DEVELOPMENT OF AFFINITY MICROCOLUMNS FOR HIGH-THROUGHPUT BIOINTERACTION ANALYSIS

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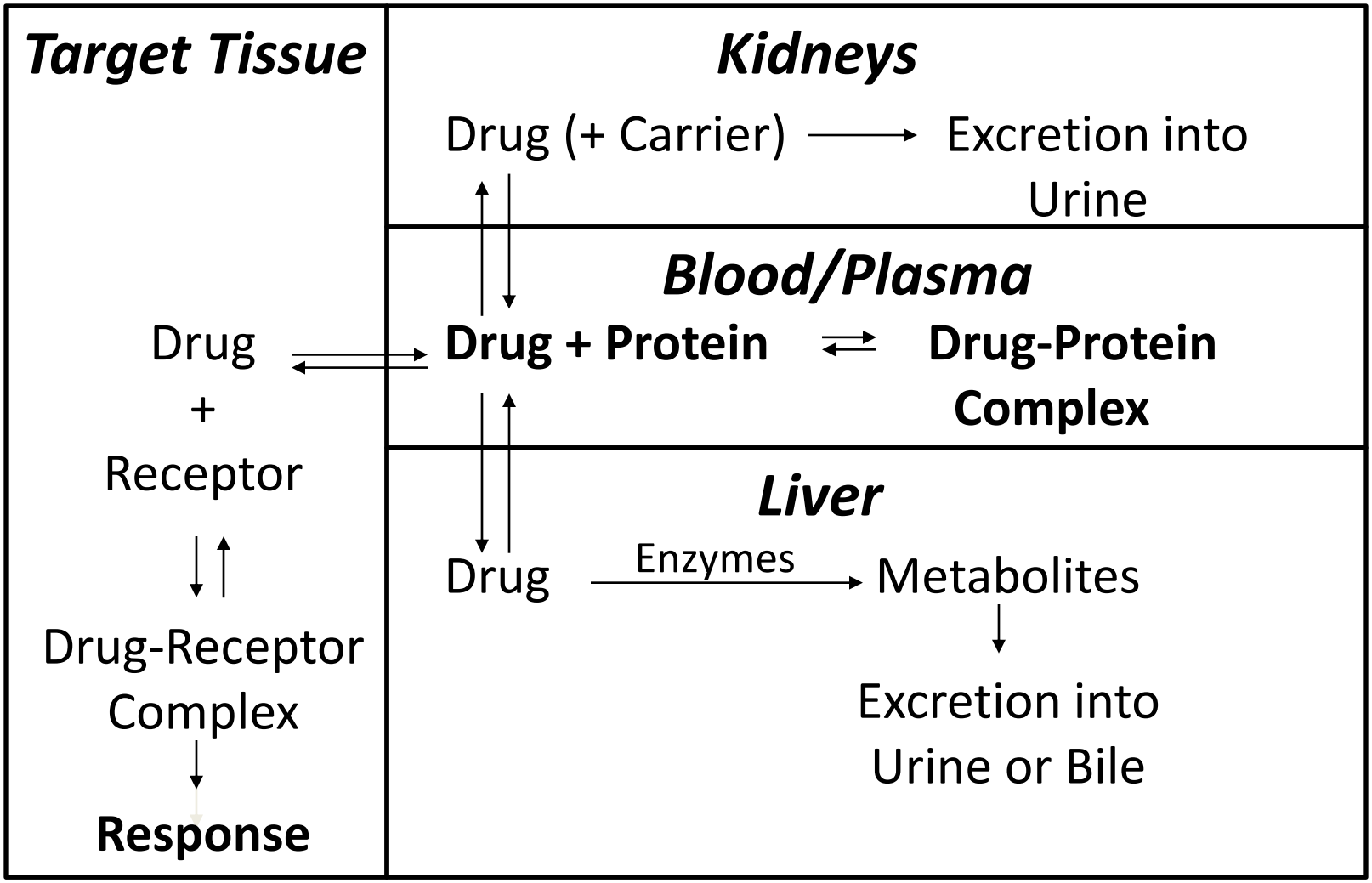
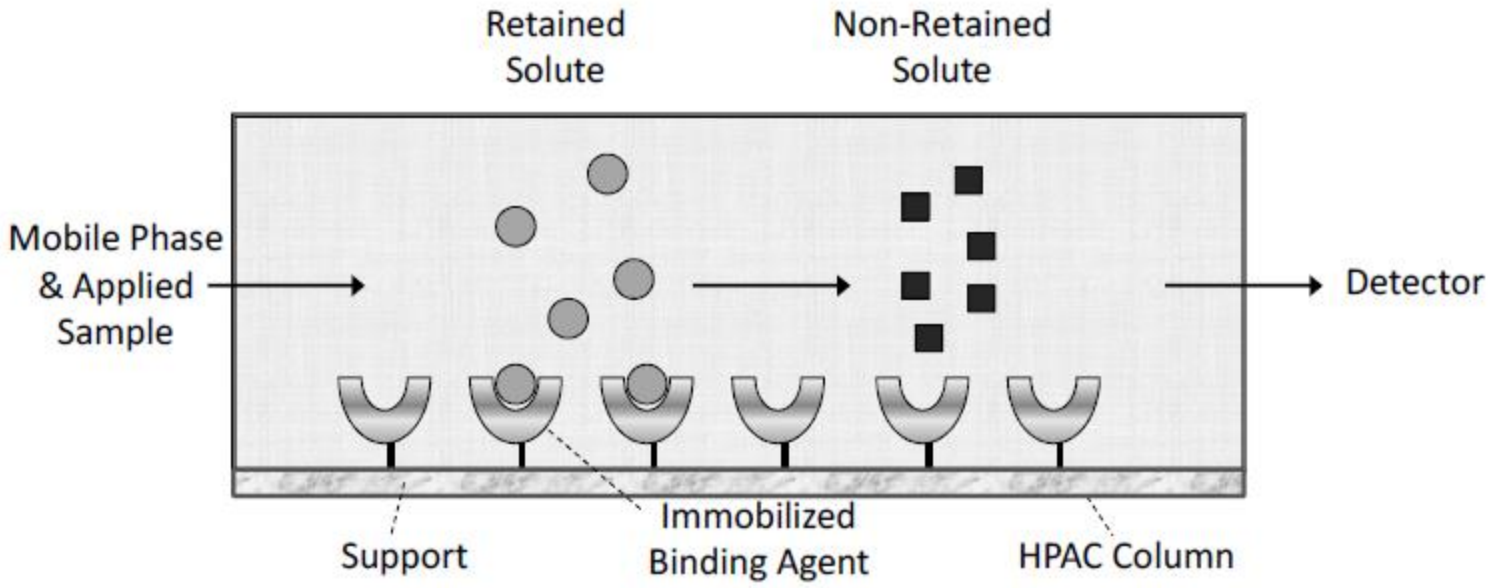
European Bioanalysis Forum 2011
17 November 2011

Drug-Protein Interactions



Hage, D.S. et al. *J. Sep. Sci.* **2009**, *32,* 835-853.

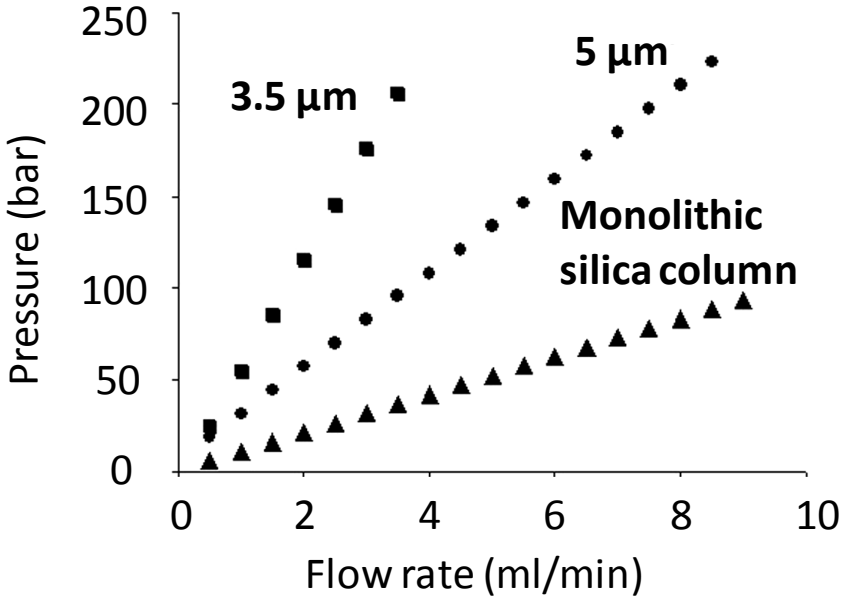
High-Performance Affinity Chromatography (HPAC)

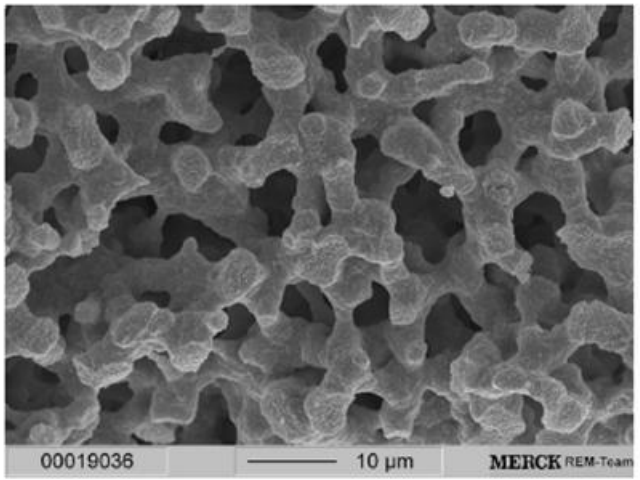


Hage, D.S. et al. *Curr. Drug Metab.* **2011**, *12,* 313-328.

Monolithic Supports

- Higher external porosity than particle-based supports
  - Higher permeability
  - Lower backpressures
  - Better efficiency
- Two types of pores
  - Flow-through pores (macropores)
  - Smaller “diffusion” pores (mesopores)





Cabrera, K. *J. Sep. Sci.* **2004**, *27,* 843-852.
Mallik, R.; Hage D.S. *J. Sep. Sci.* **2006**, *29,* 1686-1704.

Silica Particles vs. Silica Monoliths

Silica Particles

- ❑ Good mechanical strength
- ❑ High surface area
- ❑ Total porosity ~65%

Silica Monoliths

- ☑ Single piece of porous silica
- ☑ Total porosity ~80%
- ☑ Lower backpressure
- ☑ Better mass transfer
- ☑ Faster separations
- ✖ Shrinkage

from www.phenomenex.com

Cabrera, K. *J. Sep. Sci.* **2004**, *27,* 843-852.
Mallik, R.; Hage D.S. *J. Sep. Sci.* **2006**, *29,* 1686-1704.

Affinity Microcolumns

Advantages

- Require small amounts of ligand
- Reduced surface area, less non-specific binding
- Reduced analysis times

Challenges

- Need new approaches for preparing and packing columns
- Need new immobilization methods to increase the activity of ligand
- Need better understanding of properties and limitations

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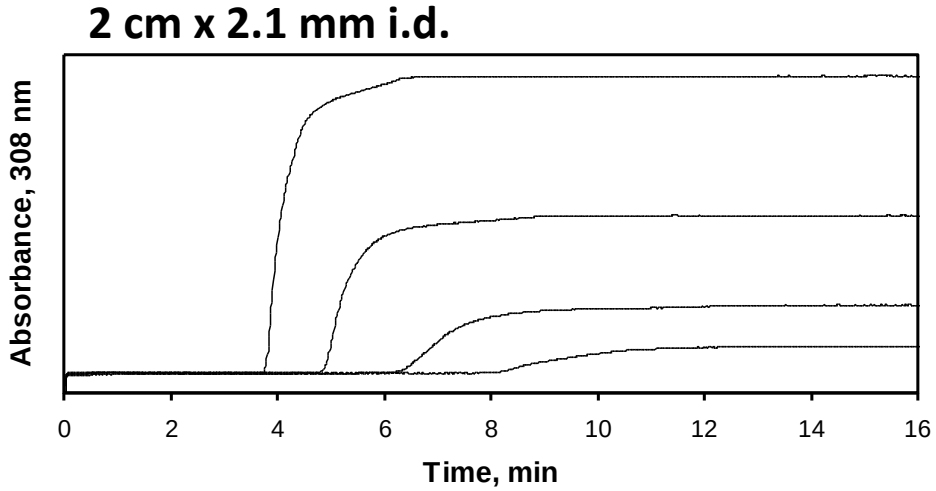
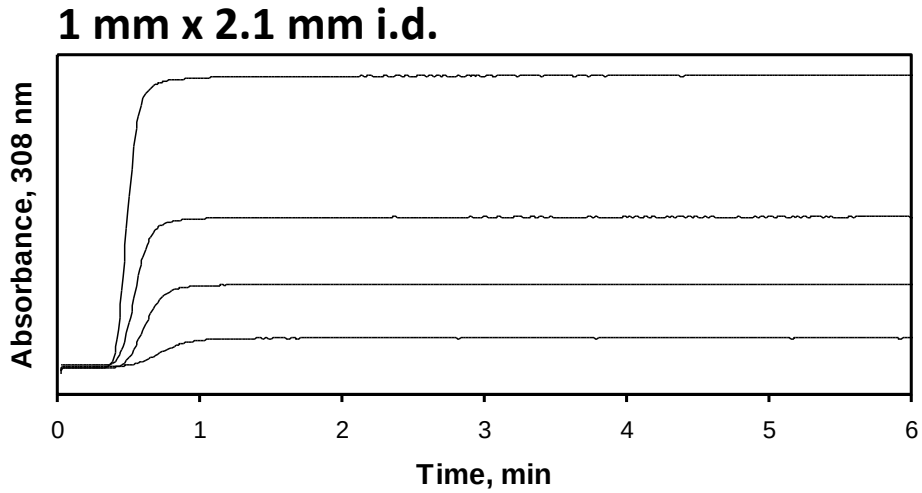
Frontal Analysis Measurements



- Breakthrough times: 4.2-9.5 min
- Breakthrough volume: 2.8 mL



- Breakthrough times: 30-45 s
- Breakthrough volume: 0.29 mL

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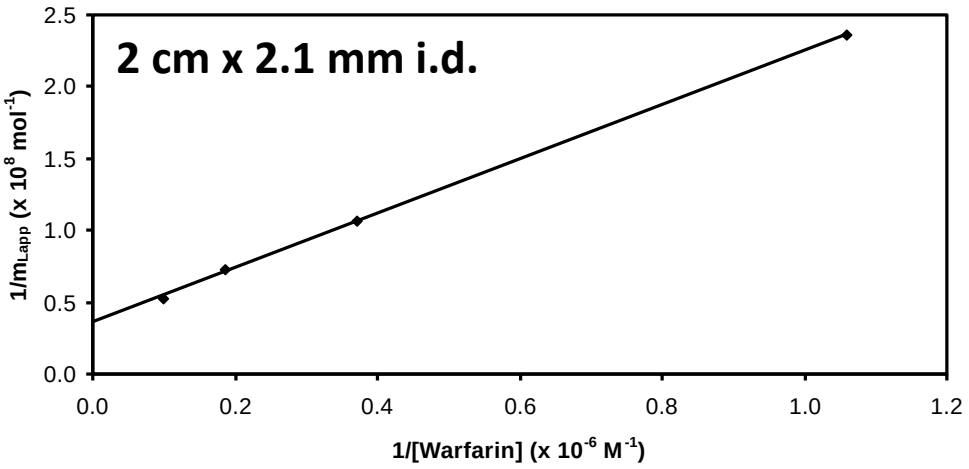
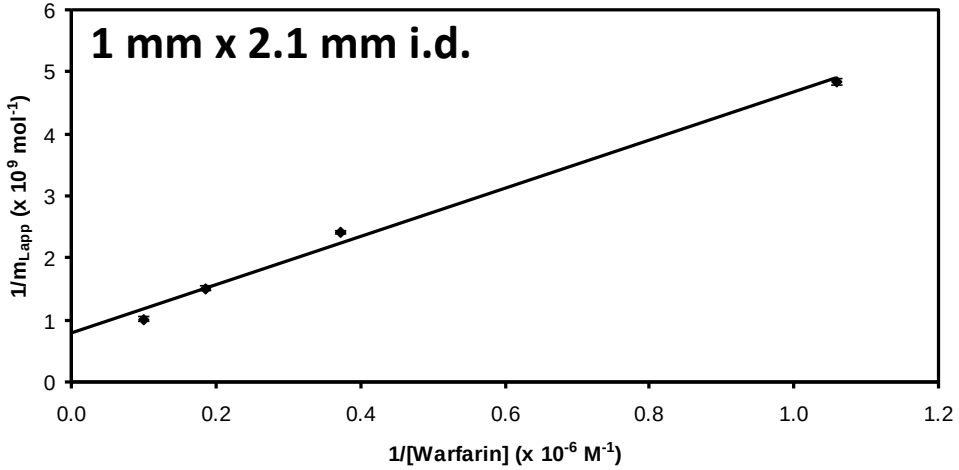
Frontal Analysis Measurements





$$\frac{1}{m_{\mathrm{Lapp}}} = \frac{1}{K_A m_L [A]} + \frac{1}{m_L}$$

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Frontal Analysis Results

Column Length (mm)	m_L (nmol)	Relative Activity (versus 20 mm column)	K_a (x 10^5 M^{-1})
20	27.6 (± 0.2)	1.00	2.0 (± 0.1)
3	3.8 (± 0.3)	0.9 (± 0.1)	2.3 (± 0.1)
2	2.6 (± 0.3)	0.9 (± 0.1)	2.1 (± 0.2)
1	1.2 (± 0.1)	0.8 (± 0.1)	2.6 (± 0.1)

Literature K_a values: 2.1 – 2.6 x 10^5 M^{-1}

- Binding capacity decreased in proportion to column size
- Decrease in precision in K_a values (2 cm: ±5% , 1 mm: ±25%) due to decrease in amount of time and stationary phase available for drug binding

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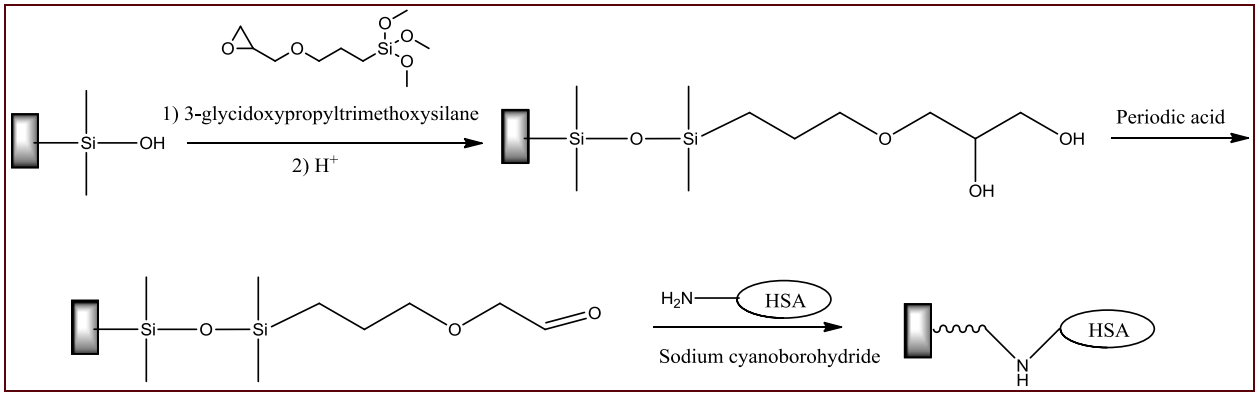
Preparation of HSA Silica Monoliths

- Chromolith™ Performance Si column was donated by Merck KGaA (4.6 mm i.d. x 10 cm)

1. Columns cut to lengths from 1 to 5 mm
2. Converted into diol silica monolith
3. Immobilized HSA by Schiff base method



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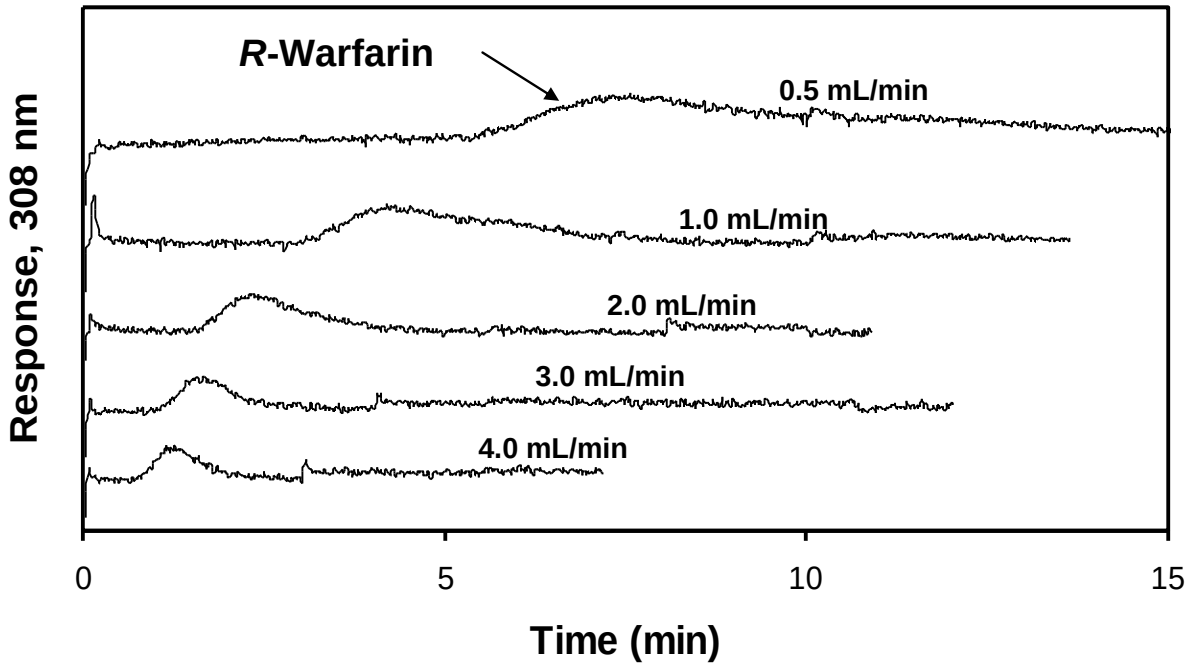
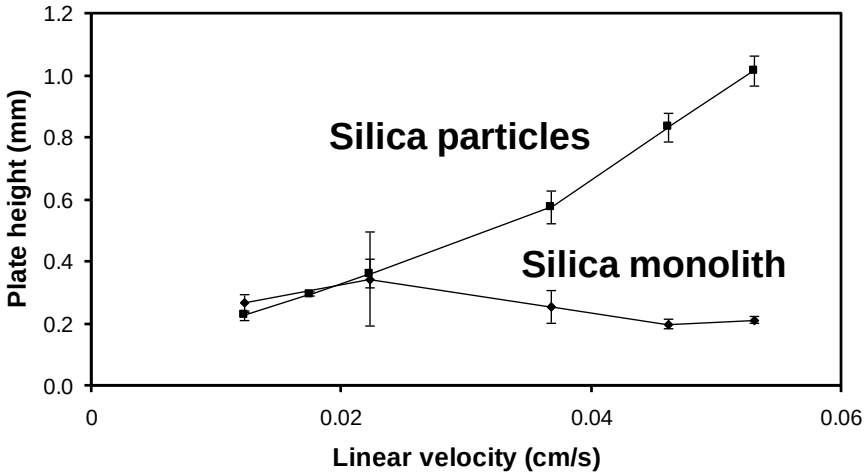
Warfarin Retention on HSA Silica Monoliths

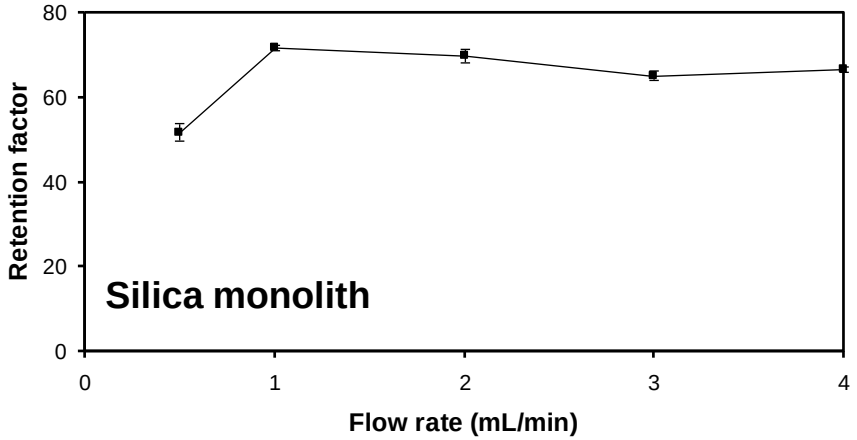
- Longer HSA columns (4-10 cm): 25-150 min
- HSA silica monolith microcolumn (3 mm): 1.4-9.5 min

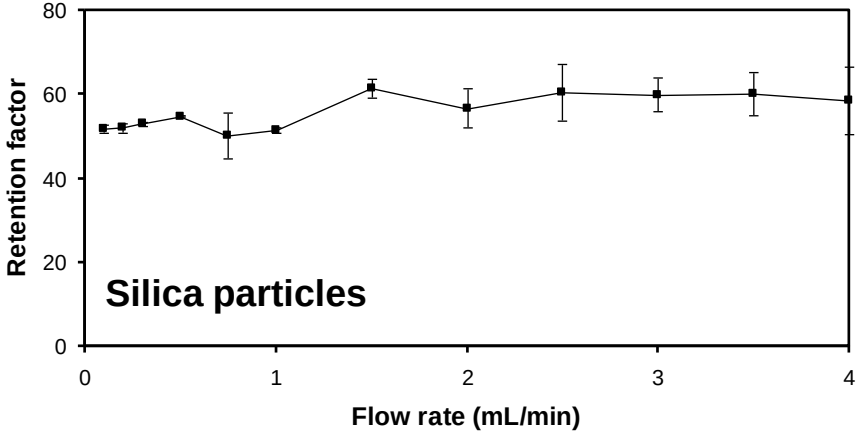


Yoo, M.J.; Hage, D.S. *J. Sep. Sci.,* **2009,** *32,* 2776-2785.

Silica Particles vs. Silica Monoliths







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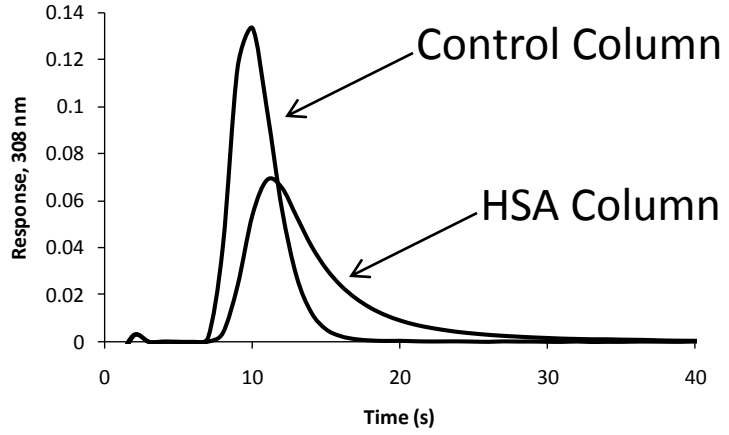
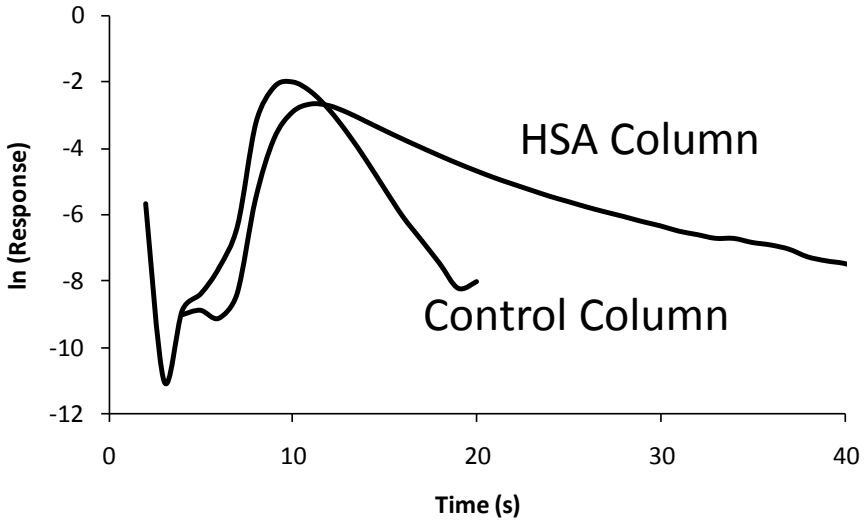
Rapid Determination of Drug-Protein Dissociation Rates





Control Column

- Washing away of non-bound analyte
- Release of any non-specifically bound analyte

HSA Column

- Release of the analyte from the immobilized HSA

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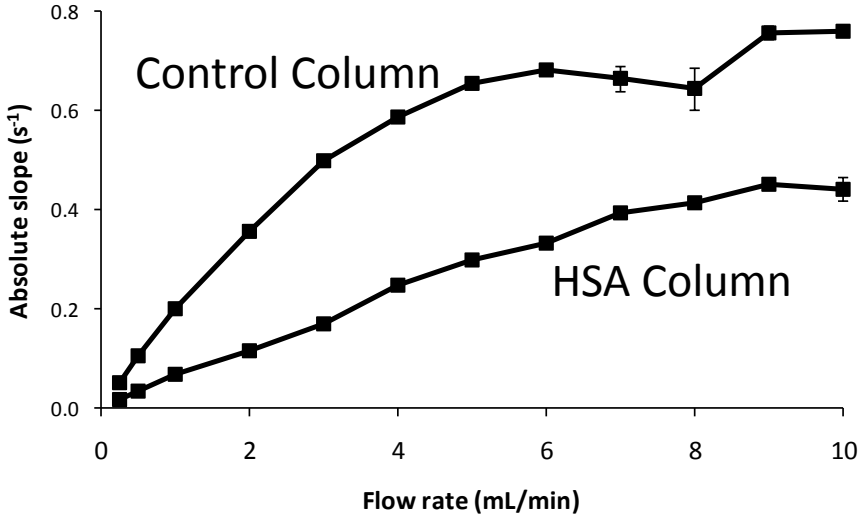
Typical Results

Drug	K_a (M^{-1})	Binding Site on HSA	Measured Value k_d (s^{-1})	Literature Value k_d (s^{-1})
Warfarin	2.1-2.6 x 10^5	I	0.41 (± 0.06)	0.35 - 0.66
Imipramine	1.6 x 10^5	II	0.29 (± 0.11)	0.41 - 0.67
Diazepam	2.2 x 10^5	II	0.44 (± 0.11)	--
Acetohexamide	0.43-1.3 x 10^5	I, II	0.58 (± 0.02)	--
Tolbutamide	5.3-5.5 x 10^4	I, II	0.49 (± 0.15)	--



Same general behavior and types of plots were seen for the other drugs that were examined.

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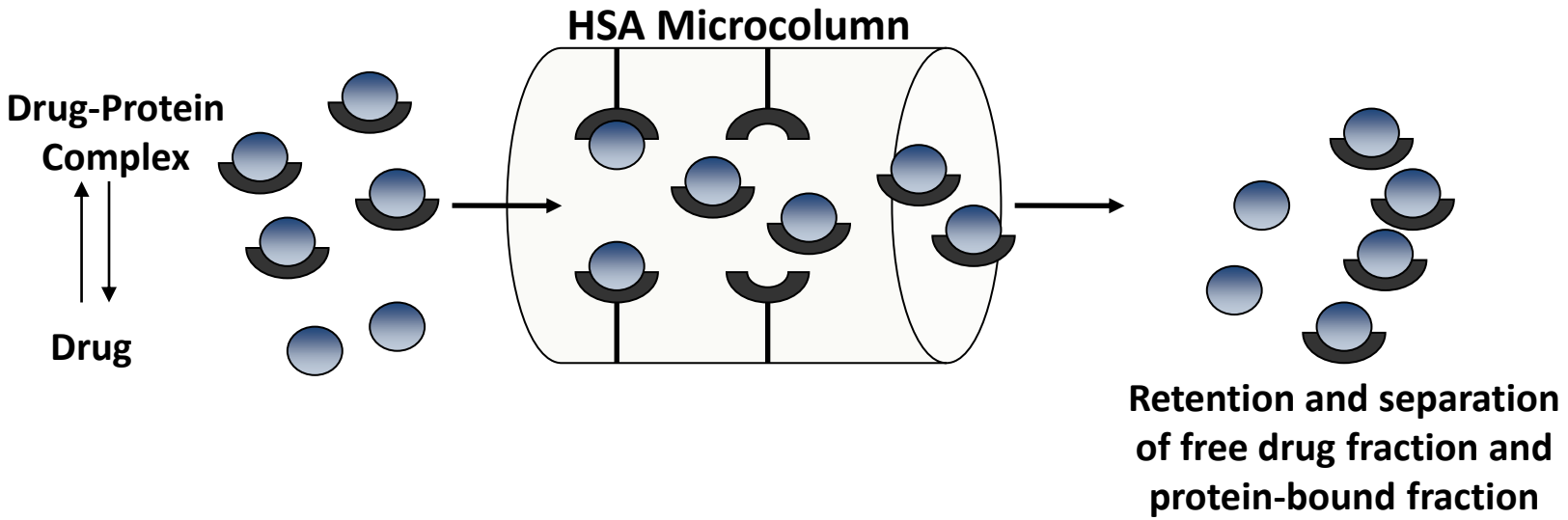
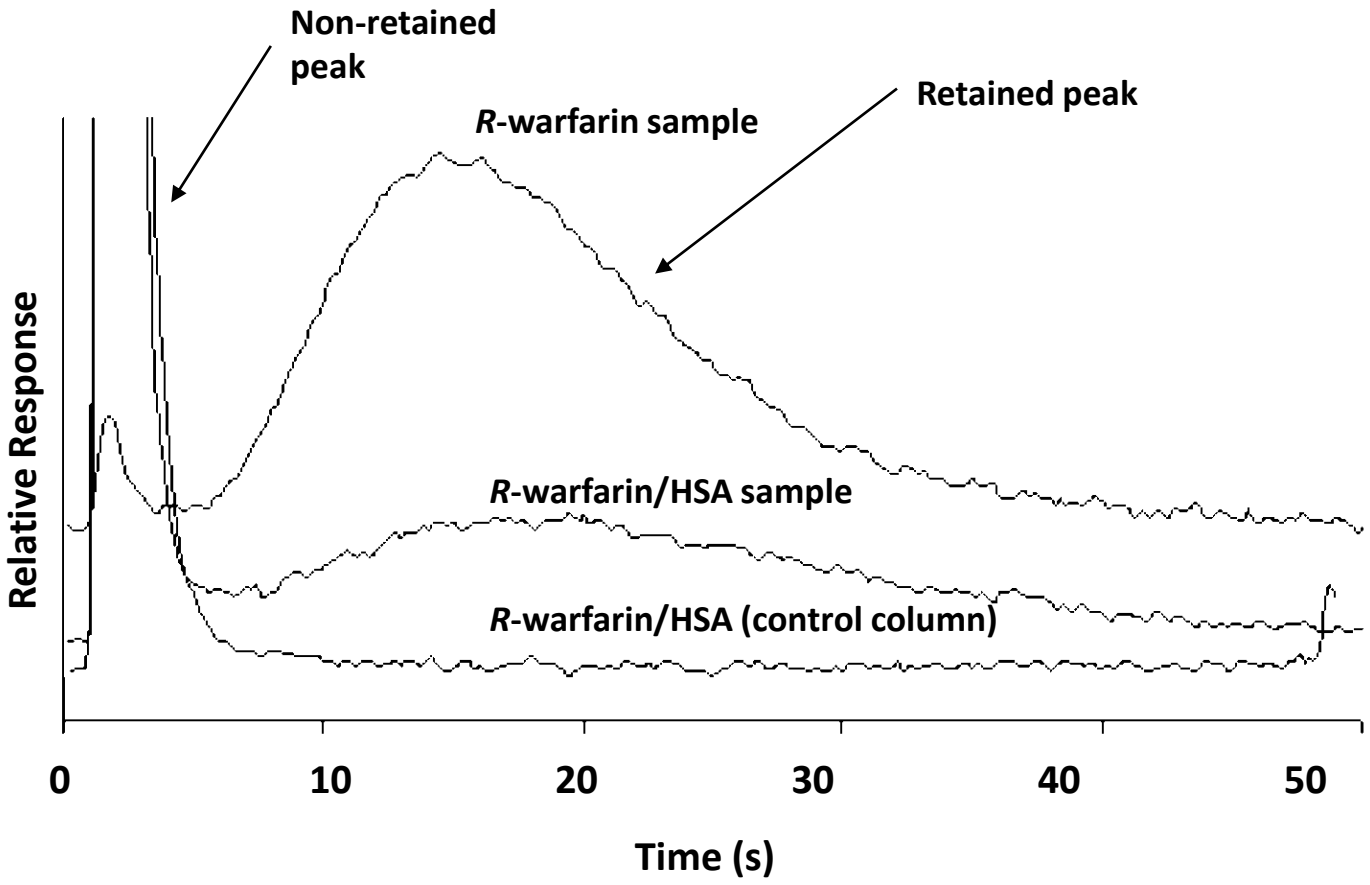
Analysis of Free Drug Fractions



- HSA microcolumns were used in ultrafast extraction and free drug fraction measurements
  - HSA is most abundant serum protein and important binding agent for a variety of drugs-use for more than analyte
  - Lower association equilibrium constants of drugs for HSA (10^3-10^5) versus antibodies (10^6-10^{12}) allows for the use of isocratic conditions
  - Less expensive to perform compared to using columns based on immobilized antibodies

Mallik, R.; Yoo, M.J.; Briscoe, C.J.; Hage, D.S. *J. Chromatogr. A,* **2010,** *1217,* 2796-2803.

Analysis of Free Drug Fractions



Analysis times of only 40 s per injection at 4.5 mL/min

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Analysis of Free Drug Fractions

Immobilization Method and Sample	HSA Microcolumns (Ultrafast Extraction)	Ultrafiltration (Reference Method)
Schiff Base Method		
S-Warfarin + HSA	0.20 (± 0.03)	0.20 (± 0.02)
R-Warfarin + HSA	0.31 (± 0.05)	0.28 (± 0.02)
SMCC Method		
S-Warfarin + HSA	0.23 (± 0.02)	0.21 (± 0.03)
S-Ibuprofen + HSA	0.28 (± 0.02)	0.25 (± 0.07)
Imipramine + HSA	0.92 (± 0.02)	0.86 (± 0.07)

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Summary

- Affinity microcolumns provide comparable results to those obtained with longer columns and can be used in rapid analysis of drug-protein binding
  - Smaller amount of protein (low nmol to upper pmol) needed for column preparation
  - Faster analysis times (net retention times are 100 times faster than traditional 10 cm column)
  - Affinity silica monolith microcolumns have better efficiency (higher flow rates, larger number of theoretical plates)
- Ultrafast extraction based on HSA microcolumns can be used for measuring the free fractions of some drugs in drug-protein mixtures.
  - Good agreement with reference method
  - Fast analysis times (40 s per injection)

References

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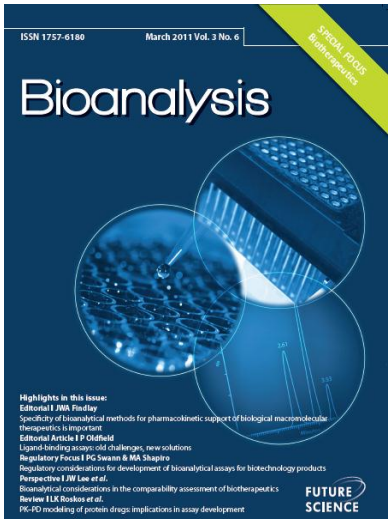
Acknowledgements

- Bioanalysis editorial board and readers
- Waters Corporation
- Prof. David S. Hage
- National Institutes of Health (R01 GM044931)
- Merck KGaA



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