

KNAUER's

Eluent Savings

Handbook





Save now ...

Reducing eluent usage in HPLC – in particular acetonitrile

Acetonitrile (ACN) is a solvent commonly used in HPLC and a number of polar organic analyses (e.g. reverse-phase mode) are performed with acetonitrile as eluent. Supply problems since Fall 2008 have resulted in a sharp increase in the price of ACN. At present, it is not known whether this general scarcity will continue.

To deal with the current situation, we have listed a number of possibilities to reduce acetonitrile usage and, in this way, sustain daily laboratory operations despite the described shortage.

The following tips for saving acetonitrile will be discussed:

- Savings Suggestion 1: **The “slim method”**
- Savings Suggestion 2: **The “slim and short method”**
- Savings Suggestion 3: **The “slim, short & fast method”**
- Savings Suggestion 4: **UHPLC or the “small particle & slim-short-fast method”**
- **Six additional tips on saving Eluent ...**

We wish you good luck with saving!

Silvia Marten
Head of Applications and Columns Department

Savings Suggestion 1: The “slim method”

If you use a **conventional HPLC system** in the laboratory, such as the KNAUER **Smartline** system, here is a simple method to make do with less acetonitrile: reduce the column inner diameter and adjust a few parameters.

With an identical linear flow rate, so-called microbore columns (2 and 3 mm ID) can be used with substantially lower flow volumes than the usual analytical columns (4 and 4.6 mm ID). Thereby, the flow is adjusted according to the changed column inner diameter so that run times remain unchanged. With this method you can **reduce acetonitrile usage by up to 75%** and thereby reduce the costs of your analyses. This is illustrated in the table below for a column length of 250 mm.

Solvent usage and volumes for various column inner diameters

ID [mm]	Flow rate [μ l/min]	Eluent usage [ml/h]	Column volume [μ l]
4.6	1500	90	2493
4.0	1000	60	1880
3.0	563	33.8	1060
2.0	250	15	471

The reduction of the column inner diameter not only reduces solvent usage, but also leads to an increase in sensitivity by narrower peaks. However, this can only be fully exploited if the HPLC system has been optimized in regard to the system dead volume. Capillary inner diameters of 0.1 mm and a detector with a low flow cell volume are obviously required. The sample injection volume must also be adjusted to prevent column overloading. The resulting lower detector signal can be countered with a higher sample concentration (when possible).



Inner diameter: 4.6 4.0 3.0 2.0 mm

Savings Suggestion 2: The “slim and short method”

In addition to using a reduced column inner diameter, it is worthwhile to simultaneously consider a reduction in column length. In many cases, a separation using a shorter column can also be realized with a significant reduction in analysis time. With this second change you can further increase solvent savings.

The measurements described in the following were performed on an analytical Smartline HPLC system with low pressure gradient configuration. The following system components were used:



HPLC-System

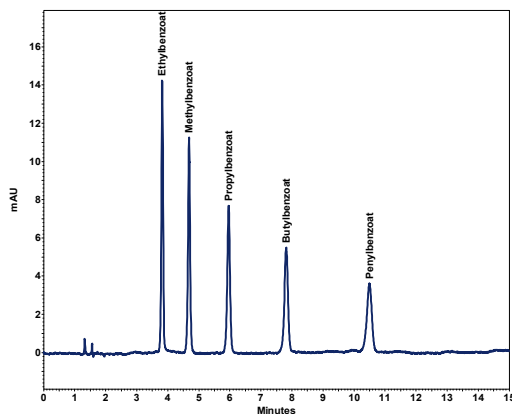
- Smartline Pump 1000, incl. 10 ml pump head
- Smartline Manager 5000 with NDG and degasser
- SmartMix static mixer, analytical version
- Smartline Autosampler 3950
- Smartline Column Oven 4050
- Smartline UV Detector 2600
- 10 mm Flow Cell
- ChromGate Software
- ChromGate PDA license for Detector 2600

Examples: Acetonitrile savings by changing the column dimension

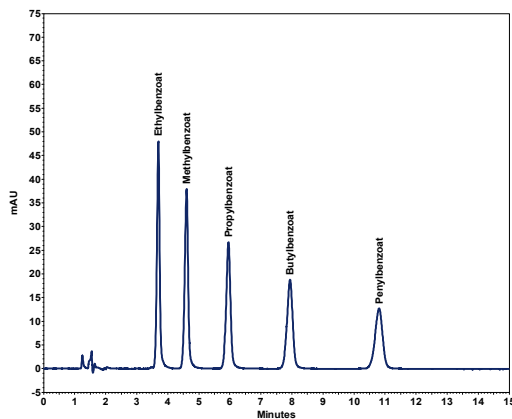
Benzoate mixture

Eluent: isocratic ACN/H₂O 75 : 25
 Temperature: 30 °C
 Injection volume: 1 µl
 Detection: 254 nm (100 Hz, 0.005 s)

Eurospher II 100-5 C18
250 x 4 mm
 flow rate: 1 ml/min



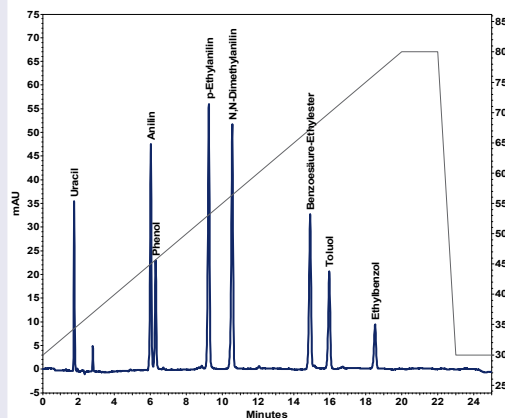
Eurospher II 100-5 C18
150 x 2 mm
 flow rate: 0.15 ml/min



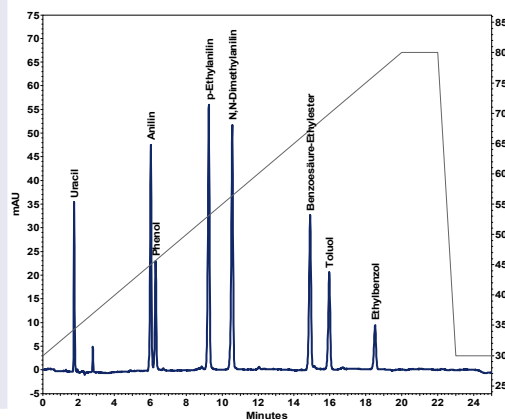
Engelhardt multipolar test mixture

Eluent A: H₂O
 Eluent B: ACN
 Gradient: 0–20 min 30–80 % B
 Temperature: 40 °C
 Injection volume: 1 µl
 Detection: 254 nm (100 Hz, 0.005 s)

Eurospher II 100-5 C18
250 x 4 mm
 flow rate: 1 ml/min



Eurospher II 100-5 C18
150 x 2 mm
 flow rate: 0.15 ml/min



The two application examples above show the same separation performance in relation to the 4 mm columns and graphically demonstrate that a column with 250 x 4 mm can be replaced with a column with the dimension 150 x 2 mm for these applications. How exactly this change will affect the acetonitrile usage is calculated here, based on the analysis in isocratic mode.



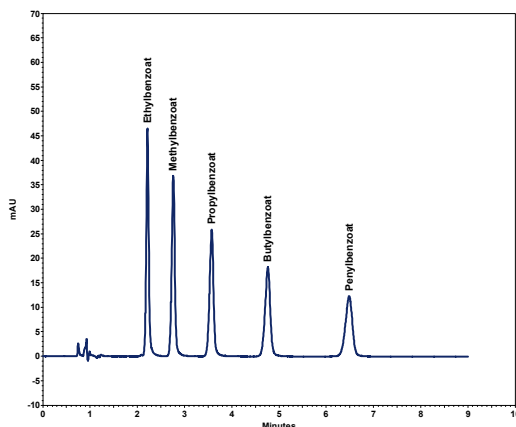
Comparison 250 x 4 mm / 150 x 2 mm

250 x 4 mm	Flow rate: 1 ml/min
Analysis time:	11 min
Eluent usage (total):	11 ml
Acetonitrile usage:	8.25 ml
150 x 2 mm	Flow rate: 0.15 ml/min
Analysis time:	11 min
Eluent usage (total):	1.65 ml
Acetonitrile usage:	1.24 ml
Savings per analysis:	85%

The comparison makes clear how great the solvent savings through the usage of shorter columns with reduced inner diameter can be. In the case above, by changing the column dimensions the **acetonitrile usage could be reduced by up to 85%**. For the gradient separation of the multipolar mixture, the usage of this procedure also permits a solvent savings. With an additional reduction in column length, care must be taken that the gradients for the analysis are also adjusted.

Savings Suggestion 3: The “slim, short & fast method”

Eurospher II 100-5 C18



150 x 2 mm

Flow rate:	0.3 ml/min
Analysis time:	8 min
Eluent usage (total):	2.4 ml
Acetonitrile usage:	1.8 ml

Savings per analysis: 78%

In the case of benzoate, the separation could be further accelerated with a higher flow rate. Crucial for this is a sufficiently high precision and adherence to the pressure limits of the HPLC system. The resulting savings is somewhat lower with 78%.



Order no.	Recommended columns
25DE181E2J	Eurospher II 100-5 C18 250 x 4 mm
15BE181E2J	Eurospher II 100-5 C18 150 x 2 mm
12BE181E2J	Eurospher II 100-5 C18 125 x 2 mm
11BE181E2J	Eurospher II 100-5 C18 120 x 2 mm
10BE181E2J	Eurospher II 100-5 C18 100 x 2 mm
05BE181E2J	Eurospher II 100-5 C18 50 x 2 mm
25BE181E2G	Eurospher II 100-3 C18 250 x 2 mm
15BE181E2G	Eurospher II 100-3 C18 150 x 2 mm
10BE181E2G	Eurospher II 100-3 C18 100 x 2 mm
05BE181E2G	Eurospher II 100-3 C18 50 x 2 mm

Additional phases and modifications are also available in all column sizes.

Savings Suggestion 4: UHPLC or the “small particle & slim-short-fast method”

Another extremely effective method to reduce solvent usage is **high-speed chromatography with UHPLC systems such as PLATINblue**. The method of high-speed chromatography is closely connected with the usage of short columns and column materials with small particle size. Stationary phases with particles under 2 µm usually produce a significantly higher separation efficiency than the 5 to 3 µm common in standard HPLC and thereby allow the columns to be shortened, while producing identical or even improved separation. The shorter columns allow for faster analyses.

This is perhaps not obvious at first glance. Shortening the columns should lead to a reduction in the number of theoretical plates and thereby negatively influence the resolution. This conclusion results from an equation for calculating the number of theoretical plates (N).

$$N = L / H$$

As the length of the column (L) decreases, the number of theoretical plates also decreases. Only the plate height (H) remains as a variable parameter. This must be reduced to make up for the reduction in the number of theoretical plates. This can be achieved by using smaller particles. The Van Deemter equation describes the parameters which affect plate height:

$$H = A + B / u + C \cdot u$$

The eddy diffusion is contained in term “A”, the longitudinal diffusion in “B” and the mass transfer in “C”. The mass transfer term “C” is also defined as the relationship between particle size (d_p^2), linear velocity (u) and the diffusion coefficient (D_m).

$$C = (d_p^2 \cdot u) / D_m$$

If the particle size (d_p^2) decreases, the mass transfer “C” also decreases as the inverse square. A reduced mass transfer term results in a decreased plate height. An increase in the diffusion coefficient (D_m) is an additional advantageous effect of small particles, which also reduces the value of C and reduces the plate height.

Most stationary phases can be produced and used with different particle sizes. Common particle sizes in the analytical realm today lie between 5 and 3 µm. The new generation of UHPLC phases includes particles which are 2 µm and smaller (e.g. **BlueOrchid 1.8 µm C18**).

Taken together, a reduction in particle diameter means a minimization of the height equivalent of a theoretical plate and therefore an increase in the number of theoretical plates. Small particle sizes provide an additional advantage. From the van Deemter curve, the possibility also arises for small particles to work at higher flow rates without losing separation efficiency. In turn, this allows faster analyses that then result in an aggregate decrease in solvent usage. An example will demonstrate the advantage of using the UHPLC system PLATINblue. An analytical method was transferred to a UHPLC system, thereby saving time and solvent.

The measurements described in the following were performed on a KNAUER PLATINblue UHPLC system. The following system components were used:



PLATINblue UHPLC-System

Pump P-1

Modular Eluent Manager M-1

SmartMix static mixer, micro version

Autosampler AS-1

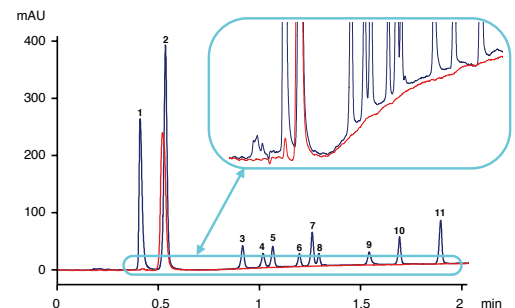
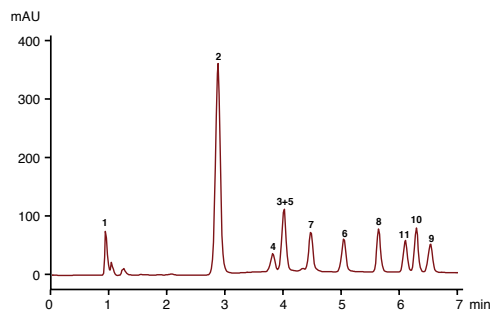
Column Temperature Manager T-1

Detector MW-1 and 2 µl Flow Cell

ChromGate PLATINblue Edition Software

UHPLC Example 1: ACN savings in a pharmaceutical application: Analysis of paracetamol and its process impurities

Eluent A:	ACN	ACN
Eluent B:	Buffer pH 2.75	Buffer pH 3.7 (200 mg/l NaH ₂ PO ₄)
Temperature:	50°C	50°C
Column:	ProntoSil 120-5 C8 ace EPS (3x250 mm)	BlueOrchid 120-1.8 C18 (2x100 mm)
Flow:	1.3 ml/min	0.8 ml/min
Injection volume:	5 µl	1 µl
Detection:	245 nm (2 Hz, 0.1 s)	245 nm (80 Hz, 0.005 s)
Gradient:	Time [min] % A % B	Time [min] % A % B
	0 10 90	0 13 87
	0.65 10 90	0.3 13 87
	6.4 60 40	2 70 30
	8 60 40	2.5 70 30



1 4-Aminophenol; 2 Paracetamol, N-(4-hydroxyphenyl)acetamide; 3 N-(4-Hydroxyphenyl)propanamide; 4 N-(2-Hydroxyphenyl)acetamide; 5 N-(3-Chlor-4-hydroxyphenyl)acetamide; 6 1-(4-Hydroxyphenyl)ethanoxime; 7 N-Phenylacetamide; 8 4-(Acetylamino)phenyl acetate; 9 4-Nitrophenol; 10 Chloracetanilide; 11 1-(2-Hydroxyphenyl)ethanone

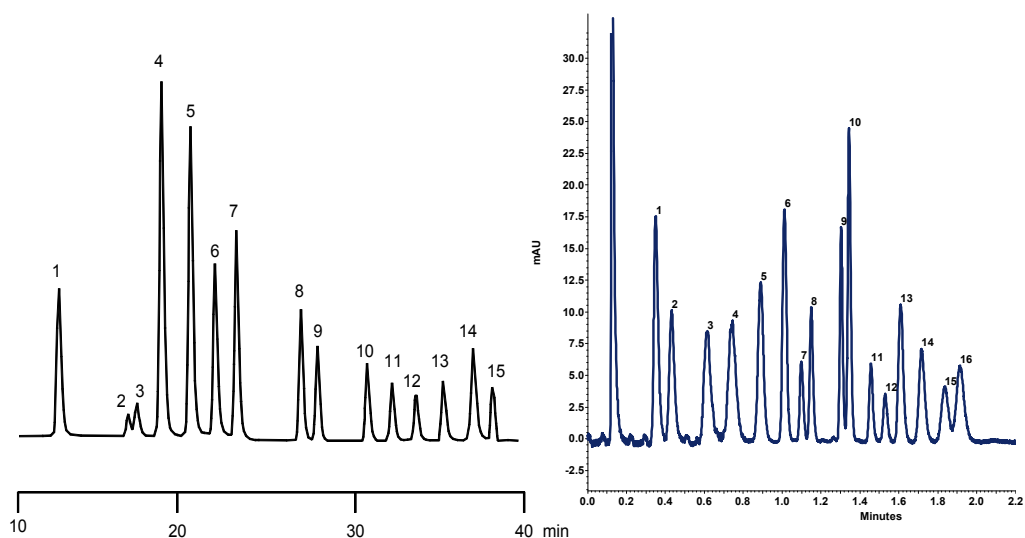
The UHPLC method employed when using a BlueOrchid 1.8 µm C18 column for the separation of the active ingredient paracetamol from its process contaminants achieves a reduction in total eluent usage of over **80 % per analysis** while working **3.5 times faster** than the already optimized method with standard HPLC. This example of a routine examination in the quality assurance of pharmaceuticals underscores the large savings potential inherent in the switch from HPLC to UHPLC.



Order no.	Recommended columns
05BI181BOE	BlueOrchid 1.8 C18 50 x 2 mm
10BI181BOE	BlueOrchid 1.8 C18 100 x 2 mm
05BI184BOE	BlueOrchid 1.8 C18A 50 x 2 mm
10BI184BOE	BlueOrchid 1.8 C18A 100 x 2 mm
05BI081BOE	BlueOrchid 1.8 C8 50 x 2 mm
10BI081BOE	BlueOrchid 1.8 C8 100 x 2 mm

UHPLC Example 2: Optimizing the separation of PAH by changing the stationary phase and column dimensions with simultaneous reduction of acetonitrile usage

Eluent A:	ACN	MeOH/H ₂ O, 75/25																																	
Eluent B:	H ₂ O	ACN																																	
Temperature:	30 °C	25 °C																																	
Column:	UltraSep ES PAH (250 x 2 mm)	BlueOrchid PAH (50 x 2 mm)																																	
Flow:	0.3 ml/min	1.0 ml/min																																	
Injection volume:	5 µl	1 µl																																	
Detection:	Fluorescence or UV 254 nm (1 Hz, 0.1 s)	254 nm (100 Hz, 0.001s)																																	
Gradient:	<table border="0"> <thead> <tr> <th>Time [min]</th> <th>% A</th> <th>% B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>60</td> <td>40</td> </tr> <tr> <td>30</td> <td>100</td> <td>0</td> </tr> <tr> <td>35</td> <td>100</td> <td>0</td> </tr> <tr> <td>45</td> <td>60</td> <td>40</td> </tr> </tbody> </table>	Time [min]	% A	% B	0	60	40	30	100	0	35	100	0	45	60	40	<table border="0"> <thead> <tr> <th>Time [min]</th> <th>% A</th> <th>% B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>90</td> <td>10</td> </tr> <tr> <td>0.5</td> <td>90</td> <td>10</td> </tr> <tr> <td>1</td> <td>0</td> <td>100</td> </tr> <tr> <td>2.5</td> <td>0</td> <td>100</td> </tr> <tr> <td>3</td> <td>90</td> <td>10</td> </tr> </tbody> </table>	Time [min]	% A	% B	0	90	10	0.5	90	10	1	0	100	2.5	0	100	3	90	10
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1	0	100																																	
2.5	0	100																																	
3	90	10																																	



1 Naphthalene; 2 Acenaphthylene; 3 Acenaphthene; 4 Fluorene; 5 Phenanthrene; 6 Anthracene; 7 Fluoranthene; 8 Pyrene; 9 Benzo(a)anthracene; 10 Chrysene; 11 Benzo(b)fluoranthene; 12 Benzo(k)fluoranthene; 13 Benzo(a)pyrene; 14 Dibenzo(a,h)anthracene; 15 Benzo(g,h,i)perylene; 16 Indeno(1,2,3-cd)pyrene

If one compares the two analyses, the **greatly reduced separation time** with the BlueOrchid phase is obvious. Where approx. 14 ml eluent is required with the standard method, after optimizing the method it is only 2.4 ml. That represents a **savings of 83% per analysis**.



Order no.	Recommended columns
05BF420BOG	BlueOrchid PAH 50 x 2 mm
10BF420BOG	BlueOrchid PAH 100 x 2 mm
05BF420BOJ	BlueOrchid PAH 50 x 2 mm
10BF420BOJ	BlueOrchid PAH 100 x 2 mm

PLATINblue – UHPLC from KNAUER

Our modular UHPLC system can operate at pressures up to 1000 bar (14500 psi) and acquire data at up to 200 Hz for high-resolution LC applications.

Since it is also capable of working under standard HPLC conditions, PLATINblue systems enable users to run established methods and migrate to high resolution or high speed methods using sub-2 μm columns at will.

The system features an intuitive user interface and the most advanced data analysis software.

Details at: www.platinblue.com



Can I save with small particles even without UHPLC?

A reduction in particle size generally has a positive influence on the separation and solvent usage – with or without a UHPLC system. The optimization steps introduced usually provide the greatest improvement when they are used in combination. For example, when the analysis allows, a reduction in the column dimensions in combination with a reduction in particle size can lead to an optimization of the analysis even with a standard HPLC system.

Limitations are:

- the greatly increased backpressure with reduced particle size
- too large system dead volume, e.g. during gradient formation or detection
- insufficient data recording rates

Additional tips for saving ACN ...

Besides reducing column dimensions and particle size, there are additional ways to reduce eluent consumption which we discuss here in brief.

Savings by eluent recycling

- Isocratic separations can employ eluent recycling to save eluent. A KNAUER switching valve can be controlled by software so that only the peak fractions containing sample are diverted to waste. The eluent savings depend upon the analysis: fewer peaks and more baseline correspond to more eluent savings.

Savings by eluent recovery

- Recovery of the eluent through distillation is costly and worthwhile only in isolated cases, generally in preparative chromatography.

Savings by optimizing equilibration time

- Column equilibration steps are often too long and are therefore amenable to optimization. Depending on the software, equilibration can be easily automated by baseline monitoring.

Savings by mobile phase substitution

- Usage of an eluent with comparable eluting strength, here methanol can be chosen.

Example: instead of an eluent with 40/60 ACN/H₂O, an eluent consisting of 55/45 MeOH/H₂O can be used – small optimizations are potentially necessary. Depending upon the stationary phase, retention times and peak order may change with methanol.

Savings by stationary phase substitution

- If the analysis allows, alternative reverse phase (RP) columns with different polarity can be considered.

Example: Increase in the phase polarity or reduction of the hydrophobicity through a different carbon content/degree of binding (e.g. C8-Phase instead of C18) → an eluent mixture containing less acetonitrile is then required for the elution of unpolar mixtures.

Savings by total substitution

- It is completely reasonable to rethink the entire analysis at some point.

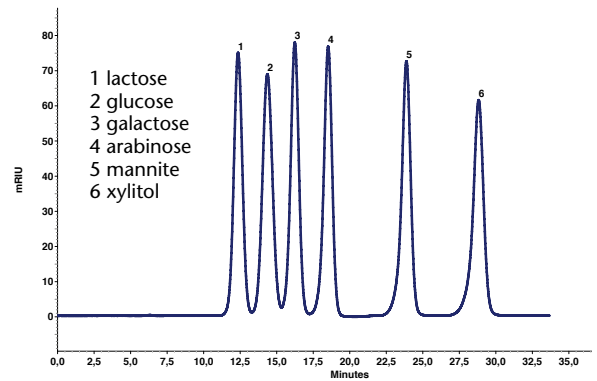
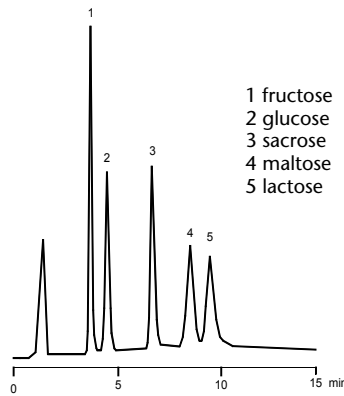
Example: The separation of carbohydrates (e.g. sucrose, glucose, fructose and maltose) is generally performed on a silica gel amino phase, such as, for example, Eurospher 100-5 NH₂. The separation requires an ACN water mixture with 75% ACN. By switching to a polymer-based Eurokat Pb or Eurokat Ca column, a **100% savings potential** for acetonitrile is possible because these polymer columns based on sulfonated polystyrene-divinylbenzene are operated exclusively with pure water.



Example: 100% savings of acetonitrile by adapting the complete analysis for the separation of carbohydrates or organic acids

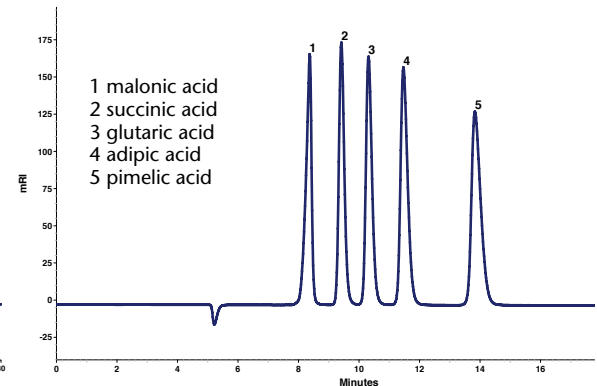
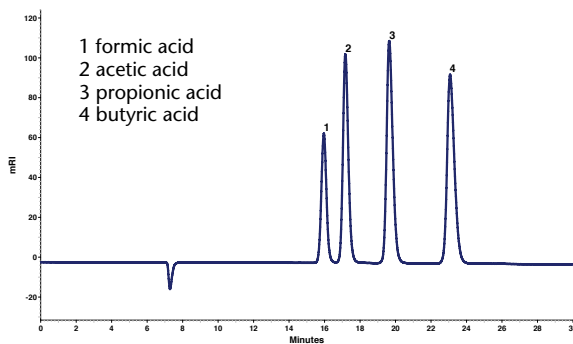
Column: Eurospher 100-5 NH₂ (125 x 4mm)
 Eluent: isocratic ACN/H₂O 75 : 25
 Flow: 1 ml/min
 Injection volume: 5 µl
 Detection: RI
 Temperature: 25 °C

Eurokat Ca (300 x 8 mm)
 isocratic H₂O
 0.5 ml/min
 20 µl
 RI
 75 °C



Column: Eurokat H (300 x 8 mm)
 Eluent: isocratic 0.01 N H₂SO₄
 Flow: 0.8 ml/min
 Injection volume: 20 µl
 Detection: RI
 Temperature: 75 °C

Eurokat H (300 x 8 mm)
 isocratic 0.01 N H₂SO₄
 0.7 ml/min
 20 µl
 RI
 75 °C



Order no. Column selection

30GX340EKN Eurokat H 300 x 8 mm
 30DX340EKN Eurokat H 300 x 4 mm

30GX350EKN Eurokat Pb 300 x 8 mm
 30DX350EKN Eurokat Pb 300 x 4 mm

30GX360EKN Eurokat Ca 300 x 8 mm
 30DX360EKN Eurokat Ca 300 x 4 mm

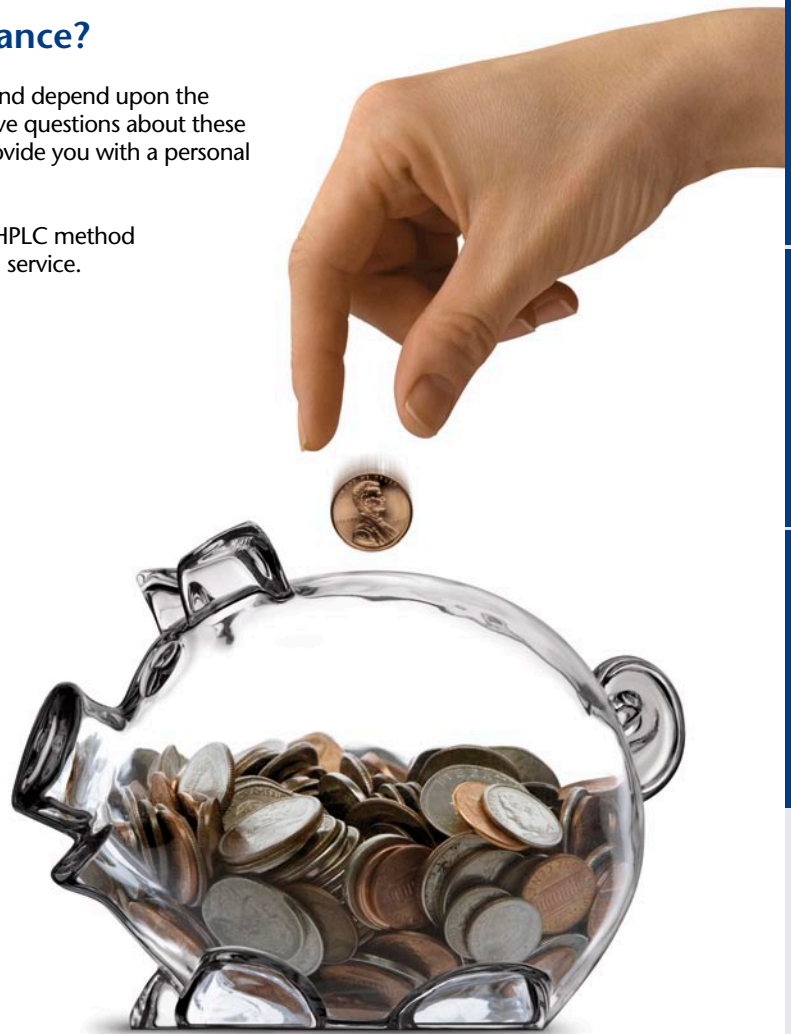
You can do it too ...

The selected examples have shown that there are many ways to deal with the current acetonitrile shortage. With a little consideration and method modification, you can generally reduce eluent usage. Your inventory manager, your time and your wallet will thank you.

Do you need assistance?

The tips shown are guidelines and depend upon the particular application. If you have questions about these topics, we would be glad to provide you with a personal consultation.

We are also happy to offer our HPLC method development and optimization service.



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