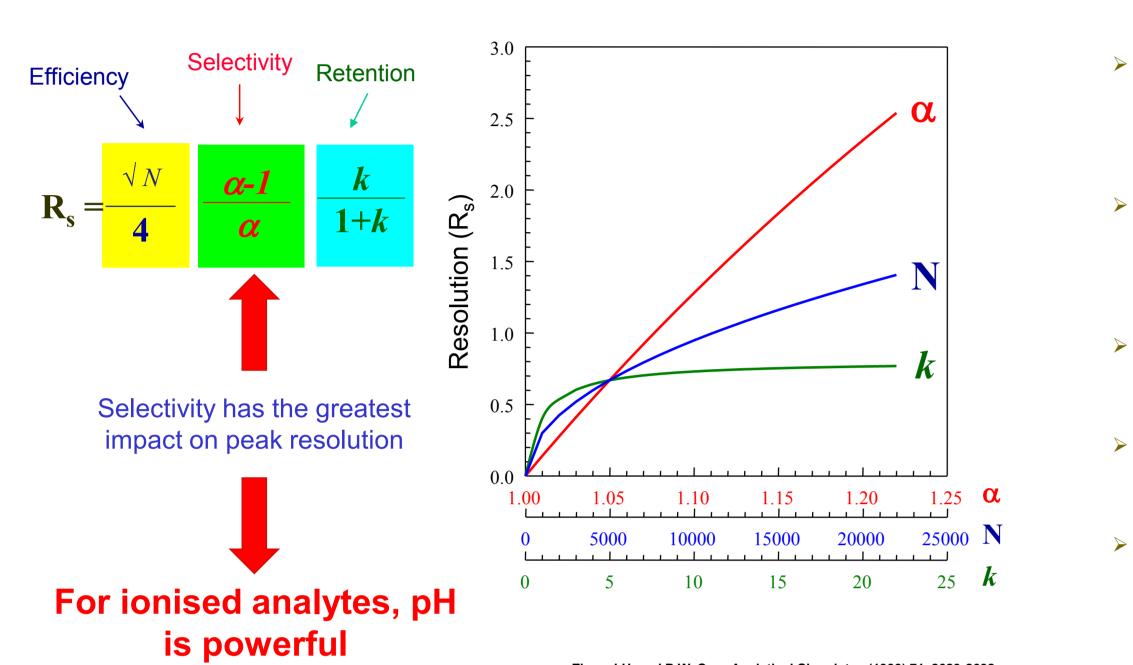


Exploring Low And High pH Eluents Using A New Silica-Based **Extended pH Range C18 Column For Analysis Of Amphetamines** & Opiates From Urine By LC-MS/MS Alan P McKeown¹, <u>Geoffrey Faden²</u>

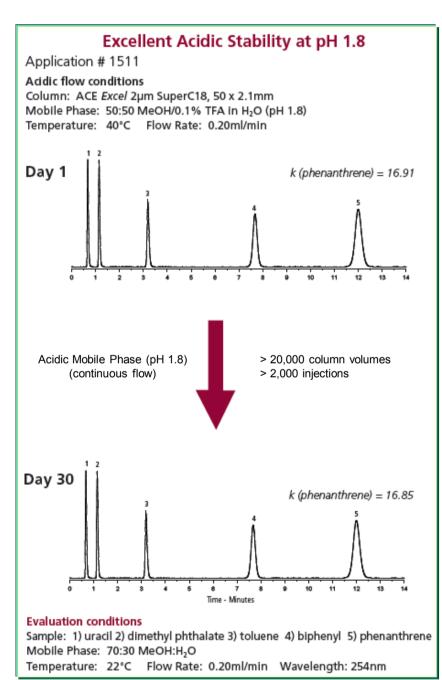
¹Advanced Chromatography Technologies Ltd, 1 Berry Street, Aberdeen, Scotland, AB25 1HF UK ²MACMOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

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



Excellent Basic Stability at pH 10.7 Application # 1512 Basic flow conditions Column: ACE Excel 3µm SuperC18, 150 x 4.6mm Mobile Phase: 50:50 MeCN/0.1% NH₃ in H₂O (pH 10.7) Temperature: 40°C Flow Rate: 1.00ml/min No.s (biphenyl) = 169,100pl/m Day 1 1 2 3 4 5 6 7 8 9 10 11 12 13 Basic Mobile Phase (pH 10.7 > 20.000 column volumes (continuous flow) > 2,000 injections Day 30 $N_{0.5}$ (biphenyl) = 168,700 pl/m 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 Time - Minutes Evaluation conditions Sample: 1) uracil 2) dimethyl phthalate 3) toluene 4) biphenyl 5) phenanthrene Mobile Phase: 80:20 MeOH:H₂O Temperature: 22°C Flow Rate: 1.00ml/min Wavelength: 254nm

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 H₂O/ACN.

Amphetamines LC-MS Conditions

- ACE Excel 3 Super C18, 75x2.1mm.
- A: 5mM Ammonium Hydroxide. pH 10.8.
- 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

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

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

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

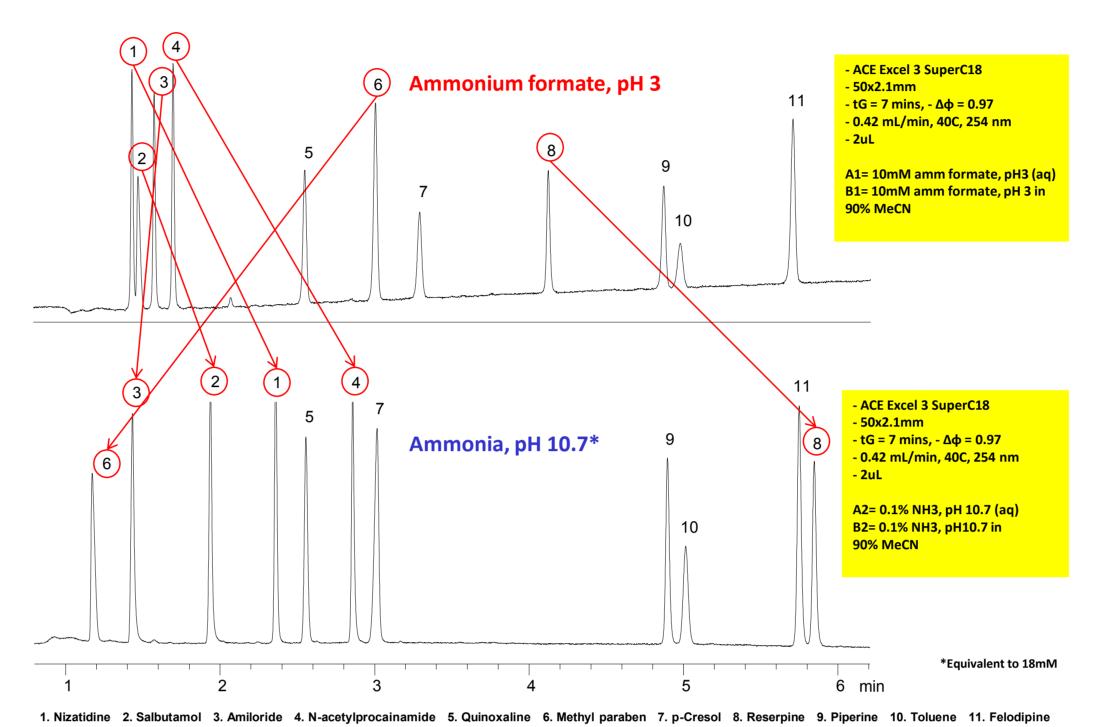
A: 5mM Ammonium Hvdroxide. pH 10.8.

Opiates LC-MS Conditions

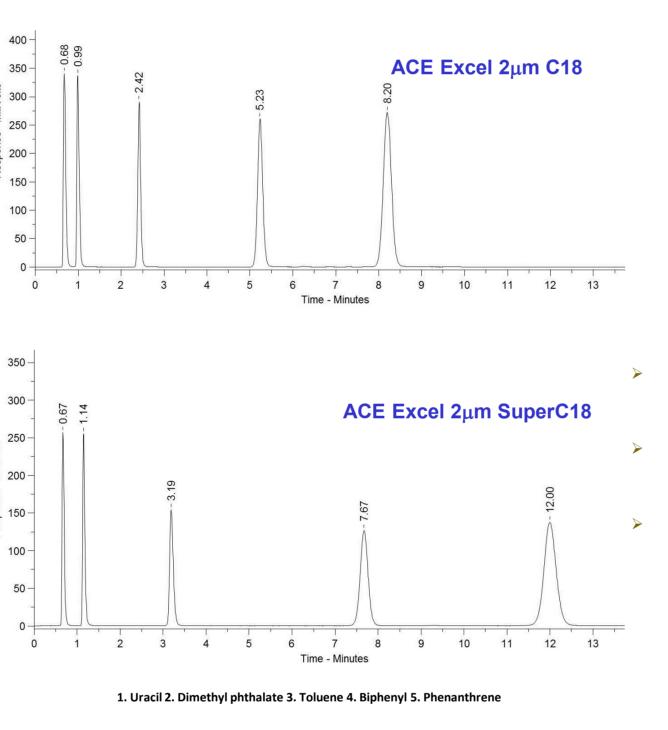
- Detector set to use Extended Dynamic Range function
- **Opiates Extraction Conditions** 1. 1mL urine spiked with 0, 100, 250 and 1000ng/mL morphine-3-β-D-glucuronide, normorphine, morphine $6-\beta$ -D-glucuronide, 6-acetylmorphone, morphine.
- 2. Centrifuged for 5 mins at 14.000rpm.
- 3. 1mL vialled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

info@ace-hplc.com

- 2. ACE[®] SuperC18[™]: 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.

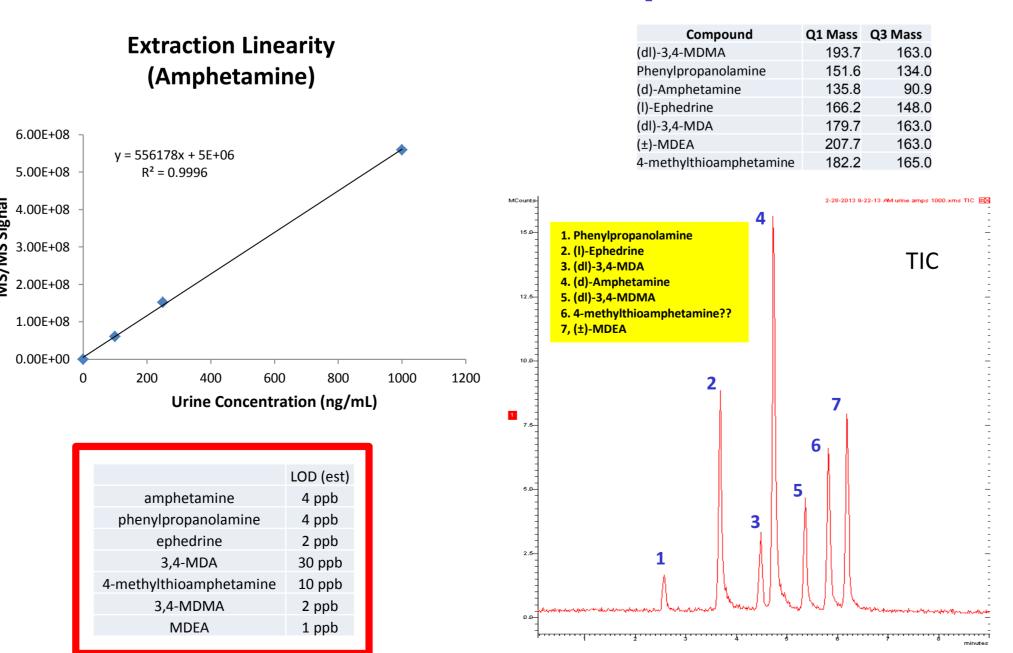


6. ACE[®] Excel SuperC18[™] SHOWS INCREASED RETENTIVITY



| | Columns: 50x2.1mm, 2µm Isocratic analysis Flow rate: 0.20 mL/min Temperature: ambient Detection: 254 nm MP: 7: 3 v/v MeOH: water | | |
|---|---|--|--|
| Same C18 elution order | | | |
| Increased retention | | | |
| Helpful for MS analyses as higher organic content can be used in eluent | | | |

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



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

| | 4.00E- |
|-------|--------|
| | 3.50E- |
| _ | 3.00E- |
| igna | 2.50E- |
| IS SI | 2.00E |
| IS/N | 1.50E |
| Σ | 1.00E |
| | 5.00E |
| | 0.00E |
| | |

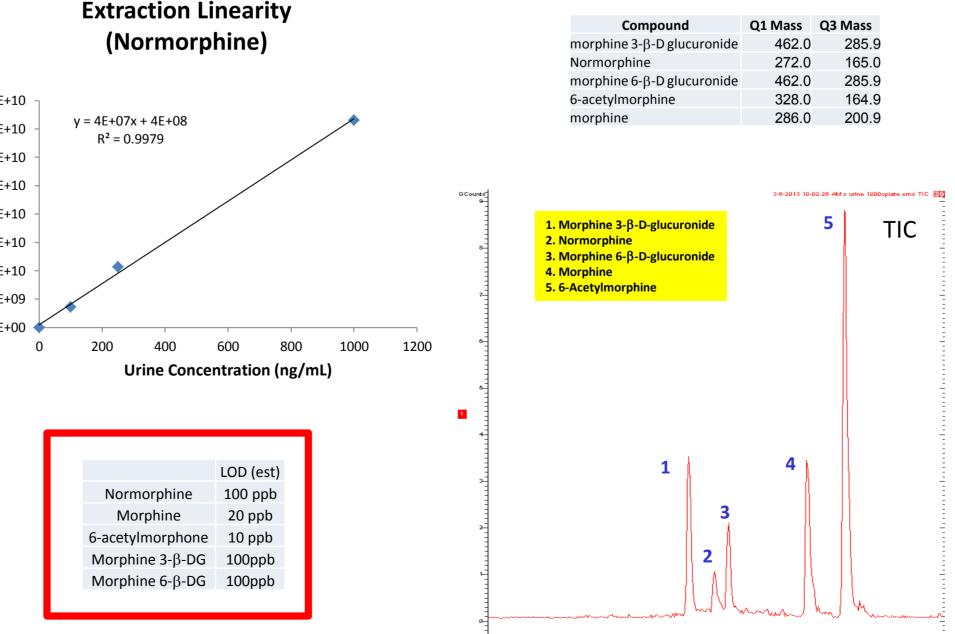
3. METHOD DEVELOPMENT: LOW / HIGH PH ELUENTS

4. PHASE INERTNESS & PERFORMANCE: HPLC AT HIGH PH

- 3 μm HPLC Dimensions
- > Column inertness
- Pyridine test at high ph

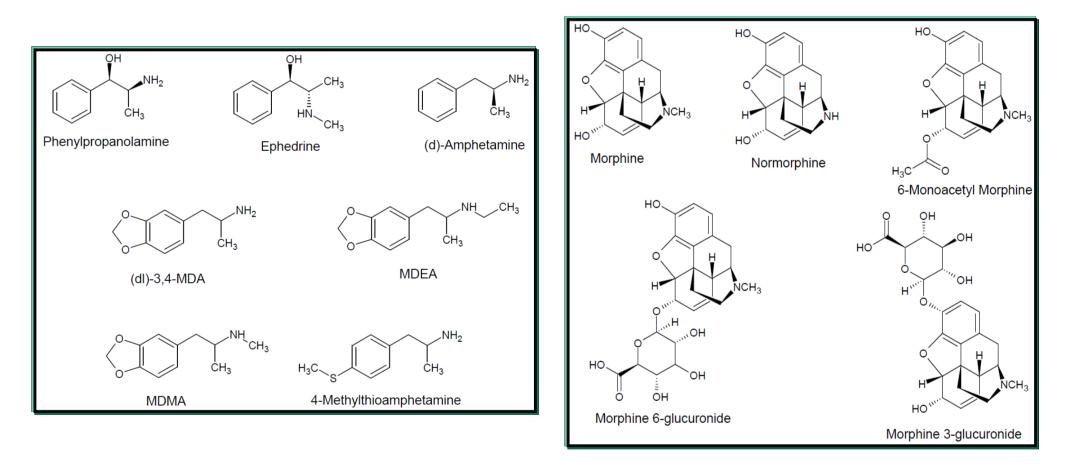
7. ANALYSIS FOR CONTROLLED SUBSTANCES

- Controlled drugs (legal & illegal) are routinely profiled in various biological matrices using LC-MS/MS.
- **Chromatography** coupled with **MS^n** 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[®] **SuperC18™** with high pH eluents for various opiate and amphetamine mixtures extracted from urine.



8. STRUCTURES OF ANALYTES IN THE MIXTURES

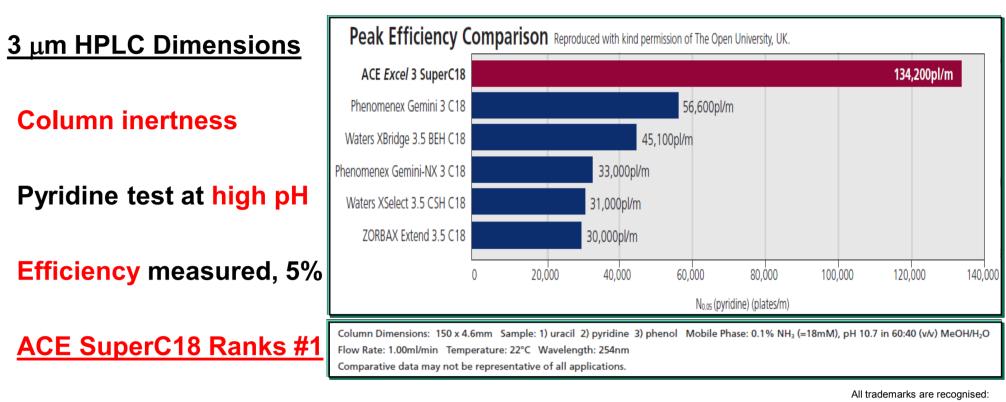
Amphetamine & Related



12. SUMMARY AND CONCLUSIONS

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Opiates & Related

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

The ACE[®] SuperC18[™] 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[®] SuperC18[™] provides chromatographers and method developers with an NEW selectivity option with an extended pH range and LC-MS analyses.

