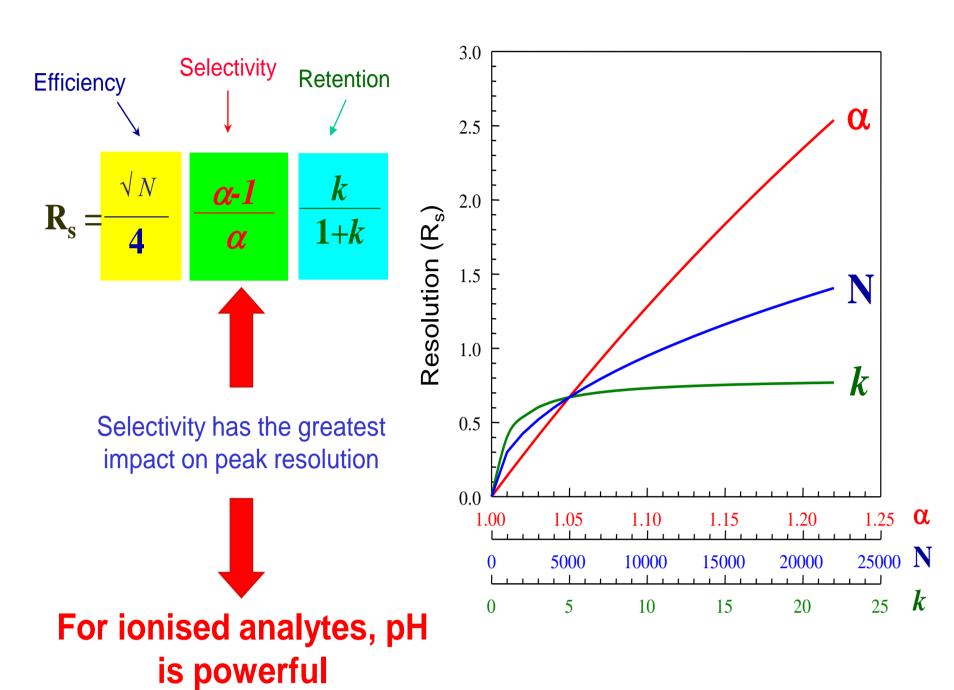


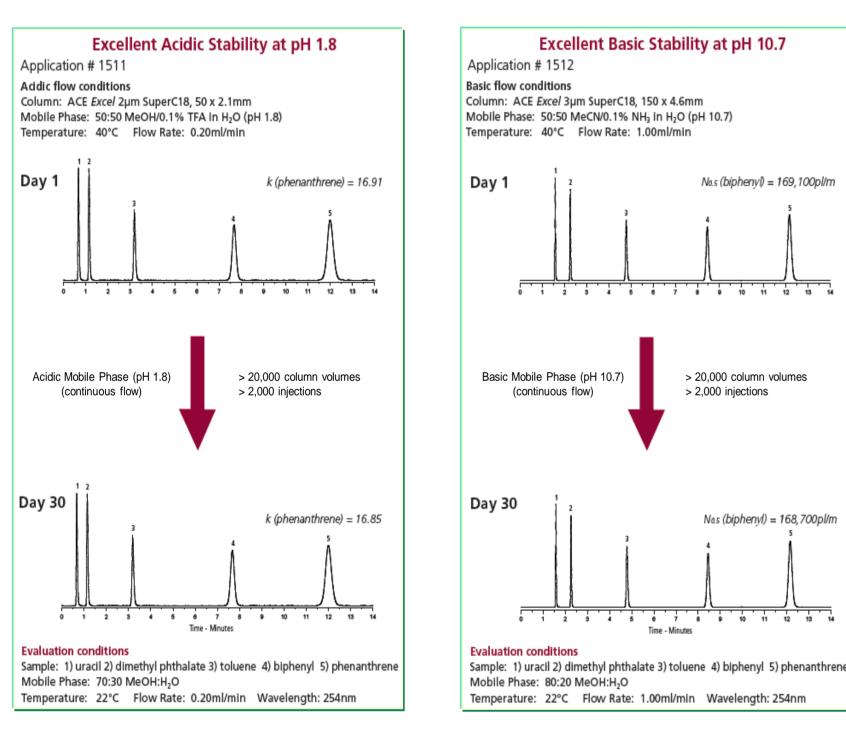


### **1. RESOLUTION: SELECTIVITY, EFFICIENCY & RETENTION**



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

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



#### **9. URINE SAMPLE EXTRACTION & TEST CONDITIONS**

**Opiates LC-MS Conditions** 

Electrospray voltage: +5 kV

Inlet capillary voltage: 30 V

H2O:MeOH.

from 5 to 17 V

60C, 2uL.

A: 5mM Ammonium Hydroxide, pH 10.8.

Gradient: 0-5-5.01-7minutes 5-95-5-5

Drying gas (nitrogen) temperature: 325 C

Nebulizing gas (nitrogen) pressure: 35 psi

Varian 320 Triple Quadrupole Mass Spectrometer

CID with argon at 1.5 mTorr; Collision cell potential ranges

Detector set to use Extended Dynamic Range function

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

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

5 to 17 V Drying gas (nitrogen) temperature: 325 C

Nebulizing gas (nitrogen) pressure: 35 psi

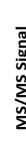
Detector set to use Extended Dynamic Range function

#### **Opiates Extraction Conditions**

1. 1mL urine spiked with 0, 100, 250 and 1000ng/mL morphine-3-β-D-glucuronide, normorphine,

- morphine 6-β-D-glucuronide, 6-acetylmorphone, morphine. 2. Centrifuged for 5 mins at 14,000rpm.
- 3. 1mL vialled up.

info@ace-hplc.com



# Low-Level LC-MS/MS Determination of Amphetamines and **Opiates From Urine Using High pH Eluent and a Novel** Extended pH Range Column Alan P McKeown<sup>1</sup>, Geoffrey Faden<sup>2</sup> <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

## **2. ACE<sup>®</sup> SuperC18<sup>™</sup>: A NEW OPTION FOR LC-MS**

Ultra-inert HPLC / UHPLC columns: extended pH stability (pH 1.5 – 11.5).

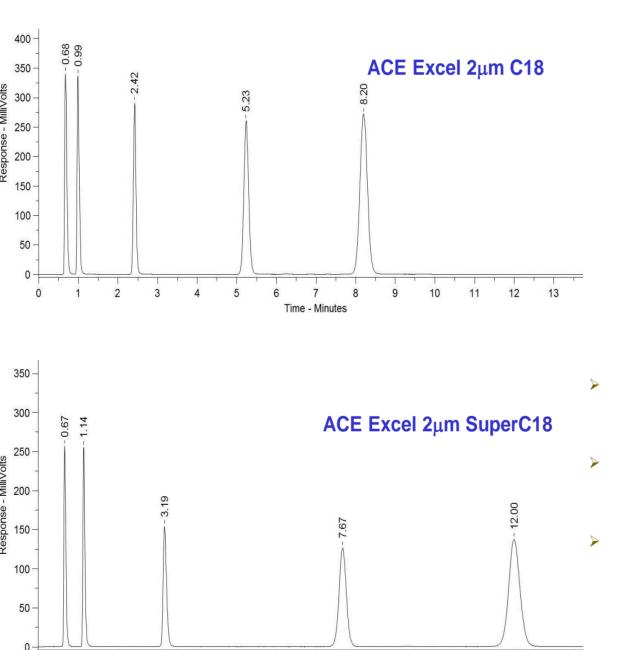
Specially designed for high and low pH mobile phases with LC-MS buffers.

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



0 1 2 3 4 5 6 7 8 9 10 11 12 13

1. Uracil 2. Dimethyl phthalate 3. Toluene 4. Biphenyl 5. Phenanthrene

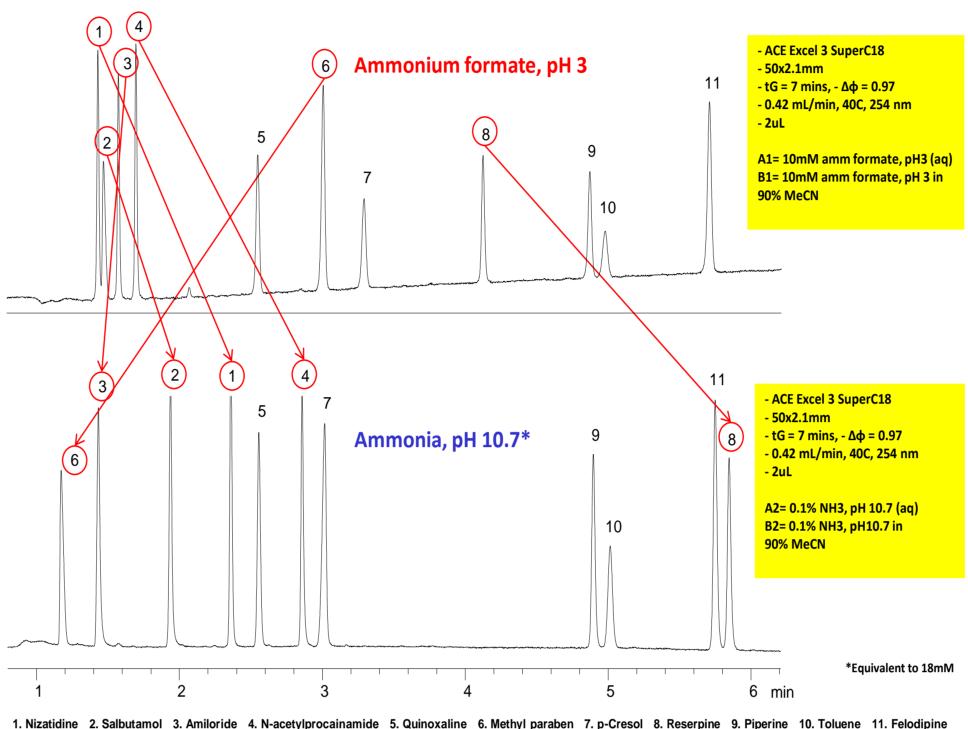
Time - Minutes

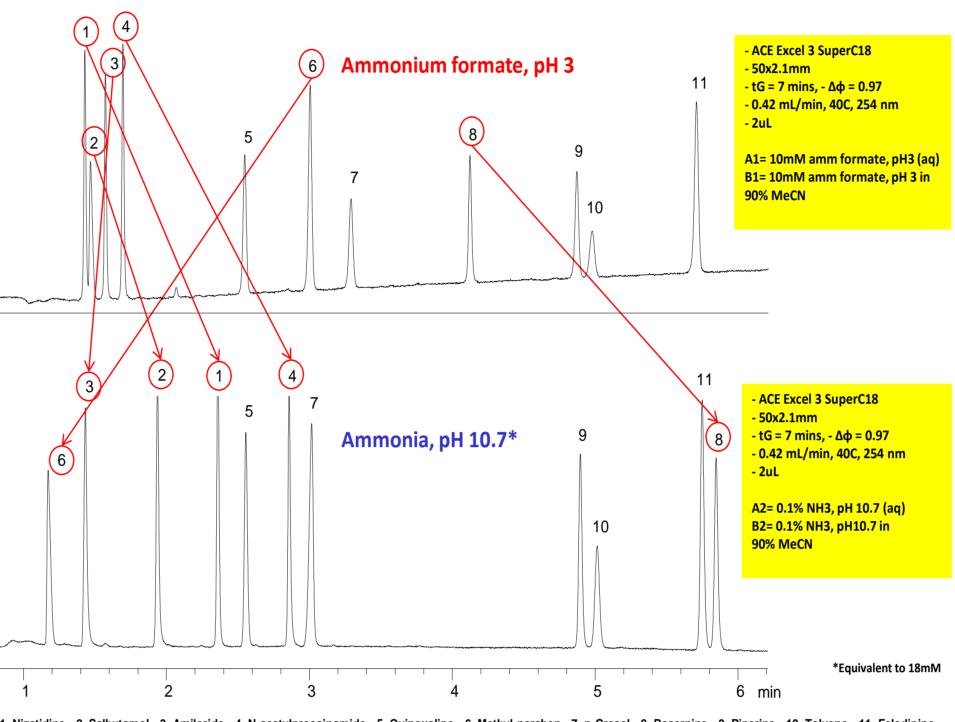
n
r

Same C18 elution order

Increased retention

Helpful for MS analyses as higher organic content can be used in eluent





## **7. ANALYSIS FOR CONTROLLED SUBSTANCES**

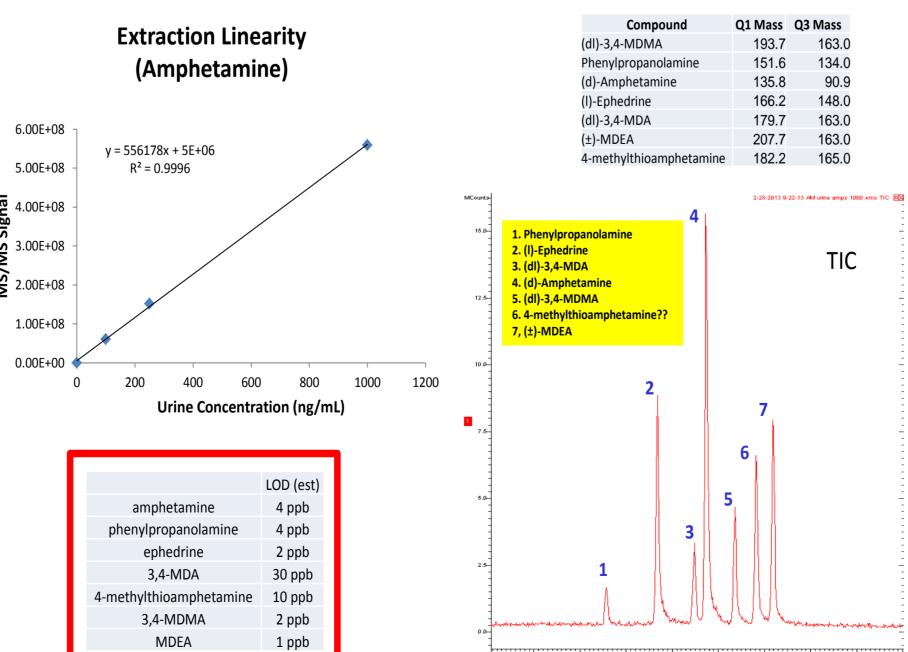
Controlled drugs (legal & illegal) are routinely profiled in various biological matrices using LC-MS/MS.

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<sup>™</sup> with high pH eluents for various opiate and amphetamine mixtures extracted from urine.

#### **10. AMPHETAMINE & RELATED LC-MS/MS pH 10.8 SUMMARY**

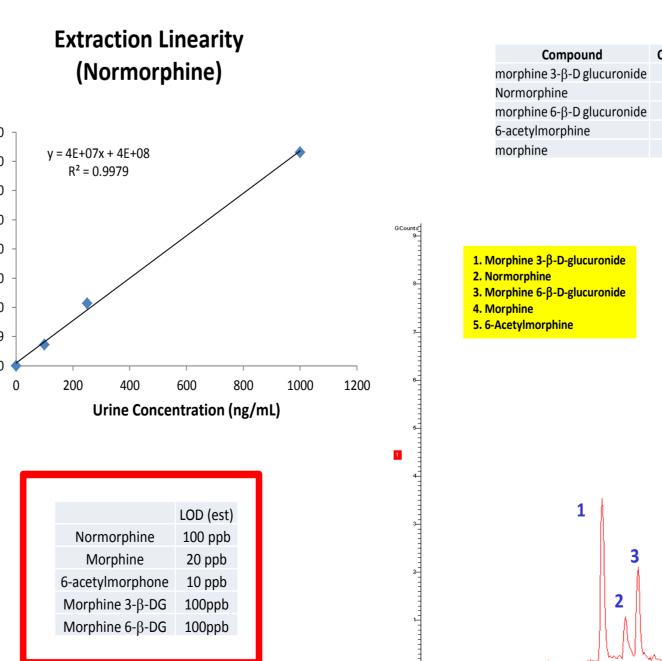


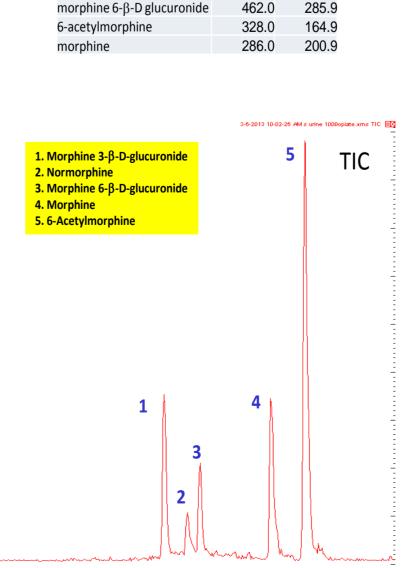
### 11. OPIATES & RELATED LC-MS/MS pH 10.8 SUMMARY

MS/MS Signal	4.00E+10
	3.50E+10
	3.00E+10
	2.50E+10
	2.00E+10
	1.50E+10
	1.00E+10
	5.00E+09
	0.00E+00

587, Chadds Ford, PA 19317 USA

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





Q1 Mass Q3 Mass

272.0

462.0 285.9

165.0

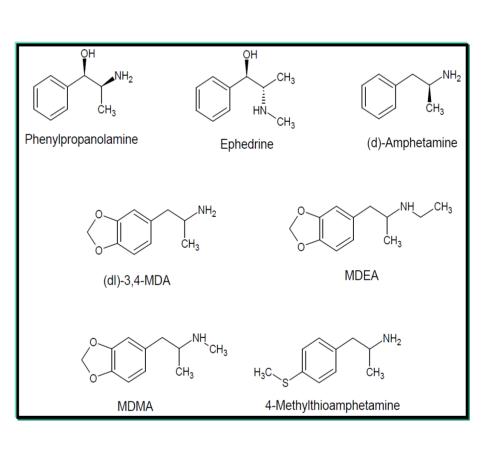
Compound

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

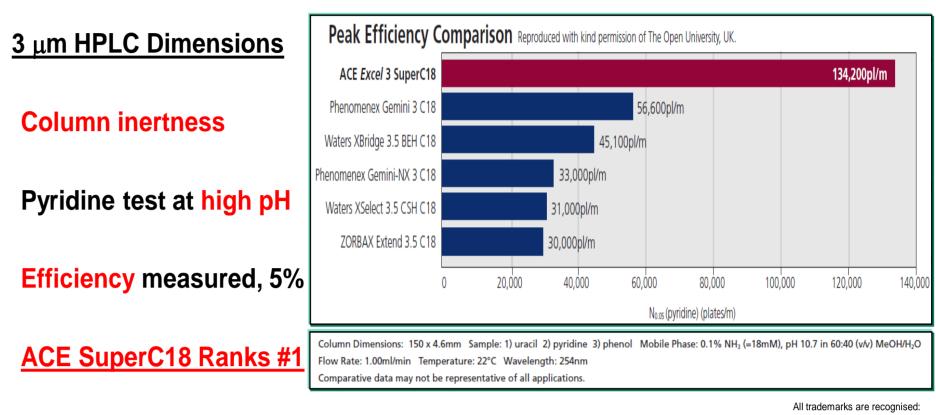
- Column inertness

## **8. STRUCTURES OF ANALYTES IN THE MIXTURES**

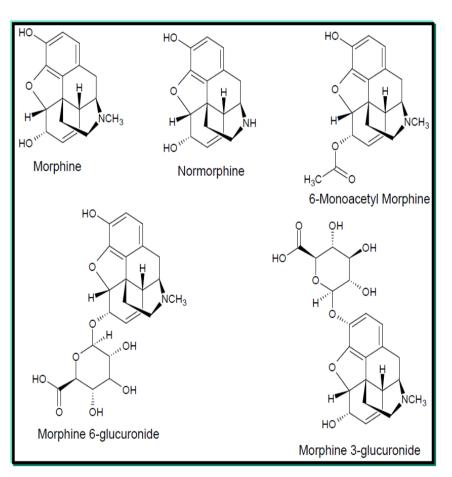
## **Amphetamine & Related**



#### **12. SUMMARY AND CONCLUSIONS**



#### **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<sup>™</sup> 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.

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