Capillary and Nano Columns

- Capillary (500µm and 300µm) and nano (100µm and 75µm) dimensions
- Wide range of bonded phases available
- 100Å and 300Å pore sizes
- High efficiency, long lifetime and guaranteed reproducibility
- LC/MS and LC/MS/MS applications

Ultra Inert High Efficiency Columns

In addition to the extensive range of analytical (1.0-4.6mm i.d.) through to preparative (21.2-50mm i.d.) columns (see pages 14-17 and 24-26), ACE columns are now available in capillary (500µm and 300µm) and nano (100µm and 75µm) dimensions. ACE capillary and nano columns are available with all ACE bonded phase chemistries in both 100Å and 300Å pore sizes. The same features that make ACE ultra-inert base deactivated columns the choice of method development chemists also make them the ideal choice for capillary and nano HPLC applications.

Improved Mass Limit of Detection

Capillary and nano HPLC is gaining acceptance for applications where limited sample amounts lead to problems in detection sensitivity. This is relevant in the areas of pharmacokinetics, trace analysis and in particular the expanding fields of bioanalytical and proteomic analysis. ACE capillary and nano columns are ideal for use with detectors requiring very low flow rates, such as electrospray LC-MS.

ACE capillary and nano HPLC columns offer high sensitivity due to their low dispersion characteristics. Table 27 shows the theoretical sensitivity increase of each i.d. column compared with a 4.6mm i.d. analytical column and 1mm i.d. microbore column. This increase in sensitivity can be important for accurate quantitation of sample limited applications.

For maximum performance, columns should be used with fully optimized HPLC systems (eg. minimize system dead volume using short lengths of <75µm connection tubing).

Table 27. Sensitivity Increase

COLUMN I.D. (mm)	TYPICAL FLOW RATE (µL/min)	THEORETICAL SENSITIVITY INCREASE[1]
4.6	1000	1
1.0	40	21
0.5	10	85
0.3	3	235
0.1	0.5	2100
0.075	0.3	3760

[1]For same sample mass

Trace Enrichment/Guard Columns

Capillary HPLC guard columns (5mm x 300µm or 500µm i.d.) prolong the lifetime of the capillary column. They are also suitable for trace enrichment and column switching applications, particularly for concentration of low abundance analytes or desalting of biological samples. These short columns can be used to separate analyte from matrix prior to analysis with detectors such as ESI-MS, where baseline resolution is not required.

Column Availability

ACE capillary and nano columns are available with all bonded phase chemistries, 100Å or 300Å pore sizes and 3, 5 or 10µm particle sizes. When ordering, replace X with the appropriate material code (see page 48).
Example: 150mm x 300µm i.d. ACE 3 C18-300 column – Part Number = ACE-211-15003.

COLUMN DIAMETER		COLUMN LENGTH (mm)					GUARD COLUMN (1 pk)
µm	mm	30	50	100	150	250[1]	
75	0.075	enquire	enquire	X-1000075	X-1500075	X-2500075	-
100	0.10	enquire	enquire	X-10001	X-15001	X-25001	-
300	0.30	X-03003	X-05003	X-10003	X-15003	X-25003	X-005003GD
500	0.50	X-03005	X-05005	X-10005	X-15005	X-25005	X-005005GD

[1]250mm column length not available with 3µm particle size.

ACE LC/MS and Rapid Analysis Columns

- **High performance – excellent peak shape for higher sensitivity**
- **Choice of 13 low bleed phases for complete optimization**
- **Ultra-inert silica enables MS compatible buffers to be used**
- **20mm, 30mm, 35mm and 50mm column lengths**
- **1.0, 2.1, 3.0, 4.0 and 4.6mm i.d.s**

Nowhere is the need for a truly ultra-inert base deactivated HPLC column more important than in high-throughput analysis or LC/MS. Even subtle differences in silanol activity between columns can markedly affect the chromatography in the very short, fast columns typically used. In addition, any peak tailing due to silanol activity can have a profound effect on detection limits in high sensitivity assays.

ACE ultra-inert base deactivated HPLC columns virtually eliminate the negative effects of silanols in HPLC separations. This unequalled performance is now available in 20, 30, 35 and 50mm length columns, with diameters from 1.0 to 4.6mm i.d. The ACE columns are suitable for high throughput and LC/MS applications, and are the ideal choice for high volume screening assays used for drug analysis and combinatorial libraries where robust, reproducible columns are essential.

> **"This is the most inert column we have tested."**
>
> Analytical Chemist, Industrial Chemical Company

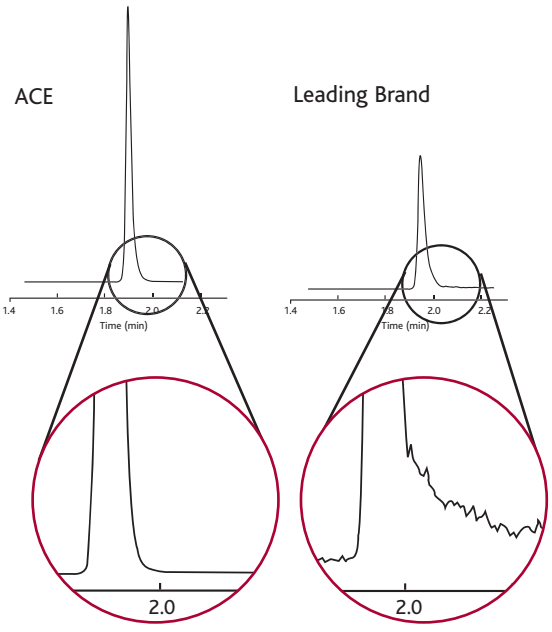
Maximum Resolution

ACE LC/MS columns, apart from being the most inert on the market, are also the most efficient, and are manufactured and validated to the same exacting standards as all ACE columns. Increased efficiency is an important benefit given the short columns typically used in LC/MS and rapid analysis applications.

In Figure 28, the top chromatograms show the LC/MS signal intensity obtained for equivalent injections on different brands of 30 x 4.6mm C8 columns. The lower chromatograms are an expanded scale, which show significant baseline tailing on the leading column brand, whereas the ACE column yields near perfect symmetry.

Such peak shape improvements may be typically seen when using high efficiency, ultra-inert columns such as ACE.

Figure 28 Effect of Peak Tailing on Signal Strength in LC/MS

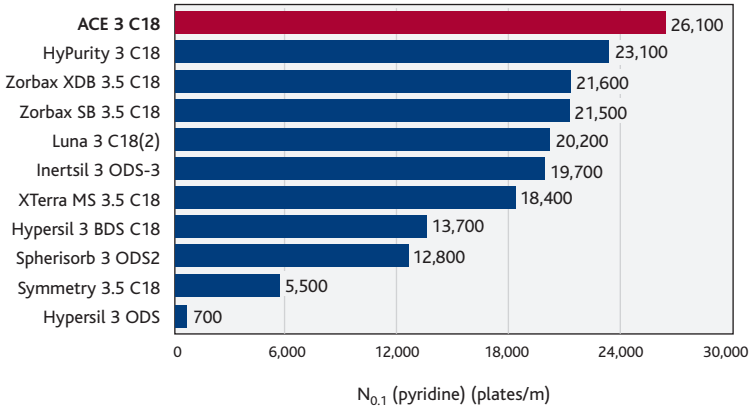


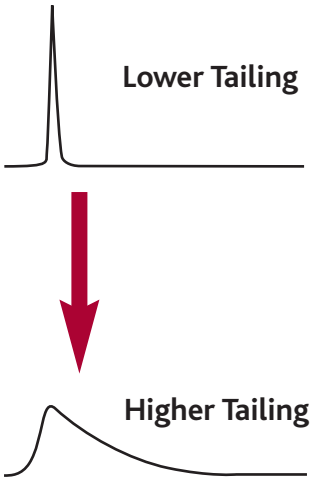
Comparison of Leading LC/MS 3µm Small Pore Columns

- Independently tested by Peter Levison Associates, UK

- Leading 3µm, small pore C18 column brands
- 50 x 2.1mm i.d. LC/MS compatible dimensions
- Basic molecule inertness test
- Peak efficiency and asymmetry investigation

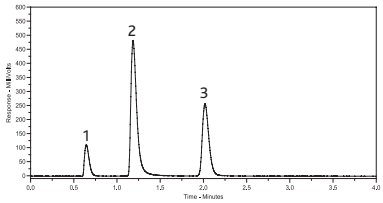
Peak Efficiency Comparison





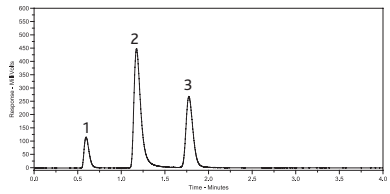
ACE 3 C18

$N_{0.1}$(pyr) = 26,100pl/m



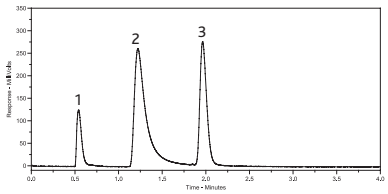
Waters XTerra MS 3.5 C18

$N_{0.1}$(pyr) = 18,400pl/m



Waters Symmetry 3.5 C18

$N_{0.1}$(pyr) = 5,500pl/m



Column: 50 x 2.1mm i.d. Sample: 1) Uracil 2) Pyridine 3)Phenol Mobile Phase: 40:60 MeOH/H_2O Flow Rate: 0.20ml/min Temperature: 22°C Wavelength: 254nm

Conclusion:

Significant differences in efficiency, peak shape and selectivity are seen when analyzing pyridine - a small highly basic molecule.

Increased tailing and retention are indicative of undesirable secondary interactions between pyridine and silanol groups on the stationary phase surface. These interactions can also result in poor column reproducibility.

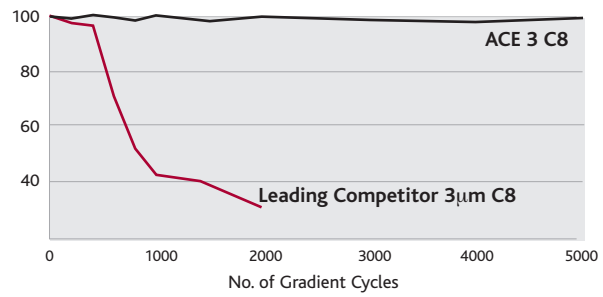
ACE LC/MS columns were independently tested and found to be the highest efficiency, most inert columns available.

ACE® Stationary Phases Virtually Eliminate the Negative Effects of Silanols on HPLC Separations

Increased Column Robustness

The requirement for long lifetime columns is essential for high throughput analyses, where mid-run column failure must be avoided. The high flow, fast gradient conditions typically employed have been shown to cause premature column bed failure due to the rapid pressure and solvent miscibility changes within the column itself. ACE LC/MS and rapid analyses columns are manufactured by a unique process which results in increased column bed stability, thus increasing column lifetime. As shown in Figure 30a, ACE columns significantly outlast a leading competitor column when tested under generic fast gradient conditions.

Figure 30a. ACE Column Robustness



Column Dimensions: 50 x 3.0mm, 3µm C8, Flow Rate: 1.25ml/min
Mobile Phase: A: 5:95 MeCN/10mM NH_4OAc, B: 95:5 MeCN/10mM NH_4OAc

Gradient: T(mins)	0	2.25	2.50	3.00
%A	0	100	0	0
%B	100	0	0	100

LC/MS Buffer Compatibility

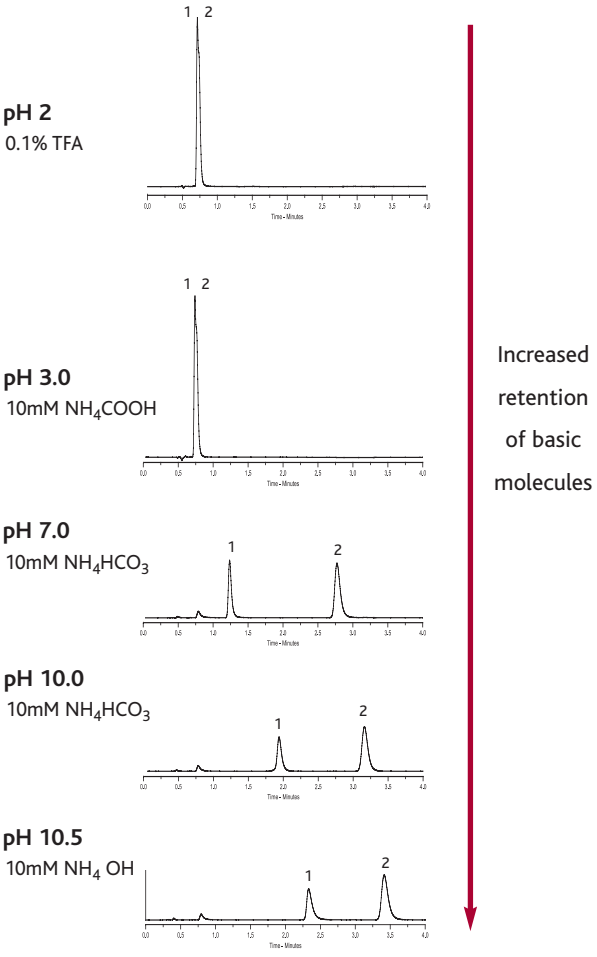
A number of independent studies (see pages 2, 3, 12, 13, 19, 23 and 29) have shown ACE columns to be the most inert on the market. This makes ACE the column of choice for LC/MS due to the extremely low requirement for buffer salts or modifiers to achieve good peak shape.

ACE columns have been demonstrated to be extremely robust at high and low pH (see pages 8 and 9). This broad pH operating range enables thorough optimization of pH and simplifies method development to a single column rather than selecting 3 different columns with low, intermediate and high pH stability.

For maximum LC/MS compatibility under acidic conditions organic acids such as formic, acetic and TFA are recommended. Under basic conditions ammonium bicarbonate, ammonium acetate and ammonium hydroxide buffers are recommended.

Figure 30b demonstrates the resolution obtained with 2 basic compounds at different mobile phase pHs on an ACE ultra-inert HPLC column.

Figure 30b. pH Investigation with LC/MS Buffers



Column: ACE 3 C18, 50 x 2.1 mm i.d., Flow Rate: 0.20ml/min Mobile Phase: 80:20 MeOH/buffer, Temperature: 22°C Sample: 1) Nortriptyline 2) Amitriptyline, Wavelength: 215 nm

ACE HPLC columns have a broad pH operating range, allowing simplified method development on a single column.

LC/MS

Reduce Analysis Costs

- Reduce analysis time
- Increase sensitivity
- Reduce waste

ACE LC/MS and Rapid Analysis columns are available in a wide range of lengths and internal diameters, to enable complete method optimization. Reduced column lengths are used to increase productivity (see Figure 31a) whereas reducing column diameter improves sensitivity and reduces solvent waste (Figure 31b).

Figure 31a. Reduce Column Length - Increase Productivity

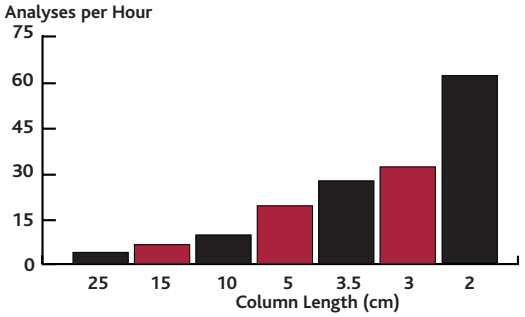
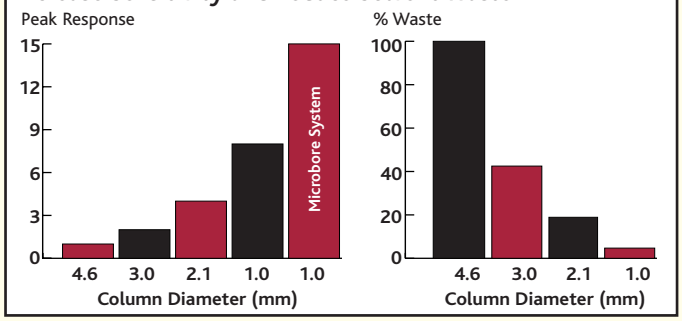


Figure 31b. Reduce Column Diameter - Increase Sensitivity and Reduce Solvent Waste

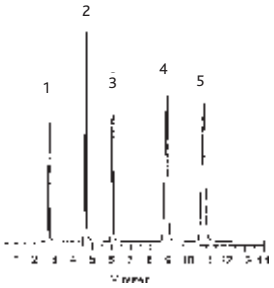


Method Optimisation

As shown in Figures 31c and 31d, the optimum phase, column dimensions and evaluation conditions can be selected to simultaneously optimize productivity, reduce waste and increase sensitivity, whilst still maintaining baseline resolution.

Figure 31c. Isocratic Method Optimisation – Baseline Resolution Maintained

Before: ACE 5 C18, 250 x 4.6mm i.d.

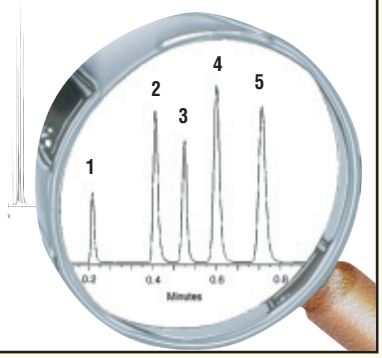


Analysis time reduced > 93%

Solvent waste reduced > 99%

Sensitivity increased x 15

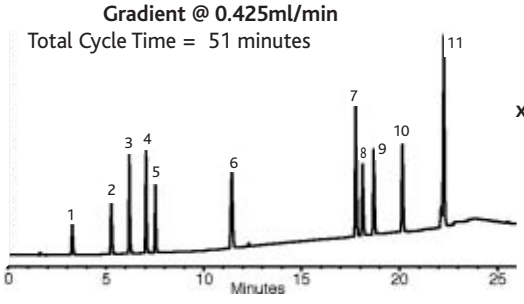
After: ACE 3 C8, 50 x 1.0mm i.d.



Sample: 1) Norephedrine 2) Nortriptyline 3) Toluene 4) Imipramine 5) Amitriptyline
Mobile Phase: Before - 80:20 MeOH/25mM KH_2PO_4 (pH6.0), After - 70:30 MeOH/25mM KH_2PO_4 (pH6.0)
Flow Rate: Before - 1.0ml/min , After - 0.15ml/min, Temperature: 22°C, Wavelength: 215nm

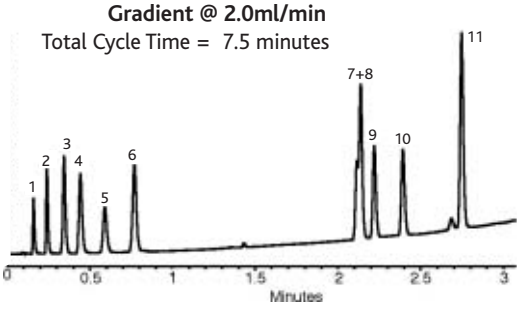
Figure 31d. Gradient Method Optimisation – High Throughput Screening

Before: ACE 3 C18, 150 x 3.0mm i.d.
Gradient @ 0.425ml/min
Total Cycle Time = 51 minutes



x7 Productivity Increase

After: ACE 3 C18, 30 x 3.0mm i.d.
Gradient @ 2.0ml/min
Total Cycle Time = 7.5 minutes



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