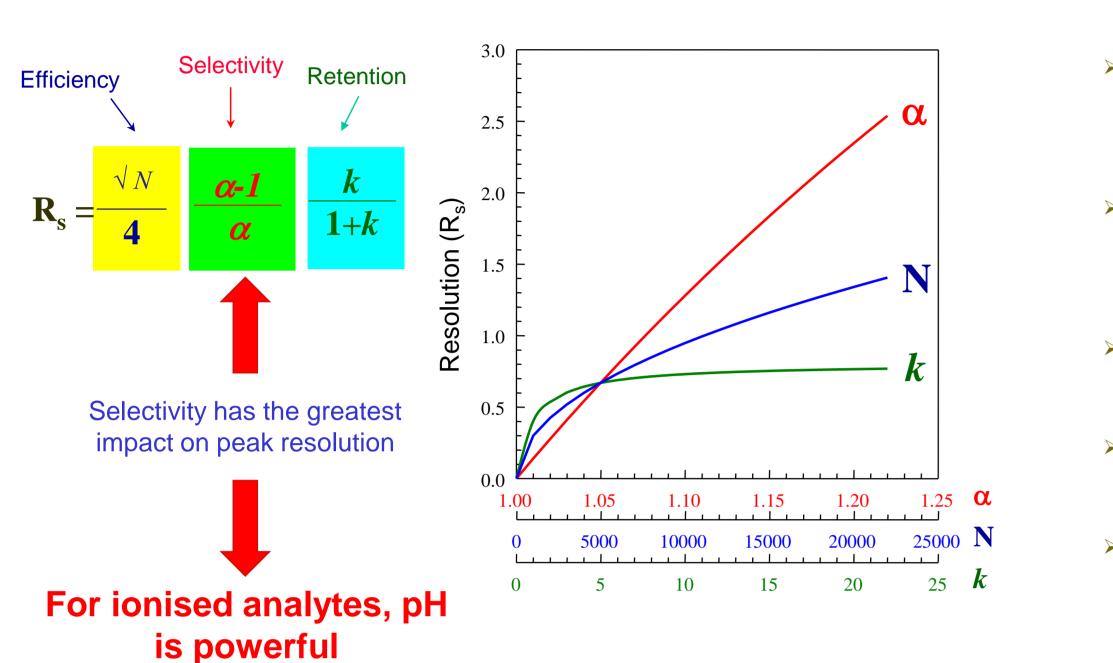
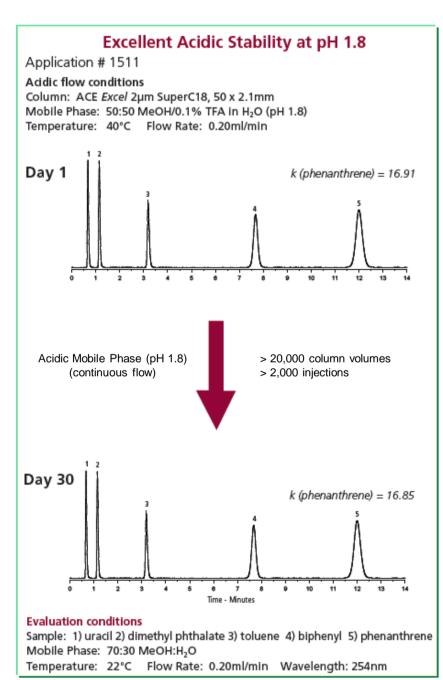


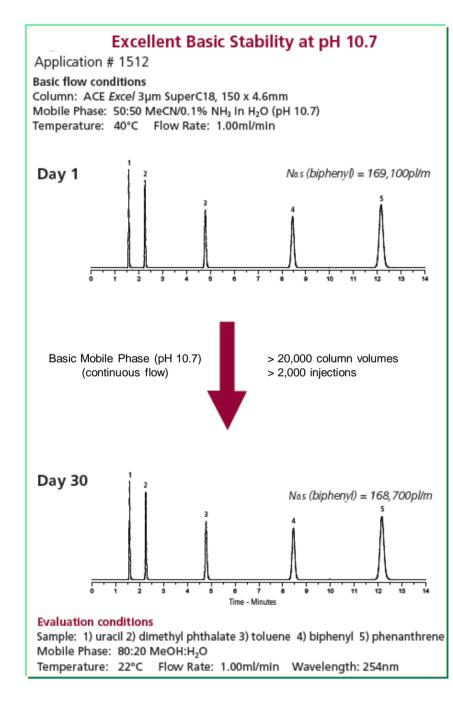
# **1. RESOLUTION: SELECTIVITY, EFFICIENCY & RETENTION**



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-263

### **5. PHASE STABILITY WITH LOW AND HIGH PH ELUENTS**





### 9. URINE SAMPLE EXTRACTION & TEST CONDITIONS

- Amphetamines Extraction Conditions
- 1. 2mLs urine spiked with 0, 100, 250 and 1000ng/mL of: 3,4-MDMA, phenylpropanolamine, amphetamine, ephedrine, 3,4-MDA, MDEA, 4-methylthioamphetamine.
- 100µL of NH₄OH added.
- 3. 2.5mL of n-butyl chloride added.
- 4. Rotated for 15min, then centrifuged for 5mins at 2000rpm.
- 5. 2mLs of n-butyl chloride supernatant vialled up for evaporation to dryness before reconstituting in 1mL 95/5 v/v H<sub>2</sub>O/ACN.

### **Amphetamines LC-MS Conditions**

- ACE Excel 3 Super C18, 75x2.1mm. A: 5mM Ammonium Hydroxide, pH 10.8.
- B: 5mM Ammomnium Hydroxide, pH 10.8 in 1:9 v/v H2O:MeOH.
- Gradient: 0-8-8.01-10minutes 30-95-30-30. 60C, 2uL.

#### Varian 320 Triple Quadrupole Mass Spectrometer

Electrospray voltage: +5 kV Inlet capillary voltage: 30 V

- CID with argon at 1.5 mTorr; Collision cell potential ranges from Inlet capillary voltage: 30 V 5 to 17 V
- Drying gas (nitrogen) temperature: 325 C
- Nebulizing gas (nitrogen) pressure: 35 psi Detector set to use Extended Dynamic Range function

H2O:MeOH. Gradient: 0-5-5.01-7minutes 5-95-5-5. 60C, 2uL. Varian 320 Triple Quadrupole Mass Spectrometer Electrospray voltage: +5 kV

B: 5mM Ammomnium Hydroxide, pH 10.8 in 1:9 v/v

ACE Excel 3 Super C18, 75x2.1mm + ACE SuperC18 guard.

CID with argon at 1.5 mTorr; Collision cell potential ranges from 5 to 17 V Drying gas (nitrogen) temperature: 325 C

Nebulizing gas (nitrogen) pressure: 35 psi

**Opiates LC-MS Conditions** 

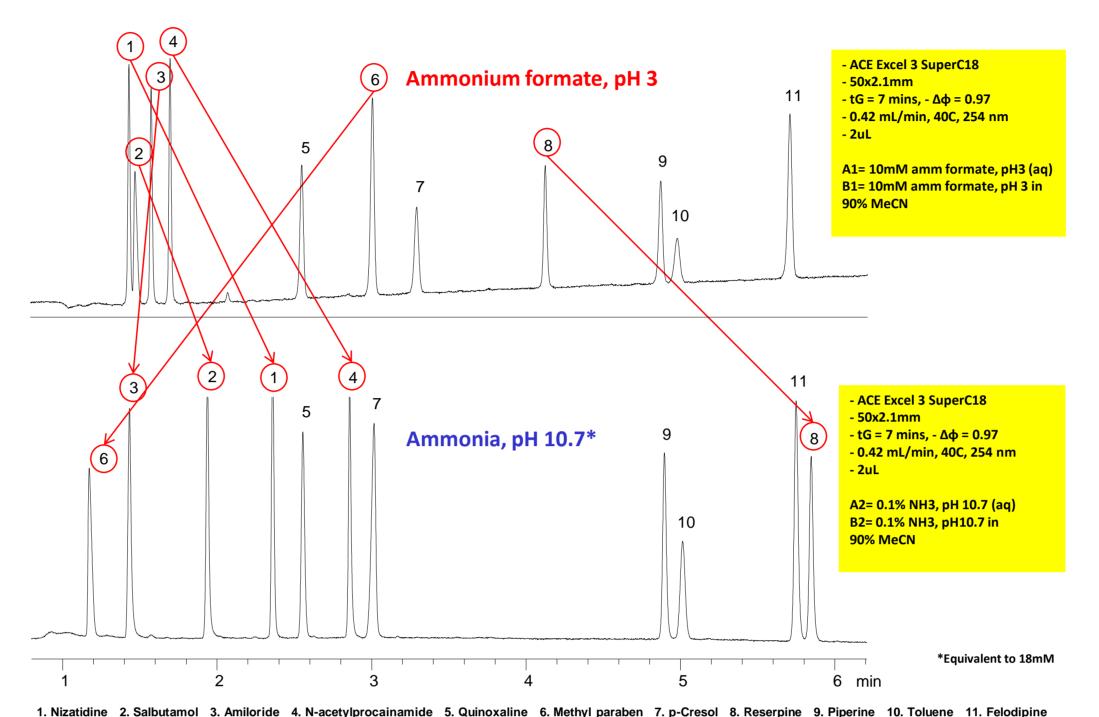
A: 5mM Ammonium Hydroxide, pH 10.8.

- Detector set to use Extended Dynamic Range function
- **Opiates Extraction Conditions** 1. 1mL urine spiked with 0, 100, 250 and 1000ng/mL morphine-3-β-D-glucuronide, normorphine, morphine  $6-\beta$ -D-glucuronide, 6-acetylmorphone, morphine.
- 2. Centrifuged for 5 mins at 14.000rpm.
- 3. 1mL vialled up.

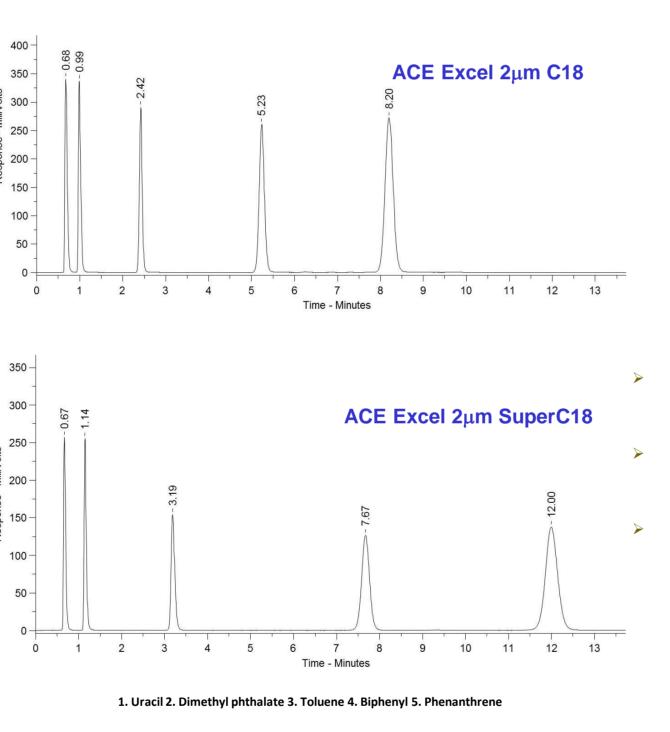
info@ace-hplc.com

# Exploring Low And High pH Eluents Using A New Silica-**Based Extended pH Range C18 Column For Analysis Of Amphetamines & Opiates From Urine By LC-MS** <u>Alan P McKeown<sup>1</sup>, Carl Zimmerman<sup>2</sup></u> <sup>1</sup>Advanced Chromatography Technologies Ltd, 1 Berry Street, Aberdeen, Scotland, AB25 1HF UK <sup>2</sup>MACMOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

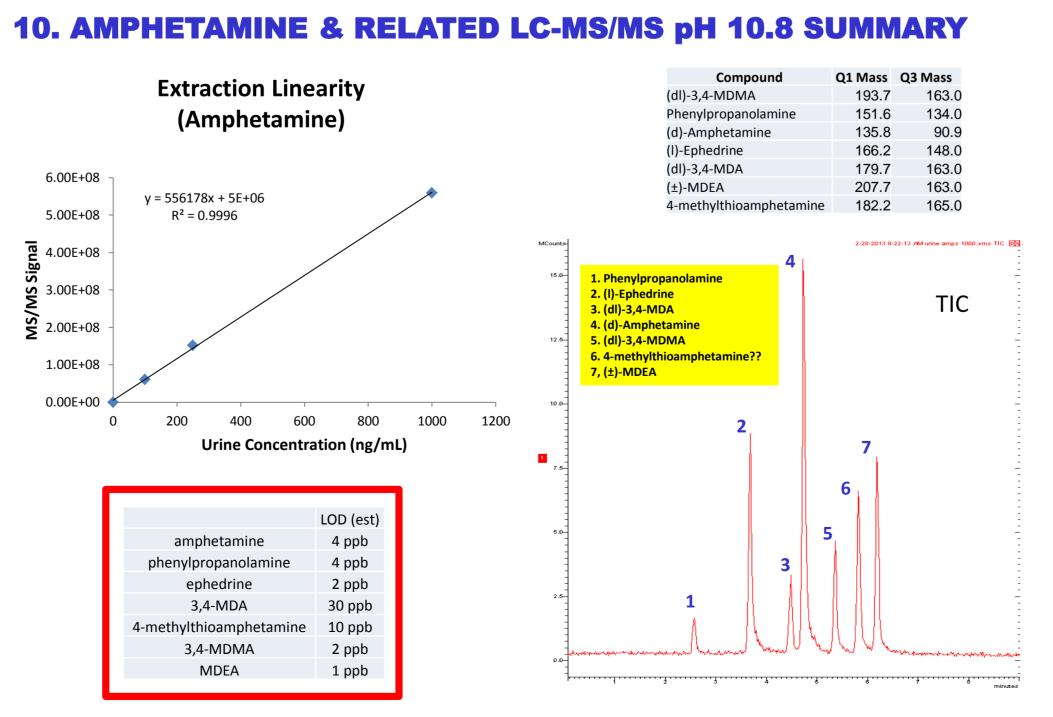
- 2. ACE<sup>®</sup> SuperC18<sup>™</sup>: A NEW OPTION FOR CHROMATOGRAPHERS
  - Ultra-inert HPLC / UHPLC columns: extended pH stability (pH 1.5 – 11.5).
  - Specially designed for high and low pH mobile phases with LC-MS buffers.
  - Ultra-low phase bleed for improved LC-MS compatibility.
  - Ideal for high pH prep apps eg isolations / purifications.
  - Stable at low, medium and high pH eluents for >20,000 column volumes.



# 6. ACE<sup>®</sup> Excel SuperC18<sup>™</sup> SHOWS INCREASED RETENTIVITY



|                       | Columns: 50x2.1mm, 2μm<br>Isocratic analysis                         |  |  |  |  |  |  |  |
|-----------------------|--|--|--|--|--|--|--|--|
|                       | Flow rate: 0.20 mL/min<br>Temperature: ambient<br>Detection: 254 nm  |  |  |  |  |  |  |  |
|                       | MP: 7: 3 v/v MeOH: water   |  |  |  |  |  |  |  |
|                       |  |  |  |  |  |  |  |  |
|                       |  |  |  |  |  |  |  |  |
| ame C18 elution order |  |  |  |  |  |  |  |  |
| CI                    | reased retention   |  |  |  |  |  |  |  |
| g                     | pful for MS analyses as<br>her organic content can<br>used in eluent |  |  |  |  |  |  |  |



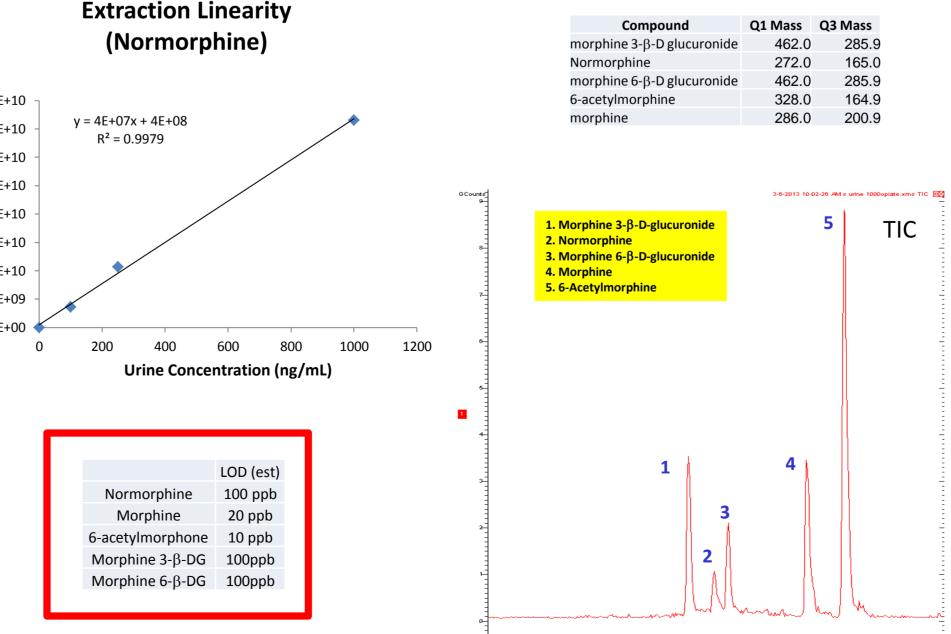
# **11. OPIATES & RELATED LC-MS/MS pH 10.8 SUMMARY**

|      | 4.00E- |
|------|--------|
|      | 3.50E- |
| =    | 3.00E- |
| igna | 2.50E- |
| MS S | 2.00E- |
| 1S/N | 1.50E- |
| 2    | 1.00E- |
|      | 5.00E- |
|      | 0.00E- |
|      |        |

# **3. METHOD DEVELOPMENT: LOW / HIGH PH ELUENTS**

# 7. ANALYSIS FOR CONTROLLED SUBSTANCES

- Controlled drugs (legal & illegal) are routinely profiled in various biological matrices using LC-MS/MS.
- **Chromatography** coupled with **MS**<sup>n</sup> is powerful for separation and low level identification / quantification.
- The ability to use a **broad pH range** during method development can be helpful for optimising a separation and getting the best peak shape.
- This LC-MS/MS works reports the use of the ACE<sup>®</sup> **SuperC18™** with high pH eluents for various opiate and amphetamine mixtures extracted from urine.

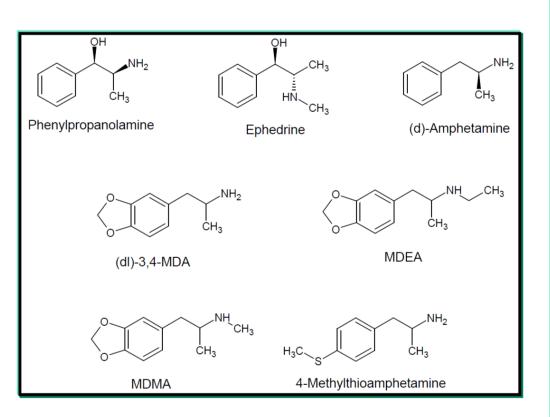


# **4. PHASE INERTNESS & PERFORMANCE: HPLC AT HIGH PH**

- <u>3 μm HPLC </u>
- Column ine
- Pyridine tes
- Efficiency r
- > ACE Super

# 8. STRUCTURES OF ANALYTES IN THE MIXTURES

# Amphetamine & Related



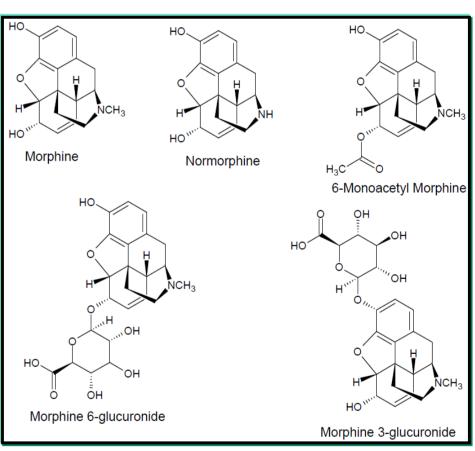
## **12. SUMMARY AND CONCLUSIONS**

ACE® is a registered trademark of Advanced Chromatography Technologies Limited. ACE Excel™ is a trademark of Advanced Chromatography Technologies Limited. Advanced Chromatography Technologies Limited acknowledges the registered and unregistered trademarks of Agilent Technologies Inc., Phenomenex Inc. and Waters Corporation and has no affiliation with any of these companies



| Dimensions           | Peak Efficiency Cor  | 1           |                       |                    |               |                  |                |                    |          |
|----------------------|--|-------------|-----------------------|--------------------|---------------|------------------|----------------|--------------------|----------|
|                      | ACE Excel 3 SuperC18   |             |                       |                    |               |                  |                | 134,200pl/r        | m        |
| rtness               | Phenomenex Gemini 3 C18  |             | 56,600pl/m            |                    |               |                  |                |                    |          |
| 111035               | Waters XBridge 3.5 BEH C18   |             |                       | 45,1               | 100pl/m       |                  |                |                    |          |
|                      | Phenomenex Gemini-NX 3 C18   |             |                       | 33,000pl/m         |               |                  |                |                    |          |
| st at high pH        | Waters XSelect 3.5 CSH C18   |             |                       | 31,000pl/m         |               |                  |                |                    |          |
|                      | ZORBAX Extend 3.5 C18  |             |                       | 30,000pl/m         |               |                  |                |                    |          |
| neasured, 5%         | 0  | 2           | 20,000                | 40,000             | 60,000        | 80,000           | 100,000        | 120,000            | 140,00   |
|                      |  |             | N <sub>0.05</sub> (py | ridine) (plates/m) |               |                  |                |                    |          |
| <u> C18 Ranks #1</u> | Column Dimensions: 150 x 4.6m<br>Flow Rate: 1.00ml/min Tempera<br>Comparative data may not be re | ature: 22°C | Waveler               | igth: 254nm        | enol Mobile P | hase: 0.1% NH₃ ( | =18mM), pH 10. | 7 in 60:40 (v/v) N | 1eOH/H₂O |

**Opiates & Related** 



Eluent pH is a powerful approach for exploring chromatographic selectivity in method development & isolations.

The ACE<sup>®</sup> SuperC18<sup>™</sup> has enhanced retentivity that can be helpful for LC-MS work as higher organic content is needed for elution which is ideal for analyte ionisation.

Amphetamine, morphine and their related species and metabolites can be measured at low levels using LC-MS/MS with the ACE® **SuperC18™** at low and high eluent pH. The high pH work is shown.

The ACE<sup>®</sup> SuperC18<sup>™</sup> provides chromatographers and method developers with an NEW selectivity option with an extended pH range and LC-MS analyses.

# www.ace-hplc.com