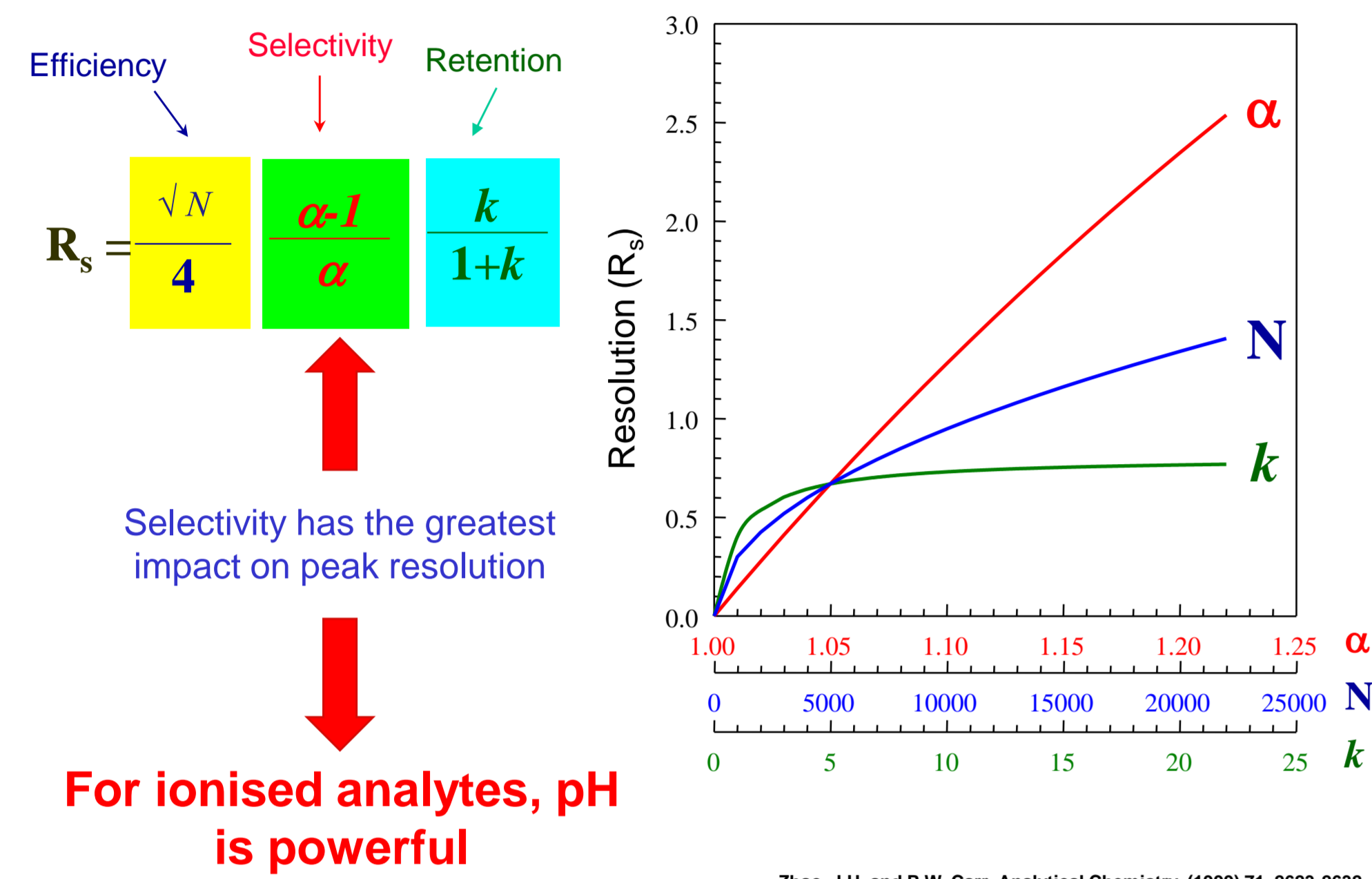


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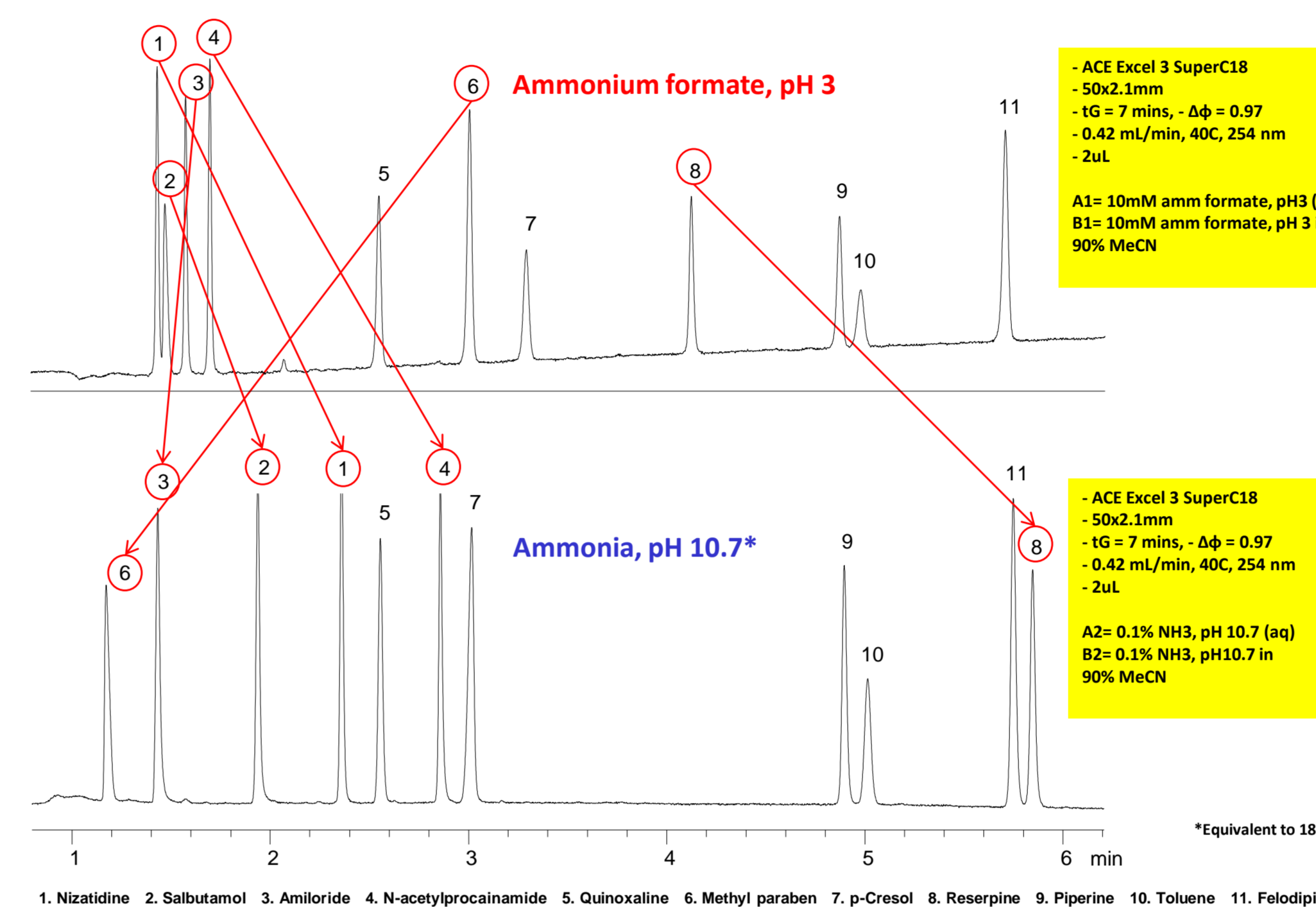
1. RESOLUTION: SELECTIVITY, EFFICIENCY & RETENTION



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.

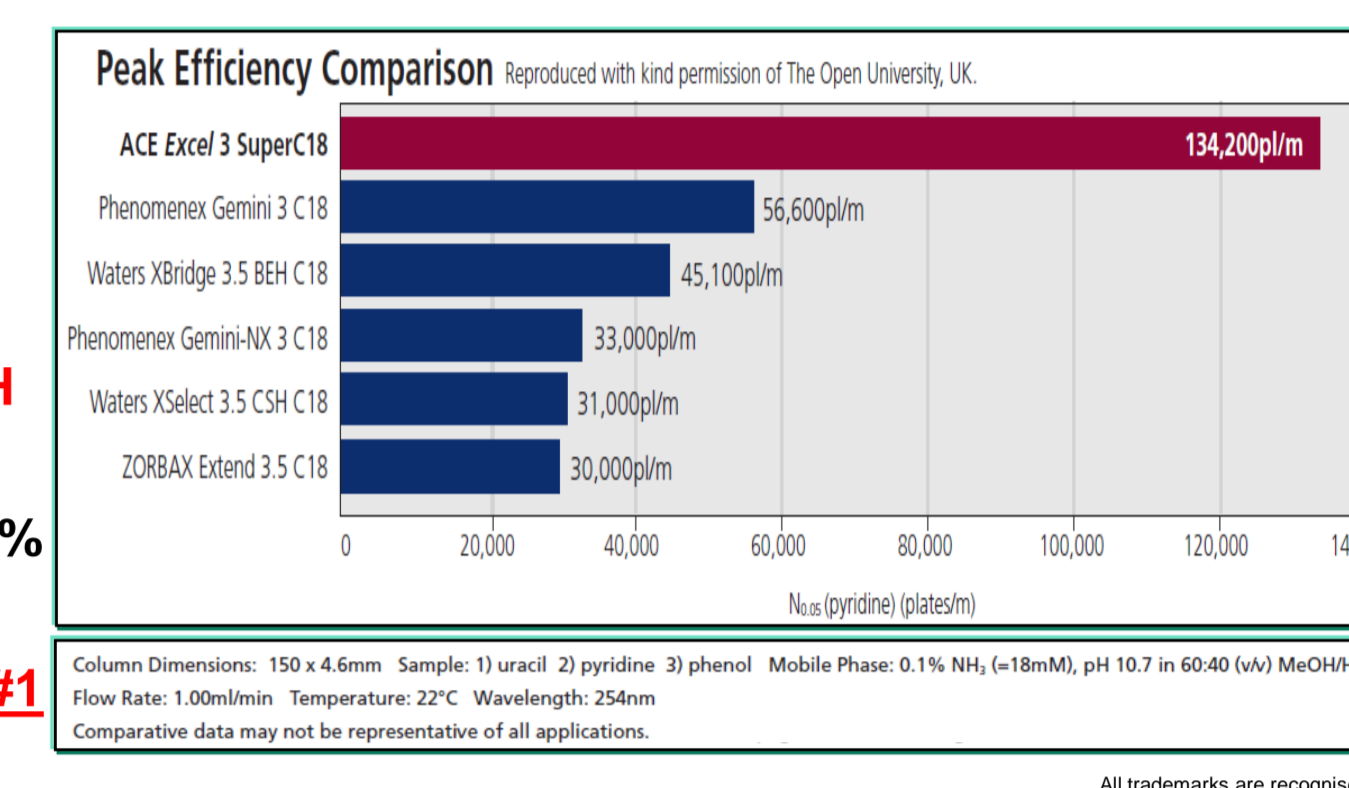
3. METHOD DEVELOPMENT: LOW / HIGH PH ELUENTS



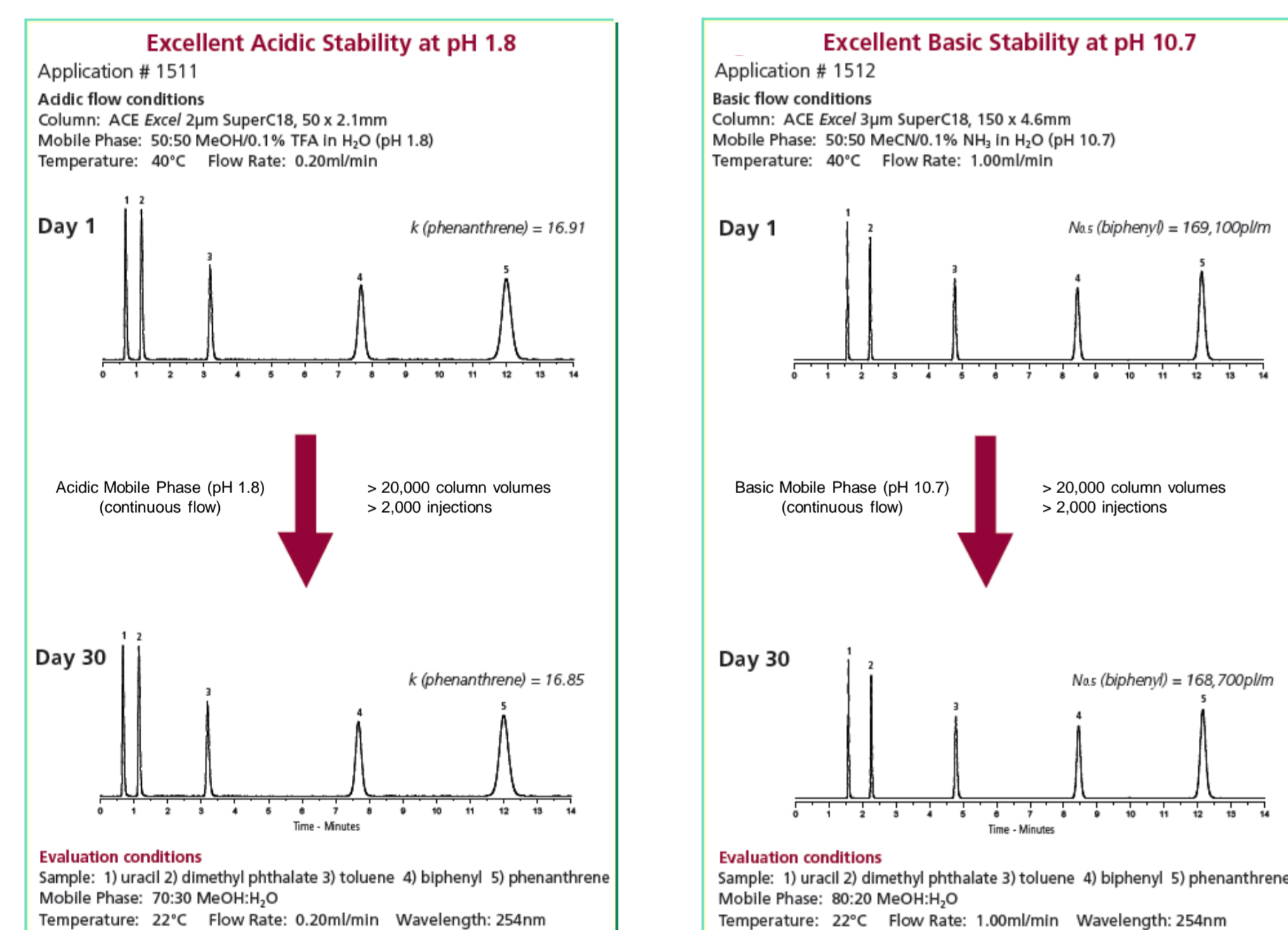
4. PHASE INERTNESS & PERFORMANCE: HPLC AT HIGH PH

3 μm HPLC Dimensions

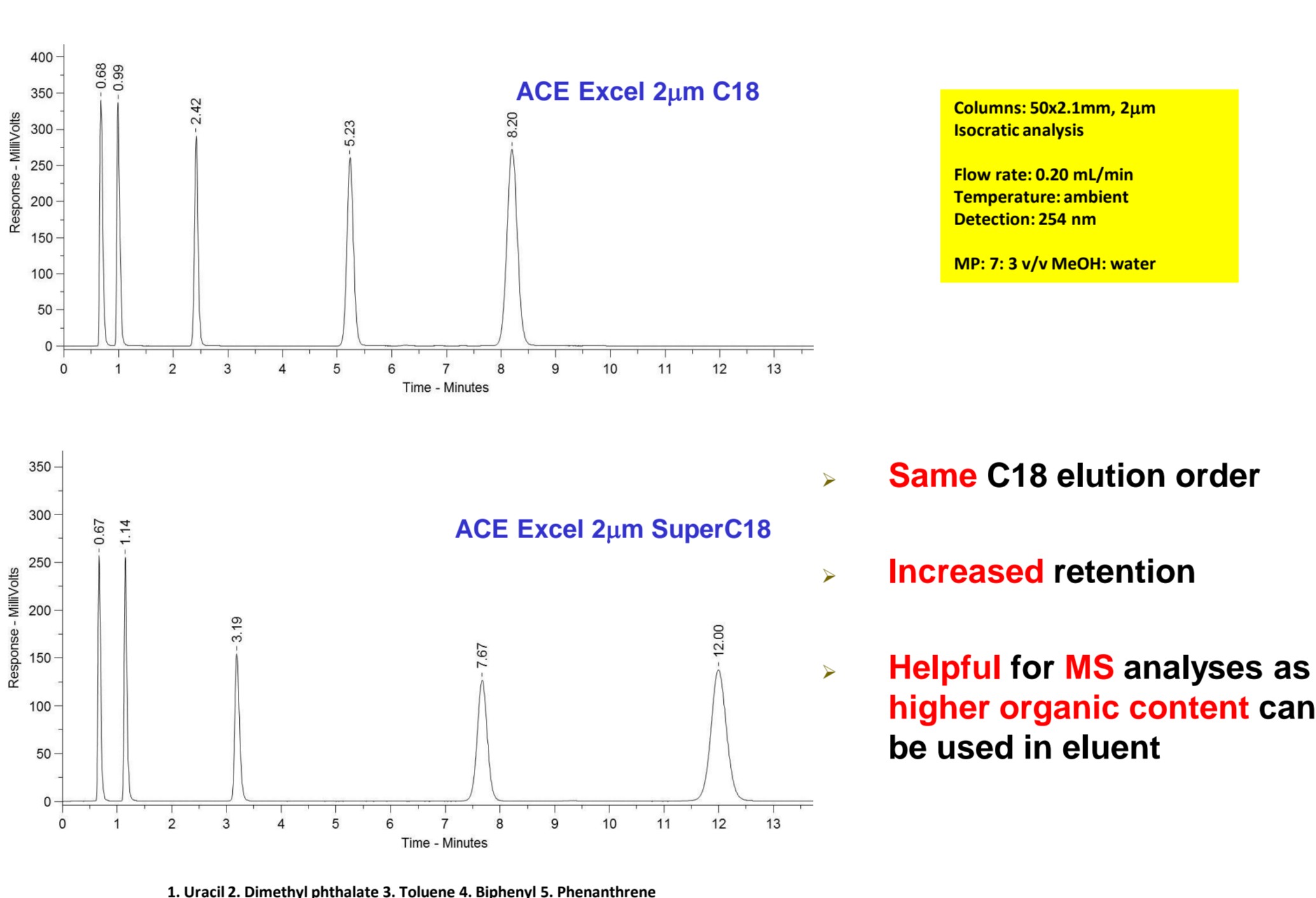
- Column inertness
- Pyridine test at high pH
- Efficiency measured, 5%
- ACE SuperC18 Ranks #1



5. PHASE STABILITY WITH LOW AND HIGH PH ELUENTS



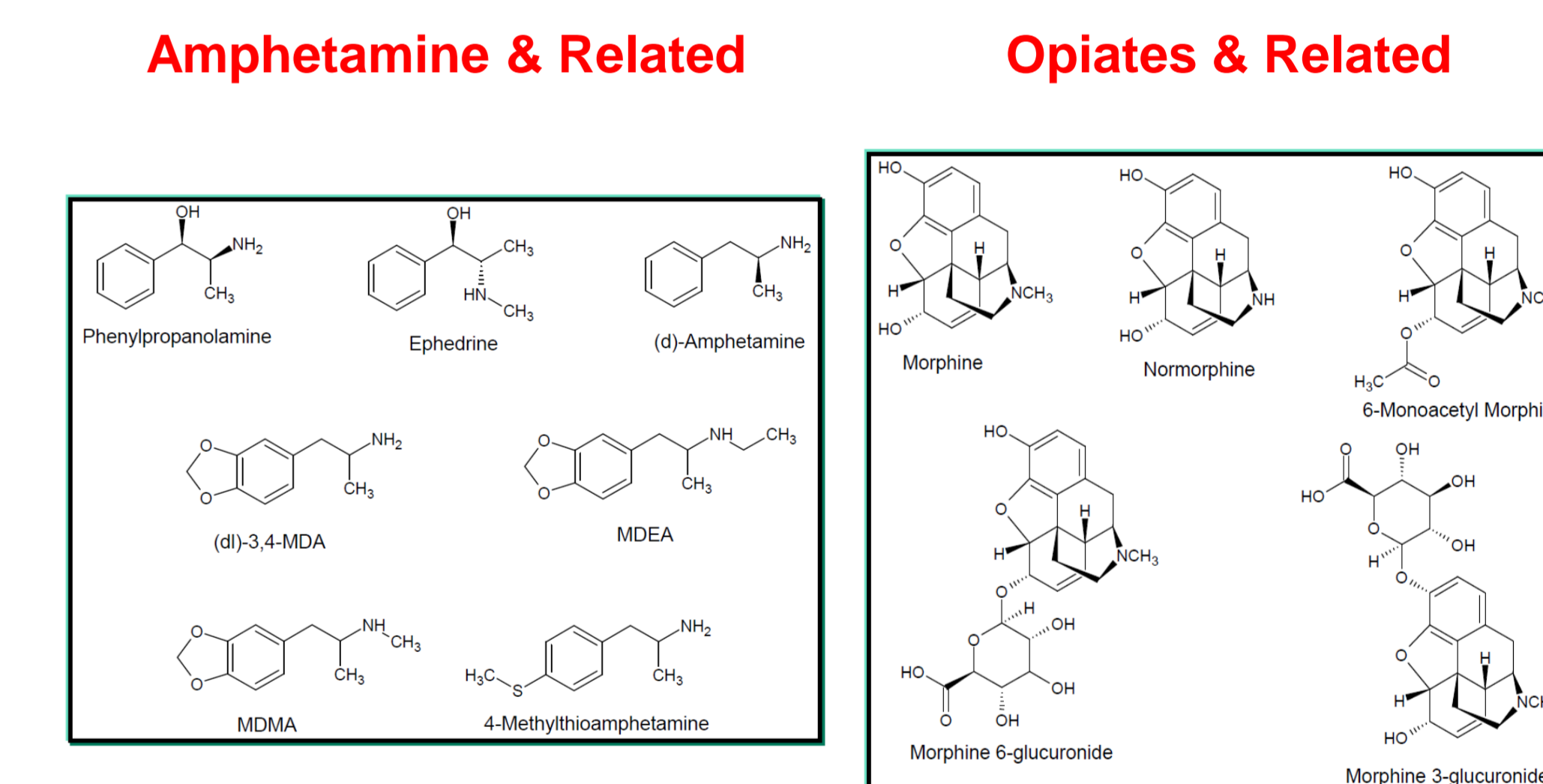
6. ACE® Excel SuperC18™ SHOWS INCREASED RETENTIVITY



7. ANALYSIS FOR CONTROLLED SUBSTANCES

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



9. URINE SAMPLE EXTRACTION & TEST CONDITIONS

Amphetamines Extraction Conditions

- 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.
- 2.5mL of n-butyl chloride added.
- Rotated for 15min, then centrifuged for 5mins at 2000rpm.
- 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.
B: 5mM Ammonium Hydroxide, pH 10.8 in 1:9 v/v H₂O:MeOH.
Gradient: 0-8-8.01-10minutes 30-95-30-30.
60C, 2uL.

Opiates LC-MS Conditions

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

A: 5mM Ammonium Hydroxide, pH 10.8.
B: 5mM Ammonium Hydroxide, pH 10.8 in 1:9 v/v H₂O:MeOH.
Gradient: 0-5-5.01-7minutes 5-95-5-5.
60C, 2uL.

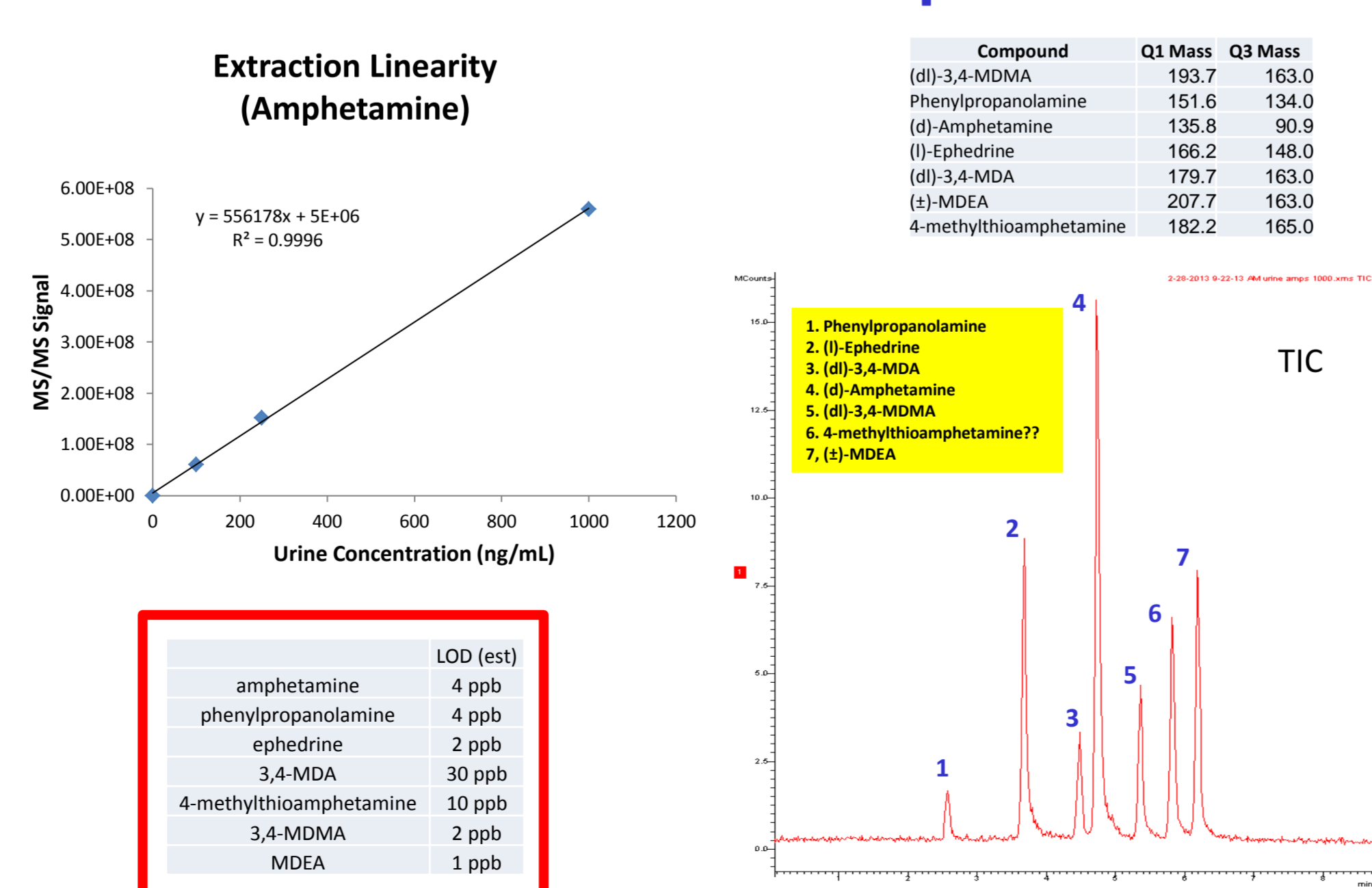
Varian 320 Triple Quadrupole Mass Spectrometer
Electrospray voltage: +5 kV
Inlet capillary voltage: 30 V
CID with argon at 1.5 mTorr; Collision cell potential ranges from 5 to 17 V
Drying gas (nitrogen) temperature: 325 C
Nebulizing gas (nitrogen) pressure: 35 psi
Detector set to use Extended Dynamic Range function

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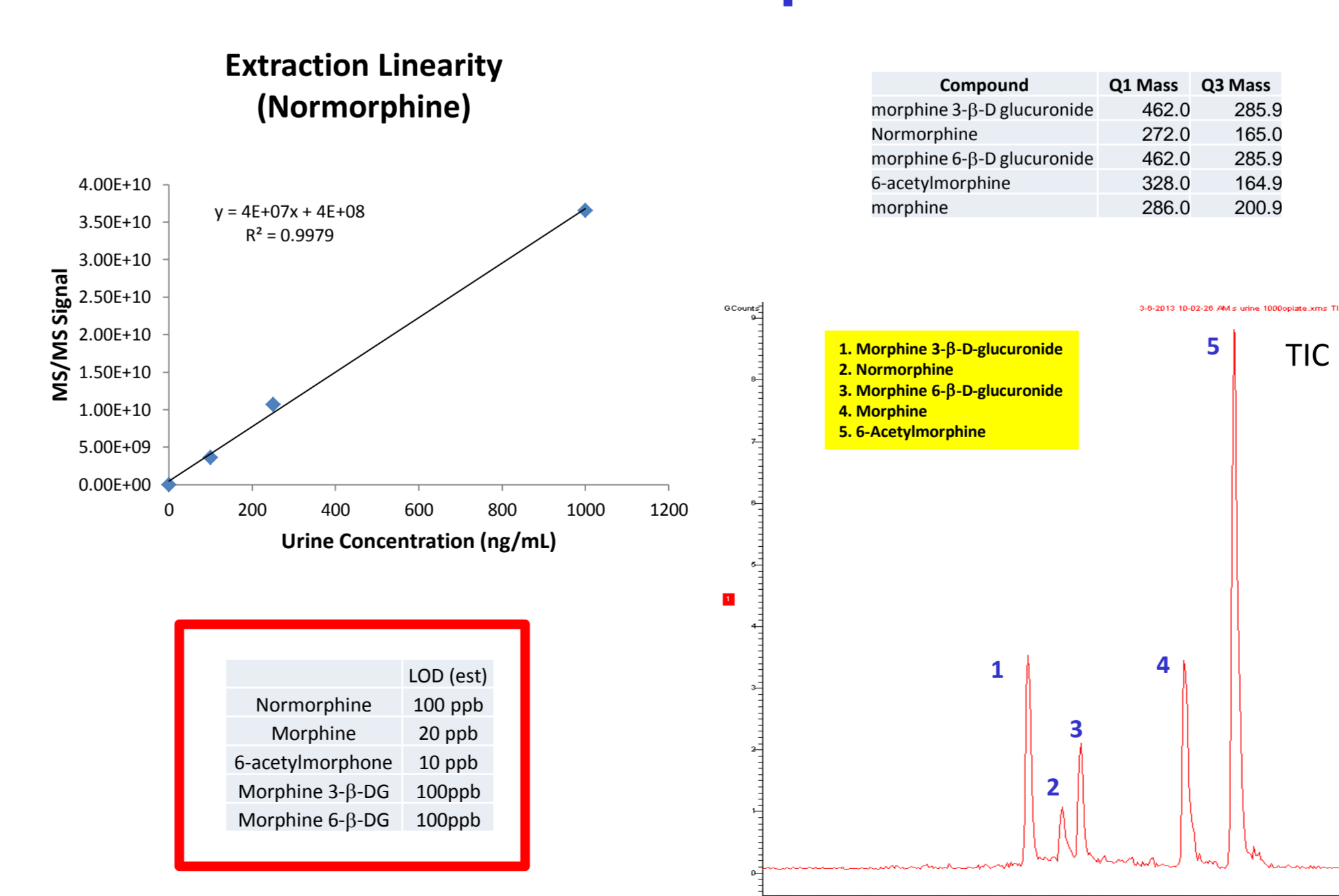
Opiates Extraction Conditions

- 1mL urine spiked with 0, 100, 250 and 1000ng/mL morphine-3-β-D-glucuronide, normorphine, morphine 6-β-D-glucuronide, 6-acetylmorphine, morphine.
- Centrifuged for 5 mins at 14,000rpm.
- 1mL vialled up.

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



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



12. SUMMARY AND CONCLUSIONS

- 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 a NEW selectivity option with an extended pH range and LC-MS analyses.

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