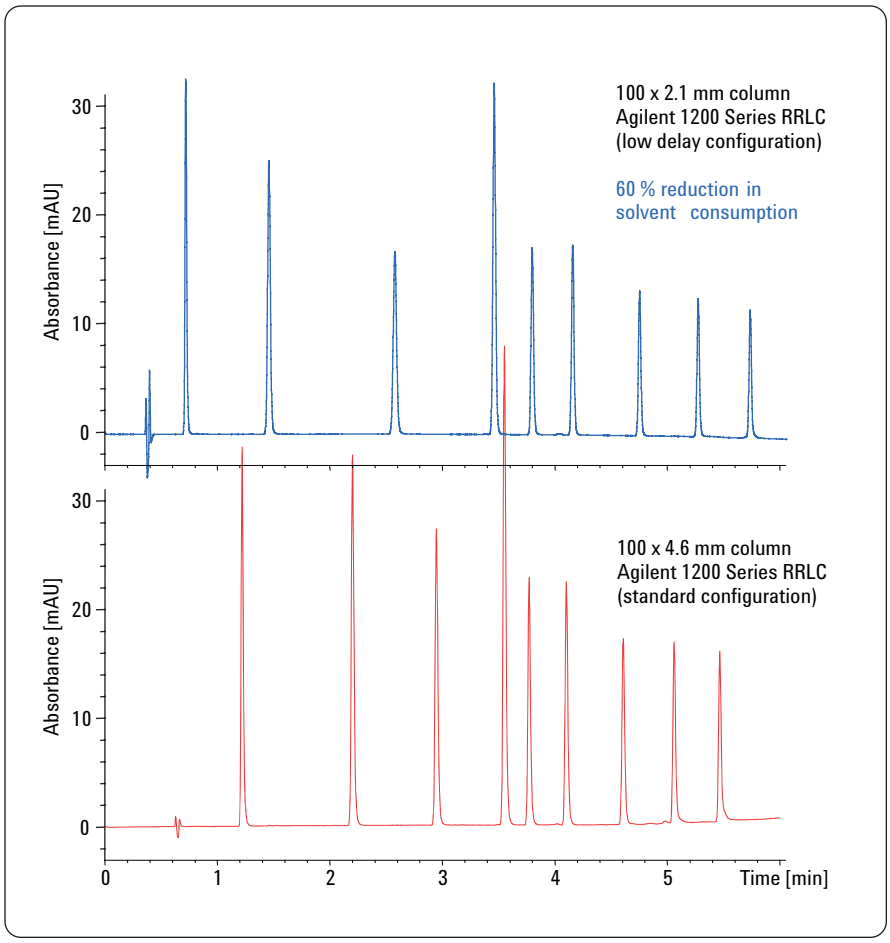


Figure 1
Solvent savings of 60 % achieved by reducing internal column diameter.

Example 1:
Reducing internal column diameter

In this example a 4.6 mm id column was replaced by a method using a 2.1 mm id column, without changing the appearance of the chromatogram and the length of the run significantly (figure 1). For this approach the LC instrumentation must be able to support the use of 2.1 mm id columns and backpressures greater than 400 bar. Significant solvent savings of 60 % were achieved by just changing to a smaller internal diameter column. Assuming the combined purchase and disposal costs of acetonitrile are 300 US$ per liter, the total cost of 100 conventional analyses amounts to 405 US$. Using a 2.1 mm id column the costs are about 162 US$, representing a 60 % reduction (table 2).

	100 x 4.6 mm column	100 x 2.1 mm column
Acetonitrile consumption per run	13.5 mL	5.4 mL
Acetonitrile costs per run	1.35 US$	0.54 US$
Disposal costs per run	2.7 US$	1.08 US$
Total costs per run	4.05 US$	1.62 US$
Costs per 100 runs	405 US$	162 US$
Costs savings per 100 runs	**243 US$**	

Table 2
Solvent and cost savings through deployment of a micro-bore column.

An additional benefit is the improvement in the resolution of peak 5. With the 4.6 mm id column the resolution was 5.09 and with the 2.1 mm id column the resolution improved to a value of 7.8.

Chromatographic conditions

Ultra fast LC with
Agilent 1200 Series RRLC system

Sample:	Phenone Test Mix (5188-6529), 1:10 diluted
Column:	ZORBAX RRHT SB C18, 50 x 4.6 mm, 1.8 µm
Gradient:	50-100 % Acetonitrile within 0.3 min
Flow rate:	5 mL/min
Stop time:	0.6 min
Column temp:	60 °C
Injection vol.:	3 µL
Detection:	VWD SL Plus, 160 Hz data acquisition rate, peak width > 0.0025 min, standard flow cell with 10 mm path length

Conventional LC with
Agilent 1200 Series HPLC system

Sample:	Phenone Test Mix (5188-6529), 1:10 diluted
Column:	ZORBAX SB-C18, 150 x 4.6 mm, 5 µm
Gradient:	35-95 % Acetonitrile within 10 min
Flow rate:	1.5 mL/min
Stop time:	10 min
Column temp:	50 °C
Injection vol.:	3 µL
Detection:	VWD, 10 Hz data acquisition rate, peak width 0.05 min, standard flow cell with 10 mm path length

Example 2:

Reducing column length and increasing flow rate

In this example a 150 x 4.6 mm column was replaced by a 50 x 4.6 mm column and a high flow rate was applied (figure 2). Using conventional LC, the total acetonitrile costs amounted 585 US$ for 100 runs. In contrast, ultra-fast LC incurred costs of only 90 US$ – a saving of 85 %. Further benefits were the analysis speed that increased by a factor of 17 and the signal-to-noise ratio that improved by about 1.5 to 2.

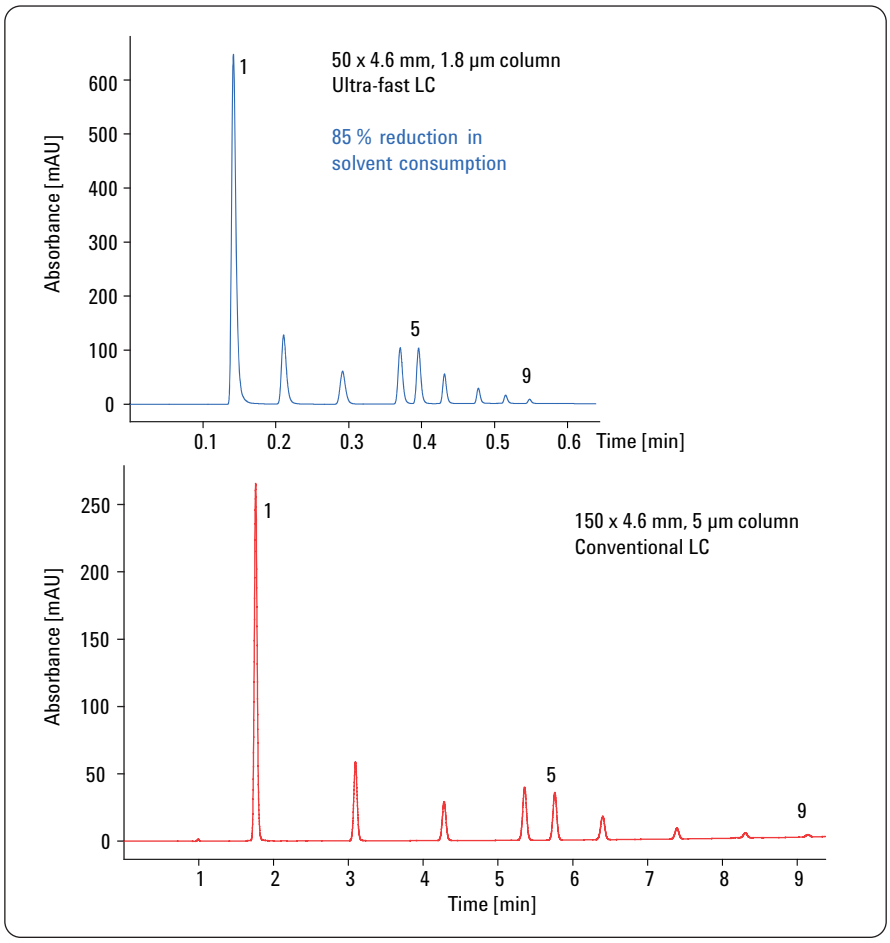


Figure 2
Solvent savings of 85 % by changing from conventional to ultra-fast LC.

	150 x 4.6 mm column, conventional LC	50 x 2.1 mm column, ultra-fast LC
Acetonitrile consumption per run	19.5 mL	3.0 mL
Acetonitrile costs per run	1.95 US$	0.30 US$
Disposal costs per run	3.90 US$	0.60 US$
Total costs per run	5.85 US$	0.90 US$
Costs per 100 runs	585 US$	90 US$
Costs savings per 100 runs	**495 US$**	

Table 3
Cost savings when changing to ultra-fast LC.

Chromatographic conditions

Narrow-bore LC with Agilent 1200 Series RRLC system

Column:	ZORBAX RRHT SB C18, 50 x 2.1 mm, 1.8 µm
Mobile phase:	Water:Acetonitrile 95:5
Flow rate:	0.5 mL/min
Gradient:	5 to 75 % Acetonitrile within 10 min
Stop time:	10 min
Post time:	2 min
Injection vol.:	1.5 µL
Column temp.:	40 °C
Detection:	VWD SL Plus, wavelength 225 nm, peak width > 0.0025 min

Conventional LC with Agilent 1120 Compact LC

Column:	HC-C18 (2), 150 x 4.6 mm, 5 µm
Mobile phase:	Water:Acetonitrile 90:10
Gradient:	0 to 90 % Acetonitrile within 15 min
Flow rate:	1.5 mL/min
Injection vol.:	20 µL
Column temp.:	40 °C
Detection:	VWD, wavelength 225 nm, peak width > 0.0025 min, response 0.06 s

Example 3:

Changing from conventional to narrow-bore LC

In this example of a pesticide application, the methodology was transferred from conventional to narrow–bore chromatography. Using short, 2.1 mm id columns achieved a reduction of solvent consumption of 80 %. The run and equilibration times were nearly the same for both methodologies (figure 3). The LC equipment must be able to support the use of narrow-bore columns. Further benefits in this case include the increase in speed and resolution for peaks 3 and 4 as well as the resolution between peak 8 and the unknown impurity.

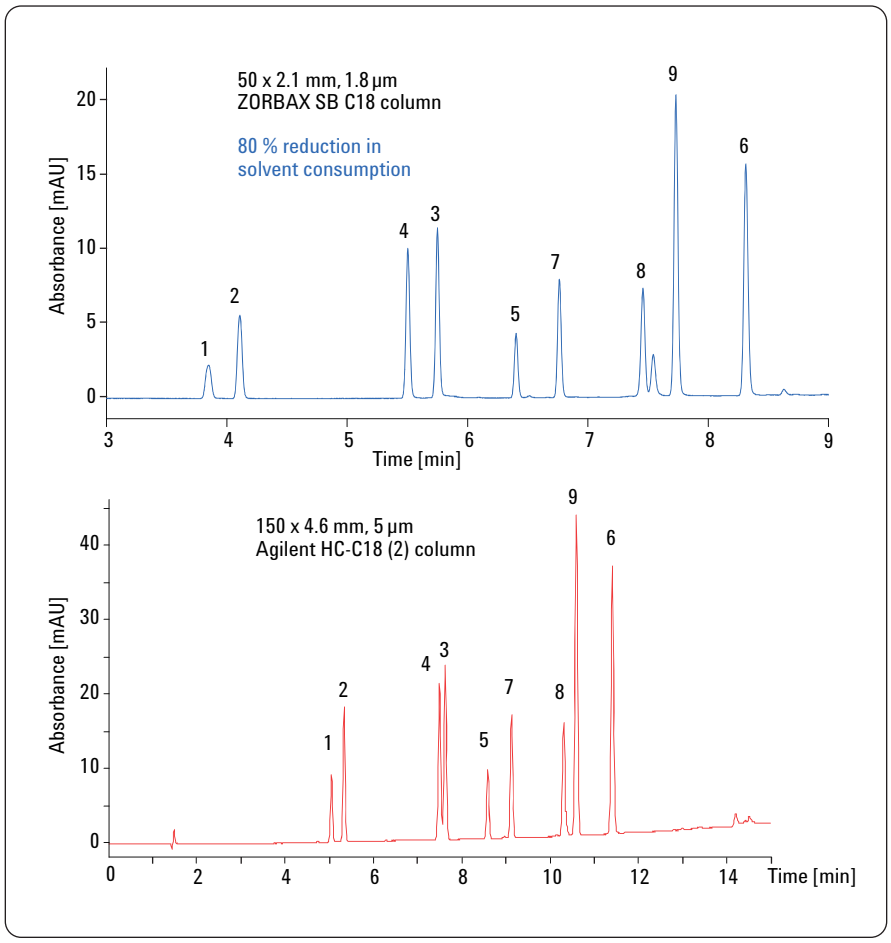


Figure 3
Solvent savings of 80 % by using narrow-bore columns.

Peak number	Compound
1	Metamitron
2	Chloridazone
3	Simazine
4	Cyanazine
5	Prometryn
6	Chlortoluron
7	Diuron
8	Propazine
9	Terbuthylazine

Chromatographic conditions

Short, narrow-bore column with Agilent 1200 Series LC

Sample:	Main compound 2 mg/mL, 4 impurities in the range 1.3 to 2.2 % (spiked)
Column:	ZORBAX SB C18, 100 x 3.0 mm, 3.5 µm
Flow rate:	0.8 mL/min
Gradient:	10 to 45 %B within 4 min
Injection vol.:	3 µL
Column temp.:	30 °C
Detection:	DAD, wavelength 270/10 nm, ref. 360/100 nm, 10 mm path length

Long, standard-bore column with Agilent 1200 Series LC

Sample:	Tramadol with 4 impurities in the range 0.7 to 1.25 %
Column:	ZORBAX SB C18, 150 x 4.6 mm, 5 µm
Mobile phase:	A: Water with 0.1 % TFA B: Acetonitrile with 0.65 %TFA
Flow rate:	1 mL/min
Gradient:	0 min, 10 %B; 8 min, 45 %B; 10.5 min, 45 %B; 11 min, 10 %B; 15 min, 10 %B
Column temp.:	30 °C
Injection vol.:	5 µL
Detection:	VWD, wavelength 270 nm, response time 0.25 s equivalent to 14 Hz

Example 4:

Reducing column length and internal diameter

In this example the column length was reduced from 150 to 100 mm and the internal diameter of the column was reduced from 4.6 to 3.0 mm (figure 4). The LC instrumentation was able to run at pressures up to 400 bar. A reduction in solvent consumption of 65 % was achieved and the run time was shortened by 50 %.

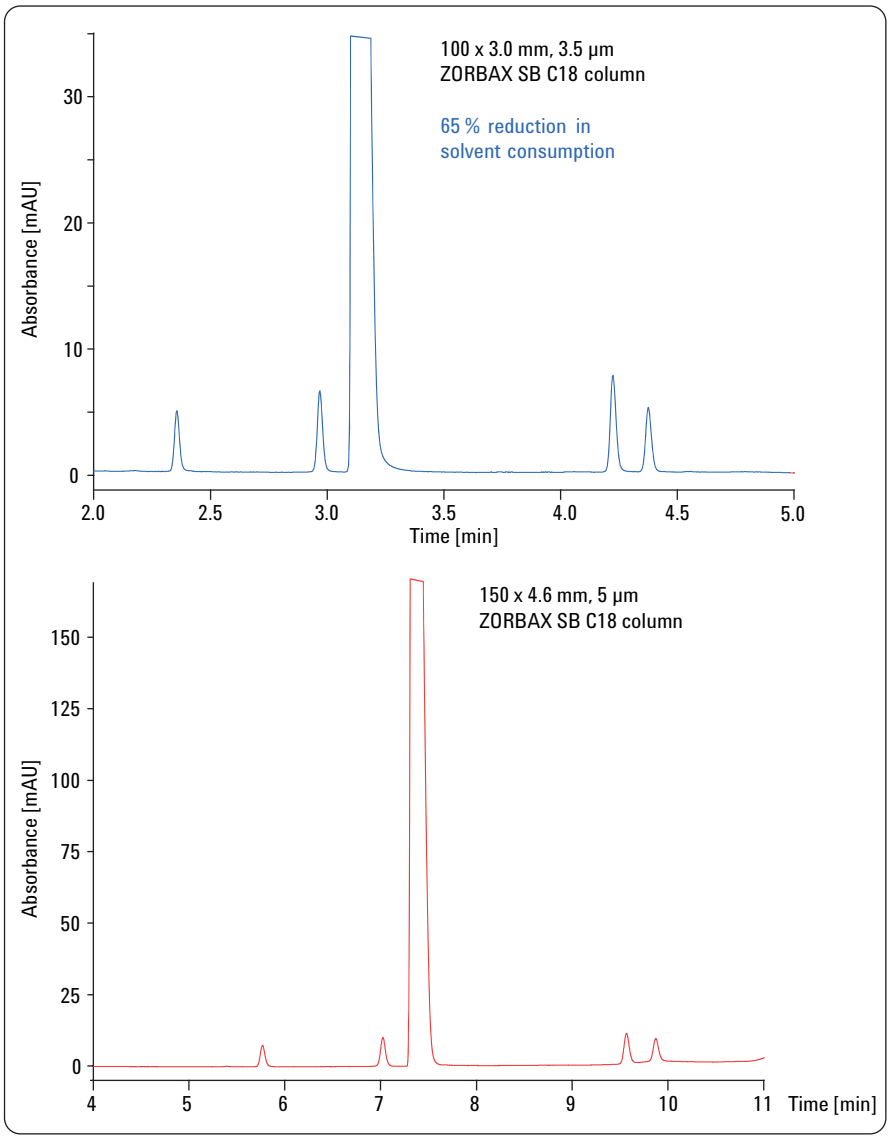


Figure 4
Solvent savings of 65 % by changing from a 4.6 mm id column to a shorter 3 mm id column.

Chromatographic conditions

Short, narrow-bore, small particle column with Agilent 1200 Series RRLC

Column:	ZORBAX SB C18, 50 x 3.0 mm, 1.8 µm
Mobile phase:	Water:Acetonitrile 95:5
Flow rate:	1.2 mL/min
Gradient:	0.5 to 75 % Acetonitrile within 5 min
Stop time:	5 min
Post time:	2 min
Injection vol.:	3 µL
Column temp.:	40 °C
Detection:	VWD, wavelength 225 nm, peak width > 0.0025 min

Long, standard-bore, large particle column with Agilent 1120 Compact LC

Column:	Agilent HC-C18 (2), 150 x 4.6 mm, 5 µm
Mobile phase:	Water:Acetonitrile 90:10
Gradient:	10 to 90 % Acetonitrile within 15 min
Flow rate:	1.5 mL/min
Injection vol.:	20 µL
Column temp.:	40 °C
Detection:	VWD, wavelength 225 nm, peak width > 0.0025 min, response time 0.06 s

Example 5:

Reducing column length, internal diameter and particle size

In this example, not only was the column length reduced from 150 to 50 mm and the internal diameter from 4.6 to 3.0 mm, but also the particle size was reduced from 5 to 1.8 µm. Deploying a sub-2-micron column achieved a reduction in solvent consumption of 72 %. Improved resolution and a decrease in run and equilibration times were additional benefits, (figure 5.)

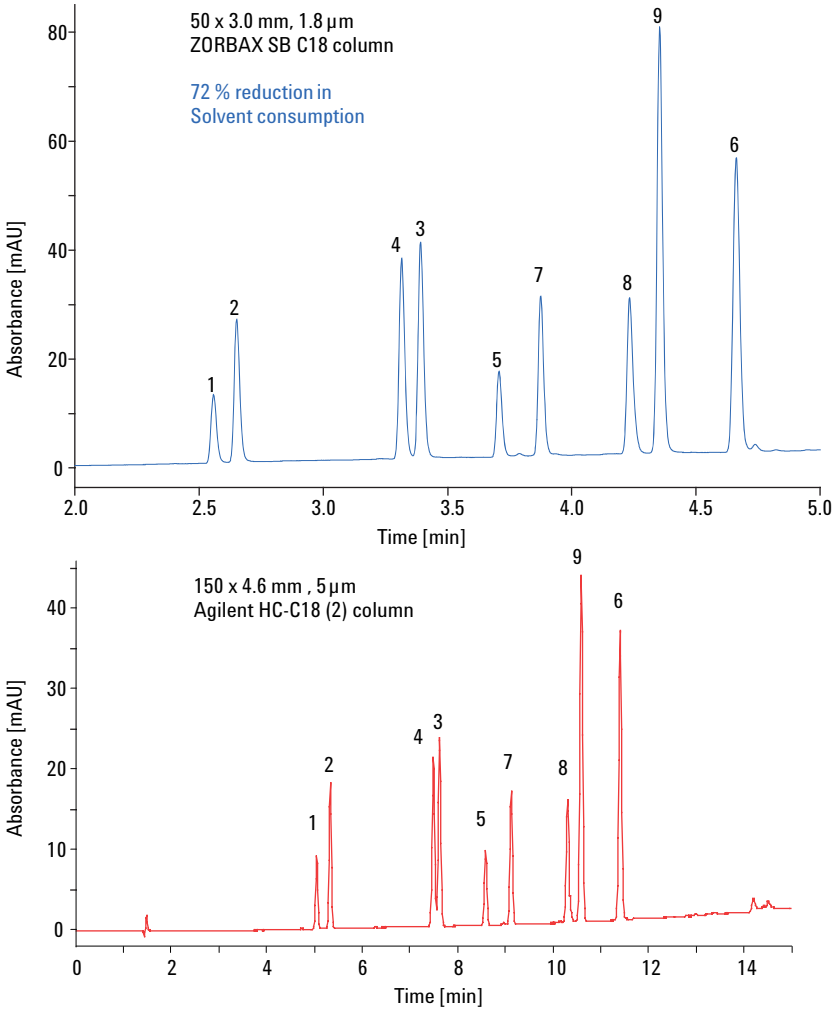


Figure 5
Solvent savings of 72 % through reduction of column length, internal diameter and particle size.

Peak number	Compound
1	Metamitron
2	Chloridazone
3	Simazine
4	Cyanazine
5	Prometryn
6	Chlortoluron
7	Diuron
8	Propazine
9	Terbuthylazine

Conclusion

There are many different strategies that can be adopted to reduce the consumption of solvents during liquid chromatographic analysis. For laboratories operating in regulated environments where the ability to modify validated methods is limited, one approach is to reduce the internal diameter and the length of the column as much as possible within the allowed limits. Adopting this approach can achieve reductions in solvent consumption of between 50 and 60 %. The corresponding savings in purchase and disposal of acetonitrile are in a similar range. When developing new methods or in operating environments that allow methods to be altered significantly, an effective approach is to deploy columns with sub-2-micron particles. Savings in solvent consumption and costs of 60 to 85 % can be achieved. At the same time, both run and equilibration times are reduced significantly. For both of these strategies it is important that the LC equipment is compatible with the altered method with respect to backpressure, and delay and dispersion volumes.

References

1.
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2.
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Published February 1, 2009
Publication Number 5990-3472EN

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