

High Throughput Enantiospecific Separations Using Multiplexed Capillary Electrophoresis with Absorbance Detection

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Outline

- Chiral Capillary Electrophoresis (CE)
- Multiplexed, 96-Capillary Electrophoresis with UV Absorbance Detection (Multiplexed CE-UV)
- Applications of Multiplexed CE-UV in Chiral Analysis
 - Combinatorial Selector Screening
 - Parallel Chiral Separations
- Assessment of Capillary-to-Capillary Reproducibility
- Detection of Low-Level Impurities
- Summary

Chiral Separations by Capillary Electrophoresis (CE)

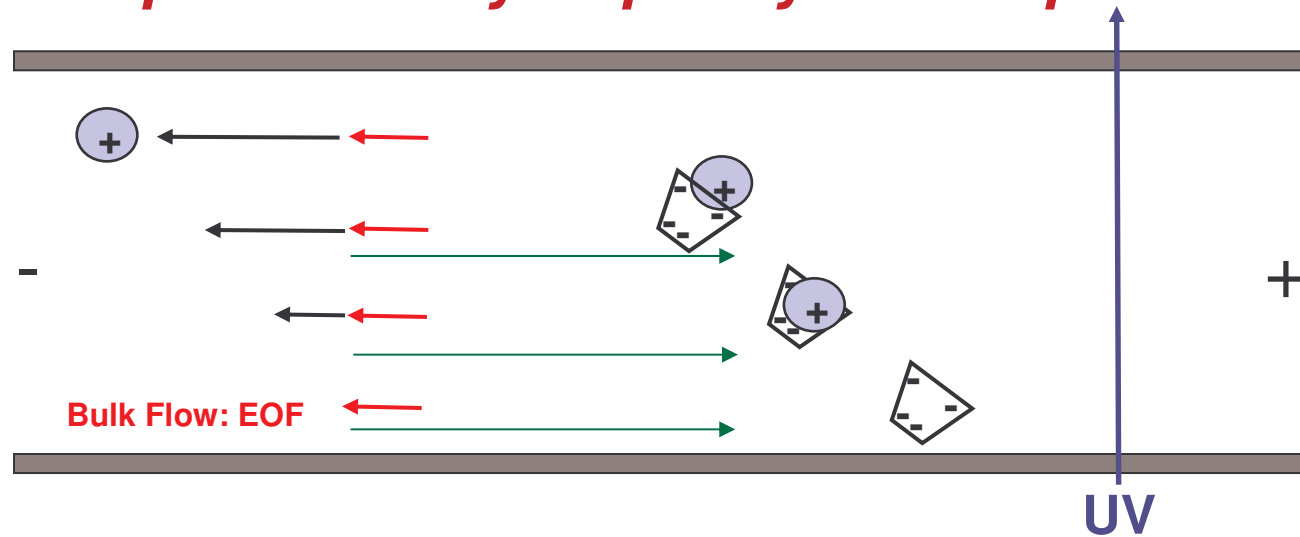
Capillary Zone Electrophoresis

- Application of high voltage (5 kV – 30 kV) across a narrow bore (50 –75 μm i.d.) bare fused silica capillary filled with conductive aqueous-based buffer
- Separation is achieved by overall differences in analyte charge/mass ratios, giving rise to different analyte velocities

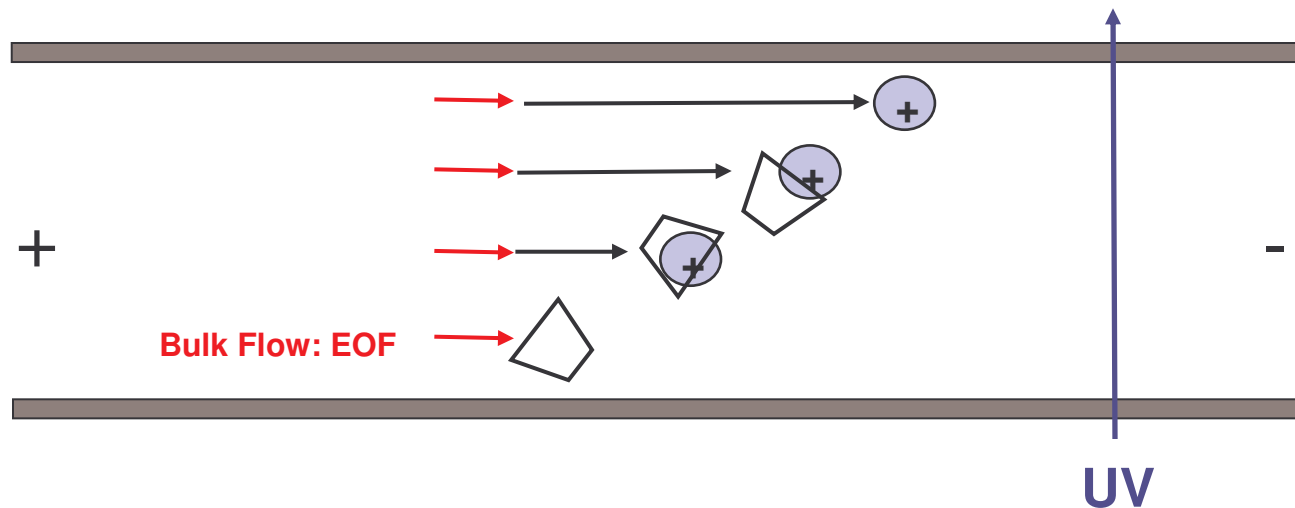
Chiral CE

- Chiral selectors (e.g., cyclodextrins (CDs)) can be added directly to the run buffer, leading to the formation of transient diastereomeric complexes with analytes
- Chiral separation is achieved by either differences in the selector affinity between enantiomers or differences in mobility of the diastereomeric complexes
- Development of chiral separation methods often involves optimization of selector type, selector concentration, different mixtures of selectors, buffer pH, or buffer concentration

Chiral Separations by Capillary Electrophoresis (CE)



1. Low pH, negative polarity: Sulfated CDs have mobility to detector, interact with positively charged and neutral compounds, imparting mobility to detector.



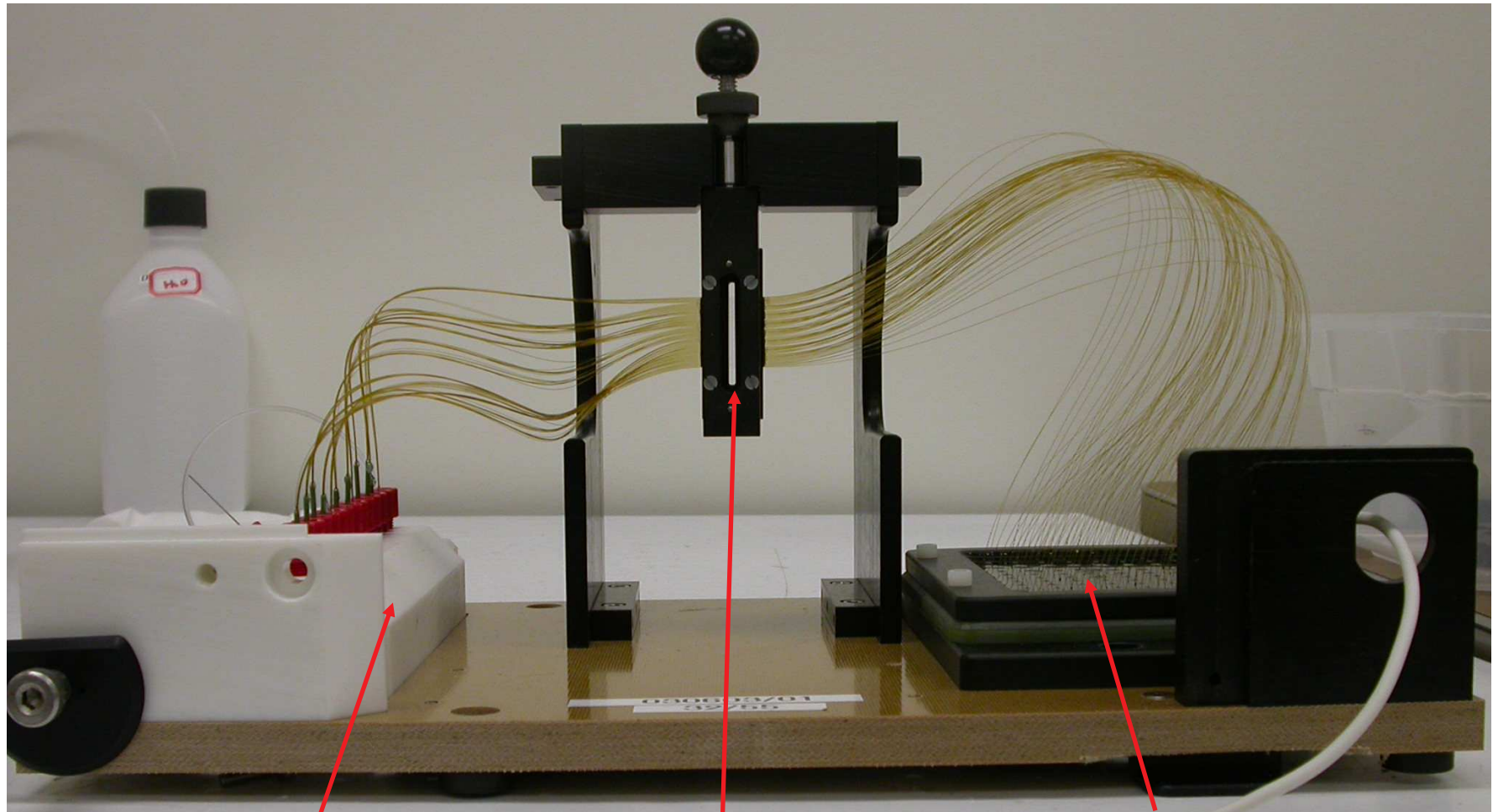
2. Low pH, positive polarity: Neutral CD moves with EOF. Neutral CDs interact with positively charged compounds, slowing their mobility towards detector.

cePRO 9600™ 96-Capillary Multiplexed CE-UV System



- 96-capillary array CE instrument with fixed wavelength UV detection
- Unattended analysis of two 96-well sample plates
- Robotic interfacing capabilities
- For chiral separations, additional capillary cooling was supplied by ducting cold air (water chilled to 4° C) across the capillary array

Capillary Array Cartridge (Viewed from Lamp Position)



Outlet Reservoir

Detection Window

Inlet Plate (96-Well Format)

- Chiral application uses 50 μm i.d., 200 μm o.d. capillaries to minimize CE current

Advantages of Multiplexed CE-UV for Drug Discovery

- **Simultaneous monitoring** of up to 96 individual CE separations
- **Low UV wavelength** (214 nm) provides more universal analyte detection
- **Multiple applications** (e.g., pK_a, log P, purity, chiral screening, drug analysis) can be performed with minimal changeover time
- Requires only **small quantities** of sample and buffer additives
- **Tolerant to sample impurities** (CE is separation technique)
- Variation of buffer conditions (e.g., pH, ionic strength, buffer additives, additive concentrations) in different capillaries can significantly **accelerate methods development**

Example:

26 Different CD Derivatives vs. 35 Compounds = > 900 Experiments!

Vescina, M.C.; Fermier, A.M.; Guo, Y. *J. Chromatogr. A* **2002**, *973*, 187-196.

Experimental Design: 8 Compounds, 4 Chiral Selectors

Sample Plate

Position	1	2	3	4	5	6	7	8	9	10	11	12
A	Atenolol											
B	Alprenolol											
C	<i>p</i> -Chloroamphetamine											
D	Isoproterenol											
E	Metaproterenol											
F	Terbutaline											
G	Nefopam											
H	Warfarin											

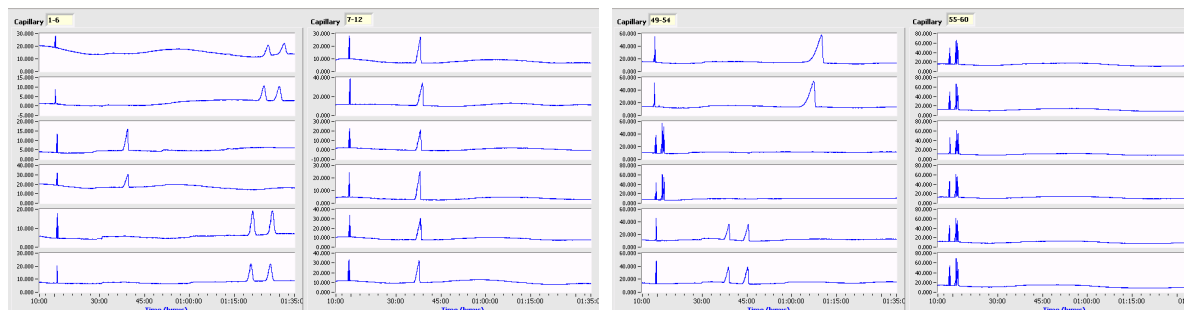
Buffer Plate

Position	1	2	3	4	5	6	7	8	9	10	11	12
A	5% HS- α -CD		5% HS- β -CD		5% HS- γ -CD		5% S- β -CD					
B												
C												
D												
E												
F												
G												
H												

- Up to 96 different experimental conditions can be evaluated simultaneously

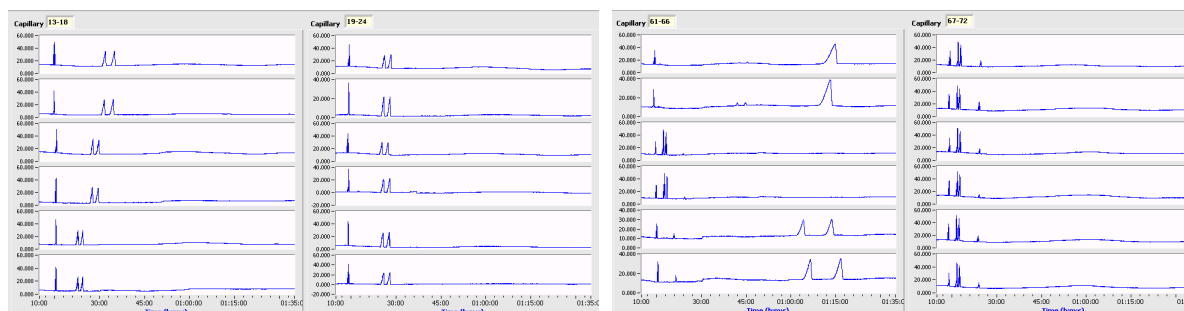
96-Capillary CE-UV: 8 Compounds, 4 Different Selectors

Atenolol



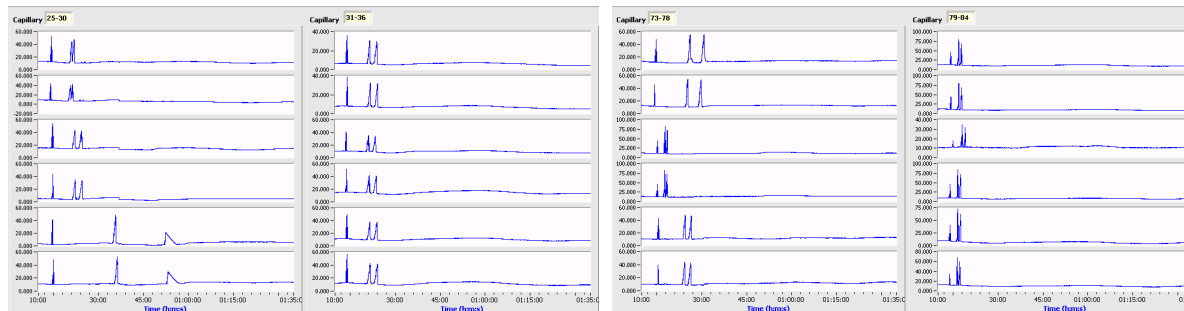
Metaproterenol

Alprenolol



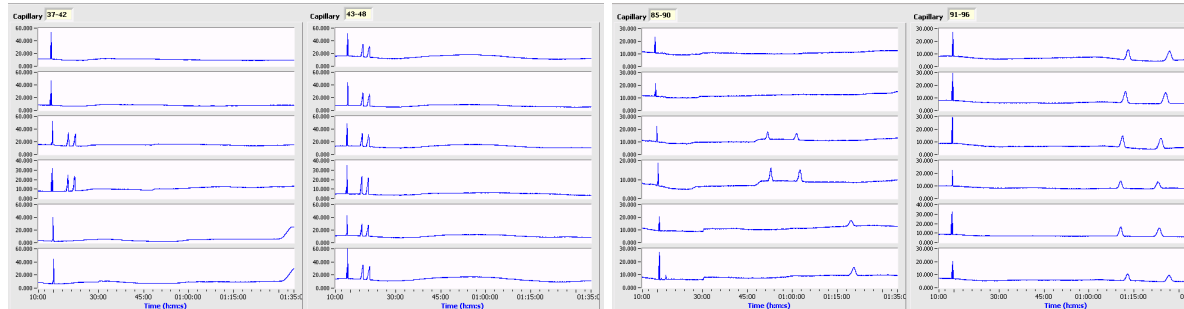
Terbutaline

p-Chloroamphetamine



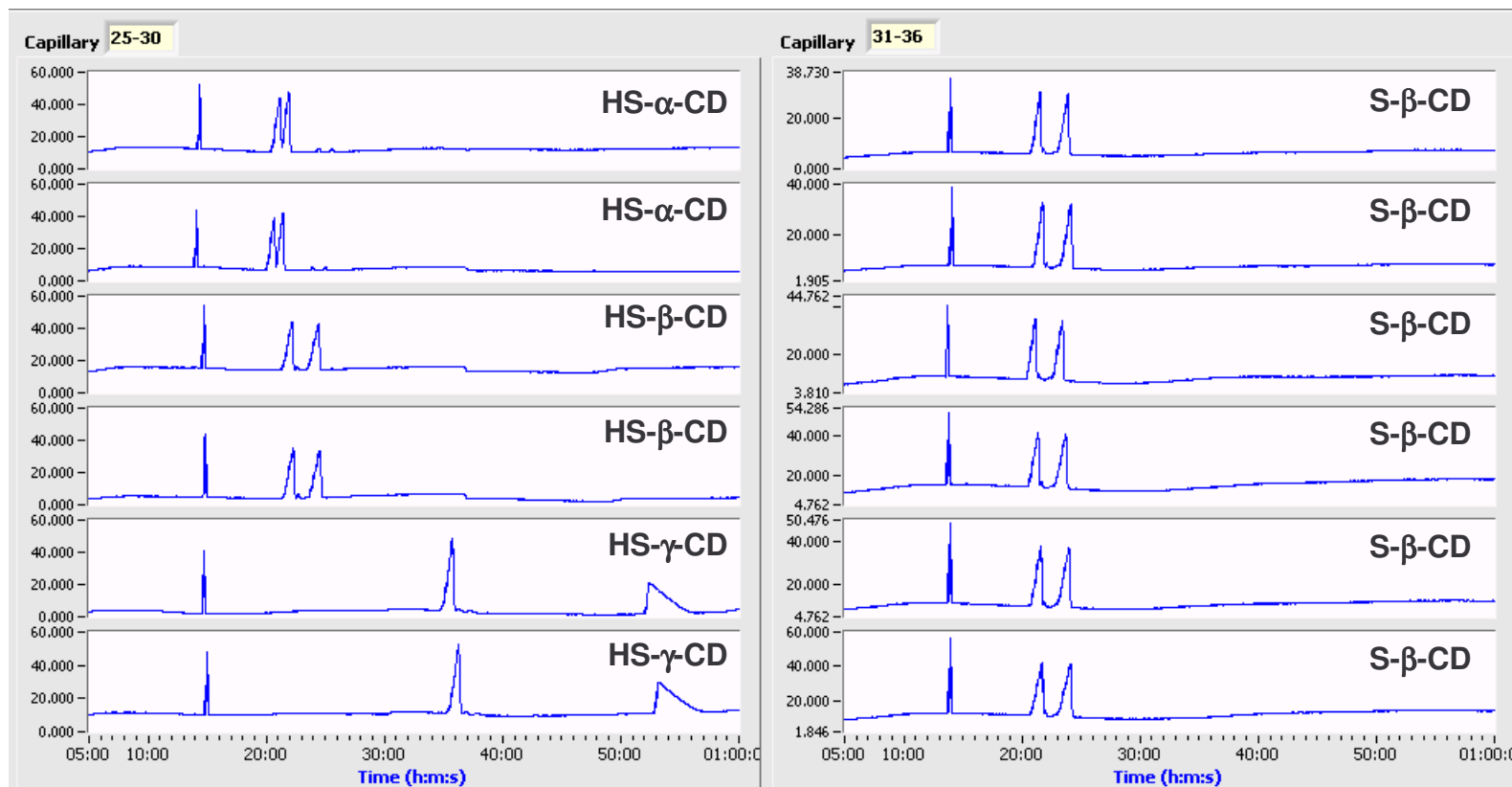
Nefopam

Isoproterenol



Warfarin

Chiral Selector Screening Results for *p*-Chloroamphetamine



	Selector	R_s	Migration Time (min)
<i>p</i> -Chloroamphetamine	HS- α -CD	0.89	23
	HS- β -CD	1.76	26
	HS- γ -CD	5.64	60
	S- β -CD	2.09	25

- Migration time of HS- γ -CD separation could be reduced by use of vacuum assisted CE

Summary of Chiral Selector Screening Results

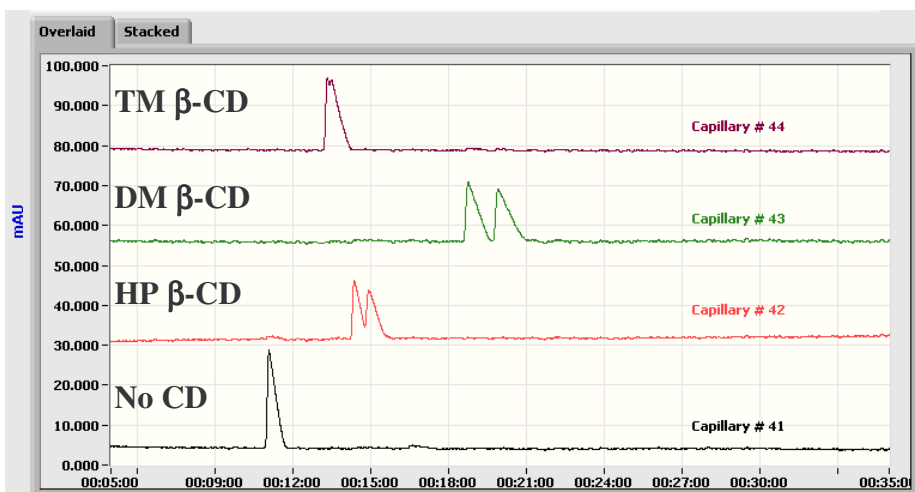
Compound	Selector	Average R_s	Migration Time (min)
Atenolol	HS- α -CD	1.68	95
	HS- β -CD		
	HS- γ -CD	2.07	90
	S- β -CD		
Alprenolol	HS- α -CD	1.88	36
	HS- β -CD	1.42	31
	HS- γ -CD	1.54	25
	S- β -CD	1.59	29
<i>p</i> -Chloroamphetamine	HS- α -CD	0.89	23
	HS- β -CD	1.76	26
	HS- γ -CD	5.64	60
	S- β -CD	2.09	25
Isoproterenol	HS- α -CD		
	HS- β -CD	2.44	24
	HS- γ -CD		
	S- β -CD	2.06	22
Metaproterenol	HS- α -CD		
	HS- β -CD	1.03	18
	HS- γ -CD	2.88	48
	S- β -CD	0.98	18
Terbutaline	HS- α -CD		
	HS- β -CD	1.55	20
	HS- γ -CD	2.79	80
	S- β -CD	1.58	19
Nefopam	HS- α -CD	2.94	34
	HS- β -CD	1.26	20
	HS- γ -CD	1.65	28
	S- β -CD	1.72	19
Warfarin	HS- α -CD		
	HS- β -CD	5.16	75
	HS- γ -CD		
	S- β -CD	4.20	90

Comparison of Multiplexed CE-UV to Single Capillary CE

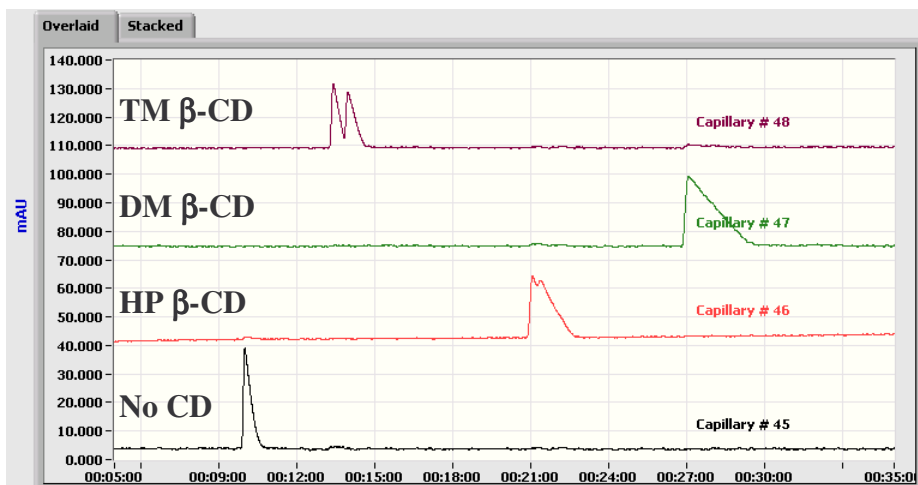
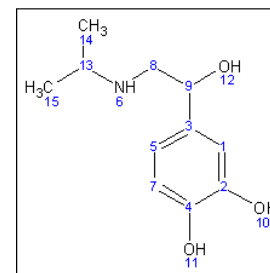
Compound	Selector	Single Capillary R_s	MT (min)	Multiplexed CE Avg. R_s	MT (min)
Atenolol	HS- γ -CD	1.8	10	2.07	90
Alprenolol	HS- α -CD	2.2	5	1.88	36
Isoproterenol	HS- β -CD	2.9	3	2.44	24
Metaproterenol	HS- γ -CD	4.1	6	2.88	48
Terbutaline	HS- γ -CD	5.2	8	2.79	80
Warfarin	HS- β -CD	3.7	5	5.16	75

- In each case, the “optimal” selector (α, β, γ) was correctly determined by multiplexed CE
- Similar resolution could be obtained for several of the compounds by multiplexed CE
- Up to a 10-fold or higher increase in throughput could be obtained without considering any additional time required for flushing steps between runs

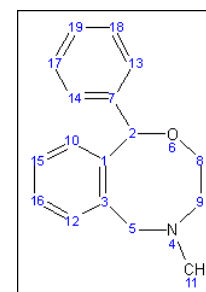
Simultaneous Chiral Selector Screening of Neutral CDs



Isoproterenol

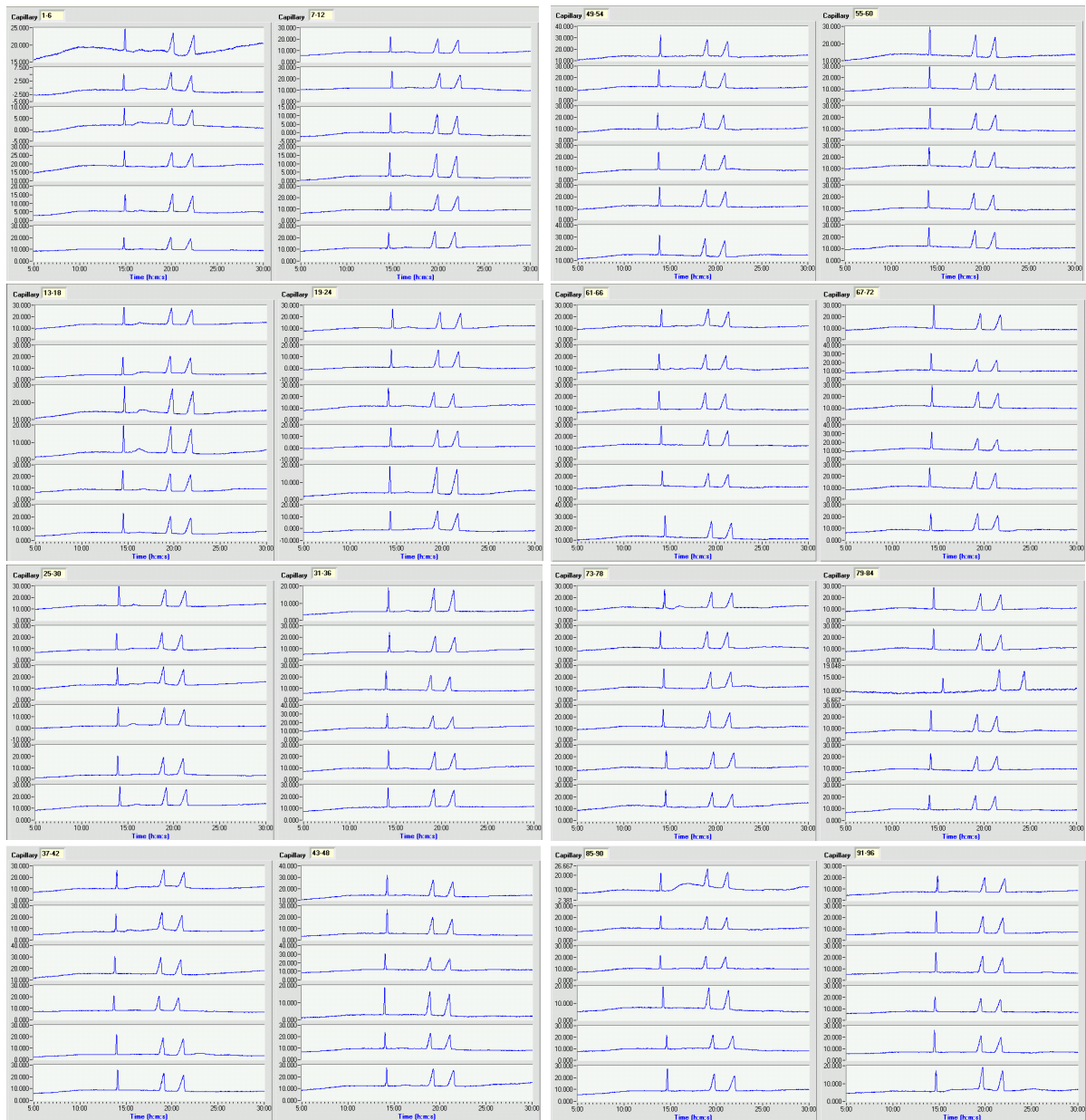


Nefopam



- Background electrolyte: 25 mM H₃PO₄, pH 2.5 + 20 mM cyclodextrin additive
- CE Separation: +12 kV (218 V/cm)

96-Capillary CE-UV: Racemic Mixture of Isoproterenol



PTS Normalized Migration Time

(+) Isoproterenol: 0.52% (n = 96)

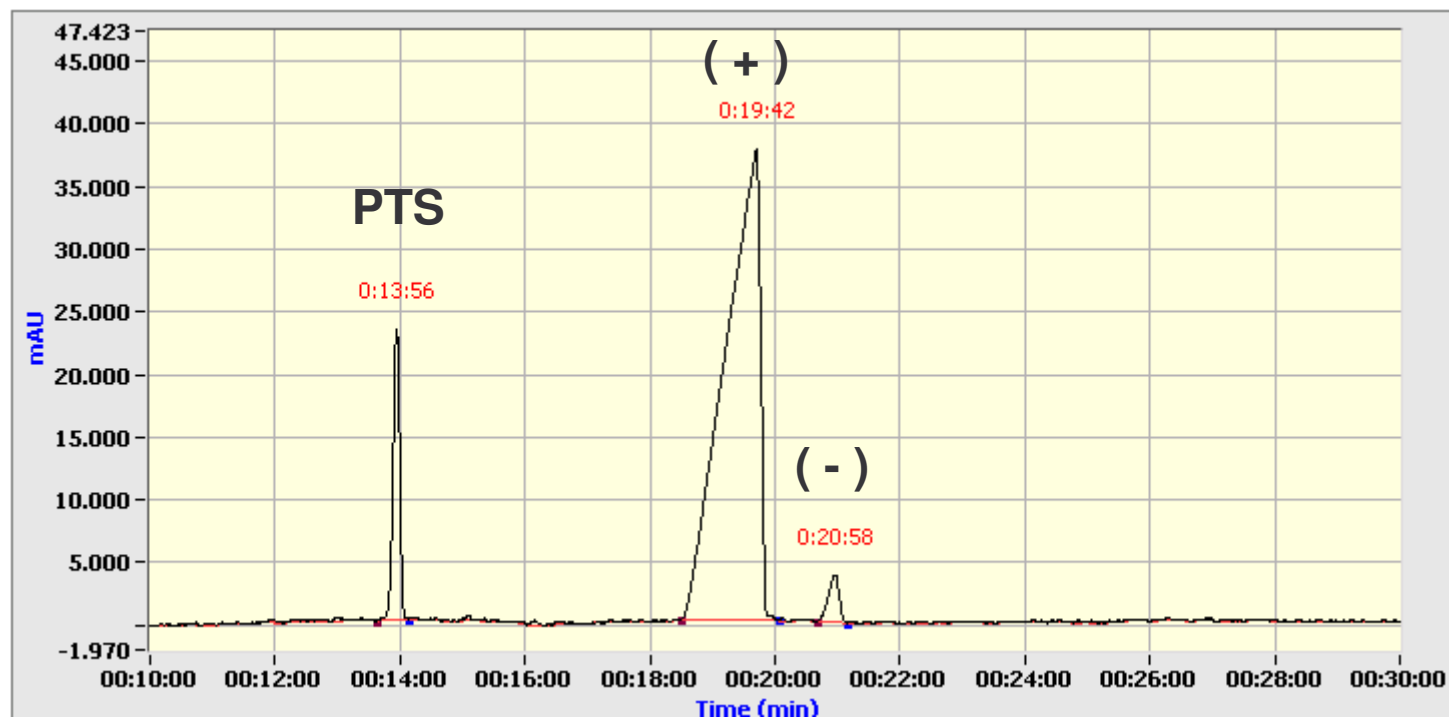
(-) Isoproterenol: 0.72% (n = 96)

(+)/(-) Normalized Peak Area

0.952 ± 0.028 (RSD = 2.68%)

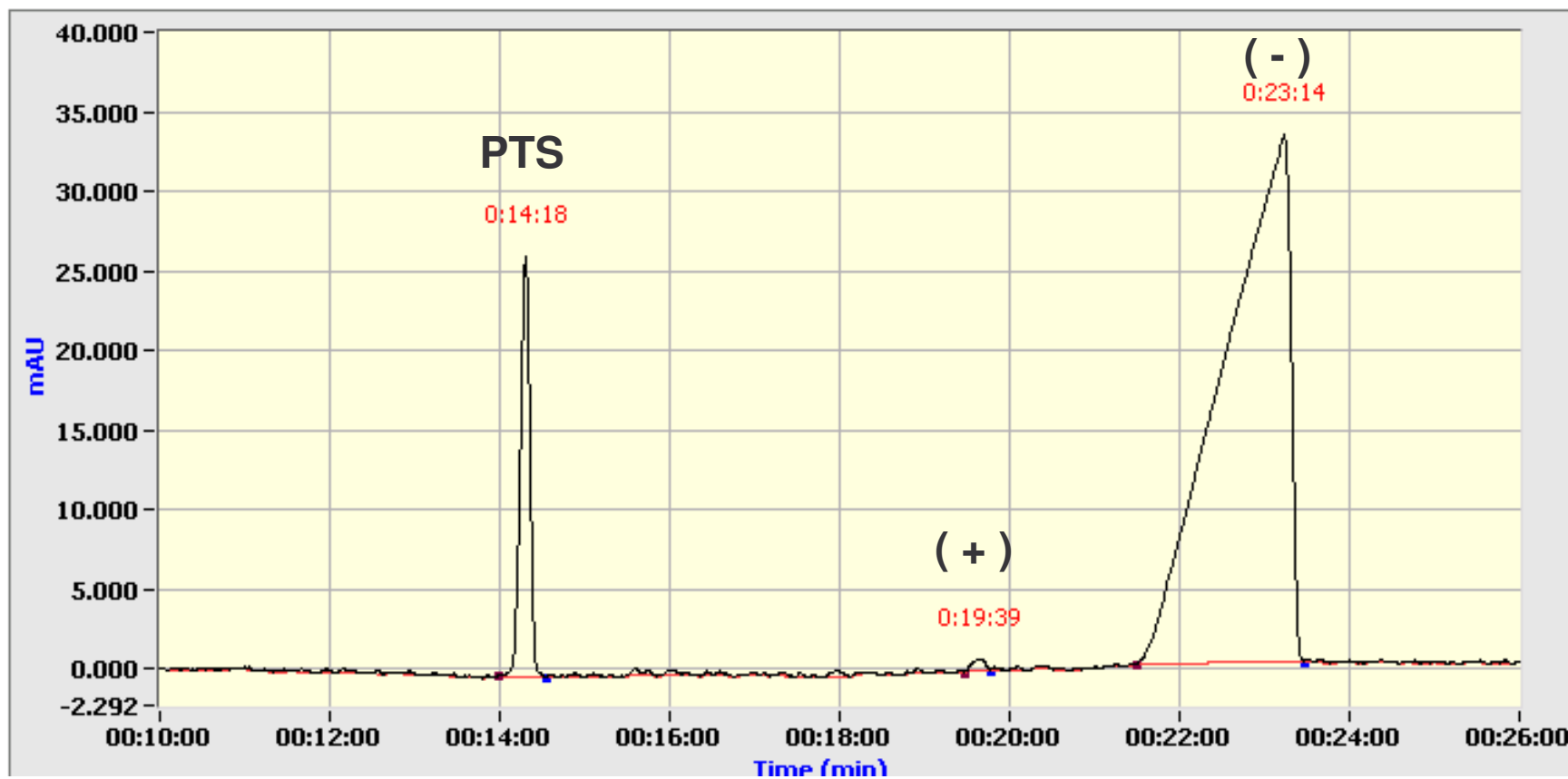
**96 samples analyzed in
< 25 min**

Capillary-to-Capillary Peak Area Reproducibility for Minor Enantiomeric Impurity



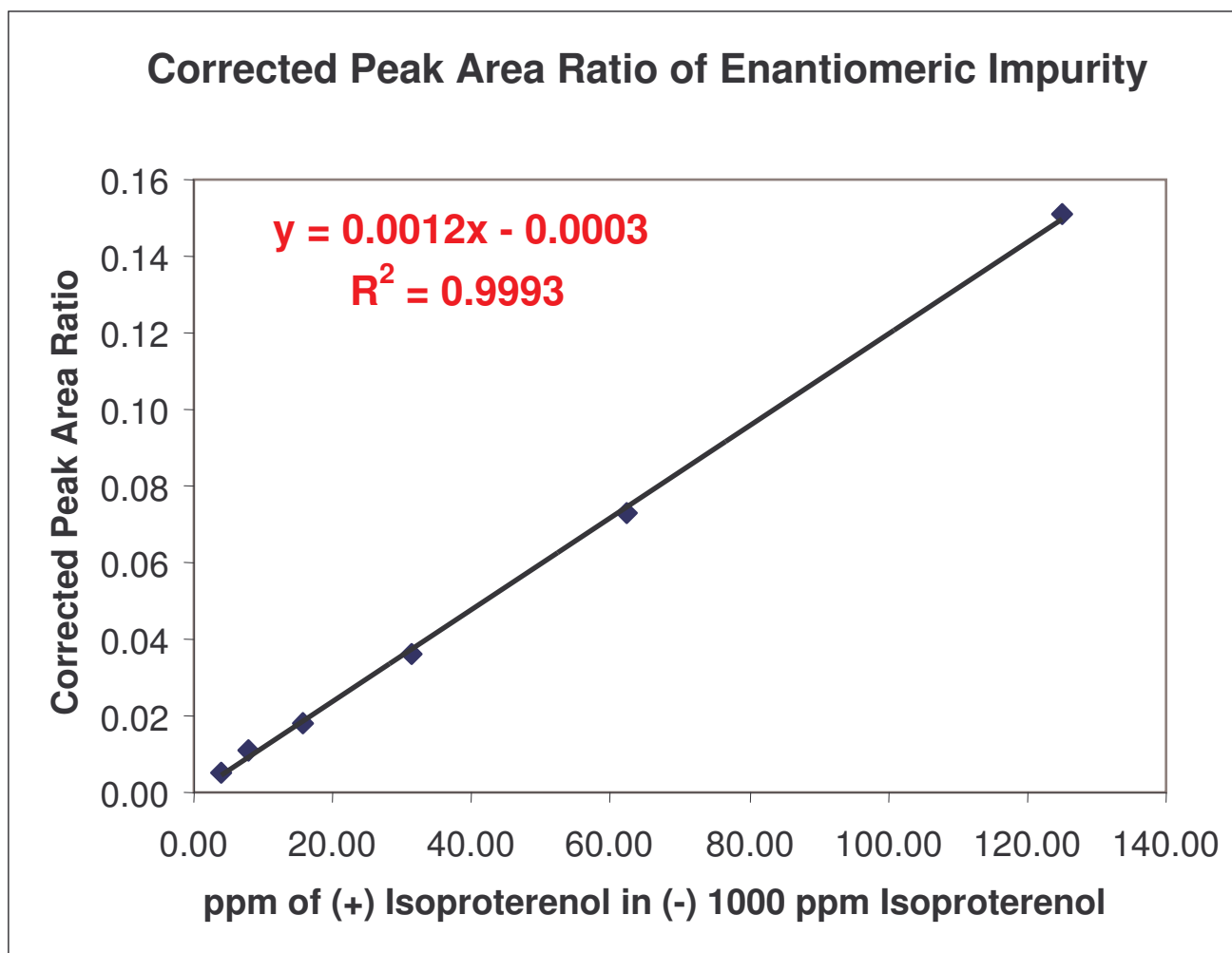
- Sample: 1000 ppm (+) isoproterenol
- BGE: 5% sulfated- β -CD (Aldrich) in 25 mM H_3PO_4 /TEA pH 2.5
- Contains a minor (-) isoproterenol enantiomer impurity
- Normalized corrected peak area of (-) impurity: 0.030 ± 0.002 (RSD = 6.30%; n = 24)

Detection of Low Level Enantiomeric Impurity



- 0.4% enantiomeric impurity of (+) Isoproterenol in (-) Isoproterenol

Plot of Impurity Velocity Corrected Peak Area Ratio vs Percent Impurity



- Linear response down to 0.4% enantiomeric impurity

Summary

- Multiplexed CE-UV is an attractive approach for performing high throughput chiral selector screening or chiral separations
- Up to 96 different selector/analyte combinations can be evaluated in a single CE experiment, significantly speeding method development
- Good migration time and peak area reproducibility can be achieved between different capillaries of the array
- Low levels of enantiomeric impurities (< 0.5%) can be detected
- Optimized methods can be performed in parallel or transferred to single capillary instruments