SPE: CITIUS, ALTIUS, FORTIUS

ISC '98

ROMA

Poster 112

15 Sept 1998

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Abstract

SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.

This year marks the twentieth anniversary of the commercialization of the first miniature cartridge columns containing silica-based adsorbents designed for SPE (1). The ideas that led to this invention, the history of column liquid-solid phase extraction [CLSE--modern SPE], and the rapid development of SPE in the last two decades as a preferred sample preparation technique will be traced.

SPE is hundreds of years old; fragrance manufacturers in Grasse to this day still extract labile oils from jasmine petals via the ancient process of embedding them in paraffin wax. Pioneering work by Schwartz in the 1950's and 1960's in which CLSE was performed on both the mini- and micro-scale (in glass melting-point capillary tubing), with on-column derivatization and/or complexation, specific for certain compound classes, is virtually unrecognized today. So, too, are some of the first laboratory-scale applications of hydrophobic polymers for reversed-phase CLSE by Bradlow in the late 1960's.

There were three characteristics of that first commercial product for CLSE/SPE that led to the rapid adoption of the technique: a convenient, efficient, disposable, miniature column format; a family of reproducible, reliable sorbents chosen and quality-controlled specially for SPE; and a package that maintained the integrity of the sorbent until it was used.

A new generation of formats and stationary phase chemistries which enable the practice of SPE to be faster, with higher sample throughput, and stronger performance will be emphasized. The unique properties of new sorbents which have spurred a renaissance in the use of polymer packings and dramatically improved SPE performance will be reviewed.

(1) P.D. McDonald, R.V. Vivilecchia and D.R. Lorenz, "Triaxially Compressed Beds," U.S. Patent #4,211,658 (1980).

Introduction

In October, 1977, principles of *radial compression technology*, developed just two years earlier [1], were extended in the invention of the first miniature cartridge columns containing silica-based adsorbents designed for SPE [2].

To commemorate the *twentieth anniversary* of the commercialization of that invention on January 15th [silica], and March 15th [C_{18}–silica], 1978, respectively, this poster will trace the ideas that led us to that invention, identifying some relatively unknown pioneers in SPE.

Then, as now, GOALS of modern solid phase extraction [SPE] are, in the language of ancient Rome:

Citius, Altius, Fortius; *OR*

Faster [throughput]

Higher [recovery & reproducibility]

Stronger [retention & selectivity].

While *formats* for SPE have evolved in response to needs based upon sample volume & matrix, automation, convenience, & safety, a reexamination of SPE *sorbent chemistries* has led us to specially design a *new* family of *copolymer* phases which perform optimally for *reversed-phase* and *mixed-mode* SPE [3].

SPE Antica

What is Solid Phase Extraction?

#1: *Gas-Solid Phase Extraction*

At the Fragonard & Cie factory in Grasse, France, they still use a 100+ year-old solid phase extraction process to isolate the volatile, but heat labile, fragrance constituents from *jasmine*. Petals are covered with molten, very low-melting *paraffin wax*. The mixture is cooled & solidified in large wooden frames. The volatiles migrate into the solid wax. After a week or more, the wax is carefully melted, the petals removed, & the "solution" extract treated in various ways, depending upon how the jasmine essence is to be used.

#2: *Liquid-Solid Phase Extraction*

For decades, first year organic chemistry students learned how to admix *charcoal* with a homogeneous reaction solution at room temp., let it stand for a few minutes, then filter out the fine carbon particles using a carefully prepared, filter-paper-supported bed of *diatomaceous earth* (washed of fines & free of cracks), thereby decolorizing the solution (removing the undesirable polymeric & highly polar *reaction by-products* via adsorption). The solvent was then evaporated, & the products recovered, via suitable workup steps [4].

SPE Pioneers

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Early Use of Polymer Sorbents, Miniaturization

#3: *Column Liquid-Solid Phase Extraction*

At the start of my postdoctoral study at Columbia U. College of Physicians & Surgeons in 1970, I first saw in our cold room a ~10 x 150 cm glass column, filled with *XAD-2 PS-DVB* resin. After prewetting the bed with MeOH & washing with water, 24-hr urine samples (~1-2 L.)were loaded under gravity flow; after washing with water to remove salts, etc., the *steroids* & *steroid conjugates* were eluted with MeOH. This reversed-phase process was first practiced by one of our New York colleagues, Leon Bradlow [5], only months after Rohm & Haas (1967) introduced this new polymer resin.

#4: *Liquid-Solid Phase Derivatization*

A true pioneer, Daniel P. Schwarz (USDA, Philadelphia), for more than two decades, combined chemical affinity, on-column derivatization, & separation of products from excess reagent for analysis. He scaled down from large open columns (g) to small glass pipets (mg) to melting-point capillaries (µg) with *siliceous* supports.

Examples: isolation of *cholesterol* from milkfat (as the digitonide complex) [6], esterification of *organic acids* [7], acetylation of *alcohols* [8], reduction of *carbonyls* [9], & isolation of *aldehydes/ketones* as DNPH derivatives [10-12].

Miniature Column Liquid-Solid Extraction becomes SPE

#5: *Column Liquid-Liquid Partition*

In Prof. Seymour Lieberman's lab (Columbia), the art of CLLP [13], as developed in the '60's by Charles Pidacks (Lederle; later, Waters), was refined for steroid separations. Pidacks' technician Pentti Sitteri brought the technique to C.U. as a doctoral student. Thousands of miniature glass pipet LLP columns were hand-packed with coated Celite® for sample cleanup prior to RIA.

Disposable 50-mL plastic syringe barrels packed with synthetic, very large-pore silica {Extrelut® columns (Merck, 1975) [14]} or cotton gauze {JET tubes (Manhattan Instruments, 1977) [15]} appeared .

#6: *Small Polymer CLSE Columns*

A system utilizing racks of 72 small plastic columns, with integral reservoirs, prepacked with *XAD-2*, & a vacuum-assisted manifold holding racks of collection tubes, miniaturized Bradlow's technique for smaller urine samples (Brinkmann Instruments, 1972) [16]. Later uses for *XAD-2*: drugs in blood [17] and urine [18].

James Fritz & coworkers began to use small columns of XAD resins for environmental water samples [19].

V. Leoni et al. [Univ. ROMA] isolated pesticides from water with 1 x 40 cm *Tenax GC*® resin columns [20].

Invention – Oct 1977

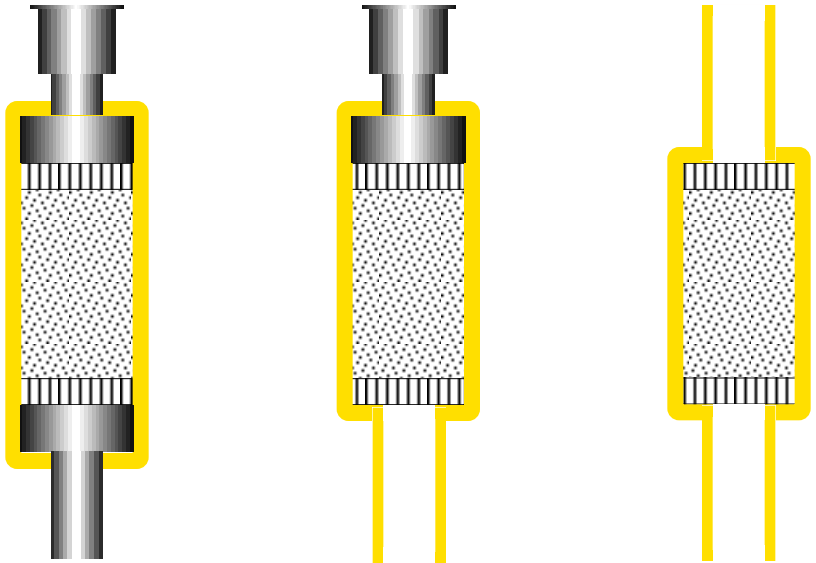
SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.

Key Experiment Defines Goals; New Format Created

Use Chromatographic Principles to Perform Sample Cleanup as well as Analysis [21]

Analytes: Vitamins A palmitate [RDA: 2.75 mg], E acetate [22 mg] and D_2 [10 µg]
Matrix: 14 g Product 19 Cereal
Extraction Solvent: 50 mL *Freon TF*⋆
CLSE Column: 2 cc silica [SA: 300 m^2/g; 3% moisture w/w] in glass pipet; load 10 mL extract (nominal 10% RDA); wash w/1 mL hexane; elute in 2 mL hexane/EtOAc 70/30
HPLC Column: µBondapak™ CN, 3.9 x 300 mm
Mobile Phase: hexane/EtOAc 95/5 [v/v]
Flow Rate: 2 mL/min
UV: 254 nm
Run Time: <4 min injection to injection

⋆ *A real discovery. If hexane was used, breakthrough occurred in CLSE step.*



1 David Lorenz assembled 2 cc of silica between porous PE frits & Yuri Tuvim-designed SS endfittings (female Luer hub inlet) in irradiation-crosslinked polyethylene tube.

Tubing was heat-shrunk tightly around components.

Assembly performed well but leaked slightly around SS fittings.

2 Richard Vivilecchia suggested removing outlet fitting & letting tubing shrink to form its own outlet.

3 David discovered that Luer syringe tip fit snugly into shrunken tubing outlet. Discarded inlet fitting. New SPE cartridge format was born [2].

David suggested brand name: SEP-Pak [Sample Enrichment & Purification].

Sep-Pak® Silica Cartridge bed: 1 x 2 cm

Later, Sep-Pak® C_{18} Cartridge bed: 1 x 1 cm

Revolutionary Benefits*

SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.

- ❒ Lower Cost
  - less solvent
  - less reagent consumption
  - less apparatus
- ❒ Greater Recoveries
  - minimal sample transfer
- ❒ Faster
  - fewer steps
- ❒ Greater Safety
  - less exposure to toxic agents
- ❒ Greater Accuracy
  - no cross contamination
- ❒ No Emulsion Problems
  - less sample handling
  - fewer steps
- ❒ No Transporting of Samples to Lab
  - direct field sampling
- ❒ Reduced Harm to Labile Samples
  - minimal evaporation
- ❒ Minimal Glass Breakage
  - less glassware used

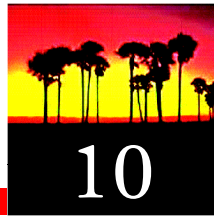
* Reprinted from [22]

Et Tu SPE – Excelsior

Keys to Rapid Growth & Acceptance of SPE

- Convenient, efficient, disposable, miniature column format
- Family of reproducible, reliable sorbents, chosen & QC'd specially for SPE
- Packaging that maintained integrity of sorbent until it was used.
- Wide Range of Initial Applications – methods use HPLC principles [23]
  - Residue Analysis [24]
  - Environmental Monitoring [25]
  - Beverage Analysis [26]
  - Toxins in Foodstuffs [27]
  - Drug Monitoring/Drug Metabolism [28]
  - Biochemical/Biomedical Research [29]
- C_{18} for Reversed-Phase ideally mated with complex, aqueous samples
  - trace enrichment of environmental samples [30]
  - biological fluids & tissue extracts: e.g., peptides [31]
- Competitive Explosion after 2.5-year induction period
  - Analytichem: ClinElut™ (miniature JET tube; 9/78); BondElut™ (silica & bonded phases; 8/80)
  - Baker: BakerBond spe* (1982) [32] **This author believes Baker's promotion of acronym SPE for miniature CLSE redefined it.*

Issues / Opportunities



Applications Attest to Utility of SPE

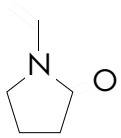
- In 1978, a small team began collecting published applications of SPE in a bibliography & electronic database. From this effort, 6 editions of *Waters Solid-Phase Extraction Applications Guide and Bibliography* were published [33], the most recent containing over 3000 fully indexed citations. Other SPE manufacturers have also created compendia [34] .

Issues

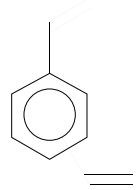
- SPE, especially for large numbers of samples, became *tedious*, *time-consuming*, *tricky*, & *irreproducible*, esp. in the hands of those with limited knowledge of chromatographic principles [35].
- Early *polymer* phases were *industrial-grade* materials (large particle size, dirty, low degree of crosslinking, swellable in organic solvents) & gradually abandoned in favor of silica-based materials.
- *Silica-bonded phases* suffered from *pH limitations*, undesirable *silanol* activity, *breakthrough* of polar compounds, *hydrophobic collapse*, *irreproducible & low recoveries*, & the need for *stopcock* manipulation [36].

HLB Vincit Omnia

SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.



Oasis® HLB Copolymer: Hydrophilic – Lipophilic Balanced Sorbent*



N-Vinylpyrrolidone — Divinylbenzene

- *Hydrophilic* monomer ["water loving"]
- Keeps sorbent surface solvated with water — *even if the bed runs dry!*
- *Lipophilic* monomer ["fat loving"]
- Provides *reversed-phase* surface for analyte retention

Optimal Properties for Reversed-Phase SPE

Specific Surface Area:	810 m^2/g
Average Pore Diameter:	80 Å
Total Pore Volume:	1.3 cm^3/g
Average Particle Diameter:	30 µm; LP 60 µm

1: Condition — 1 mL MeOH

2: Equilibrate — 1 mL water

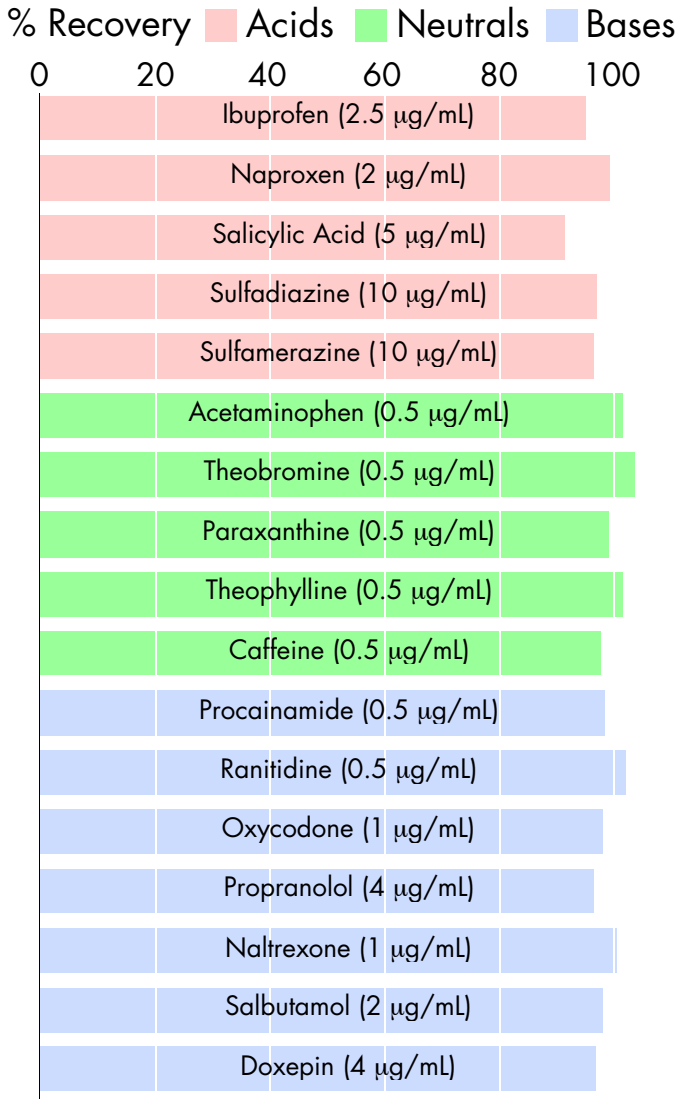
3: Load — 1 mL spiked serum sample

4: Wash — 1 mL 5% MeOH in water

5: Elute — 1 mL MeOH

6: Evaporate & Reconstitute — 40° C/200 µL mobile phase

One Simple, Generic Protocol: Many Applications



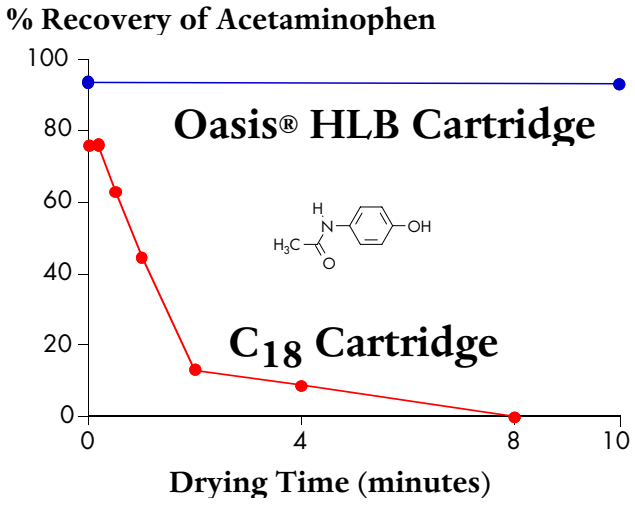
* US Patents 5,882,521; 5,976,367; 6,106,721, 6,254,780 [37]

Wettability Advantage

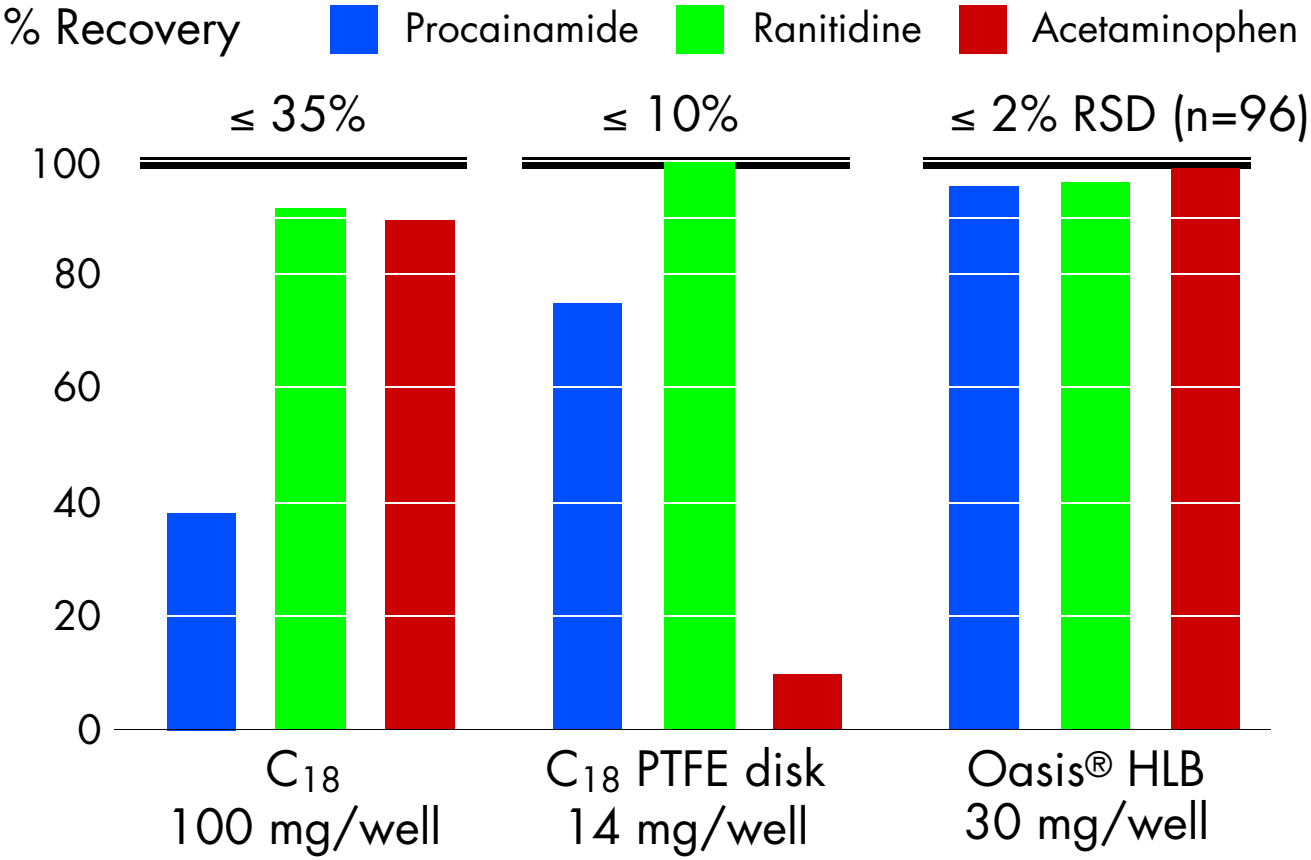
SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.

Oasis® HLB Sorbent: the *Enabling Technology* for High Performance SPE in 96-Well Plates

Effect of Drying on Recovery– Oasis® HLB vs. C_{18} Sorbent*



* Details in [38]



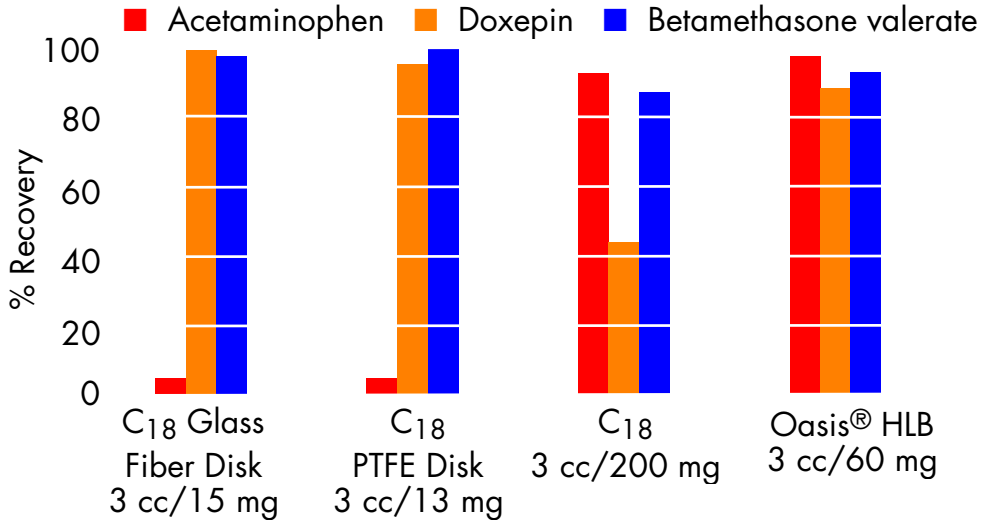
Oasis® HLB sorbent maintains its capability for higher retention & excellent recoveries, even if the bed runs dry. No more stopcocks on cartridge vacuum manifolds! Low RSD's are now possible in 96-well plate format!

[37-38]

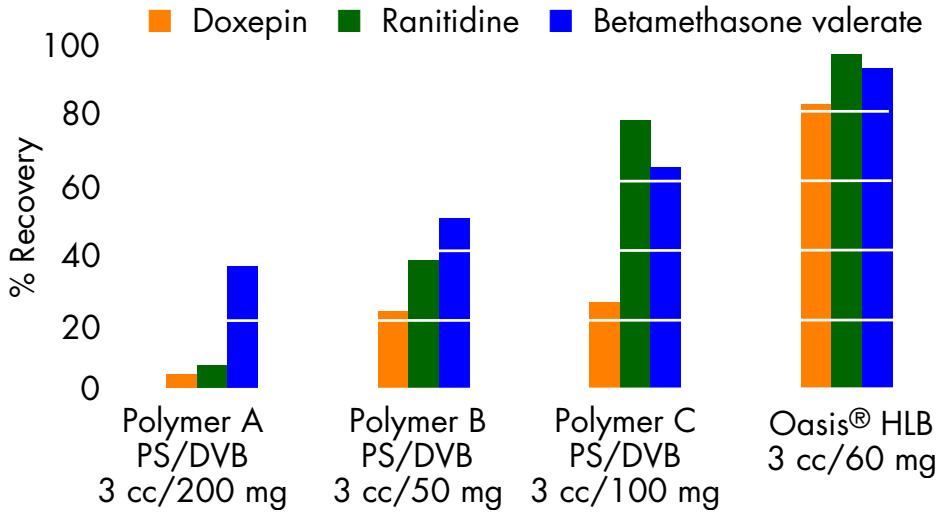
Higher Performance

Oasis® HLB Sorbent: *Superior Recovery & Capacity* – *Cartridges* (vs. Bonded-Silica or Other Polymers) *or* *Plates* (10 mg/well)

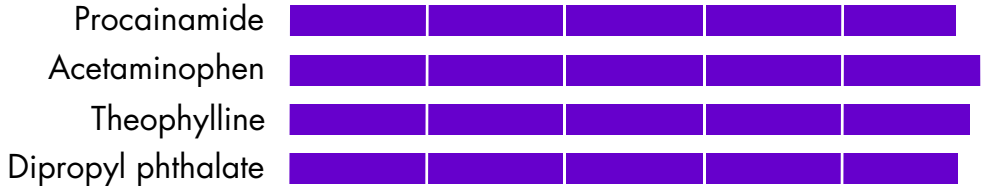
C_{18}–Bonded Silica:



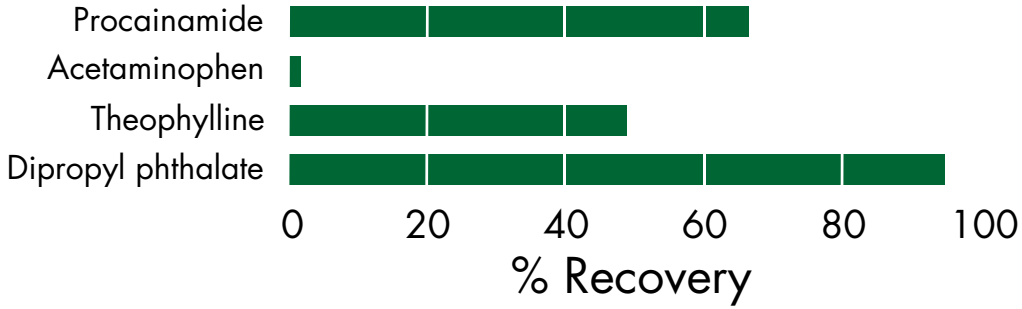
Other Polymers:



Oasis® HLB Plate (10 mg/well)



C_{18} PTFE Disk Plate



Elution in <150 µL can eliminate time-consuming, tedious evaporation & reconstitution step from your SPE method

2D for Selectivity

SPE: Citius, Altius, Fortius, ISC'98, Roma, Poster #112.

Tricyclic Antidepressants in Porcine Plasma: Lower UV Background

Since HLB sorbent is stable from pH 1 to 14 & unaffected by organic solvents, more options are available for SPE method optimization [39].

Optimized SPE Method

Condition/Equilibrate
1 mL Methanol/1 mL Water
▼
Load
1 mL Spiked Sample
▼
Wash 1
1 mL 5% Methanol in Water
▼

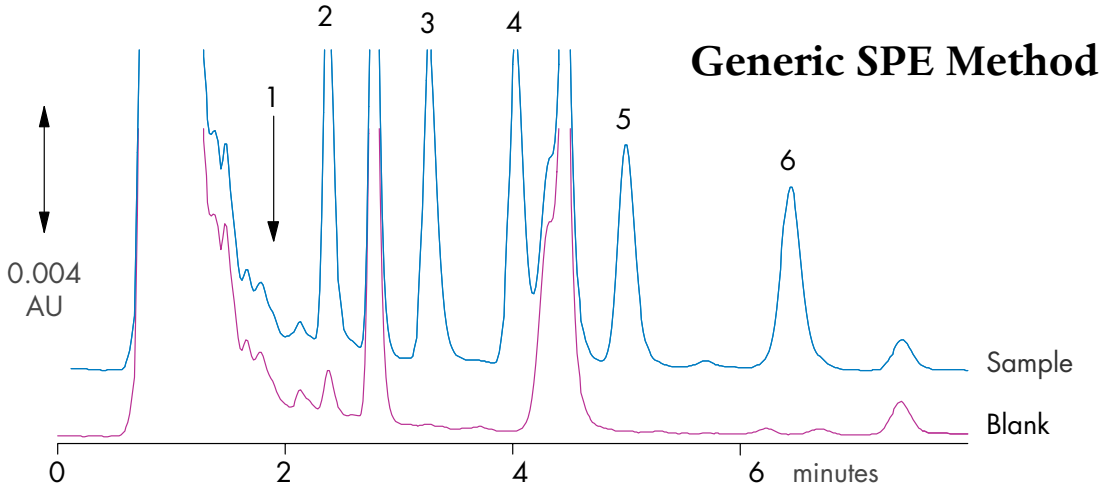
Acidic Analytes	Basic Analytes
Wash 2	*Wash 2*
Acid in Methanol/Water	Base in Methanol/Water
▼*	▼*
Elute	*Elute*
Base in Methanol/Water	Acid in Methanol/Water

*Additional washes may be required

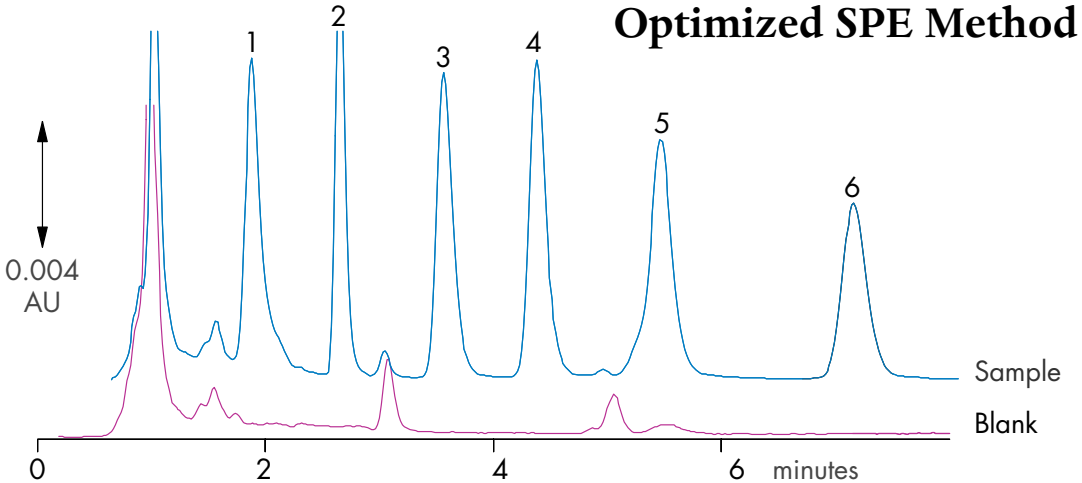
Details of Optimized Method

***Wash 1*:** 2% NH_4OH in 5% MeOH
***Wash 2*:** 2% NH_4OH in 65% MeOH
***Wash 3*:** 2% HOAc in 5% MeOH
***Elute*:** 65% MeOH in water or omit Wash 3 & elute with 2% HOAc in 65% MeOH

Generic SPE Method



Optimized SPE Method



***Column*:** SymmetryShield™ RP_8, 3.5 µm, 4.6 x 75 mm ***Detection*:** UV at 254 nm
***Mobile Phase*:** 50 mM phosphate, pH 7: MeOH (33.6:66.4); 1.4 mL/min
***Plasma Extracts Spiked at 500 ng/mL*:** 140 µL (Generic) or 110 µL (Optimized) injected

1. Nordoxepin
2. Nortriptyline
3. Doxepin (IS)
4. Imipramine
5. Amitriptyline
6. Trimipramine

More Details in [38-39]

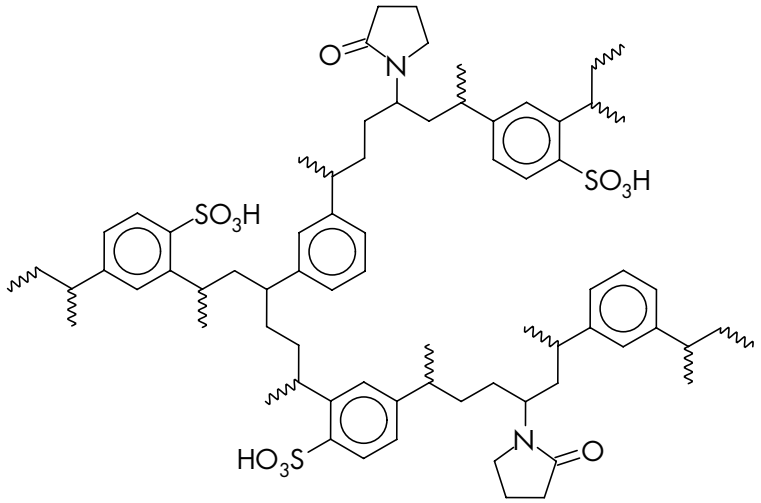
New Selectivity – MCX

SPE: Citius, Altius, Fortius; ISC'98, Roma, Poster #112.

Oasis® MCX sorbent⋆:

Mixed-mode Cation eXchange & reversed-phase

- Water-wettable copolymer
- No silanols to complicate retention mode or method development
- Stable from pH 1 to 14 & in organic solvents
- Selective retention of basic drugs by a single type of cation exchange group.
- All the benefits of HLB – clean, reproducible batch-to-batch – with a new hook.



Sulfonation of poly(divinylbenzene-co-N-vinylpyrrolidone) is done at a tightly controlled level of 1.0 meq/gram, producing a unique strong cation exchange sorbent.⋆

⋆patent pending

MCX Performance

SPE Method for Urine

Load
3 mL spiked, acidified urine

Wash 1
2 mL 0.1 N HCl

Wash 2
2 mL MeOH

Wash 3 (optional)
2 mL 5% NH_4OH in 60% MeOH

Elute
2 mL 5% NH_4OH in MeOH

Evaporate & Reconstitute
300 µL 20% MeOH

Results for Methadone

		Oasis® MCX 60 mg/3 cc Cartridge		C_8/SCX 300 mg/ 3 cc	
Compound	*[µg/mL]*	*% Recovery*	*% RSD*	*% Recovery*	*% RSD*
Methadone	0.5	97.2	0.3	53.8	2.4
Methadone metabolite [EDDP]	0.2	93.2	0.7	55.9	3.2
Propranolol	0.4	97.7	0.5	88.7	3.2

HPLC Conditions for Methadone & metabolite:

Column: SymmetryShield™ RP18, 5 µm, 3.9 x 150 mm
Mobile Phase: 0.1% TFA/MeOH 60/40 (v/v)
Flow Rate: 1.0 mL/min
Detection: UV at 210 nm
Injection volume: 100 µL
Temperature: 30° C.
Internal Standard: Estazolam

SPE *Quo Vadis?*

CITIUS

- Further miniaturization to marry SPE to specific, sensitive detectors for rapid analysis.

ALTIUS

- Smaller means higher sample throughput, &, inevitably, a higher level of automation.

FORTIUS

- Stronger, more capable chemistries for selective isolation &/or affinity/chemical conversion/complexation, all to enhance detection sensitivity & specificity.

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* To get a PDF copy of these references, go to www.waters.com, click on "Applications", then on "Waters Applications Library". Enter number indicated here in red into the search criteria form, & click on the "Search" button.

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Amici Gratias

Thanks – to my past & present colleagues & coinventors whose names appear on the patents & references cited herein, & especially to three individuals⋆ pictured here who have worked with me on SPE activities since January, 1978! – for your great vision, skill, creativity, dedication & friendship – semper fidelis!

Bibliography & Database Team

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