



**Advances in sample handling,  
preparation and separation**

**The world leader in serving science**

# Outline

- Simplify sample handling: Thermo Scientific™ Virtuoso™ Vial Identification System
- SPE sample preparation using Thermo Scientific™ SOLA Cartridges and Plates and Thermo Scientific™ SOLA $\mu$  plates
- Thermo Scientific™ Accucore™ and Thermo Scientific™ Accucore™ Vanquish™ UHPLC columns
- Summary

**Simplify sample handling**  
**Virtuoso Vial Identification System**



# Why vial identification?



## Current Method

- ▶ **Unreliable labeling process**
  - Adhesive labels aren't permanent or tolerant of common solvents
  - Hand marking isn't permanent
- ▶ **Temporary, hand written labels**
  - Smudges, smears and illegibility with hand written labels
- ▶ **Limited space for information on the vial**
  - Especially the case with hand written labels
- ▶ **Time spent creating temporary labels**
  - Hand labeling with log book
  - Time creating and affixing adhesive labels

## Thermo Scientific Virtuoso Vial Identification System

- ▶ Removes manual labeling, nearly eliminating labeling errors
- ▶ Improves data accuracy by providing reliable identification
- ▶ User-defined, customizable template provides more detail
- ▶ System frees resources for revenue generating activities

# No smudge, no smear, no question

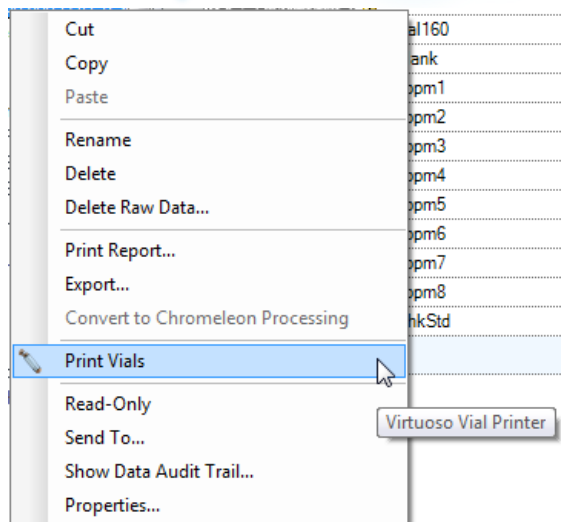
## Virtuoso Vial Identification System

- Create customizable vial identification directly on vial
- Increase vial labelling through-put up to 8 times
- Improves confidence in data by removing labelling errors

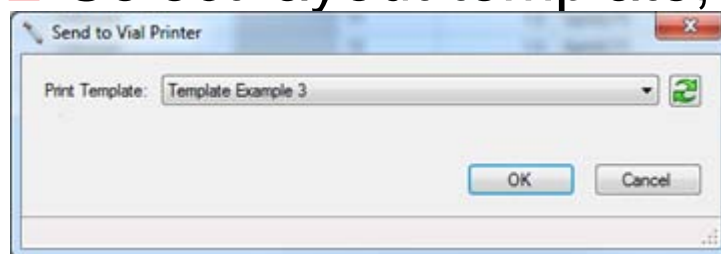


# Integrated into Chromeleon 7.2 for Vanquish UHPLC system

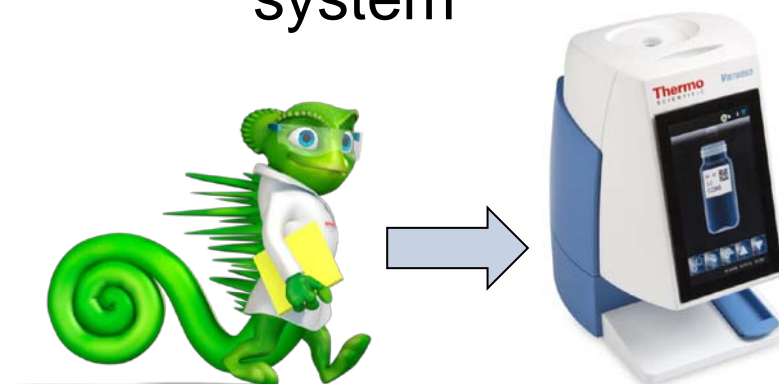
1. Click "print vials" in context menu



2. Select layout template, click OK



3. Walk to a Virtuoso system



# Allowing 5 easy steps

4. Select sequence from list, hit OK



5. Drop in empty vials



Ready!



**SPE sample preparation using  
SOLA and SOLA $\mu$  technology**





# Solid Phase Extraction

- Column chromatography technique
- Solid media (sorbent)
- Liquid phase including analyte(s) and matrix
- Preferential interaction analyte(s)
- Based on difference in physicochemical properties of;
  - Analyte(s)
  - Matrix



# SPE choice

- Base material: polymer, silica, carbon
- Format: 96 well plates, cartridges, QuEChERS, on-line, MEPS
- Selectivity options
  - Reversed phase    Hydrophobic phases
  - Normal phase    Hydrophilic phases
  - Ion exchange    Electrostatic attraction (+  $\leftrightarrow$  -)
  - Mixed mode    Hydrophobic & ionic retention
  - Hypercarb
- Products: SOLA, SOLA $\mu$ , Retain, Servo +/-Servo, Verify, QuEChERS



# Classic Layout of SPE cartridge



HDPE frits

+



Sorbent material

+



Polypropylene cartridge/plate

Frits keep the sorbent material in place in the cartridge they perform no other function

# Which could lead to issues such as

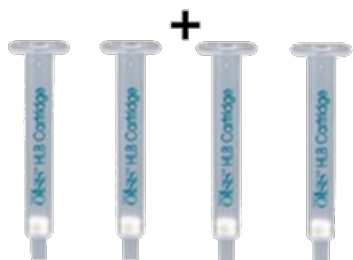


HDPE frits

+

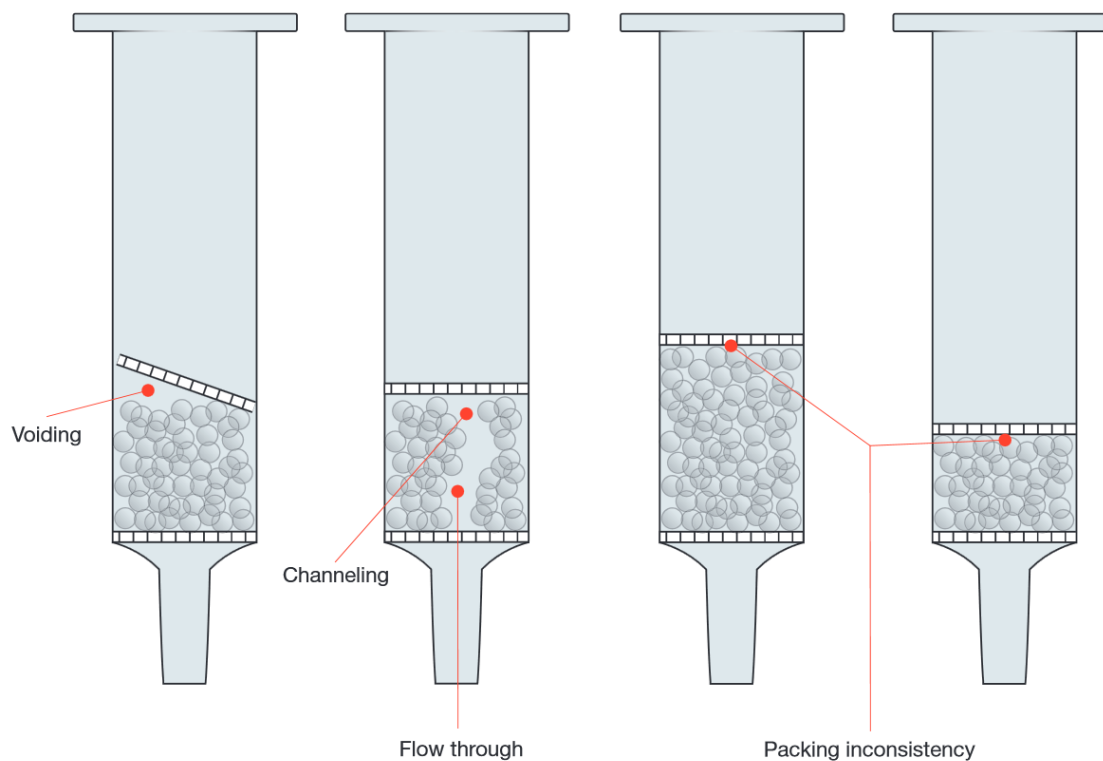


Sorbent material



Polypropylene cartridge/plate

Frits keep the sorbent material in place in the cartridge they perform no other function



***Less consistency and less reproducible results***

# SOLA SPE – what is the difference?



HDPE powder

+



Polymeric material

+



Polypropylene cartridge/plate

**SOLA is a solid single unit with integrated frit material**

# SOLA SPE – offering consistency and reproducibility



HDPE powder

+



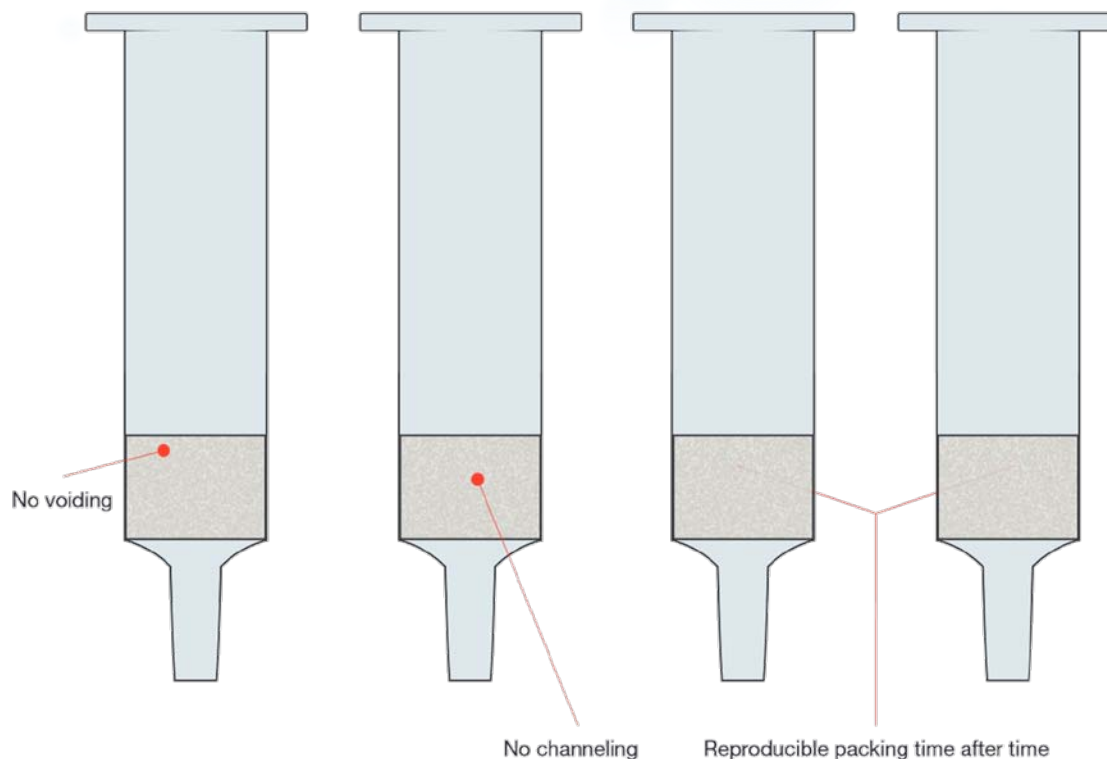
Polymeric material

+



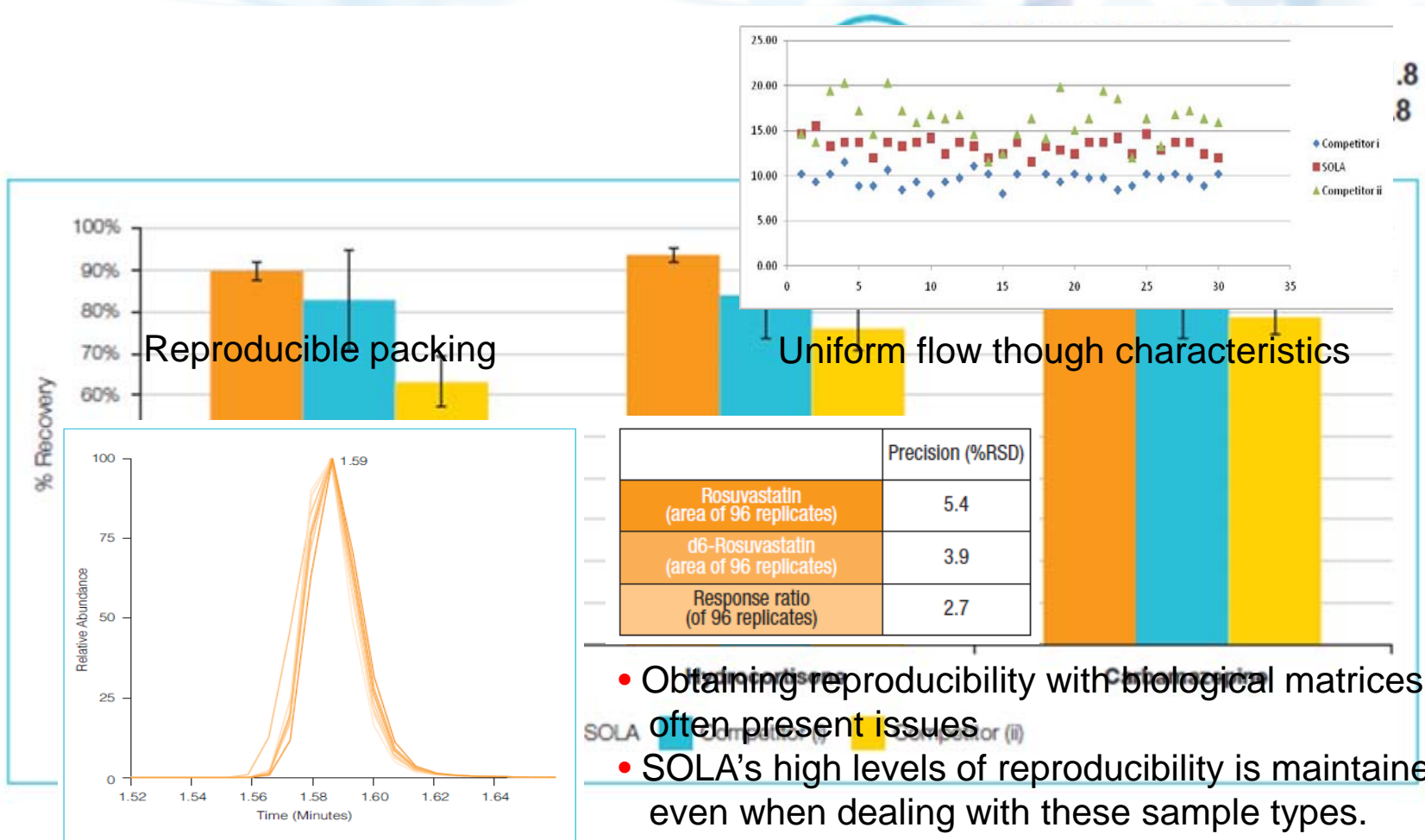
Polypropylene cartridge/plate

**SOLA is a solid single unit with integrated frit material**



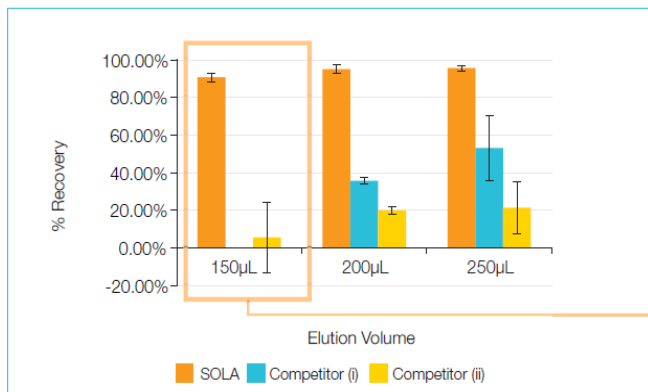
***More consistency and more reproducible results***

# SOLA – reproducibility



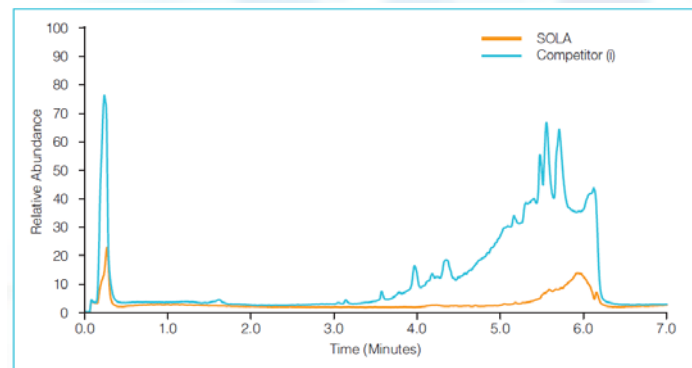
- Obtaining reproducibility with biological matrices often present issues
- SOLA's high levels of reproducibility is maintained even when dealing with these sample types.

# Less solvent / higher sensitivity



Significantly higher recovery levels are achieved with SOLA products at an elution volume of 150µL for caffeine, hydrocortisone and carbamazepine compared to competitor loose-packed SPE products.

Hydrocortisone



Dichloromethane extract comparison: SOLA products versus competitor (i)

Integrating the frit material reduces hold up volume allowing reproducible elution in lower volumes than conventional loose-packed SPE

SOLA's unique production removes impurities from frit material and sorbent



# SOLA $\mu$ for Micro Elution SPE

- Bioanalytical processes are being driven towards:
  - Higher efficacy/potency drugs - increased sensitivity requirements
  - Lower sample/elution volumes <100 $\mu$ L of sample
  - High levels of reproducibility with low failure rates
  - Large/volatile molecule stability (Biopharma)
  - High throughput efficient workflows
- Micro elution - deliver on these requirements:
  - Provides increased sensitivity (due to pre-concentration on plate)
  - Able to process low volume samples (25 $\mu$ L)
  - Reduces adsorption and solvation issues associated with large molecules
  - 96 well formats (amenable to high throughput automation)

# SOLA $\mu$ Based on Unique SOLA SPE Design



HDPE powder

+



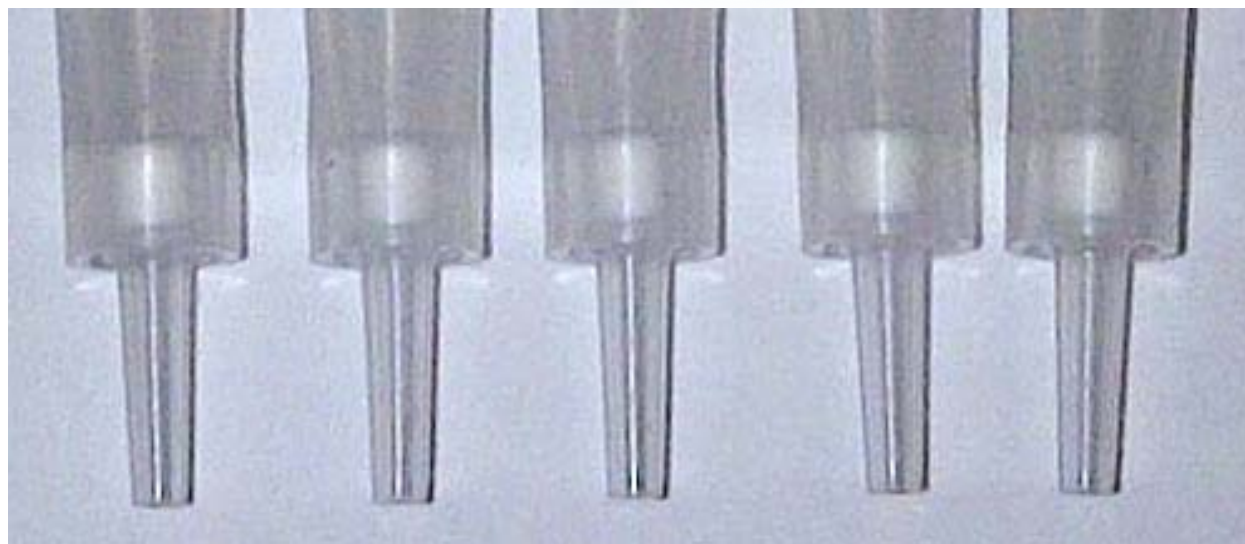
Polymeric material

+



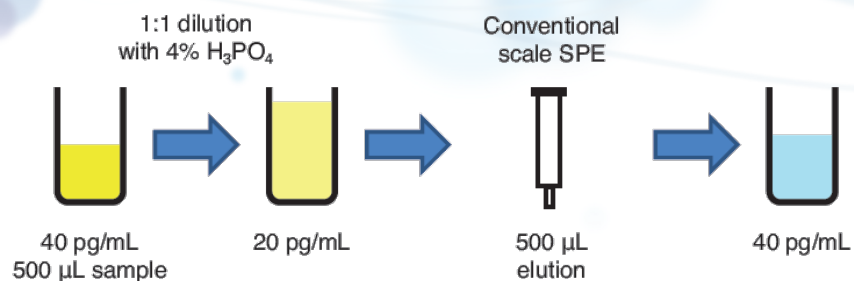
Polypropylene plate

SOLA $\mu$  is a solid single unit with integrated frit material

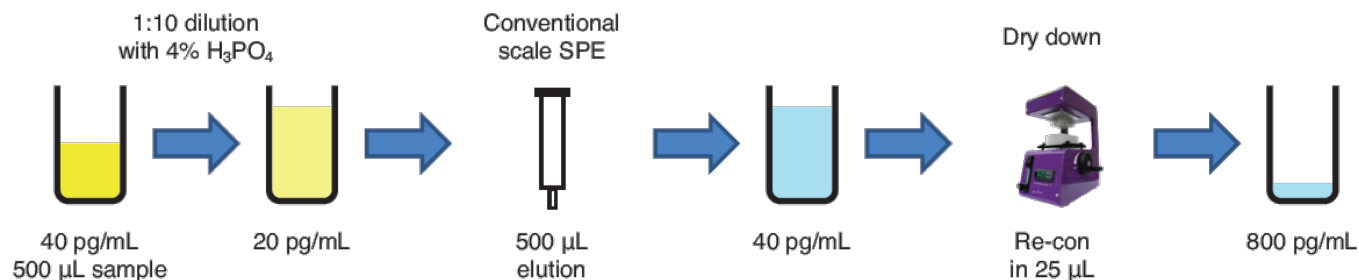


*Fewer failures*  
*Improved reproducibility*  
*Improved throughput and efficiency*

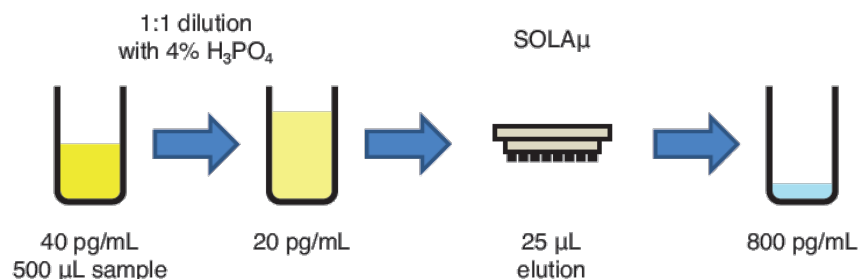
# Further Advantages of SOLA $\mu$



**Problem;**  
improvement in assay  
sensitivity required



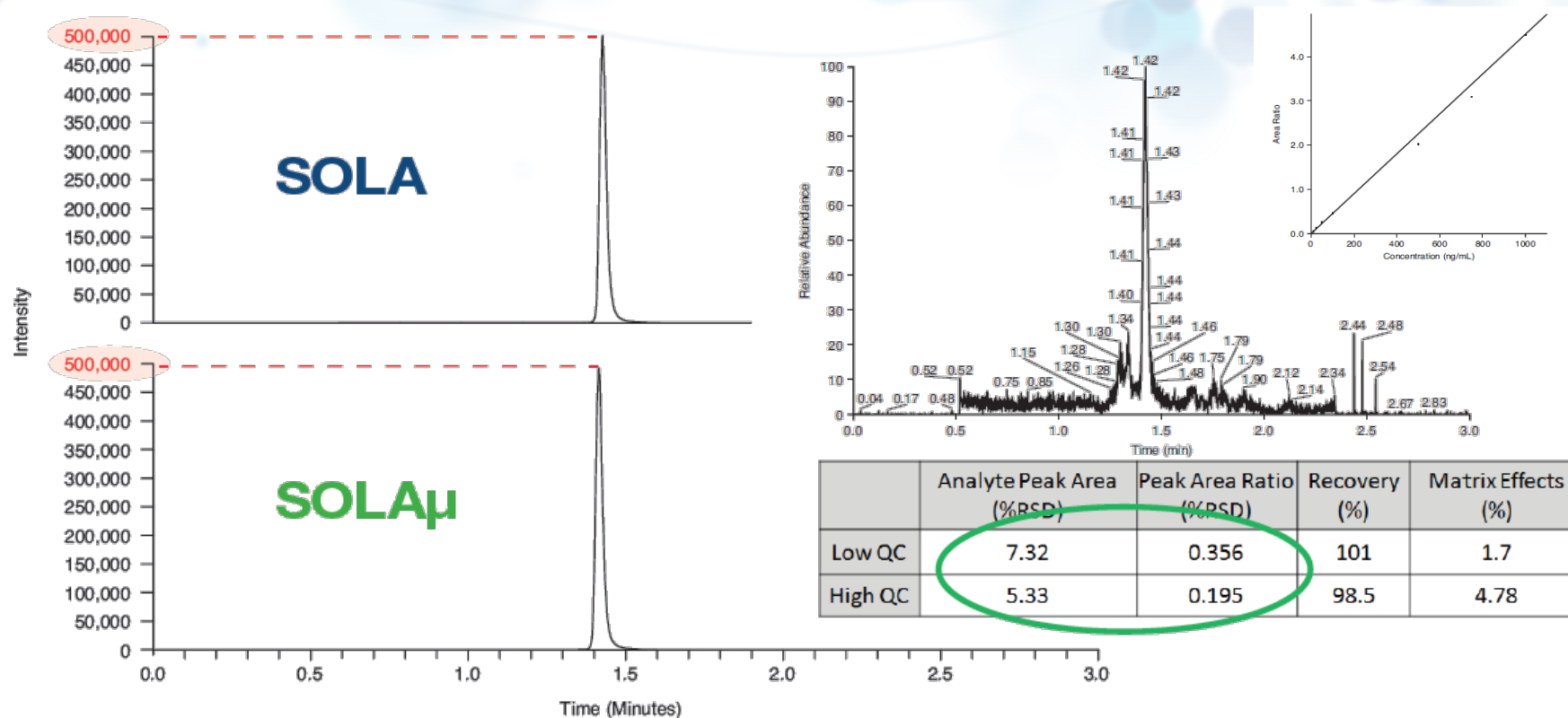
**Option 1;** introduce  
additional steps with  
associated issues



**Option 2;** SOLA $\mu$  provides  
twenty fold increase in  
sample concentration  
without changes to workflow

***Less Handling and Increased Concentration***

# Results of SOLA $\mu$ clean-up and concentration

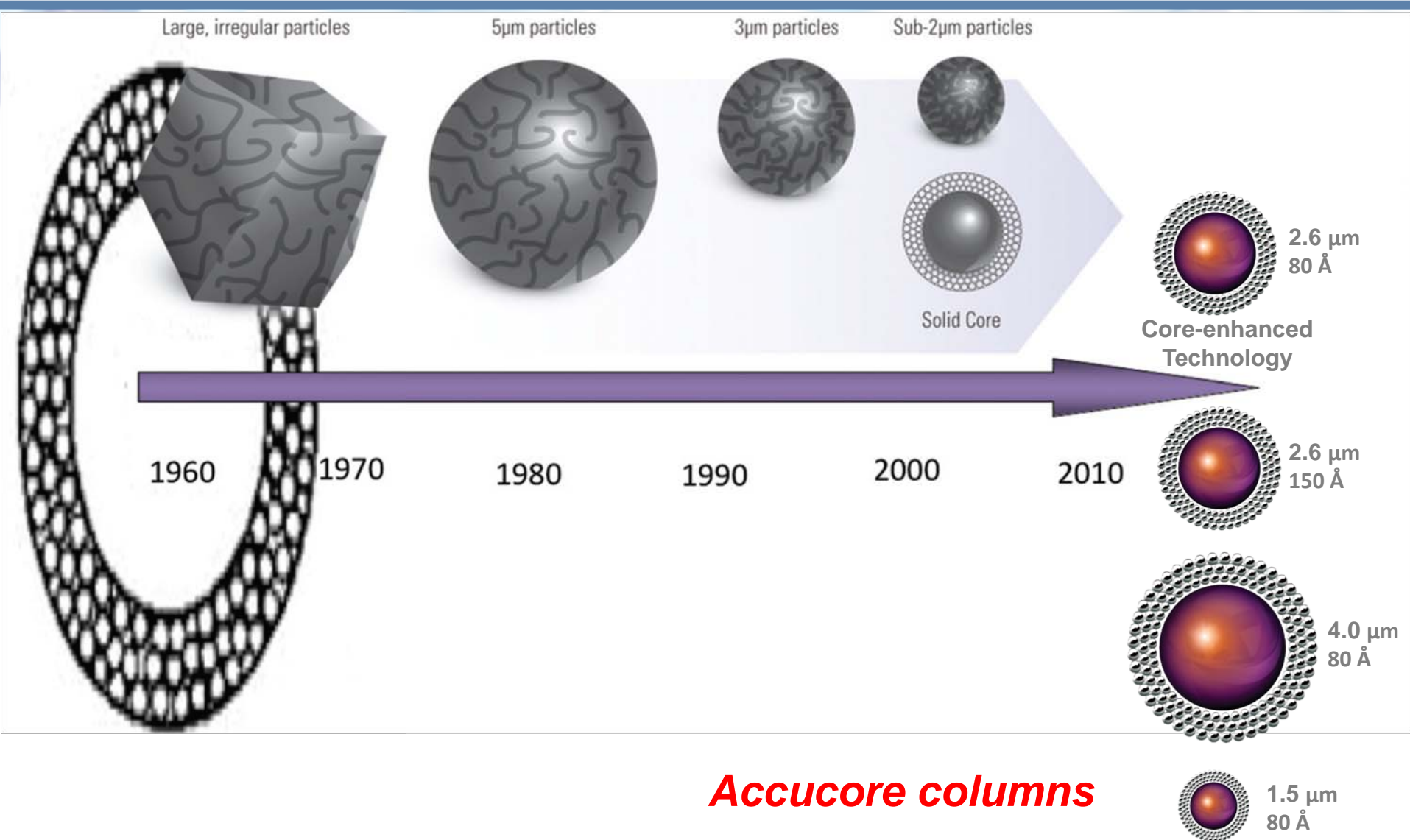


Comparison of Niflumic acid extracted with 10 mg SOLA WAX using 250  $\mu$ L of sample and from SOLA $\mu$  WAX using 25  $\mu$ L of sample. Both methods show equivalent assay performance.

**Accucore and Accucore Vanquish  
UHPLC Columns**

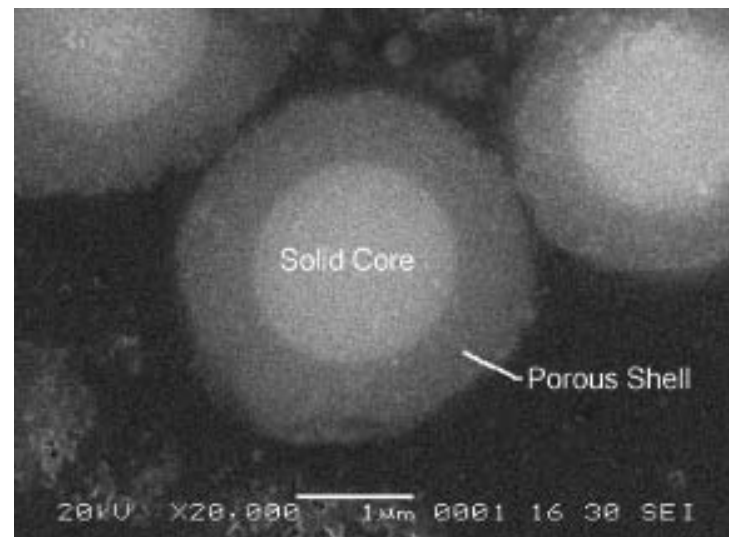
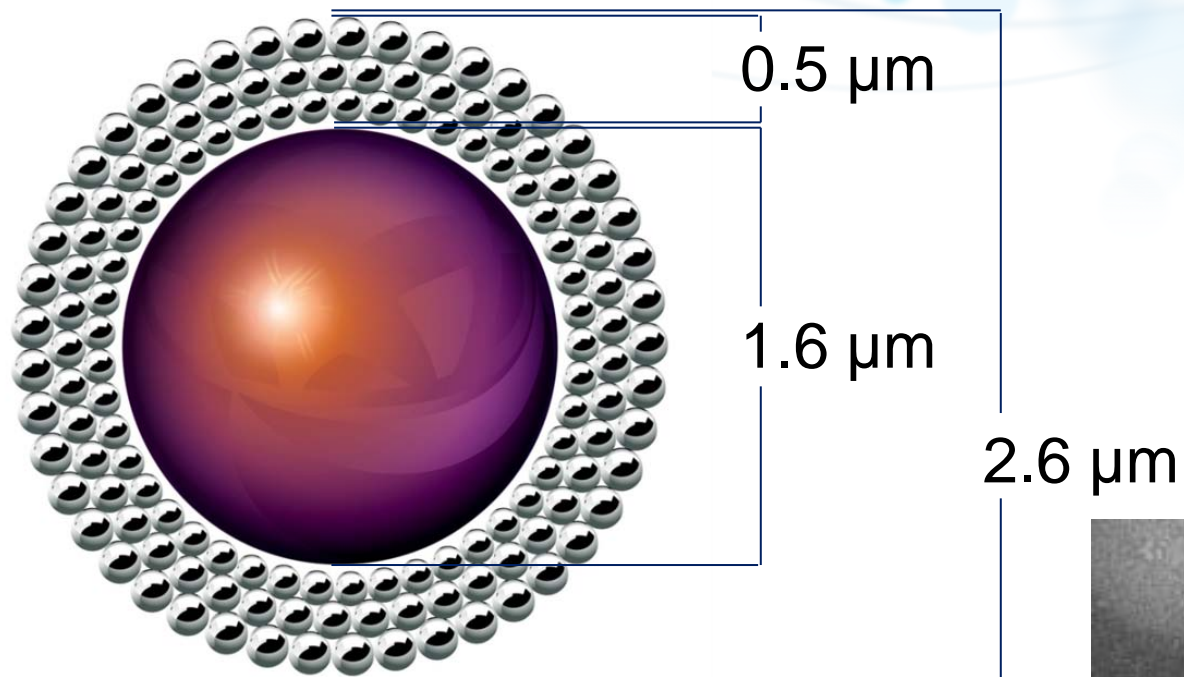


# Development of LC particle design

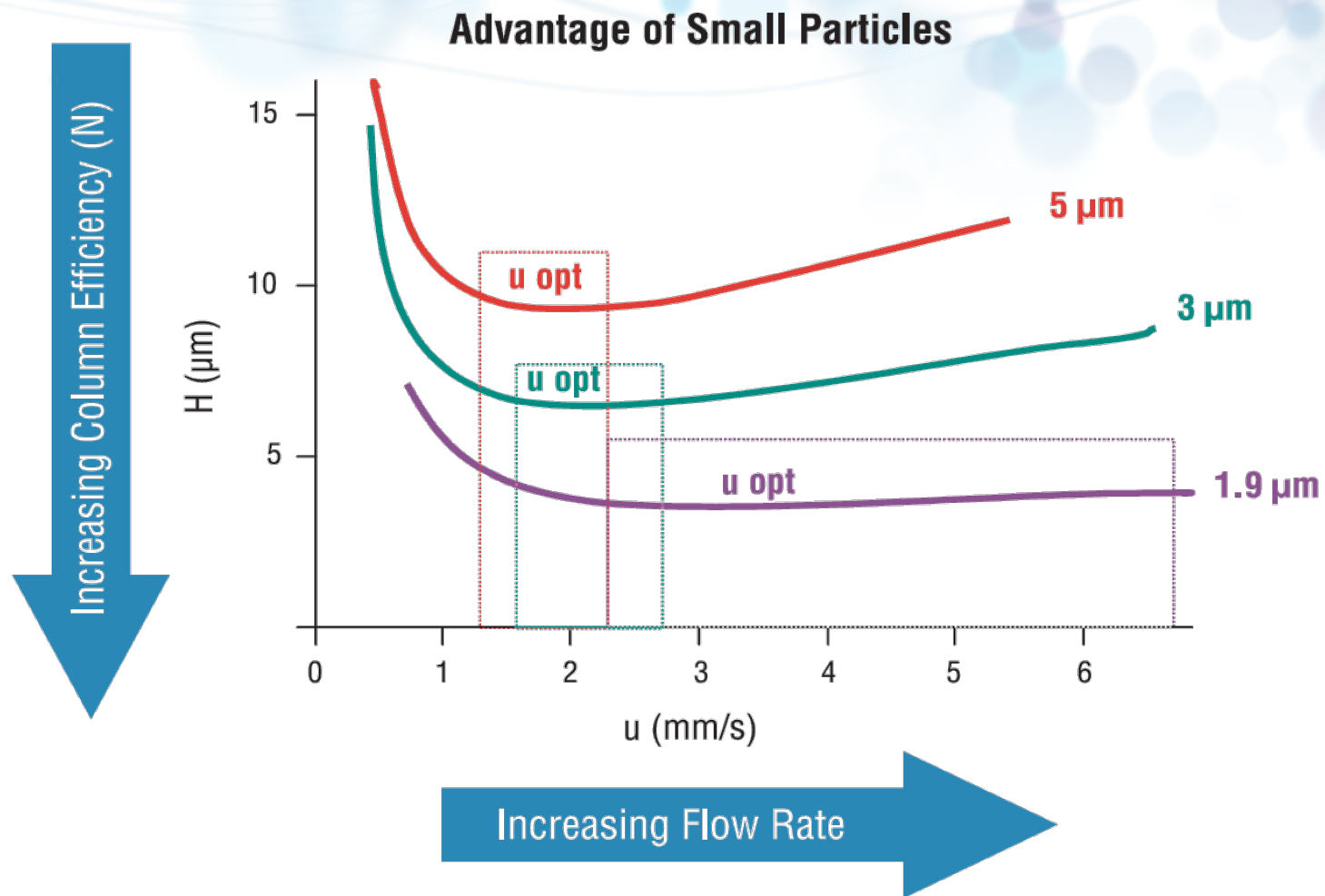


**Accucore columns**

# Solid Core - particle layout

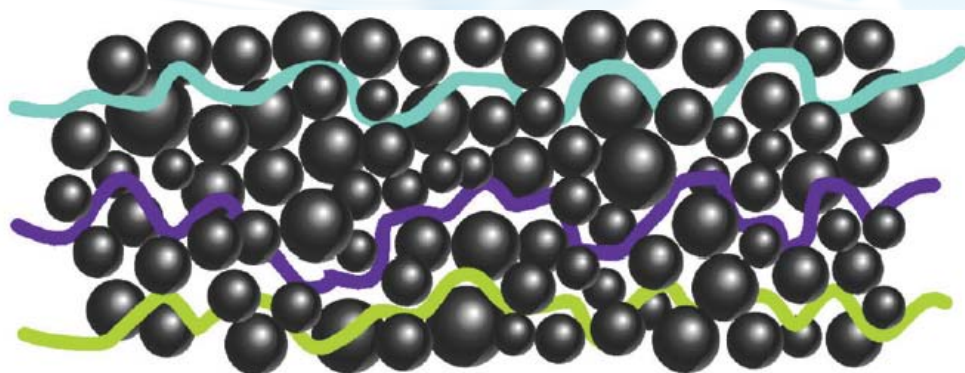


# Development of smaller particles

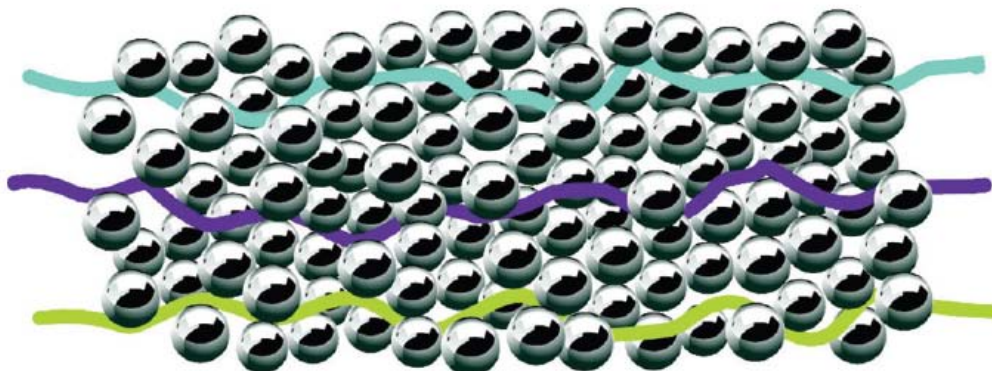




# Effect of particle size distribution



Fully Porous Particles  $D_{90/10} \sim 1.5$



Core Enhanced Technology  $D_{90/10} \sim 1.1$

# Core-Enhanced / Solid Core Technology

- Features

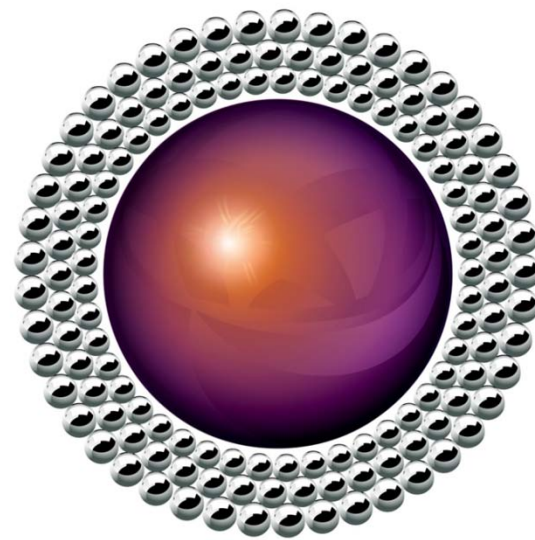
- More uniform particle sizing
- Better packing of particles
- Reduced pore depth
- Reduced mass transfer effects in mobile phase

- Benefits

- More efficient chromatography
- Allows the use of low pressure systems

- Competitive edge

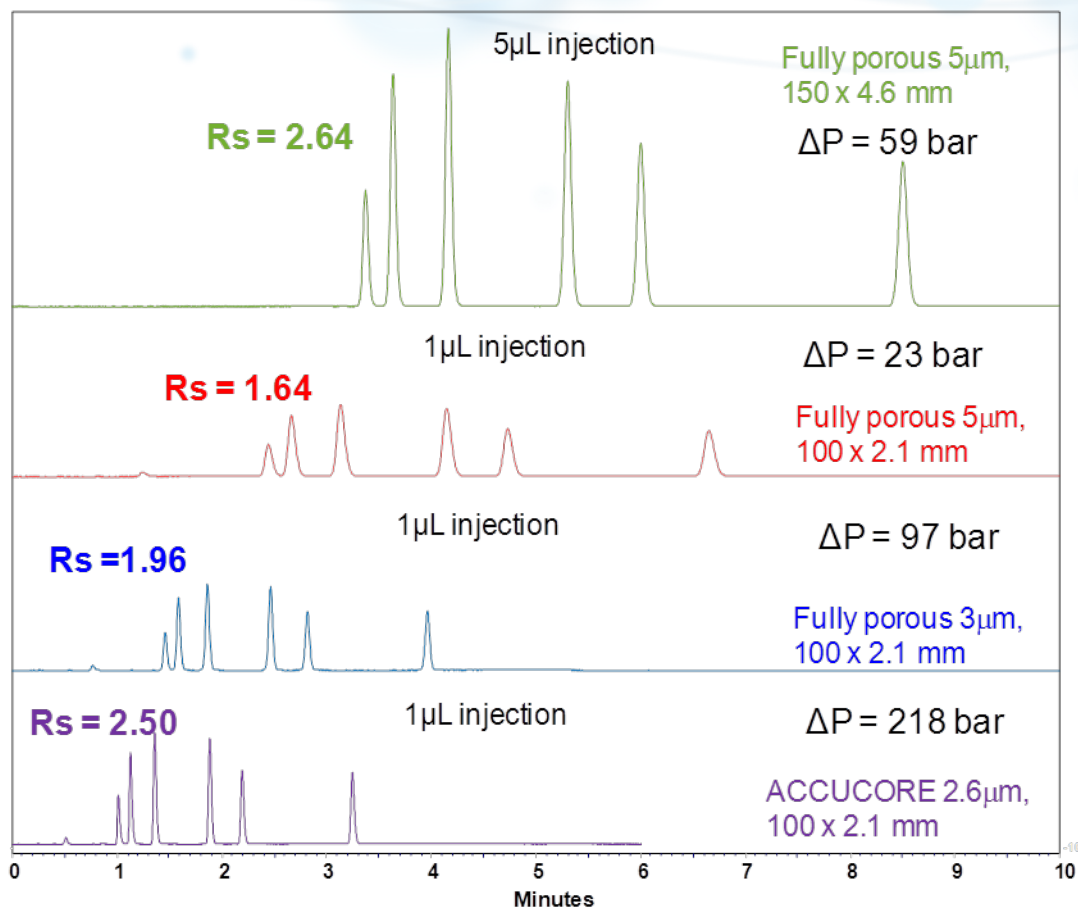
- Bar for bar gives better separations than porous materials



# Wide range of Accucore separation functionalities

- Accucore RP-MS
- Accucore Polar Premium
- Accucore C18
- Accucore Phenyl-X
- Accucore C8
- Accucore C30
- Accucore aQ
- Accucore HILIC
- Accucore Urea-HILIC
- Accucore Phenyl-Hexyl
- Accucore PFP
- Accucore 150-C18
- Accucore 150-C4
- Accucore 150-Amide-HILIC
- Accucore XL C18
- Accucore XL C8
- **NEW:** Accucore Vanquish C18

# Faster than fully porous 5 $\mu$ m and 3 $\mu$ m

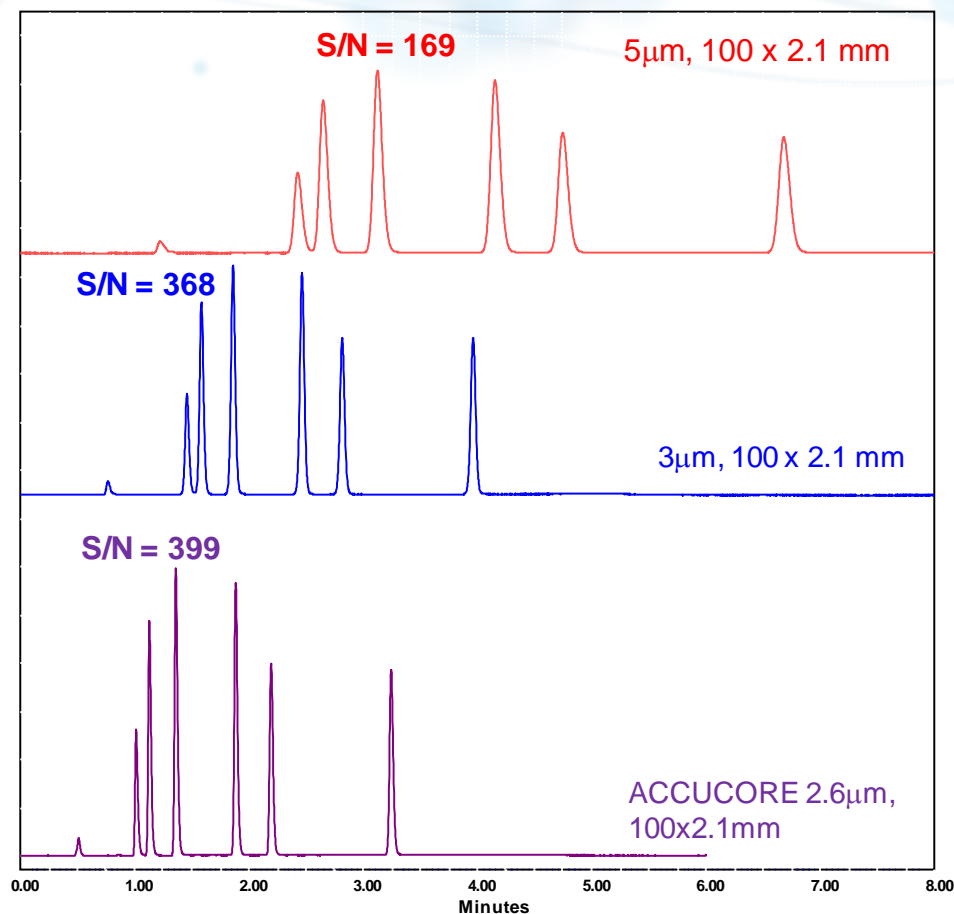


Method Transfer Calculator:  
[www.thermoscientific.com/crc](http://www.thermoscientific.com/crc)

- Gradient and flow rate:
  - Fully porous 5  $\mu$ m 150 x 4.6 mm  
35 – 60 %B in 10.0 min  
1000  $\mu$ L/min solvent used 17 mL
  - Fully porous 5  $\mu$ m, 100 x 2.1 mm  
35 – 60 %B in 6.7 min  
210  $\mu$ L/min solvent used 2.4 mL
  - Fully porous 3  $\mu$ m, 100 x 2.1 mm  
35 – 60%B in 4.0 min  
350  $\mu$ L/min solvent used 2.4 mL
  - Accucore RP-MS 2.6  $\mu$ m, 100 x 2.1 mm  
35 – 60 %B in 3.5 min  
400  $\mu$ L/min solvent used 2.4 mL

**Reduced analysis time and solvent costs**

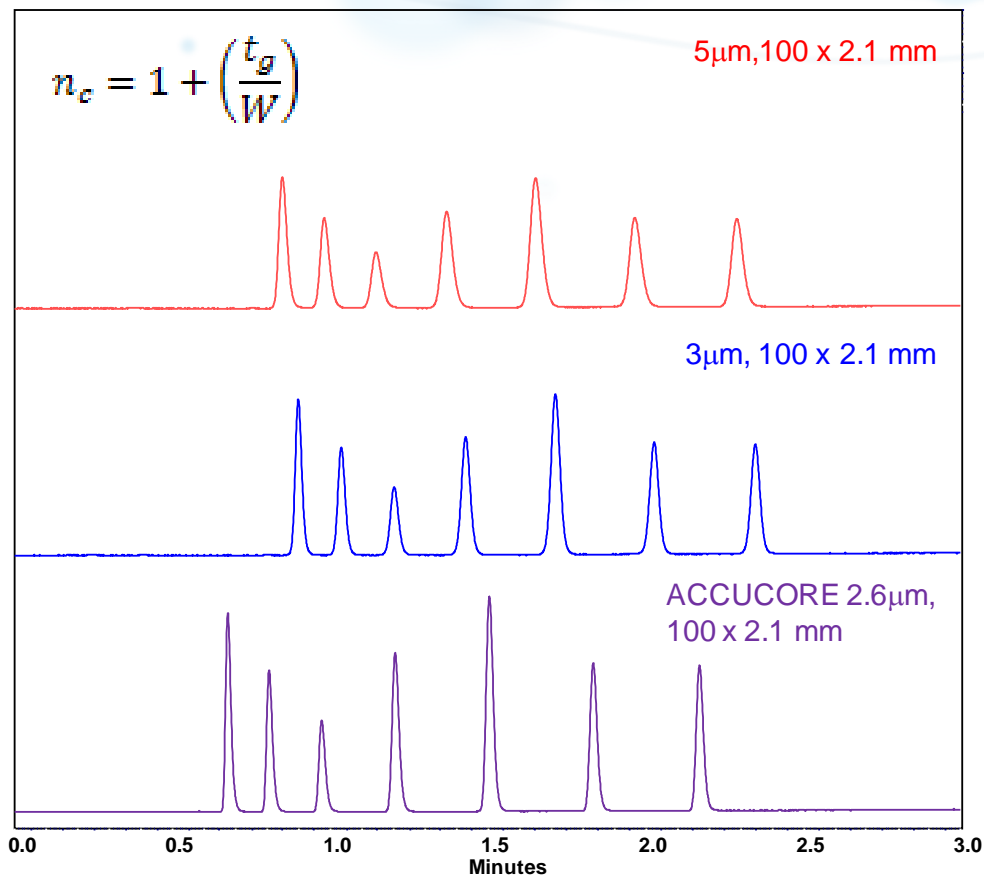
# More sensitive than fully porous 5 $\mu$ m and 3 $\mu$ m



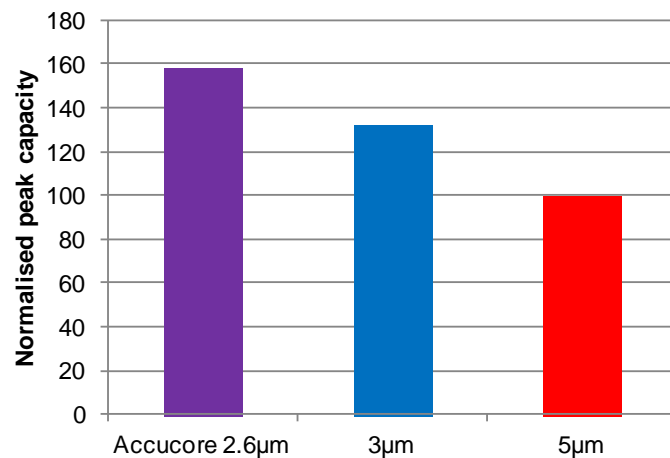
- Gradient and flow rate:
  - **5 $\mu$ m, 100 x 2.1mm**  
35 – 60 %B in **6.7 min**  
**210  $\mu$ L/min**
  - **3 $\mu$ m, 100 x 2.1mm**  
35 – 60 %B in **4.0 min**  
**350  $\mu$ L/min**
  - Accucore RP-MS **2.6 $\mu$ m, 100 x 2.1mm**  
35 – 60 %B in **3.5 min**  
**400  $\mu$ L/min**

***Higher S/N ratios – detection and quantitation of low level impurities***

# More peak capacity than fully porous 5 $\mu$ m or 3 $\mu$ m

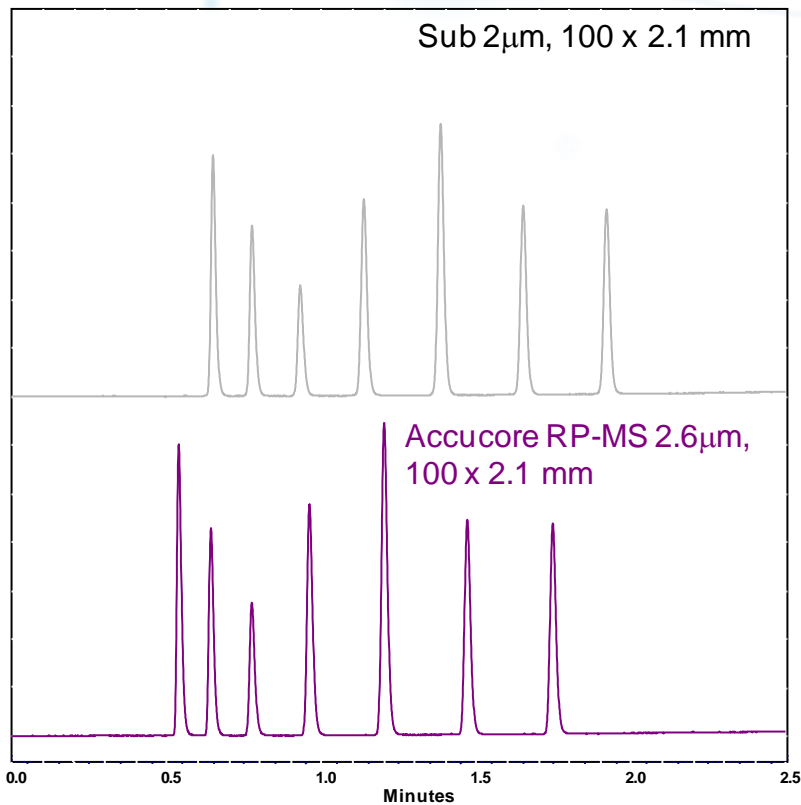


- Gradient: 65 – 95%B in 2.1 min, 95% B for 0.4 min
- Flow rate: 400  $\mu$ L/min



**Higher peak capacity**

# Lower backpressure than fully porous sub- $2\mu\text{m}$



- Flow rate: 500  $\mu\text{L}/\text{min}$
- Mobile phase: A - Water; B – Acetonitrile

	Accucore RP-MS 2.6 $\mu\text{m}$ , 100 x 2.1 mm	Sub 2 $\mu\text{m}$ , 100x2.1mm
Maximum pressure (bar)	171	338

***Equivalent performance, lower pressure (50% lower)***

# Accucore application examples

Analysis of Intact Proteins on a Thermo Scientific Accucore 150-C4 150 Å Pore Diameter HPLC Column

Application Note 1

Analysis of Bovine Serum Albumin (BSA) Protein Digest on a Thermo Scientific Accucore 150-C18 150 Å Pore Diameter HPLC Column

Application Note 2

Analysis of 3 Nonsteroidal Anti-inflammatory Drugs Using a Solid Core HPLC Column

Application Note 3

Separation of a Mixture of Vitamin K Isomers Using a Solid Core HPLC Column at Sub-ambient Temperature

Application Note 4

Quantitative Forensic Opioids, and The Urine Without Hydrolysis

Seash Rair Wandland, Kary Maxwell, Joseph...

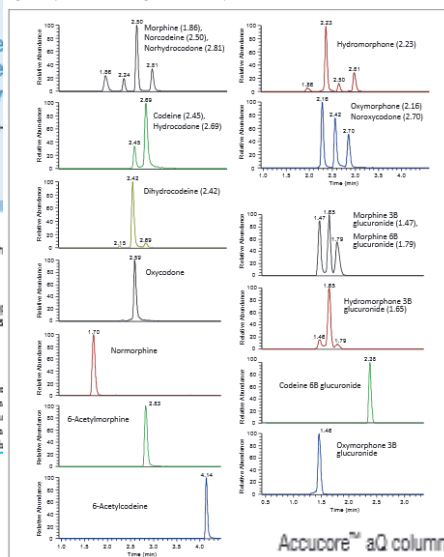
**Key Words:** Opiates, opioids, metabolites, sample preparation liquid chromatography (BPLC), mass spectrometry (MS), forensic toxicology

**Goal:** To develop a quantitative forensic method for analysis of opiates and their metabolites in human urine without the time-consuming hydrolysis.

**Introduction:**

Analysis of opiates and opiate metabolites often done with a hydrolysis step that can take up to 24 hours. The authors have eliminated the hydrolysis step by using a conjugated metabolite instead using a Thermo Scientific Accucore 150-C4 150 Å Pore Diameter HPLC Column

Figure 2. Representative chromatograms for all 19 compounds



Analysis of 2-aminobenzamide Labeled Dextran Ladder on a Solid Core nanoLC Column

Application Note 5

Fast, Quantitative SPE LC-MS/MS Analysis of Montelukast in Human Plasma

Application Note 6

Analysis of Ibuprofen and Valerophenone Using a Thermo Scientific Accucore XL C18 4 μm HPLC Column

Application Note 7

High-Throughput Analysis of Dextromethorphan and Dextrorphan

Kimberly Phipps, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Application Note 8

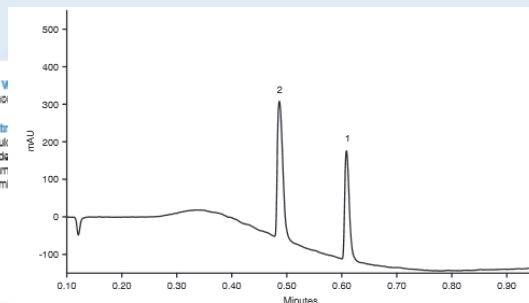
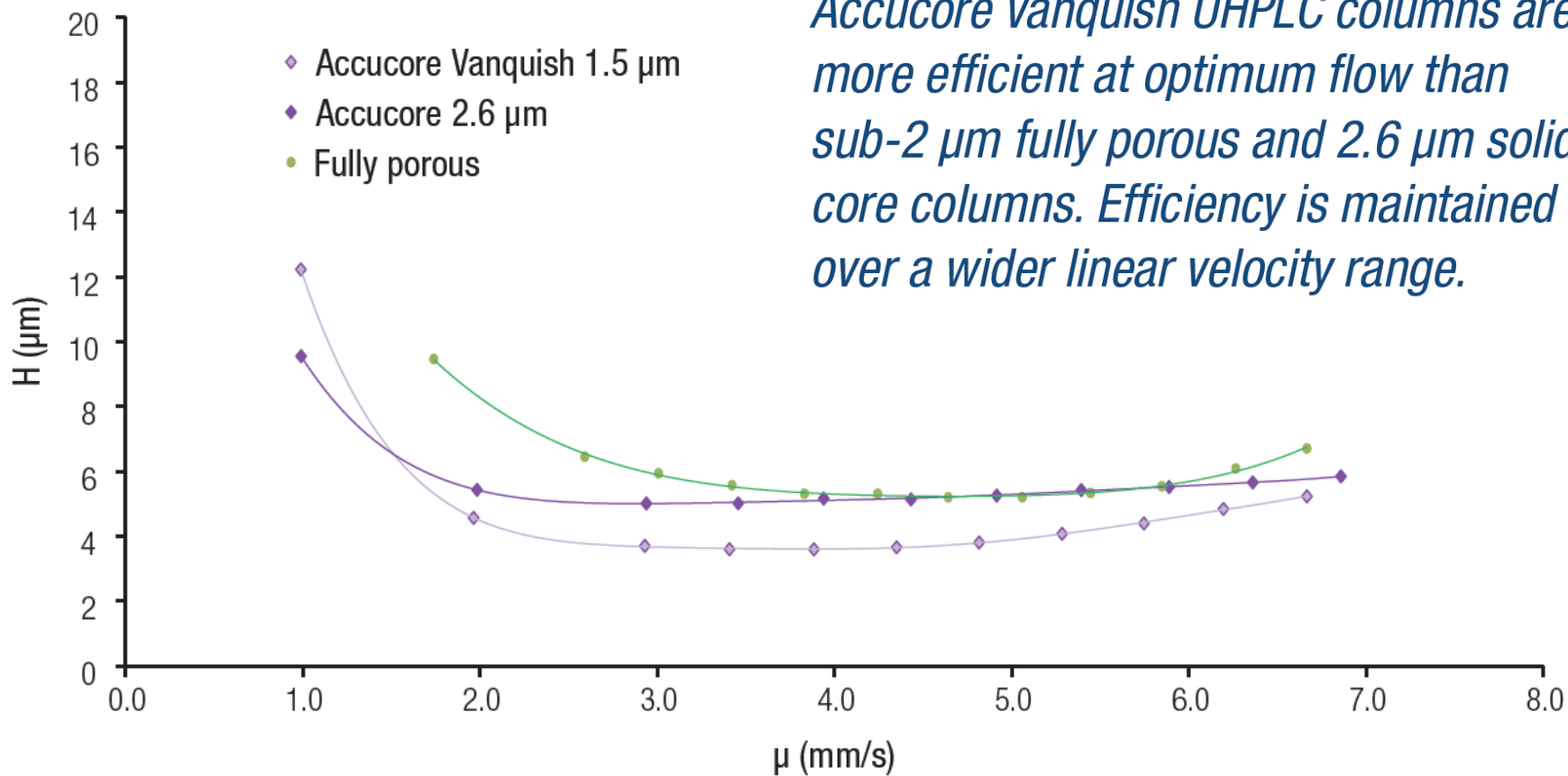


Figure 3: Chromatogram for dextromethorphan (1) and dextrorphan (2)



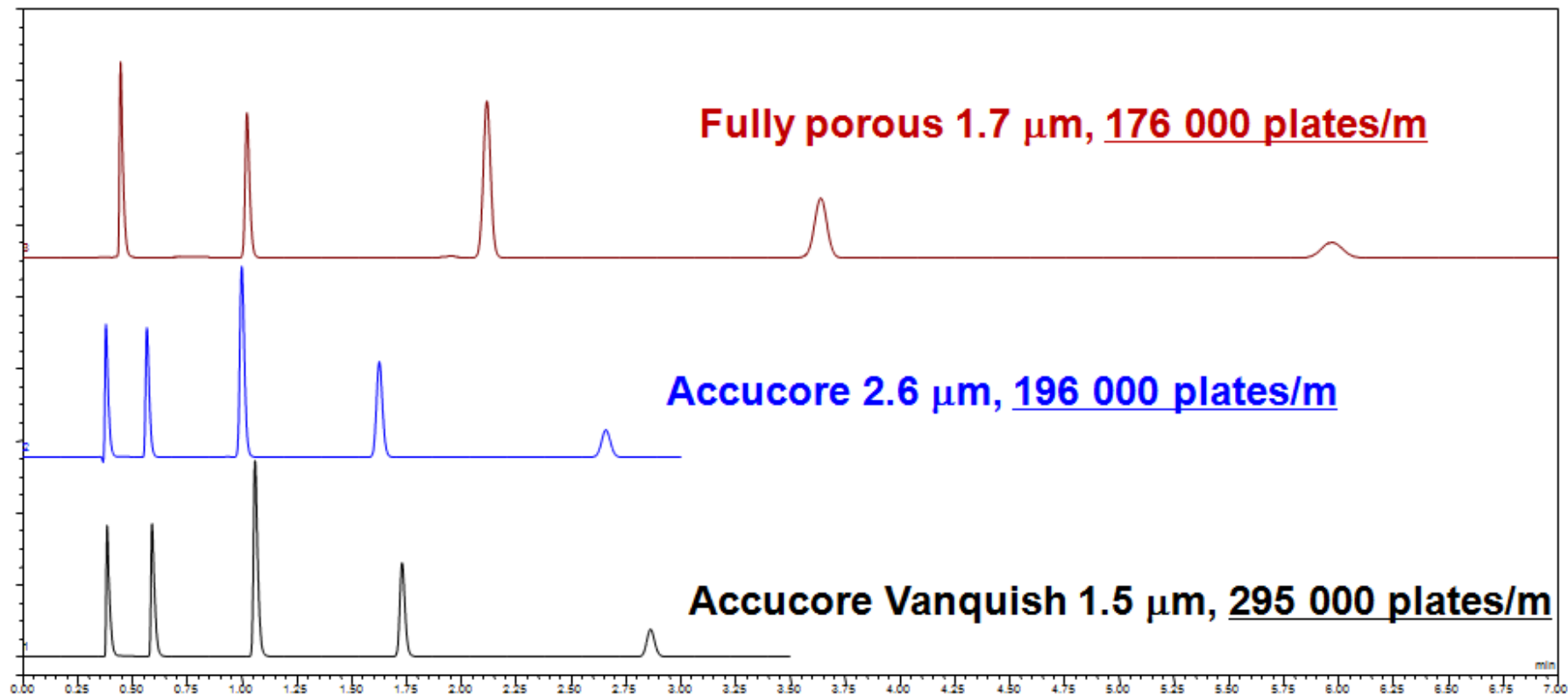
# New 1.5 $\mu\text{m}$ column technologies

- Efficiency = better separations



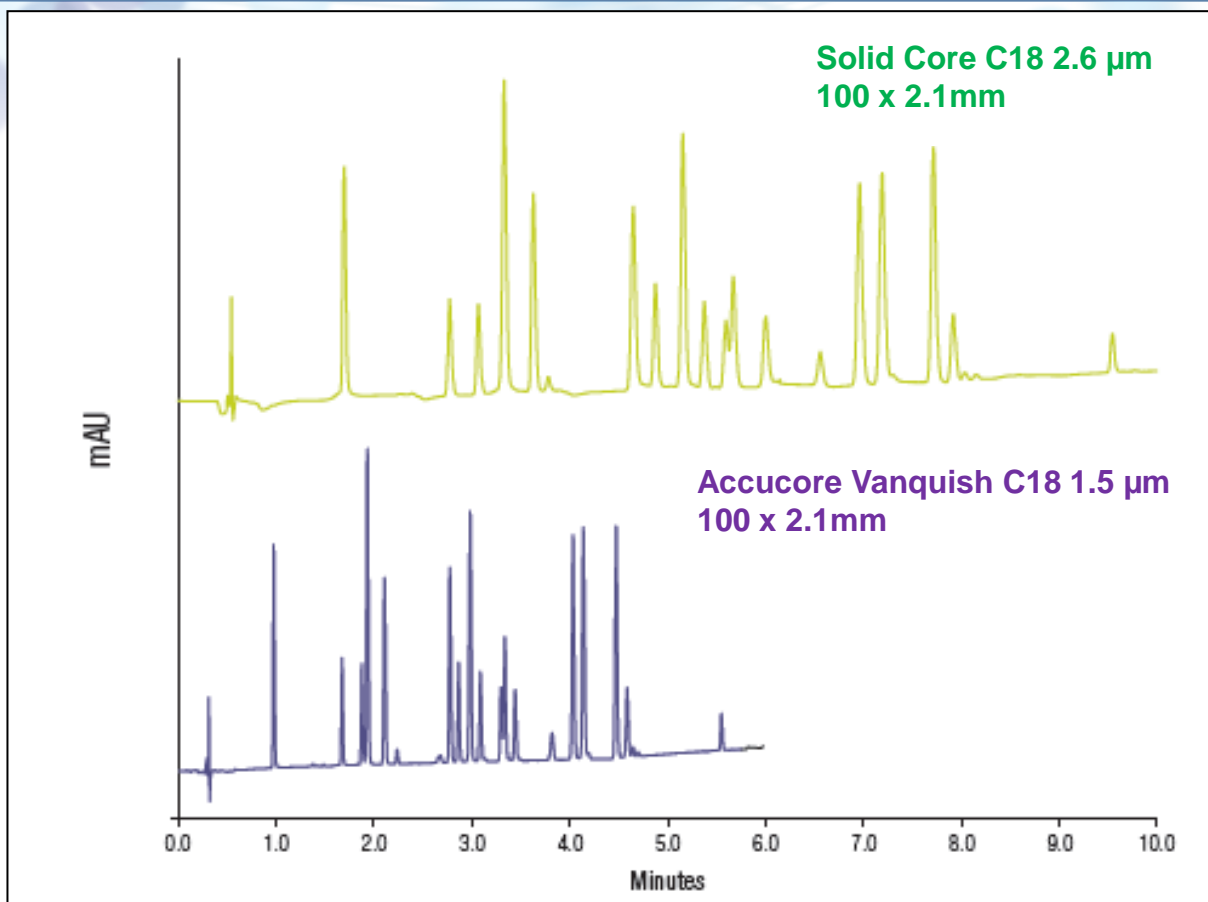
*Accucore Vanquish UHPLC columns are more efficient at optimum flow than sub-2  $\mu\text{m}$  fully porous and 2.6  $\mu\text{m}$  solid core columns. Efficiency is maintained over a wider linear velocity range.*

# Why 1.5 $\mu\text{m}$ provides: better separations



***40% efficiency gain***

# Accucore Vanquish columns deliver: more results



Mobile phase A:	water	
Mobile phase B:	acetonitrile	
Gradient:	Accucore Vanquish C18 1.5 µm 100 × 2.1 mm	
	Time (min)	%B
	0	20
	4	40
	7	80
	Solid core C18 2.6 µm 100 × 2.1 mm	
	Time (min)	%B
	0	20
	6.9	40
	12.1	80
Flow Rate:	Solid Core C18 2.6 µm 100 × 2.1 mm = 380 µL/min	
	Accucore Vanquish C18 1.5 µm 100 × 2.1 mm = 650 µL/min	
Inj. Volume:	0.5 µL	
Temp.:	43 °C	
Detection:	UV at 230 nm (0.1s rise time, 50 Hz)	
Analytes:	1. Desethylatrazine	10. Diuron
	2. Metoxuron	11. Isoproturon
	3. Hexazinone	12. Metobromuron
	4. Simazine	13. Metazachlor
	5. Cyanazine	14. Sebutylazin
	6. Methabenzthiazuron	15. Propazine
	7. Chlorotoluron	16. Terbutylazine
	8. Atrazine	17. Linuron
	9. Monolinuron	18. Metolachlor

**Average increase in resolution of 24%**

**40% time saving and better separations**

**Average increase in peak capacity of 220%**

# Accucore Vanquish columns - application examples

## Rapid Screening Method for Illicit Drugs, Using an Advanced Solid Core UHPLC Column and UHPLC System with MS/MS Detection

Derek Hillbeck, Thermo Fisher Scientific, Runcorn, UK

Application Note 1

### Key Words

Vanquish, Accucore, opiates, benzodiazepines, amphetamines, drugs of abuse, forensic, toxicology



### Abstract

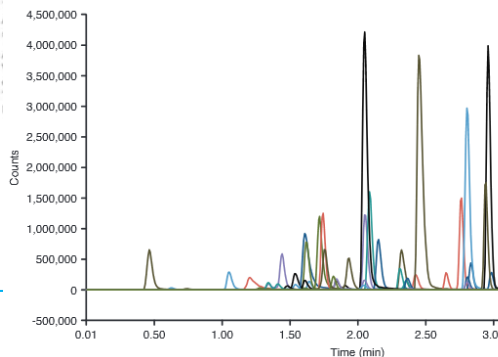


Figure 1: Overlaid selected-reaction monitoring chromatograms showing detection of 47 compounds in a 4 minute detection window

## Rapid Screening Method for Veterinary Antibiotics Using an Advanced Solid Core UHPLC Column and UHPLC System with MS/MS Detection

Gordon Wilson and Derek Hillbeck, Thermo Fisher Scientific, Runcorn, UK

Application Note 2

### Key Words

Vanquish, Accucore, antibiotics, sulfonamides, penicillins, macrolides,

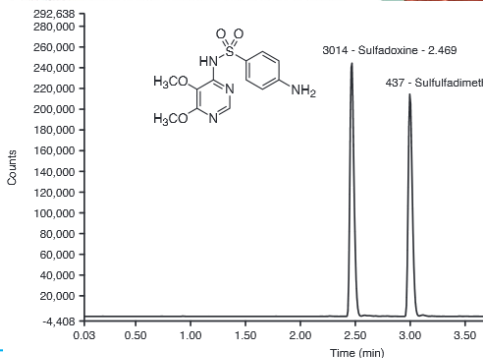
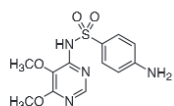


Figure 5: Selected-reaction monitoring chromatogram showing the chromatographic separation of Sulfadoxine and Sulfafadimeth isomers

## Rapid Separation of Eighteen Herbicides Using Advanced UHPLC and Sub-2 µm Solid Core Column Technologies

Kristina Kirkham, Luisa Pereira, Derek Hillbeck, Thermo Fisher Scientific, Runcorn, UK

Application Note 2001

### Key Words

Accucore Vanquish, Vanquish UHPLC, pesticides, herbicides, rapid separation, high resolution

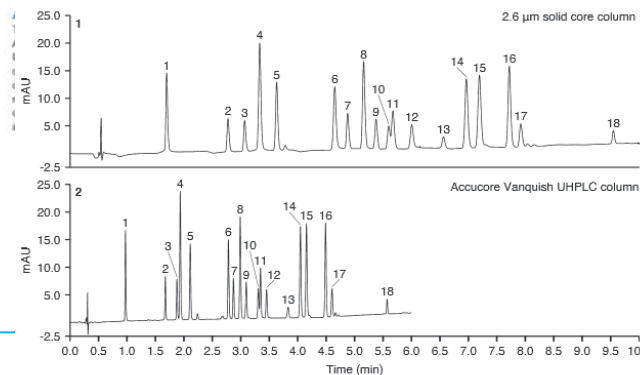


Figure 6: Chromatographic separation of 18 herbicides on a 2.6 µm solid core column (top) and the Accucore Vanquish UHPLC column (bottom)

# Summary

- Virtuoso Vial Identification System
  - Perfect for sample traceability
  - Easy to use, without information loss
- SOLA & SOLA $\mu$  cartridges and plates SPE solutions
  - Fritless design, for optimized bed
  - Minimized sample handling steps
  - Highest recoveries and cleanliness
- Accucore & Accucore Vanquish UHPLC columns
  - Core-enhanced or solid core technology
  - UHPLC resolution and separation times
  - New Accucore Vanquish to take full advantage of Vanquish 1500 bar

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Introducing Next Generation UHPLC Columns - Accucore Vanquish UHPLC Columns deliver better separations, more results and easier interactions.

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- ▶ LC Column Selection Tool

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*Partners in driving value creation*

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