

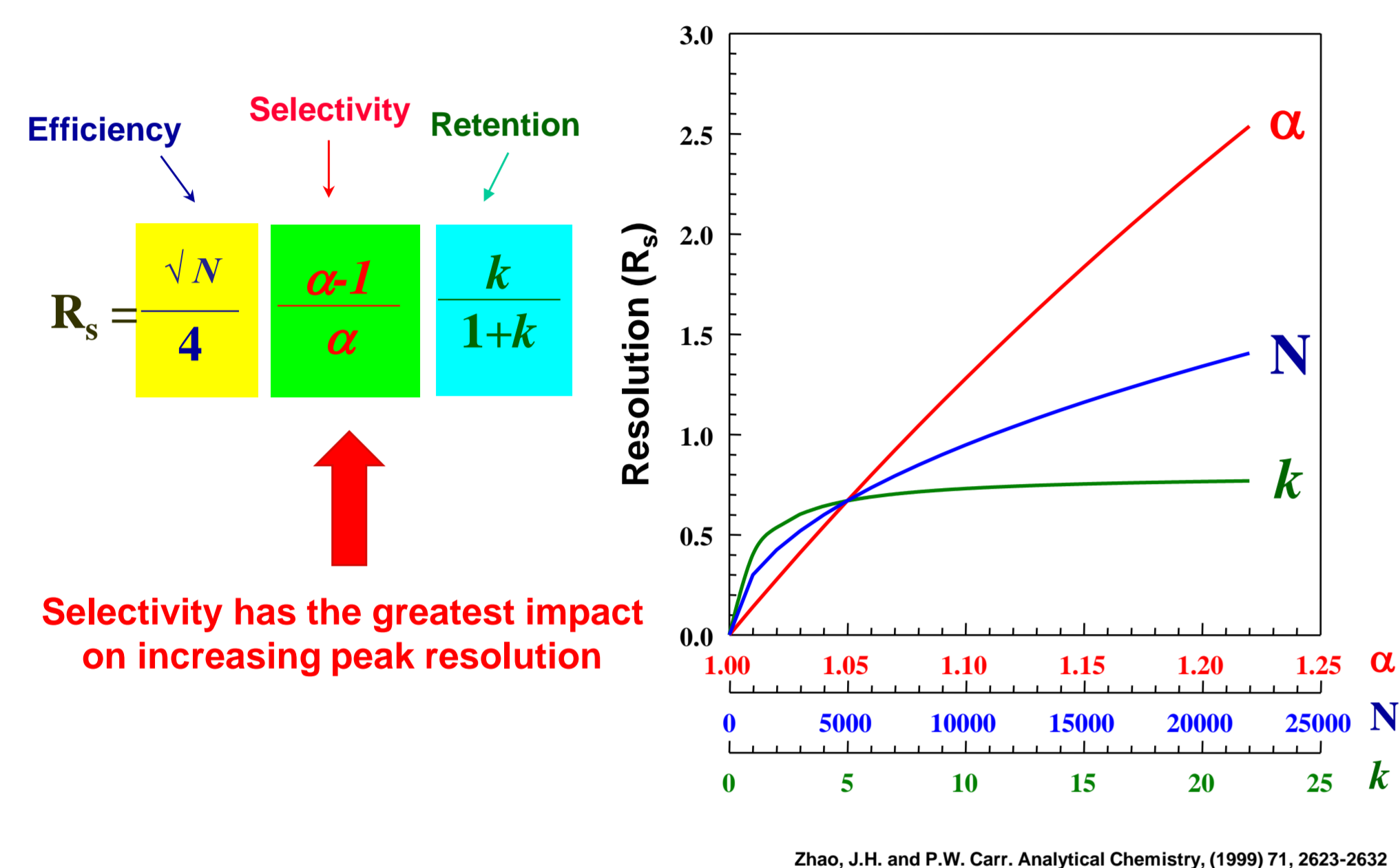
Alan P McKeown<sup>1</sup>, Carl Zimmerman<sup>2</sup>

<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 587, Chadds Ford, PA 19317 USA

## THE CHALLENGE

Engineer new HPLC / UHPLC phases based upon the robust C18 ligand with alternative selectivities that are reproducible, robust, exhibit low bleed and give efficient peaks

### 1. CHROMATOGRAPHIC PEAK RESOLUTION



### 2. HPLC SURVEYS<sup>2</sup> ...LISTENING TO THE ANALYST

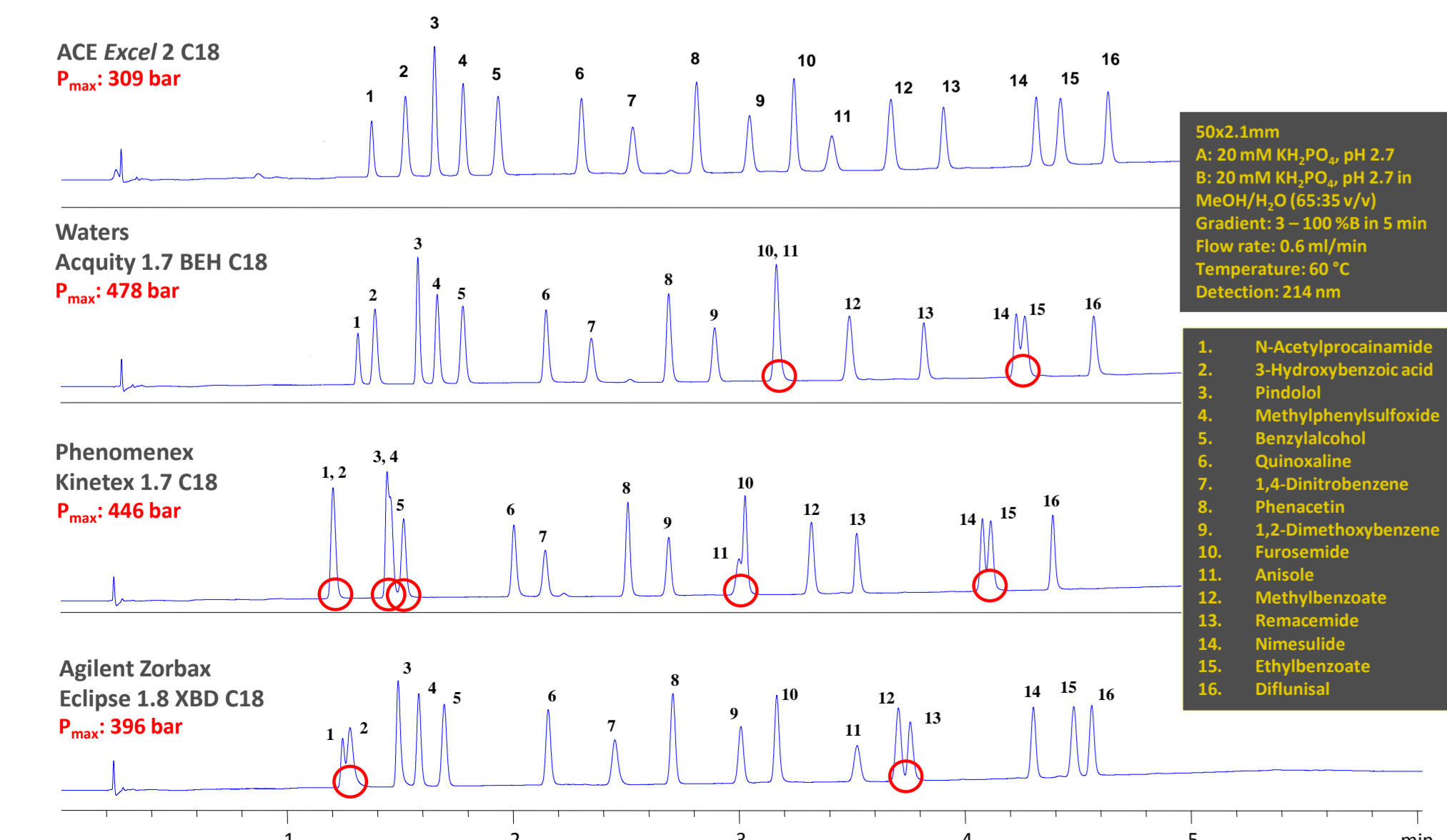
Column reproducibility and column lifetime are major factors for analysts

- Have been the top 2 feedback points since 2007
- Critical in pharmaceutical and other major industries for method transfers / consistency and long term performance

Reversed-phase is the dominant separation mode

- C18 & C8 = 60%; phenyl = 16%; CN = 9.5%; fluorinated = 5.9%
- 92% analysts use C18 at some time in their work...they typically meet the above criteria
- BUT limited selectivity

### 3. C18 PHASES PROVIDE SIMILAR SELECTIVITY



All trademarks are recognised...comparative separations may not be representative of all applications

### 4. THE BENEFITS OF AROMATIC FUNCTIONALITY

Phases with aromatic functionality include phenyl and pentafluorophenyl (PFP) based ligands

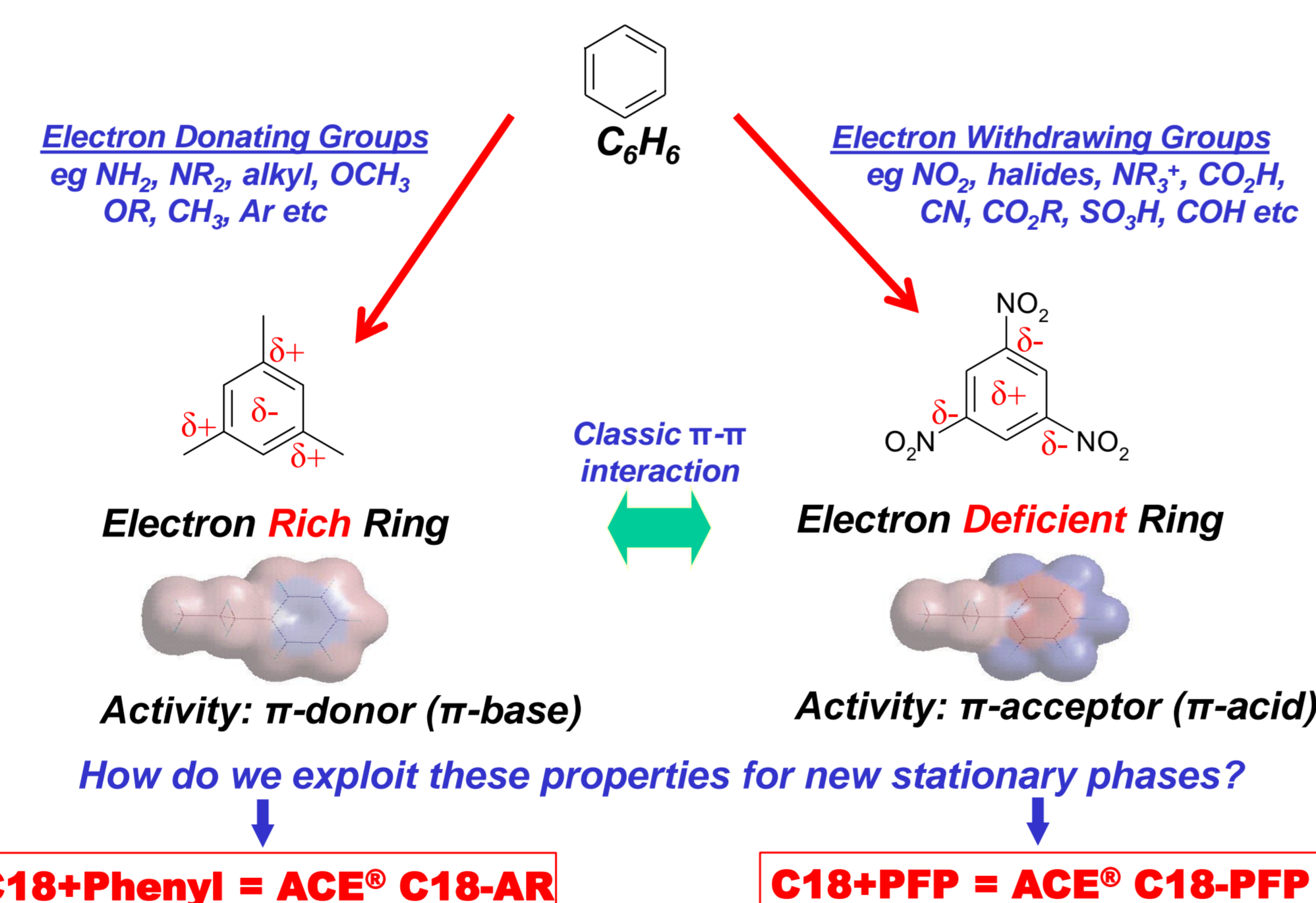
Advantages

- Aromatic functionality potentially offer unique interactions with analytes (c.f. C18) giving alternative selectivity
- Provides enhanced retention of polar compounds
- Many aromatic functionality-based phases can be used in 100% aqueous eluents

Disadvantages

- Phenyl / PFP phases may suffer phase bleed
- Batch-to-batch reproducibility & robustness traditionally weak

### 5. THE POWER OF $\pi$ ...SCIENTIFIC LED PHASE DESIGN



### 6. ACE<sup>®</sup> PHASES : MULTI-MODE SEPARATION MECHANISMS

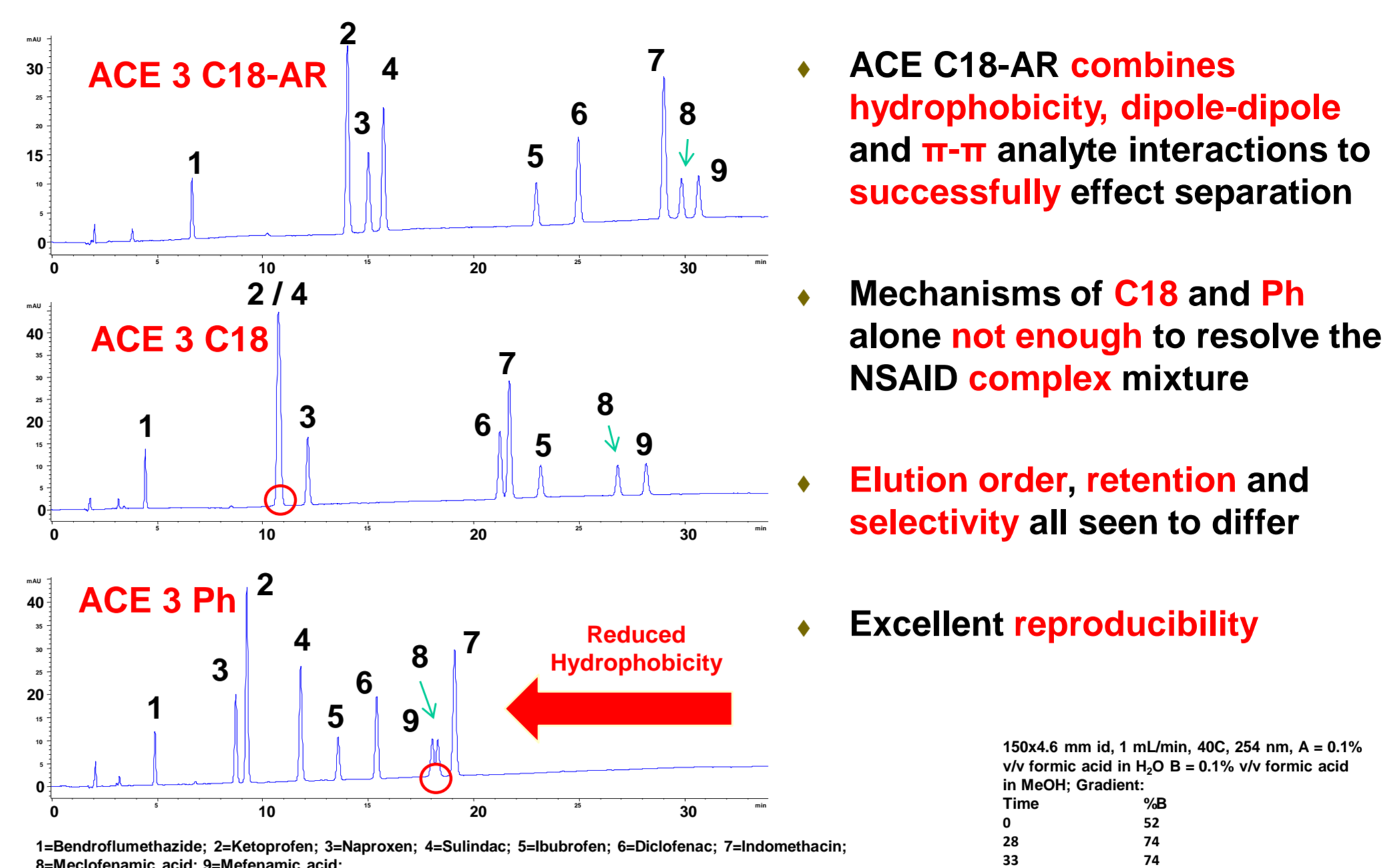
Combining the character of C18+phenyl and C18+PFP into single phases harnesses the best of each ligand type for unique selectivity

Separation mechanism	ACE <sup>®</sup> C18-AR			ACE <sup>®</sup> C18-PFP		
	Typical C18	Typical Phenyl	ACE <sup>®</sup> C18-AR	Typical C18	Typical PFP	ACE <sup>®</sup> C18-PFP
Hydrophobicity	++++	+ / ++	++++	Hydrophobicity	++++	+ / ++
$\pi$ - $\pi$ Interaction	-	+++	+++	$\pi$ - $\pi$ Interaction	-	+++
Dipole - Dipole	-	+	+	Dipole - Dipole	-	+++
Hydrogen Bonding	-	++	++	Hydrogen Bonding	-	+++
Shape Selectivity	++	++	++ / +++	Shape Selectivity	++	+++

The predominance of each retention mechanism will be dictated by the analyte's physicochemical properties, its structure and the chromatographic conditions applied

Reproducible Multi-Mode Interactions Offer the Chromatographer More

### 7. ACE<sup>®</sup> C18-AR SELECTIVITY : NSAIDS



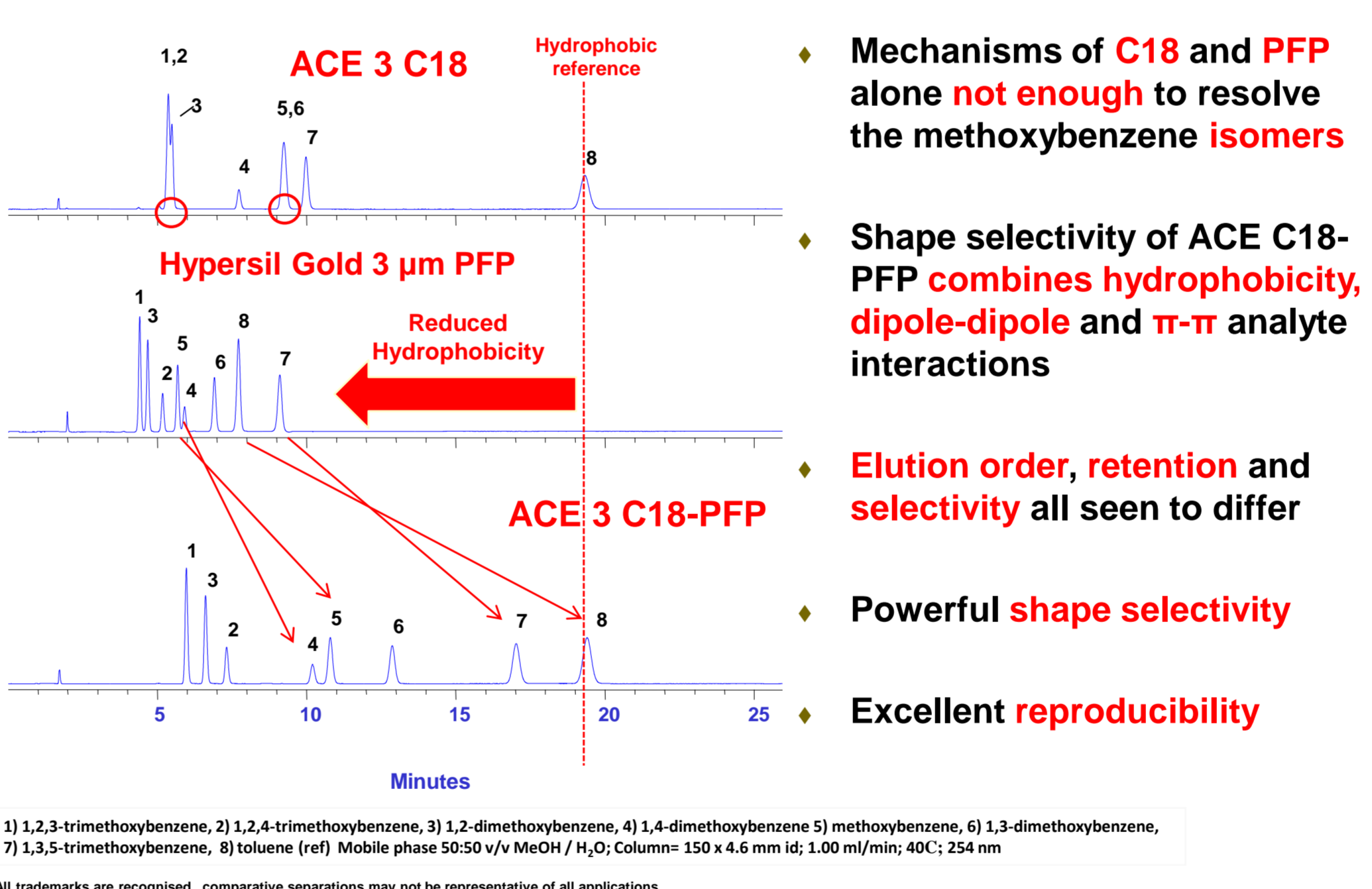
ACE C18-AR combines hydrophobicity, dipole-dipole and  $\pi$ - $\pi$  analyte interactions to successfully effect separation

Mechanisms of C18 and Ph alone not enough to resolve the NSAID complex mixture

Elution order, retention and selectivity all seen to differ

Excellent reproducibility

### 8. ACE<sup>®</sup> C18-PFP SELECTIVITY : STRUCTURAL ISOMERS



Mechanisms of C18 and PFP alone not enough to resolve the methoxybenzene isomers

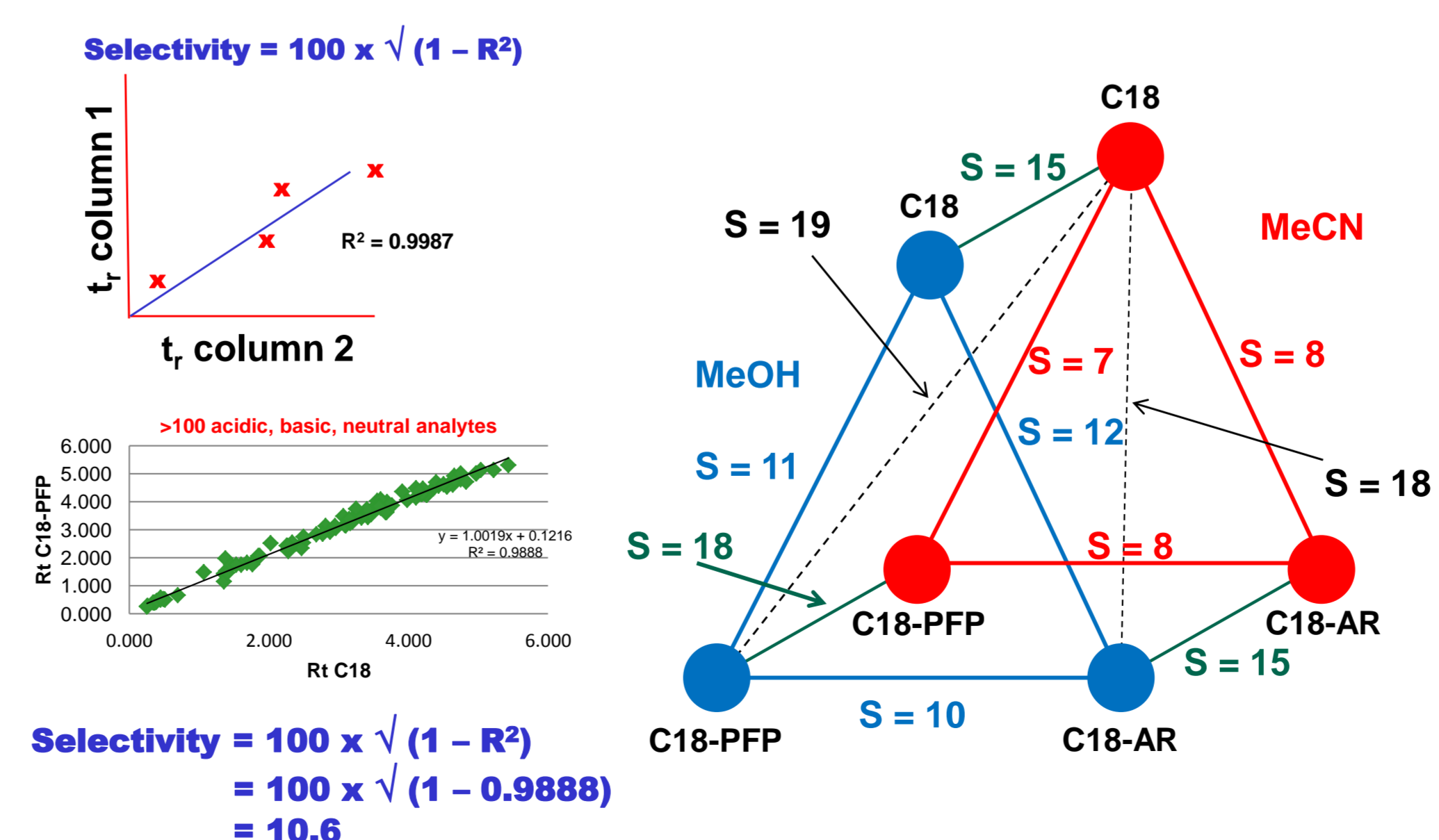
Shape selectivity of ACE C18-PFP combines hydrophobicity, dipole-dipole and  $\pi$ - $\pi$  analyte interactions

Elution order, retention and selectivity all seen to differ

Powerful shape selectivity

Excellent reproducibility

### 9. ACE<sup>®</sup> PHASE COMPARISONS WITH SELECTIVITY 'S' VALUES\*



\* Neue, O'Gara, Méndez "Selectivity in Reversed-Phase Separations: Influence of the Stationary Phase", J. Chromatogr. A 1127 (2006), 161-174

### 10. ACE<sup>®</sup> SELECTIVITY RANKING : MEOH & MeCN

For >100 acidic, basic and neutral analytes assessed

MeOH			MeCN		
Column 1	Column 2	Selectivity 'S'	Column 1	Column 2	Selectivity 'S'
C18	C18-AR	12	C18	C18-AR	8
C18	C18-PFP	11	C18-AR	C18-PFP	8
C18-AR	C18-PFP	10	C18	C18-PFP	7

MeOH	MeCN	Selectivity Value
C18-PFP	C18	19
C18-AR	C18	18
C18-AR	C18-PFP	18
C18-PFP	C18-AR	18
C18-PFP	C18-PFP	18
C18	C18-AR	17
C18	C18-PFP	17
C18	C18	15
C18-AR	C18-AR	15

Shows value of using the 3 phases in a 2 solvent screen for method development work

### 11. WHAT DO I USE THESE NOVEL PHASES FOR?

- ACE<sup>®</sup> C18-AR
  - Useful for analytes that contain electron withdrawing moieties eg  $-\text{NO}_2$ , -halides,  $-\text{NR}_3^+$ ,  $-\text{SO}_2$ ,  $-\text{CO}_2\text{H}$ ,  $-\text{SO}_3\text{H}$ ,  $-\text{CO}_2\text{R}$ ,  $-\text{CHO}$  etc
  - Moderate shape selectivity
- ACE<sup>®</sup> C18-PFP
  - Useful for analytes that contain electron donating moieties eg  $-\text{NH}_2$ ,  $-\text{NR}_2$ ,  $-\text{OCH}_3$ ,  $-\text{OH}$ , -alkyl, -Ar etc
  - Excellent shape selectivity...good for regioisomers

### 12. CONCLUSIONS

- The unique ACE<sup>®</sup> C18-AR and ACE<sup>®</sup> C18-PFP phases have been engineered based upon aromatic functionality
- These phases give complementary, yet alternative selectivity and offer the chromatographer new choices for method development
- The phases offer high peak efficiency, reproducibility, robustness and with low UV & MS bleed
- The phases are available for HPLC (ACE<sup>®</sup> 3, 5, 10  $\mu\text{m}$ ) and for UHPLC (ACE<sup>®</sup> Excel<sup>™</sup> 2  $\mu\text{m}$ )

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