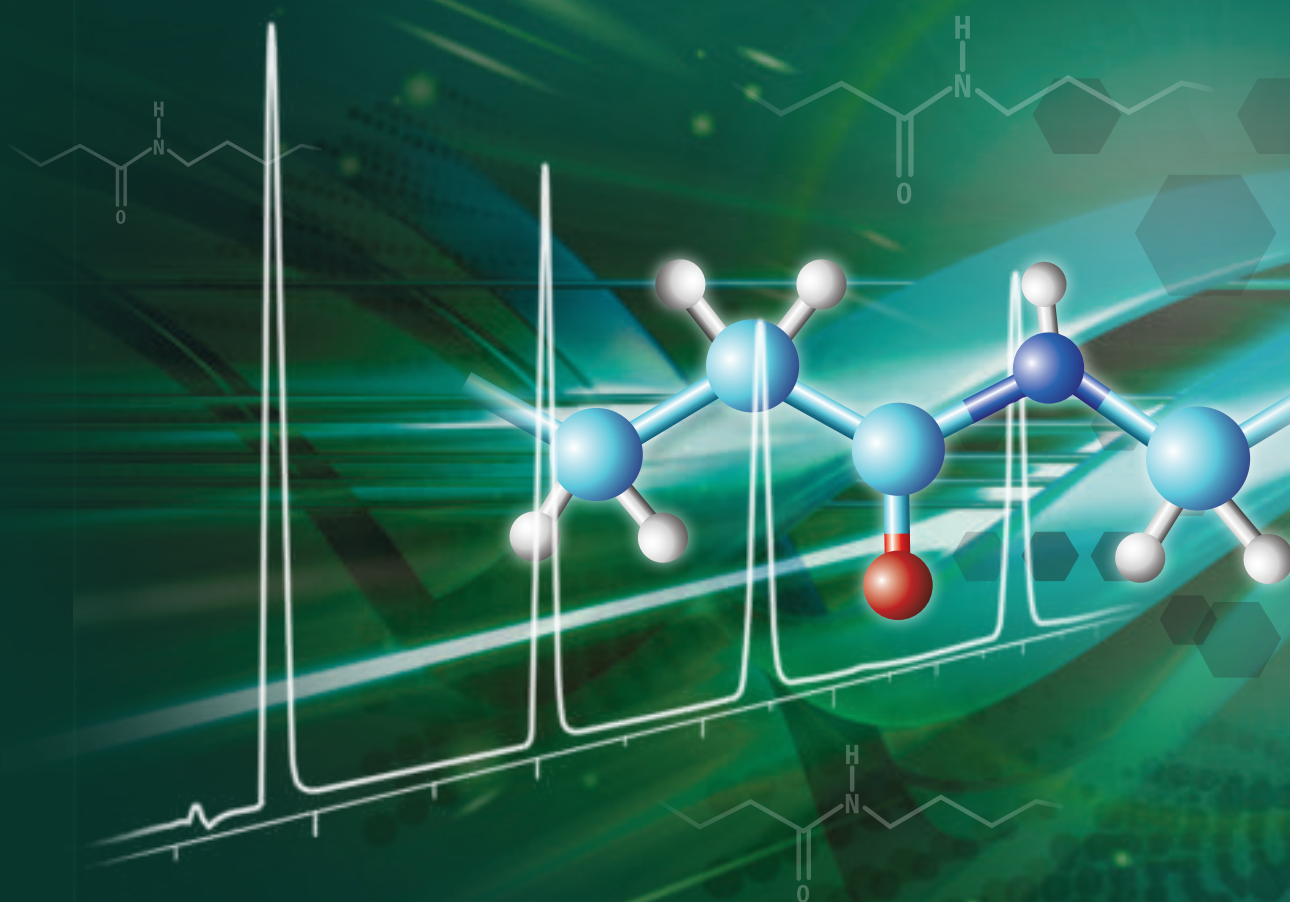


# ACE<sup>®</sup> C18-Amide

For increased polar retention and alternative selectivity



- Alternative selectivity for method development
- Improved separations with polar, acidic, basic and phenolic compounds
- High efficiency 2 $\mu$ m, 3 $\mu$ m, 5 $\mu$ m and 10 $\mu$ m particles for UHPLC and HPLC
- Ultra-inert for maximum performance and reproducibility

# ACE® C18-Amide

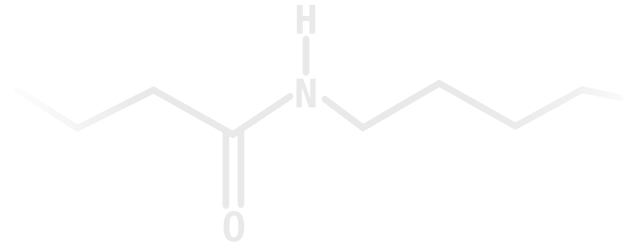
For increased polar retention and alternative selectivity

## Ideal Column Choice for Method Development

- Alternative selectivity to C18 and C8 columns with polar molecules - especially for acids
- Compatible with 100% aqueous mobile phases
- High efficiency 2µm, 3µm, 5µm and 10µm particles for UHPLC, HPLC and preparative separations
- Low bleed for UV and LC/MS compatibility

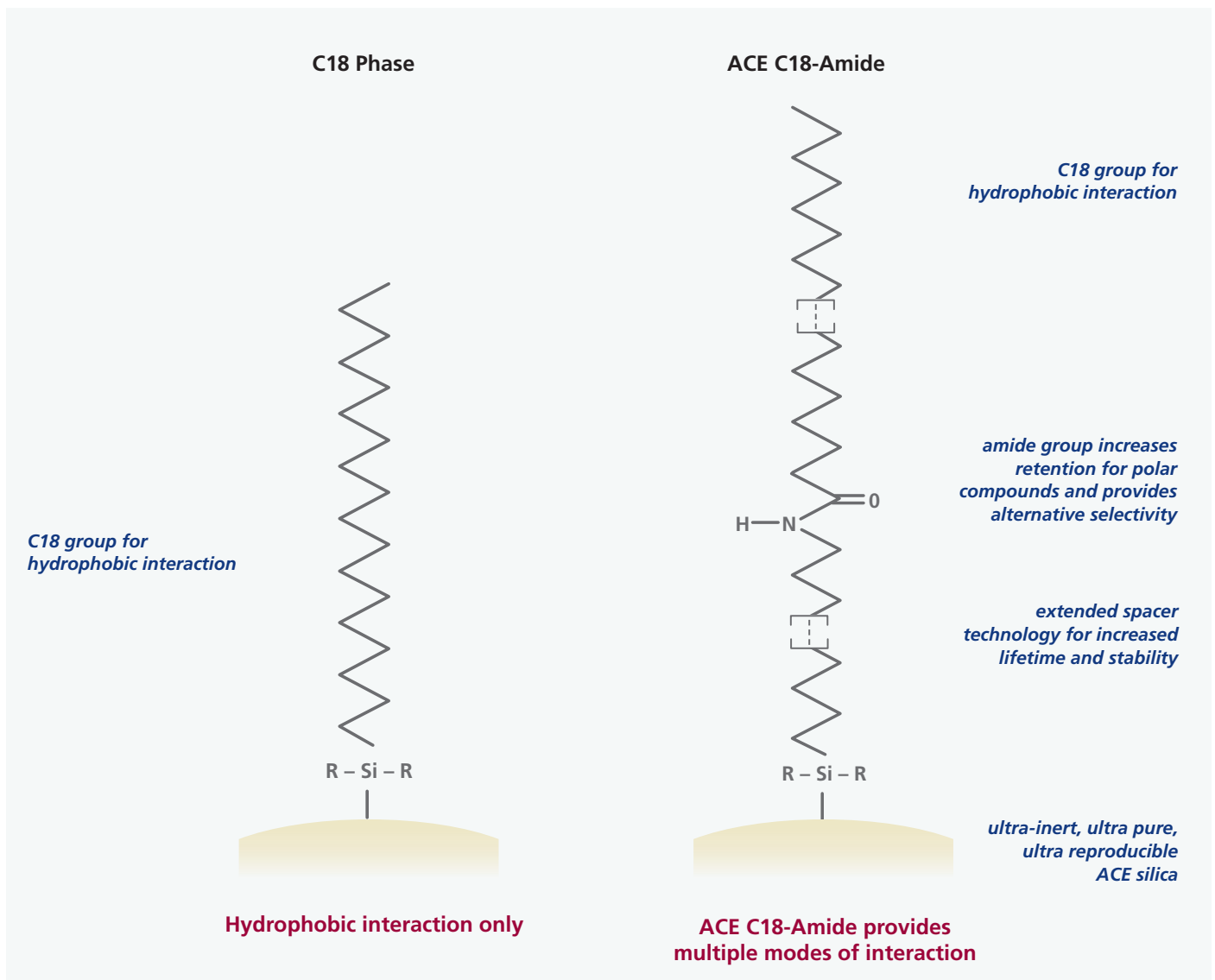
## Recommended Applications

- Small water soluble analytes and polar compounds
- H-bond donors, acids, bases and phenolic compounds
- Small peptides



## Why does ACE C18-Amide Provide Alternative Selectivity?

- ACE C18-Amide combines a C18 with a polar amide group on a single ligand
- Extended spacer technology additionally provides extended column lifetime



## Leverage the Power of Selectivity with ACE C18-Amide to pull peaks apart

The resolution equation determines the parameters that contribute to resolution;  $N$ ,  $\alpha$  and  $k$ . In recent years there has been a significant focus on the use of ultra efficient "UHPLC" columns (such as ACE Excel 2 $\mu$ m columns) as a means of achieving separation goals.

$$R_s = \left(\frac{1}{4}\right) N^{0.5} \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k}{1 + k}\right)$$

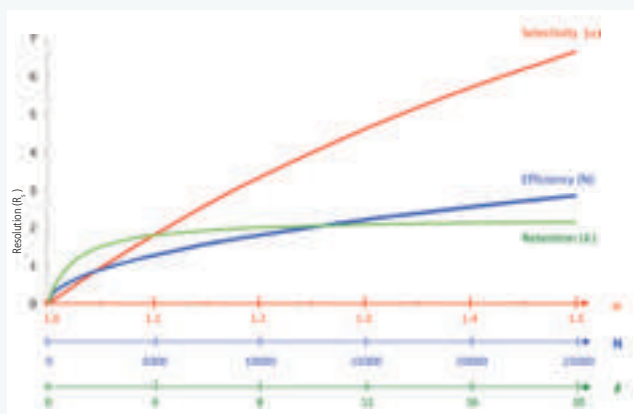
Resolution                      Efficiency                      Selectivity                      Retention Factor

However, selectivity is often overlooked. This is unfortunate as of the three parameters that affect resolution, selectivity is the most powerful (see Figure 1). By leveraging both efficiency and selectivity, better and faster separations can often be achieved.

ACE C18-Amide is the latest addition to the ACE range of bonded phases which offer both complementary selectivity and the option of 2 $\mu$ m, 3 $\mu$ m, 5 $\mu$ m and 10 $\mu$ m particle sizes - to enable development of rugged, robust UHPLC and HPLC methods.

**Fig. 1 - The Effect of  $N$ ,  $\alpha$  and  $k$  on Resolution ( $R_s$ )**

For a typical separation where  $N = 10,000$ ,  $k = 4$  and  $\alpha = 1.1$



Increasing  $N$ ,  $\alpha$  or  $k$  increases resolution ( $R_s$ ), but as seen from the above plots, increasing either  $N$  or  $k$  suffers from quickly diminishing returns. Increasing selectivity ( $\alpha$ ) does not have this problem and is therefore the most powerful of these three variables to optimise when developing a separation.



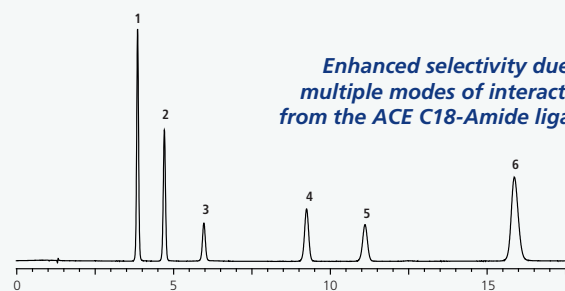
## Leverage the power of bonded phase selectivity to pull peaks apart

Figure 2 illustrates the difference between two ACE bonded phases, C18-Amide and C18. Although both phases offer the possibility of strong hydrophobic interaction from their respective C18 chains, the amide group embedded within the C18-Amide phase introduces additional modes of interaction which ultimately increases retention for polar compounds and provides alternative selectivity.

**Fig. 2 - Use ACE C18-Amide to Enhance Selectivity**

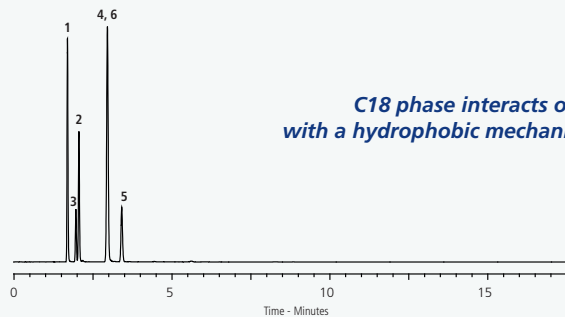
Application # 1601

### ACE Excel 3 C18-Amide



*Enhanced selectivity due to multiple modes of interaction from the ACE C18-Amide ligand*

### ACE Excel 3 C18



*C18 phase interacts only with a hydrophobic mechanism*

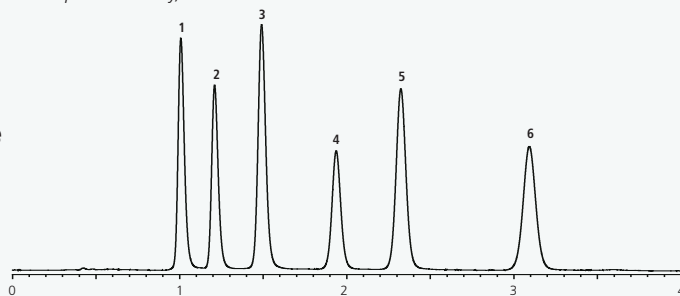
Sample: 1) resorcinol 2) catechol 3) 2-methyl resorcinol 4) 4-methyl catechol  
5) 3-methyl catechol 6) 4-nitro catechol Mobile Phase: 25:75 MeCN/27mM H<sub>3</sub>PO<sub>4</sub> in H<sub>2</sub>O  
Column Dimensions: 150 x 4.6mm Flow Rate: 1.50ml/min Temperature: 30°C  
Wavelength: 270nm

# ACE C18-Amide Provides Enhanced Polar Selectivity

**Fig. 3 - Comparison of ACE C18-Amide to Leading C18 UHPLC Columns** Application # 1602

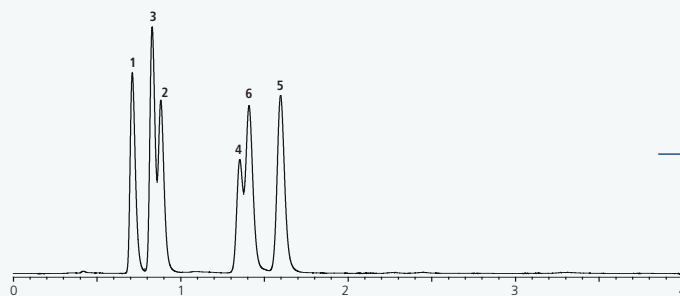
Reproduced with kind permission of The Open University, UK

**ACE Excel 2 C18-Amide**  
(ultra-inert fully porous silica)

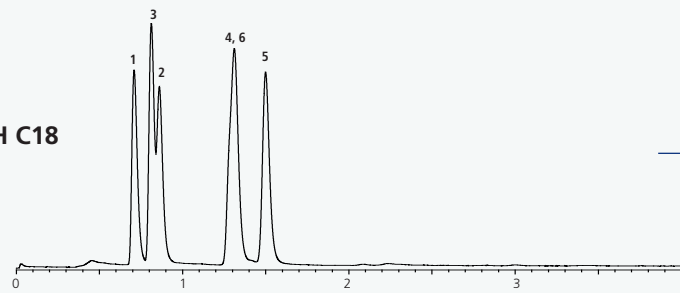


*ACE C18-Amide provides increased retention and improved separation due to enhanced polar selectivity*

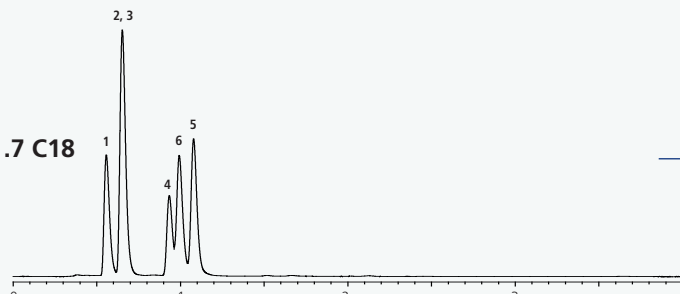
**ACE Excel 2 C18**  
(ultra-inert fully porous silica)



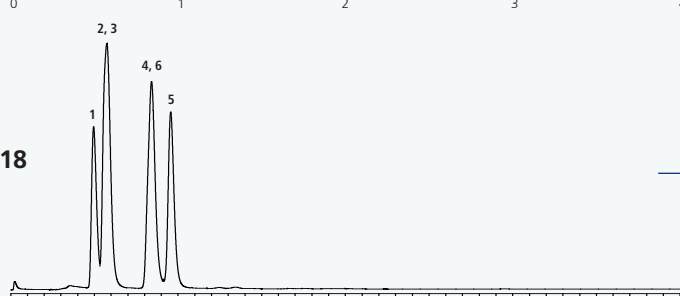
**Waters Acquity 1.7 BEH C18**  
(hybrid particle)



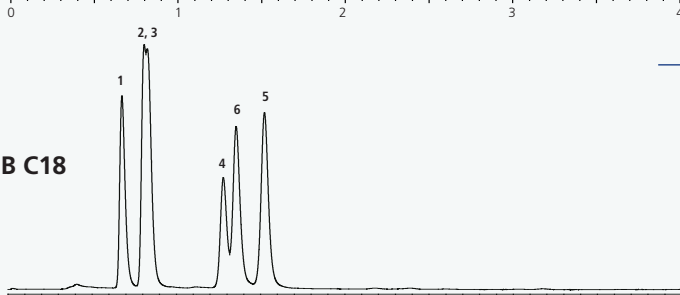
**Phenomenex Kinetex 1.7 C18**  
(superficially porous particle)



**Thermo Accucore 2.6 C18**  
(superficially porous particle)



**ZORBAX Eclipse 1.8 XDB C18**  
(fully porous silica)



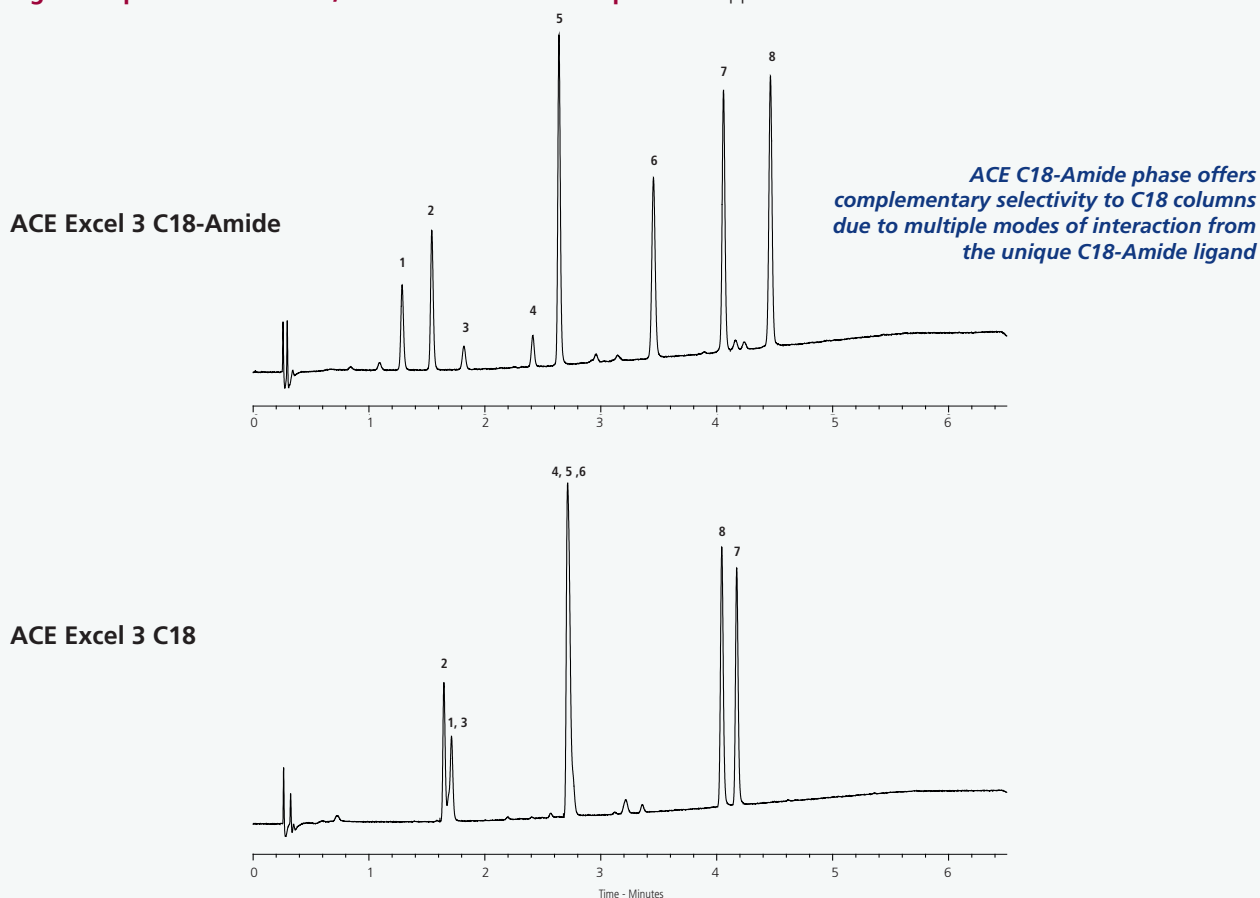
*Leading C18 brands provide similar selectivity*

Column Dimensions: 50 x 2.1mm  
Sample: 1) resorcinol 2) catechol  
3) 2-methyl resorcinol 4) 4-methyl catechol  
5) 3-methyl catechol 6) 4-nitro catechol  
Mobile Phase: 25:75 MeOH/25mM H<sub>3</sub>PO<sub>4</sub> in H<sub>2</sub>O  
Flow Rate: 0.30ml/min  
Temperature: 30°C Wavelength: 214nm

Comparative data may not be representative of all applications. Please see back page for acknowledgement of trademarks.

## Exploit Alternative Selectivity for Method Development

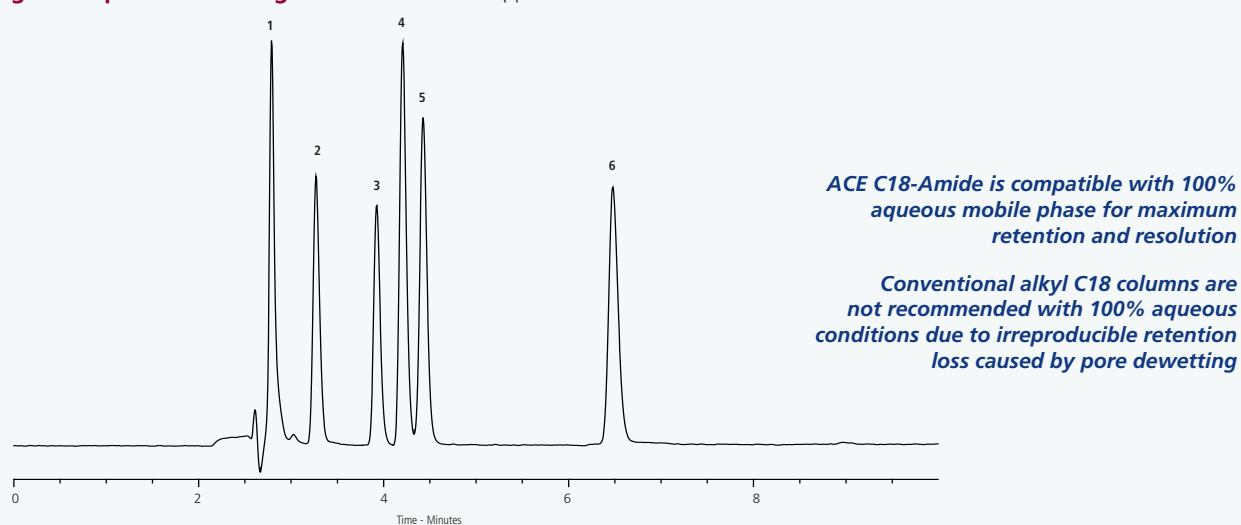
Fig. 4 - Separation of Acidic, Basic and Neutral Compounds Application # 1603



Sample: 1) methylphenylsulphoxide 2) pindolol 3) 3-hydroxybenzoic acid 4) 1,2-dimethoxybenzene 5) berberine 6) myricetin 7) piperine 8) chrysin  
Mobile Phase: A = 20mM ammonium formate in H<sub>2</sub>O (pH 3.0) B = 20mM ammonium formate (pH 3.0) in 90:10 (v/v) MeOH/H<sub>2</sub>O Gradient: 3 – 100% B in 5 minutes  
Column Dimensions: 50 x 2.1mm Flow Rate: 0.60ml/min Temperature: 40°C Wavelength: 254nm

## Compatible with 100% Aqueous Mobile Phases

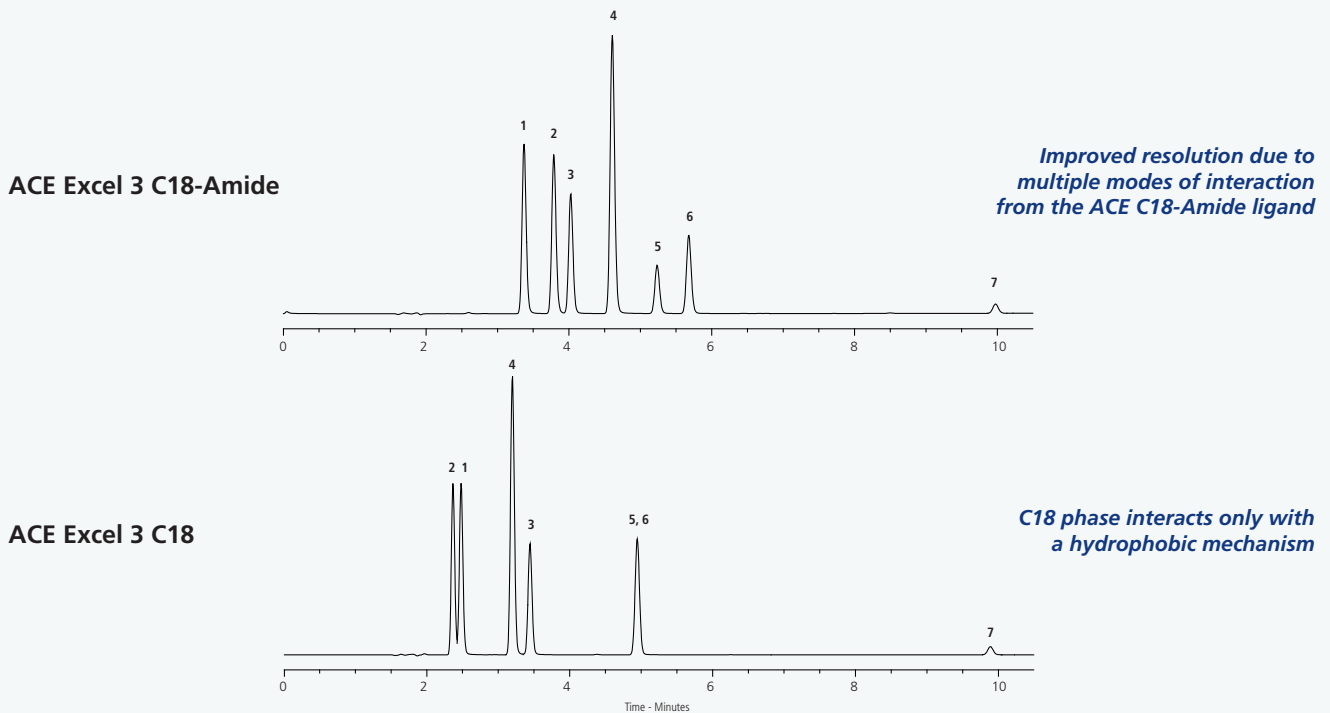
Fig. 5 - Separation of Organic Wine Acids Application # 1604



Sample: 1) oxalic acid 2) tartaric acid 3) malic acid 4) lactic acid 5) ascorbic acid 6) citric acid  
Mobile Phase: 40mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> in H<sub>2</sub>O (pH 2.5)  
Column: ACE Excel 3 C18-Amide, 250 x 2.1mm Flow Rate: 0.21ml/min Temperature: 25°C Wavelength: 214nm

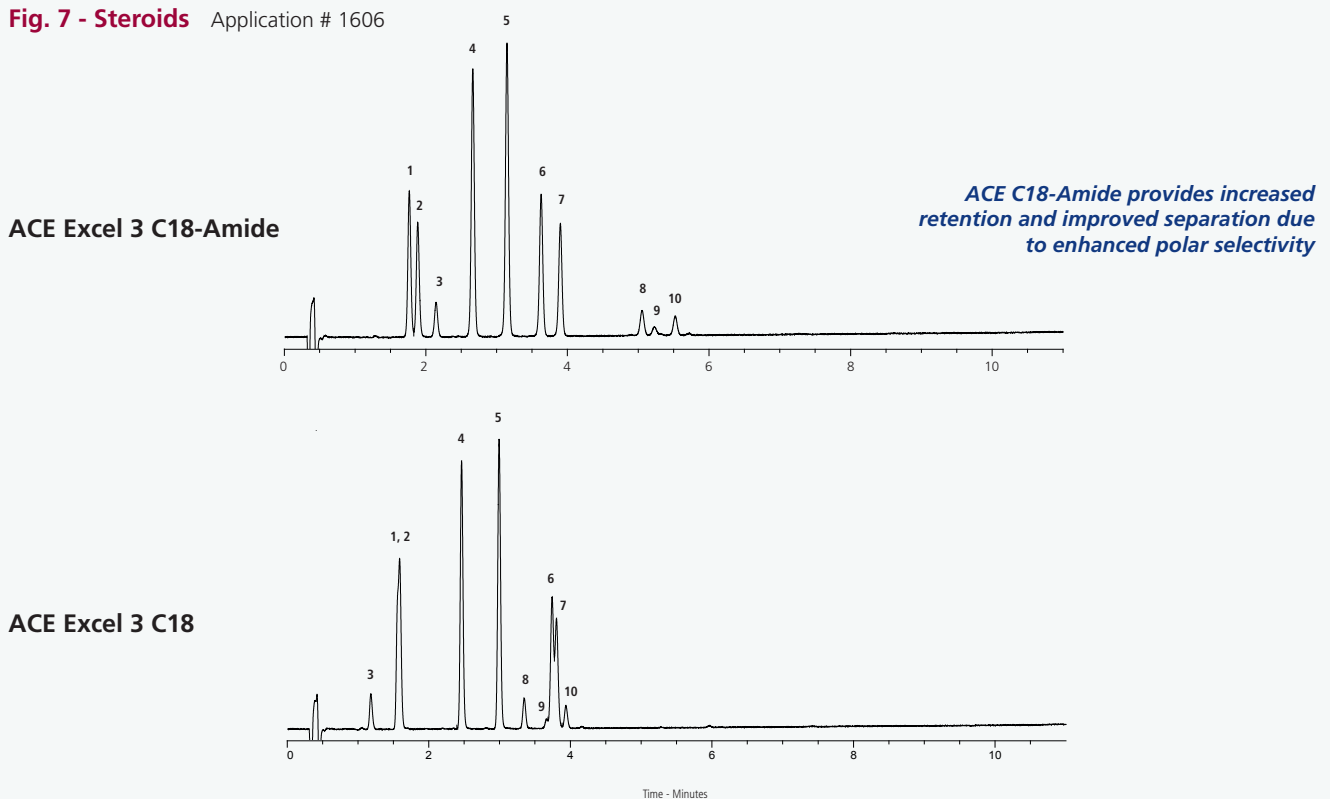
## ACE C18-Amide Provides Alternative Selectivity

**Fig. 6 - Food and Beverage Additives - Vanillins** Application # 1605



Sample: 1) vanillic acid 2) 4-hydroxybenzoic acid 3) vanillin 4) 4-hydroxybenzaldehyde 5) guaiacol 6) ethyl vanillin 7) eugenol  
 Mobile Phase: A = 0.1% formic acid in H<sub>2</sub>O B = 0.1% formic acid in MeCN Gradient: 30 – 55% B in 10 minutes  
 Column Dimensions: 150 x 4.6mm Flow Rate: 1.00ml/min Temperature: 40°C Wavelength: 260nm

**Fig. 7 - Steroids** Application # 1606

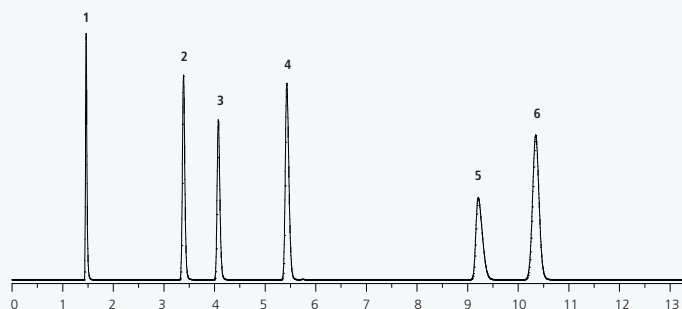


Sample: 1) prednisone 2) prednisolone 3) estriol 4) corticosterone 5) 11 $\alpha$ -hydroxyprogesterone 6) 11-ketoprogesterone  
 7) 21-hydroxyprogesterone 8)  $\beta$ -estradiol 9) 17 $\alpha$ -estradiol 10) 17 $\alpha$ -ethynylestradiol  
 Mobile Phase: A = 0.1% formic acid in H<sub>2</sub>O B = 0.1% formic acid in MeCN Gradient: 25 – 80% B in 10 minutes  
 Column Dimensions: 50 x 2.1mm Flow Rate: 0.40ml/min Temperature: 25°C Wavelength: 260nm

## Guaranteed Reproducibility and Fully Scalable

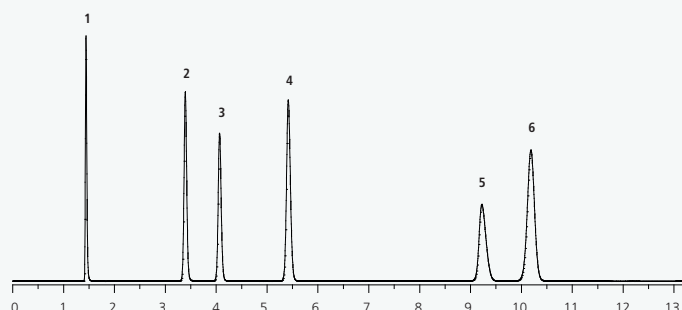
**Fig. 8 - Reproducible Scale Up with ACE C18-Amide** Application # 1607

### ACE Excel 2 C18-Amide



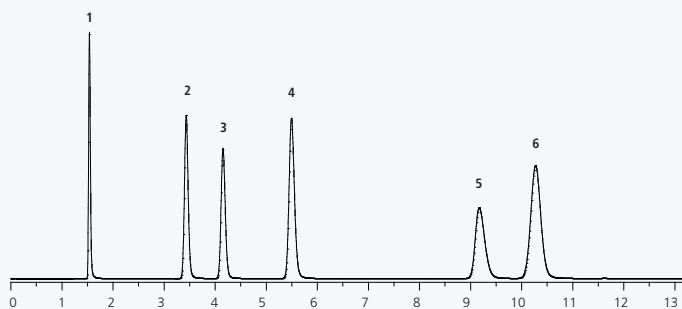
**LC/MS**  
**150 x 2.1mm**  
**0.21ml/min**  
(Silane Batch #1)

### ACE Excel 3 C18-Amide



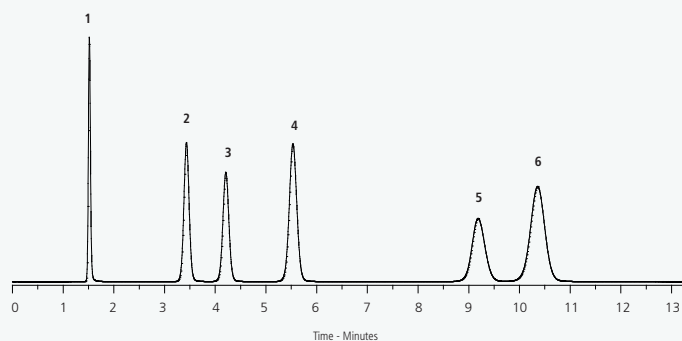
**Analytical**  
**150 x 3.0mm**  
**0.42ml/min**  
(Silane Batch #2)

### ACE Excel 5 C18-Amide



**Analytical**  
**150 x 4.6mm**  
**1.00ml/min**  
(Silane Batch #3)

### ACE 10 C18-Amide



**Preparative**  
**150 x 21.2mm**  
**21.2ml/min**  
(Silane Batch #4)

Sample: 1) uracil 2) 4-hydroxybenzoic acid 3) acetylsalicylic acid 4) benzoic acid 5) 2-hydroxybenzoic acid 6) ethyl paraben  
Mobile Phase: 35:65 MeCN/0.1% TFA in H<sub>2</sub>O Temperature: 22°C Wavelength: 254nm

The availability of 2µm, 3µm, 5µm and 10µm particle sizes combined with a range of dimensions from UHPLC through to preparative HPLC scale ensures that methods can be reproducibly scaled up or down.

The chromatograms in figure 8 demonstrate the excellent reproducibility achieved when changing both silica batch and silane batch, and the reproducible scalability obtained when changing particle size and column diameter.

## Product Availability and Specifications

| Phase         | Functional Group                          | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area (m <sup>2</sup> /g) | Carbon Load (%) | Maximum pH Range     | USP Listing |
|---------------|---|-----------|--------------------|---------------|----------------------------------|-----------------|----------------------|-------------|
| ACE C18-Amide | Octadecyl with integral amide polar group | Yes       | 2, 3, 5, 10        | 100           | 300                              | 16.4            | 2.0-8.0 <sup>a</sup> | L1 / L60    |

<sup>a</sup>For optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at higher pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC/MS Buffer Selection" by John Dolan – please contact your distributor to request your FREE copy or visit [www.ace-hplc.com](http://www.ace-hplc.com).

To further extend column lifetime under HPLC conditions (up to 5000psi/350bar), ACE guard cartridges or ACE HPLC pre-column filters are recommended.

To further extend column lifetime under UHPLC conditions (up to 15000psi/1000bar), ACE UHPLC pre-column filters are recommended.

For HPLC column connections up to 5000psi (350bar), PEEK fingertight fittings (p/n ACE-CC10) are recommended.

For UHPLC column connections up to 15000psi (1000bar), reuseable fittings (p/n EXL-CC10) are recommended.

For further details please contact your distributor or visit [www.ace-hplc.com](http://www.ace-hplc.com).

### ACE Excel 2µm C18-Amide UHPLC/HPLC Columns (supplied in dual compatible UHPLC/HPLC "Excel" hardware format with 1000bar/15000psi pressure limit)

| Column Diameter | Column Length  |                |                |                |                |                |                |                |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                 | 20mm           | 30mm           | 35mm           | 50mm           | 75mm           | 100mm          | 125mm          | 150mm          |
| 2.1mm           | EXL-1012-0202U | EXL-1012-0302U | EXL-1012-3502U | EXL-1012-0502U | EXL-1012-7502U | EXL-1012-1002U | EXL-1012-1202U | EXL-1012-1502U |
| 3.0mm           | EXL-1012-0203U | EXL-1012-0303U | EXL-1012-3503U | EXL-1012-0503U | EXL-1012-7503U | EXL-1012-1003U | EXL-1012-1203U | EXL-1012-1503U |
| 4.6mm           | EXL-1012-0246U | EXL-1012-0346U | EXL-1012-3546U | EXL-1012-0546U | EXL-1012-7546U | EXL-1012-1046U | EXL-1012-1246U | EXL-1012-1546U |

### ACE Excel 3µm C18-Amide UHPLC/HPLC Columns (supplied in dual compatible UHPLC/HPLC "Excel" hardware format with 1000bar/15000psi pressure limit)

| Column Diameter | Column Length  |                |                |                |                |                |                |                |                |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                 | 20mm           | 30mm           | 35mm           | 50mm           | 75mm           | 100mm          | 125mm          | 150mm          | 250mm          |
| 2.1mm           | EXL-1112-0202U | EXL-1112-0302U | EXL-1112-3502U | EXL-1112-0502U | EXL-1112-7502U | EXL-1112-1002U | EXL-1112-1202U | EXL-1112-1502U | EXL-1112-2502U |
| 3.0mm           | EXL-1112-0203U | EXL-1112-0303U | EXL-1112-3503U | EXL-1112-0503U | EXL-1112-7503U | EXL-1112-1003U | EXL-1112-1203U | EXL-1112-1503U | EXL-1112-2503U |
| 4.6mm           | EXL-1112-0246U | EXL-1112-0346U | EXL-1112-3546U | EXL-1112-0546U | EXL-1112-7546U | EXL-1112-1046U | EXL-1112-1246U | EXL-1112-1546U | EXL-1112-2546U |

### ACE Excel 5µm C18-Amide UHPLC/HPLC Columns (supplied in dual compatible UHPLC/HPLC "Excel" hardware format with 1000bar/15000psi pressure limit)

| Column Diameter | Column Length  |                |                |                |                |                |                |                |                |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                 | 20mm           | 30mm           | 35mm           | 50mm           | 75mm           | 100mm          | 125mm          | 150mm          | 250mm          |
| 2.1mm           | EXL-1212-0202U | EXL-1212-0302U | EXL-1212-3502U | EXL-1212-0502U | EXL-1212-7502U | EXL-1212-1002U | EXL-1212-1202U | EXL-1212-1502U | EXL-1212-2502U |
| 3.0mm           | EXL-1212-0203U | EXL-1212-0303U | EXL-1212-3503U | EXL-1212-0503U | EXL-1212-7503U | EXL-1212-1003U | EXL-1212-1203U | EXL-1212-1503U | EXL-1212-2503U |
| 4.6mm           | EXL-1212-0246U | EXL-1212-0346U | EXL-1212-3546U | EXL-1212-0546U | EXL-1212-7546U | EXL-1212-1046U | EXL-1212-1246U | EXL-1212-1546U | EXL-1212-2546U |

### ACE 5µm C18-Amide Semi-Prep and Preparative HPLC Columns

| Column Diameter | Column Length |               |               |               |               |               |
|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                 | 50mm          | 75mm          | 100mm         | 125mm         | 150mm         | 250mm         |
| 7.75mm          | ACE-1212-0508 | ACE-1212-7508 | ACE-1212-1008 | ACE-1212-1208 | ACE-1212-1508 | ACE-1212-2508 |
| 10.0mm          | ACE-1212-0510 | ACE-1212-7510 | ACE-1212-1010 | ACE-1212-1210 | ACE-1212-1510 | ACE-1212-2510 |
| 21.2mm          | ACE-1212-0520 | ACE-1212-7520 | ACE-1212-1020 | ACE-1212-1220 | ACE-1212-1520 | ACE-1212-2520 |

### ACE 10µm C18-Amide Analytical, Semi-Prep and Preparative HPLC Columns Please enquire

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**ACE® UHPLC and HPLC columns are available through our international distributor network**  
Available from:

