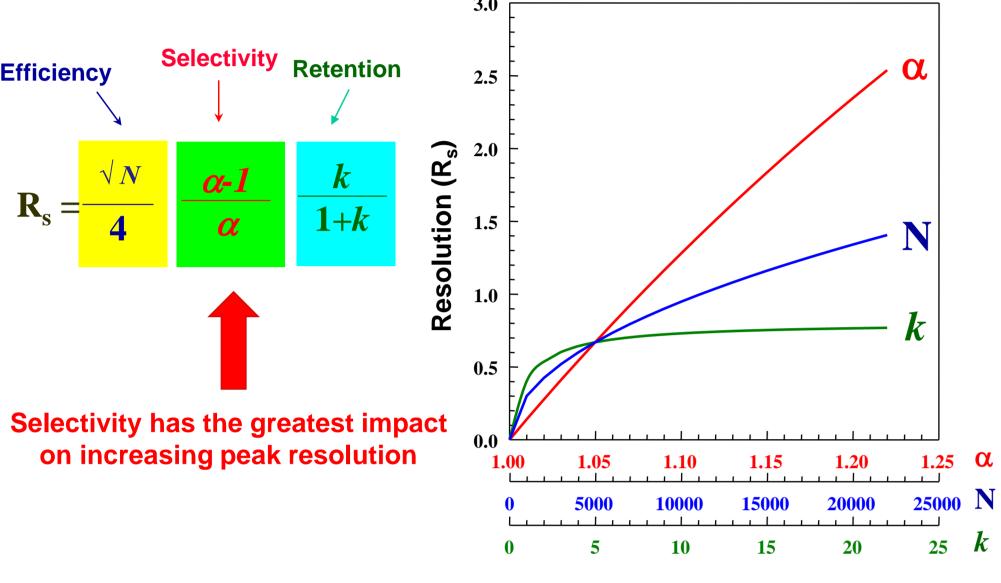


Exploring and Leveraging Mixed Mode Interactions To Maximise Chromatographic Selectivity With Uniquely **Designed HPLC / UHPLC Stationary Phases** <u>Alan P McKeown¹, Carl Zimmerman²</u> ¹Advanced Chromatography Technologies Ltd, 1 Berry Street, Aberdeen, Scotland, AB25 1HF UK ²MACMOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA **THE CHALLENGE** Engineer new HPLC / UHPLC phases based upon the robust C18 ligand with alternative selectivities that are reproducibile, robust, exhibit low bleed and give efficient peaks **10. ACE® SELECTIVITY RANKING : MEOH & MECN** 7. ACE[®] C18-AR SELECTIVITY : NSAIDS 4. THE BENEFITS OF AROMATIC FUNCTIONALITY Phases with aromatic functionality include phenyl and ACE 3 C18-AR ACE C18-AR combines For >100 acidic, basic and neutral analytes assessed pentafluorophenyl (PFP) based ligands and π - π analyte interactions to successfully effect separation C18 Advantages (R_s) C18 Mechanisms of C18 and Ph 2/4 C18-AR C1 - Aromatic functionality potentially offer unique interactions with ACE 3 C18 alone not enough to resolve the **NSAID** complex mixture analytes (c.f. C18) giving alternative selectivity - Provides enhanced retention of polar compounds Elution order, retention and Many aromatic functionality-based phases can be used in selectivity all seen to differ **100% aqueous eluents** ⁴⁰ ACE 3 Ph ² **Excellent** reproducibility 1.15 1.20 Disadvantages 150x4.6 mm id, 1 mL/min, 40C, 254 nm, A = 0.1% v/v formic acid in $H_2O B = 0.1\% v/v$ formic acid - Phenyl / PFP phases may suffer phase bleed in MeOH; Gradient: 25 Batch-to-batch reproducibility & robustness traditionally weak =Bendroflumethazide; 2=Ketoprofen; 3=Naproxen; 4=Sulindac; 5=Ibubrofen; 6=Diclofenac; 7=Indomethacin =Meclofenamic acid: 9=Mefenamic acid: Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632 **11. WHAT DO I USE THESE NOVEL PHASES FOR?** 5. THE POWER OF TT...SCIENTIFIC LED PHASE DESIGN 8. ACE® C18-PFP SELECTIVITY : STRUCTURAL ISOMERS Mechanisms of C18 and PFP 1,2 **ACE 3 C18** alone not enough to resolve Electron Withdrawing Groups **Electron Donating Groups** C_6H_6 the methoxybenzene isomers ♦ ACE[®] C18-AR eg NH₂, NR₂, alkyl, OCH₃ eg NO₂, halides, NR₃⁺, CO₂H, factors for analysts OR, CH₃, Ar etc CN, CO₂R, SO₃H, COH etc Useful for analytes that contain electron withdrawing moieties – Have been the top 2 feedback points since 2007 Shape selectivity of ACE C18eg -NO₂, -halides, -NR₃⁺, -SO₂, -CO₂H, -SO₃H, -CO₂R, -CHO etc Hypersil Gold 3 µm PFP PFP combines hydrophobicity, - Critical in pharmaceutical and other major industries for method Moderate shape selectivity dipole-dipole and π - π analyte Reduced transfers / consistency and long term performance interactions Classic π-π $O_2 N$ ____ ♦ ACE[®] C18-PFP interaction Elution order, retention and **Electron Deficient Ring** Electron Rich Ring Useful for analytes that contain electron donating moieites selectivity all seen to differ ACE 3 C18-PFP - C18 & C8 = 60%; phenyl = 16%; CN = 9.5%; fluorinated = 5.9% eg -NH₂, -NR₂, -OCH3, -OH, -alkyl, -Ar etc 3 Excellent shape selectivity...good for regioisomers Powerful shape selectivity - 92% analysts use C18 at some time in their work...they typically meet 2 the above criteria Activity: π -acceptor (π -acid) Activity: π -donor (π -base) **Excellent reproducibility** How do we exploit these properties for new stationary phases? BUT limited selectivity 1) 1,2,3-trimethoxybenzene, 2) 1,2,4-trimethoxybenzene, 3) 1,2-dimethoxybenzene, 4) 1,4-dimethoxybenzene 5) methoxybenzene, 6) 1,3-dimethoxybenzene) 1.3.5-trimethoxybenzene, 8) toluene (ref) Mobile phase 50:50 v/v MeOH / H₂O; Column= 150 x 4.6 mm id; 1.00 ml/min; 40C; 254 nm C18+PFP = ACE[®] C18-PFP C18+Phenyl = ACE[®] C18-AR trademarks are recognised...comparative separations may not be representative of all applications a Current Trends in HPLC Column Usage, R.E. Majors, 1Jan12, Modern Medicine website, accessed on 15Feb12: http://www.modernmedicine.com/modernmedicine/article/art

1. CHROMATOGRAPHIC PEAK RESOLUTION

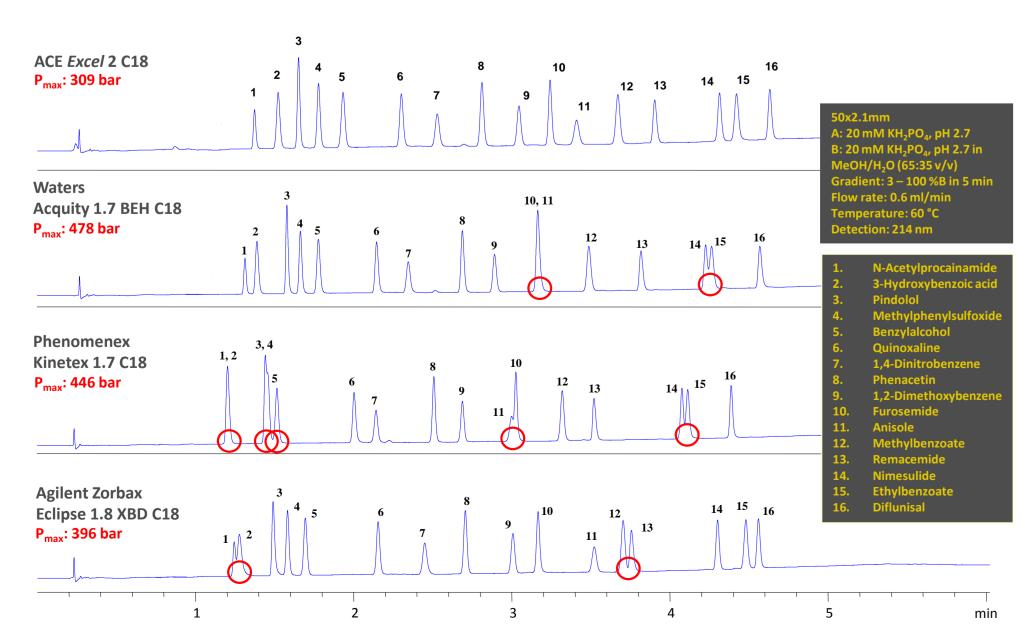


2. HPLC SURVEYS^a ... LISTENING TO THE ANALYST

Column reproducibility and column lifetime are major

Reversed-phase is the dominant separation mode

3. C18 PHASES PROVIDE SIMILAR SELECTIVITY



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

- 6. ACE® 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

ACE [®] C18-AR				ACE [®] C18-PFP			
Separation mechanism	Typical C18	Typical Phenyl	ACE [®] C18-AR	Separation mechanism	Typical C18	Typical PFP	ACE [®] C18-PFP
Hydrophobicity	++++	+ / ++	++++	Hydrophobicity	++++	+ / ++	++++
π - π Interaction	-	+++	+++	π - π 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

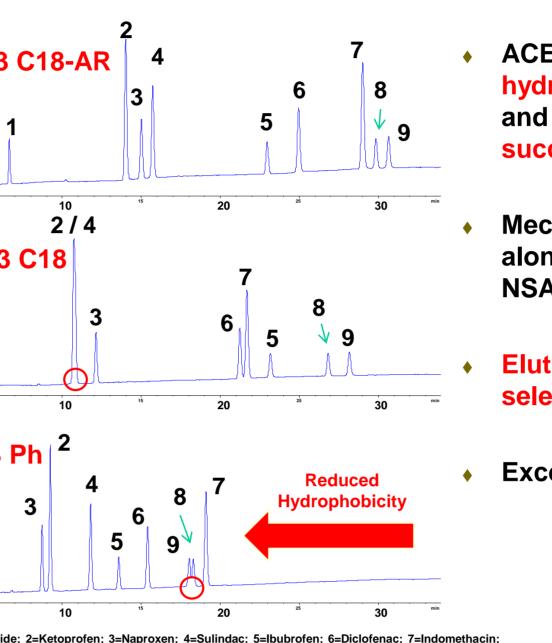
Reproducible Multi-Mode Interactions Offer the Chromatographer More

6.000

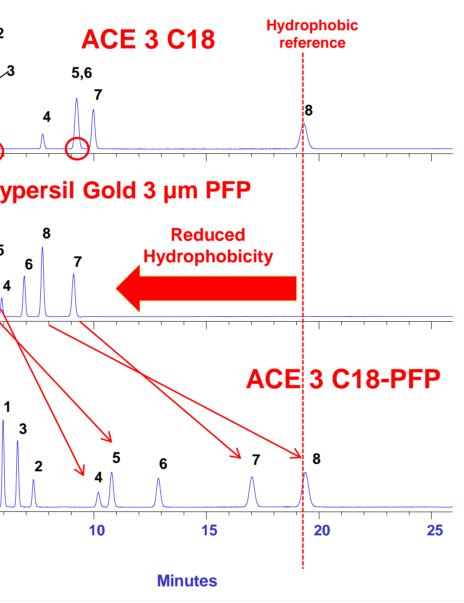
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5 3.000 2.000 1.000



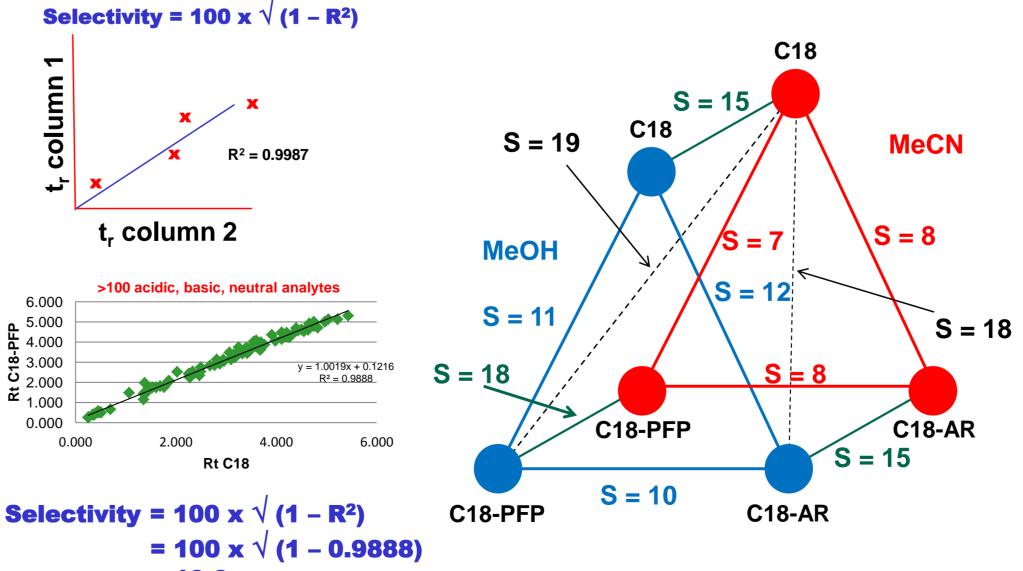




12. CONCLUSIONS

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= 10.6



9. ACE® PHASE COMPARISONS WITH SELECTIVITY 'S' VALUES



MeOH		MeCN			
olumn 2	Selectivity 'S'		Column 1	Column 2	Selectivity 'S'
218-AR	12		C18	C18-AR	8
18-PFP	11		C18-AR	C18-PFP	8
18-PFP	10		C18	C18-PFP	7
МеОН		MeCN	Selectivity Value		
C18-PFP		C18		19	

C18-AR	C18	18	
C18-AR	C18-PFP	18	
C18-PFP	C18-AR	18	show
C18-PFP	C18-PFP	18	using the 3 value of in a 2 solvent devel
C18	C18-AR	17	in a 2 solvent screen for method development work
C18	C18-PFP	17	spment work
C18	C18	15	
C18-AR	C18-AR	15	

The unique ACE[®] C18-AR and ACE[®] 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[®] 3, 5, 10μm) and for UHPLC (ACE[®] Excel ™ 2μm)

www.ace-hplc.com