

CE of Basic Drugs

Using a New Capillary Surface Treatment System.

Robust, Reliable Methods that Transfer.



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Introduction

Traditional CZE, like early HPLC silica column assays once did, lacks reproducibility in the lower pH range.

Variation of EOF with pH change and electrodispersion result in a lack of robustness of CE methods.

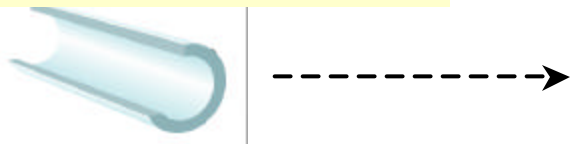
The fused-silica capillary surface variations which causes large fluctuations in EO Flow and electrodispersion can be controlled using a new, patented, surface treatment involving a rinse of the capillary with a buffered **polycation** followed by a rinse with a buffered **polyanion**;
Dynamic Coating.

This dynamic coating buffer solutions allow precise pH adjustment to your run (both up and down) without hysteresis or memory effect which classically occurs with buffer changes.

Further Optimization is easily done by finely tuning the pH of the run buffer, by adding organics, neutral or amphoteric surfactants or different cyclodextrins.

1. Principle of Dynamic Coating:

Use fused silica capillary,
rinse 1 min. with NaOH 0.1N



The principle of dynamic coating is as follows:
The Initiator Solution (A) containing polycations is first injected. The polycations bond to the capillary wall due to a large number of charge interactions. The Accelerator Solution (B) (run buffer) is then injected, it contains polyanions that stick to the first layer of polycations to form a double layer coating.

The Accelerator layer, containing polyanionic sulphate groups is insensitive to pH variation and confers a large number of negative charges to the capillary wall, creating a stable and elevated EOF in presence of an electric field.

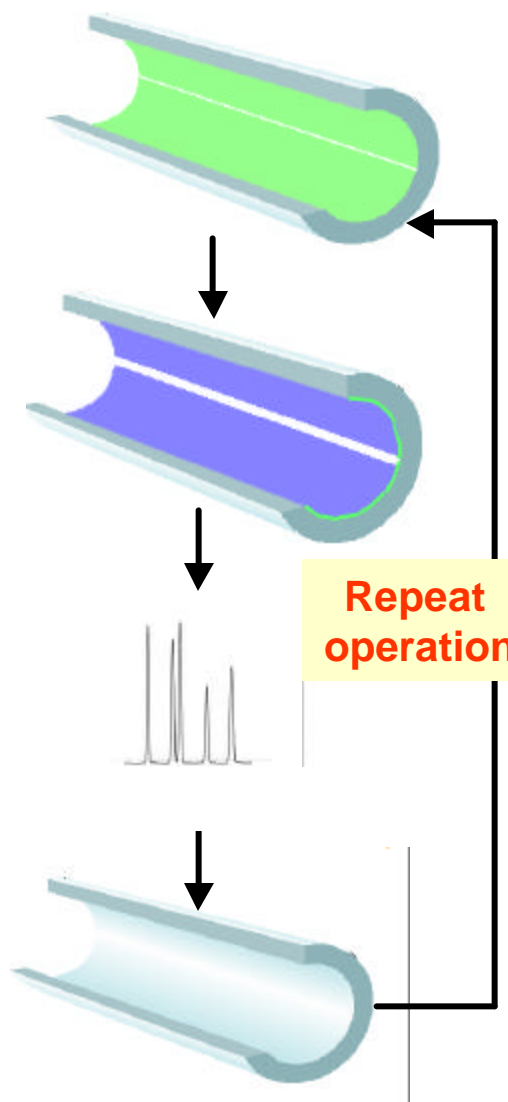
The coating is dynamic, after each electrophoresis, a simple 30 sec. rinse with NaOH 0.1N removes it and the operation can start again.

Rinse 30 sec. with
polycation

Rinse 1 min. with
polyanion
at desired pH

Inject sample and
separate at desired
voltage

Rinse 30 sec.
with
NaOH 0.1N



2. Materials

- CElixir™ Solutions, pH (MicroSolv Technology USA)
 - Initiator Solution (A)
 - pH 2.5 - 75 mM Phosphate Buffer, Accelerator (B)
 - pH 4.3 - 60 mM Malic Buffer, Accelerator (B)
 - pH 6.2 - 50 mM Phosphate Buffer, Accelerator (B)
 - pH 8.2 - 40 mM Phosphate Buffer, Accelerator (B)
 - pH 9.2 - 150 mM Borate Buffer, Accelerator(B)
- Instruments:
 - P/ACE MDQ (Beckman Coulter Inc. Fullerton Ca)
 - P/ACE 5500 (Beckman Coulter Inc. Fullerton Ca)
- Additives:
 - SB12-Amphoteric Surfactant
 - BDCD-beta dimethyl Cyclodextrin
 - GCD-gamma Cyclodextrin
 - HPBCD-hydroxy-propyl beta Cyclodextrin

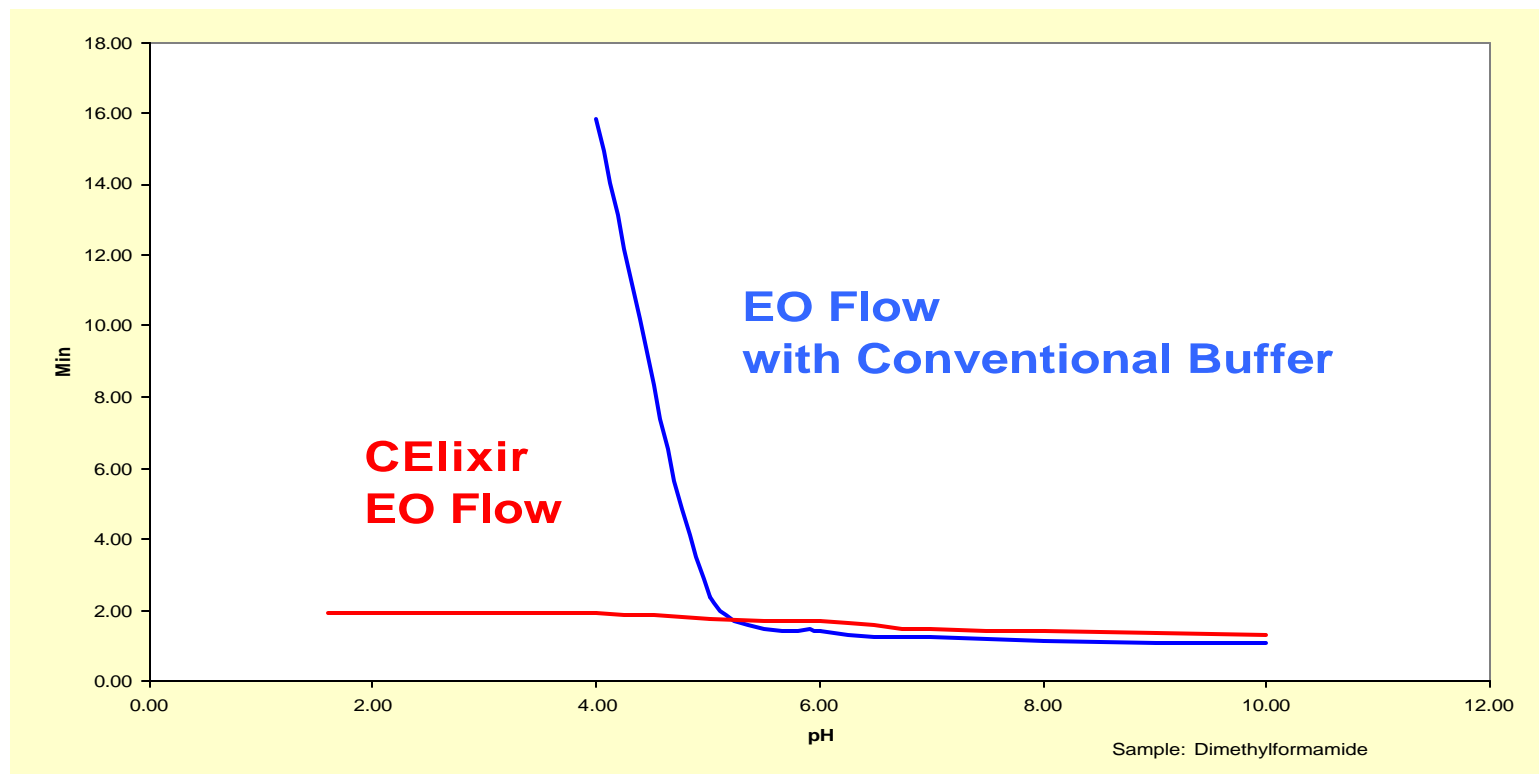


3. Features

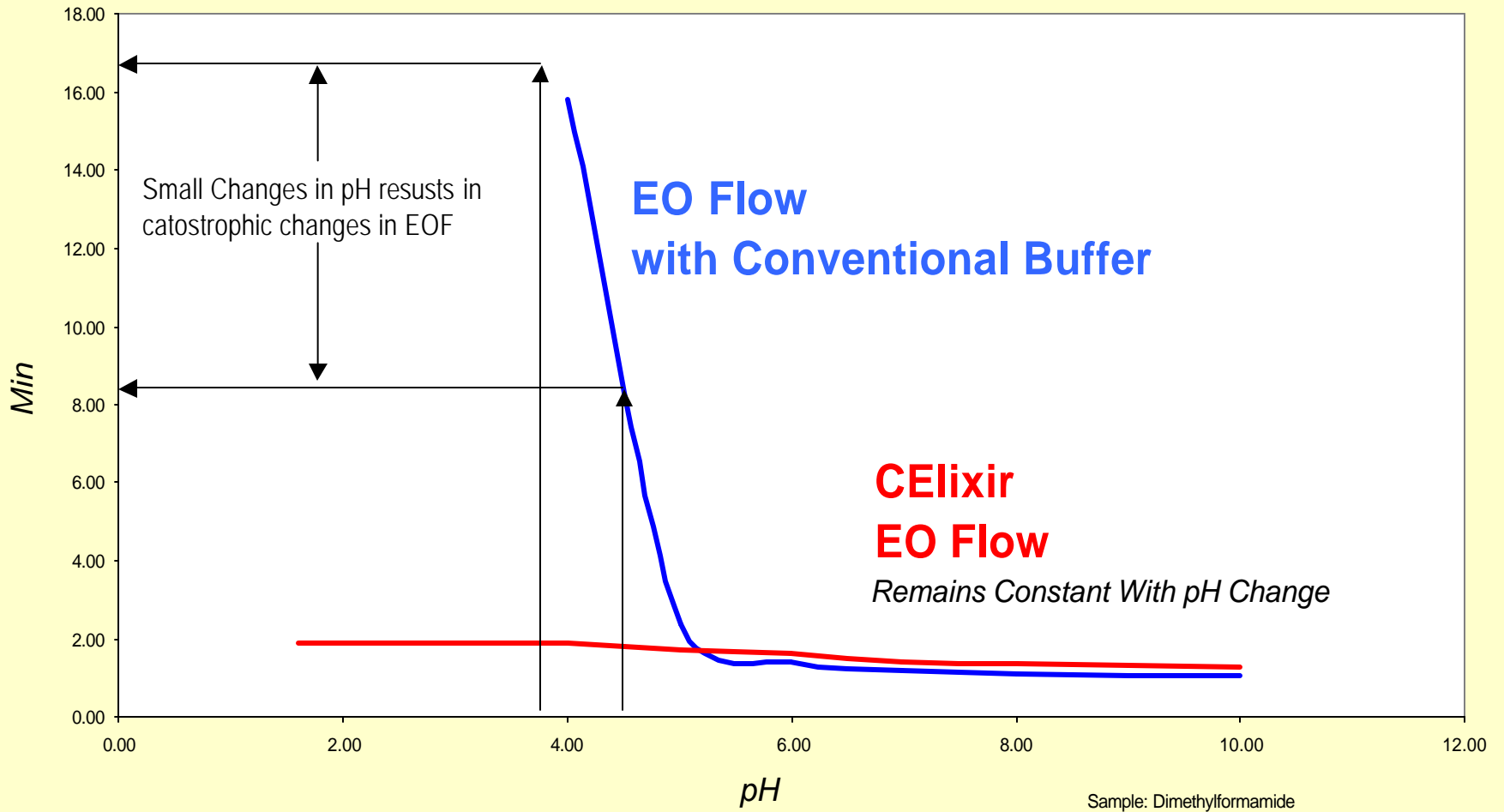
A. Stabilization of the EO Flow (Electro Osmotic Flow)

The internal wall of the capillary has a negative charge function of the pH of the buffer. This charge disappears at low pH.

When using the dynamic coating technique a constant charge over the pH range is obtained.



3. Features



3. Features

B. Intra- and Intercapillary Reproducibility Means Robustness.

A standard mixture (N-Benzyl dimethylamine, 3-Amino-1-Phenylbutane, Dibenzylamine) was analysed 3 times on three different capillaries with CElixir™ pH 2.5.

Reproducibility of migration time is:

< **0.5% CV** within the same capillary during different runs.

< **1% CV** with different capillaries during different runs.

Capillary	Mean Migr. Time	%CV	Mean Migr. Time	%CV	Mean Migr. Time	%CV
CAP1	2.57	0.16	2.57	0.80	2.92	0.10
CAP2	2.55	0.12	2.55	0.15	2.9	0.12
CAP3	2.54	0.23	2.54	0.27	2.87	0.28
%CV CAP1-3		0.60		0.60		0.87

3. Features

C. Absence of pH Hysteresis or memory effect (Reproducibility).

Changing from a phosphate buffer to borate and back to phosphate normally minimizes reproducibility.

By using CElixir™, where the capillary is rinsed after each separation and a new dynamic coating is applied, there is no memory or hysteresis effect on the capillary wall. One capillary can be used for all pH's.

Reproducible and Robust

<i>pH</i>	<i>μAmp.</i>	<i>Peak 3</i>	<i>Peak 4</i>	<i>Peak 5</i>
2.5	48	3.55	3.61	3.63
4.3	48	3.70	3.73	3.82
6.2	41	3.69	3.72	3.80
7.3	55	3.78	3.88	3.96
8.2	47	3.84	3.87	4.06
9.2	43	4.04	4.07	4.15
8.2		3.85	3.89	4.08
7.3		3.79	3.89	3.96
6.2		3.68	3.72	3.80
4.2		3.73	3.75	3.85
2.5		3.56	3.62	3.69

Migration time in minutes, of a mixture of peptides at different pH, please note also the similar current at different pH.

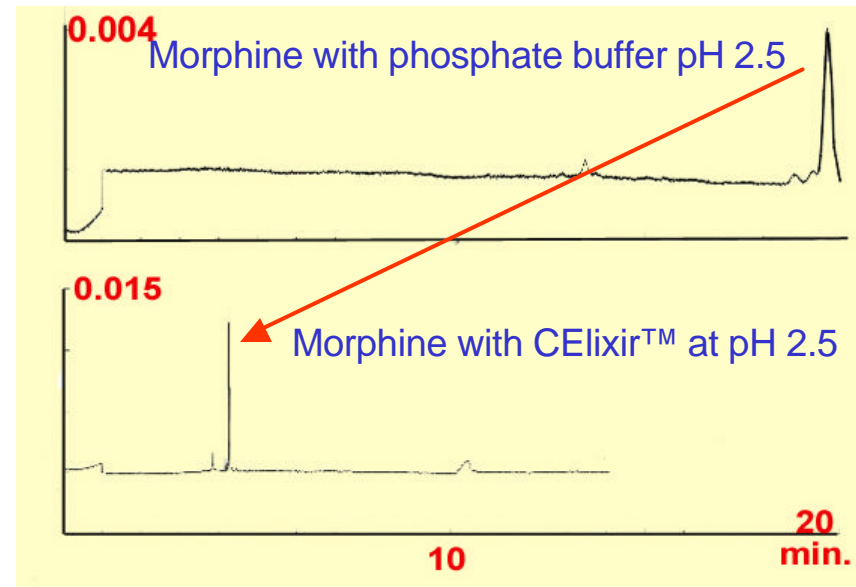
3. Features

D. Shorter Analysis Time

When working at low pH with CELixir™ we create a high EOF at low pH. This means that migration time is shorter and the signal to noise ratio is much higher.

E. No interaction with capillary wall

Between each run the dynamic coating is renewed. There is no sticking of analytes on the capillary wall and sample carry over.



4. Methods and Applications

Quick Method Development: During method development it is possible to screen, within the same run, the unknown analyte at **different pH**; for example pH 2.5 followed by 6.2. It is also possible to have a separation done with CELixir™ pH 4.3 without changing the capillary or spending time equilibrating.

Optimized Methods: The fact that the EOF is stabilized over the pH range makes it easy to control the analysis time, especially at low pH. The pH of CELixir can be adjusted to an optimum pH by mixing CELixir solutions or by adding phosphoric acid to get *pH as low as 1.7*. You can also add NaOH to reach pH 10.5. Addition of **organic solvents** and/or **amphoterics surfactants** is routinely done with good results.

Cyclodextrins as a chiral agent or as selectivity agent is possible. Neutral cyclodextrins exhibit excellent solubility with CELixir; up to 16% is possible.

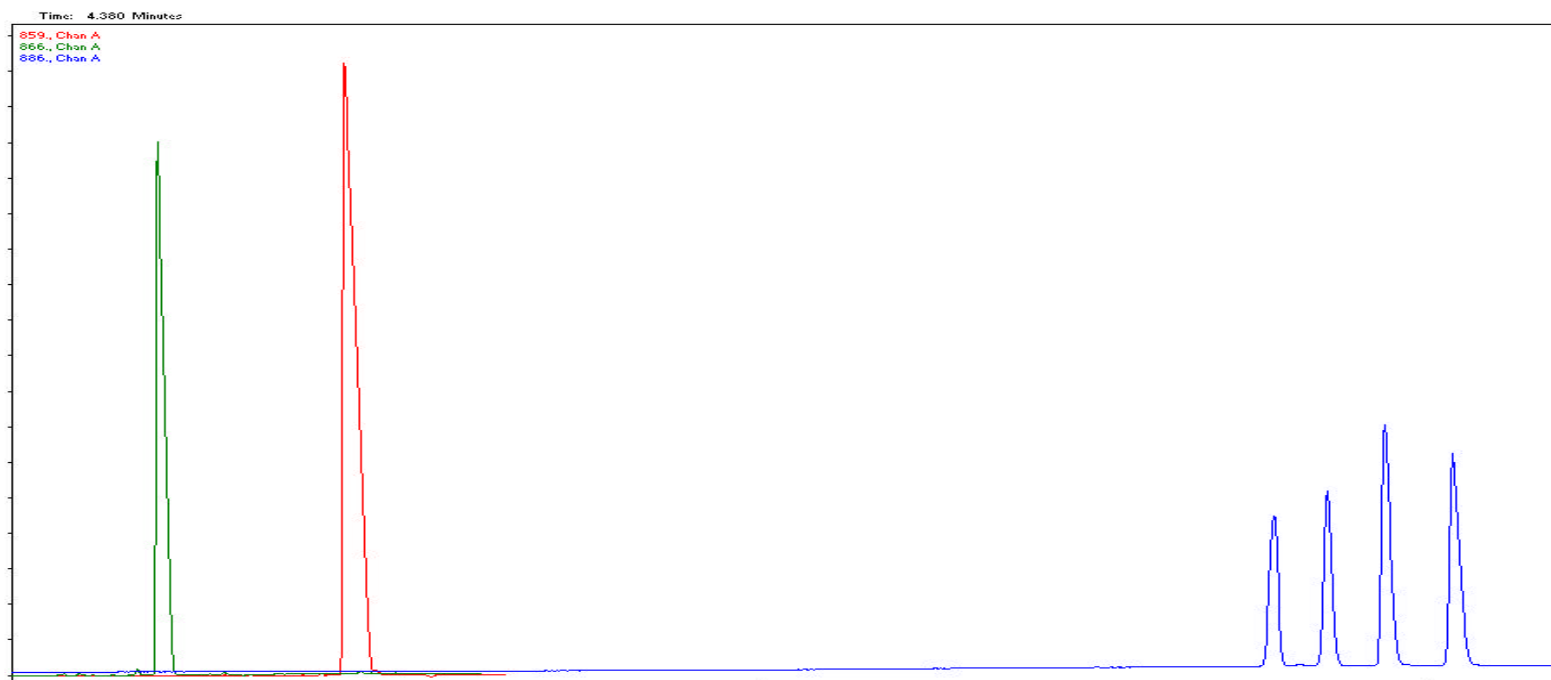
4. Methods and Applications

In capillary Electrophoresis analytes migrate as a function of the ratio of their net charge (given by the pH of the buffer) to their hydrodynamic size.

Separate Analytes of different charge; if analytes differ by at least **7% in their size** (roughly their MW) they are easily separated by CElixir at any pH where they are positively charged. See Epinephrine:

4. Methods and Applications

NOREPINEPHRINE, EPINEPHRINE and MIXTURE.



Capillary 50 u 40 cm

Injection 5 sec 25 Kv

CElixir™ pH 2.5

Capillary 75 u 60 cm

Injection 5 sec 25 Kv

CElixir™ pH 2.5 with 13 % BDMCD

4. Methods and Applications

In capillary Electrophoresis analytes migrate as a function of the ratio of their net charge (given by the pH of the buffer) to their hydrodynamic size.

Separate analytes of same charge; if your analytes differ by less than 7% in charge to mass ratio or for positional isomers, to achieve the separation one can:

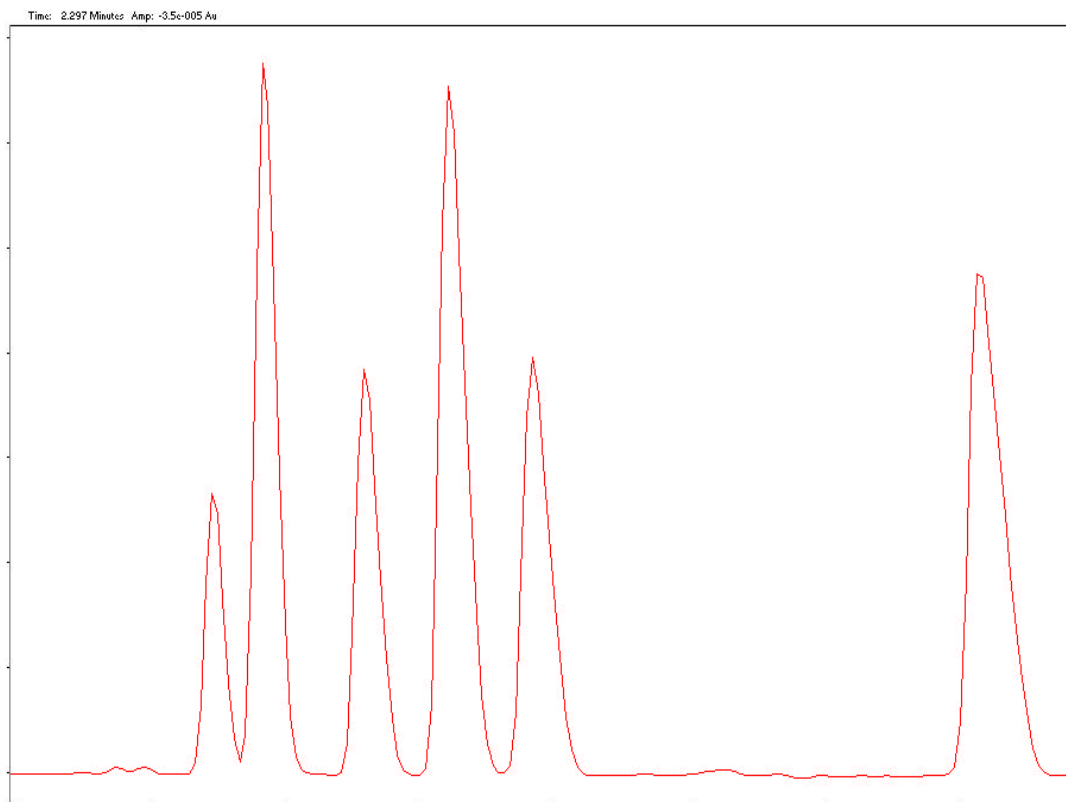
a. Manipulate the pH (as for the separation of the 6 Lutidines) to create a difference in charge quantity

or

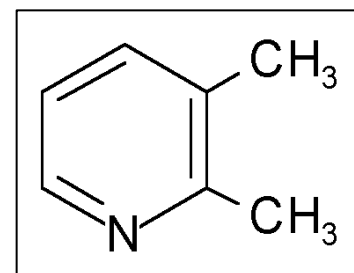
b. Modify the hydrodynamic size of the molecule, with organic additives (ie. opioids component with ethyleenglycol, tyrothricin with propanol).

4. Methods and Applications

Lutidines *Positional Isomers*



Position	pK	CV(n 9)
2.6	6.65	0.14
2.4	6.99	0.14
2.3	6.57	0.16
3.4	6.46	0.16
2.5	6.40	0.15
3.5	6.15	0.17



Capillary: 50 μ m x 40 cm

Injection 5 sec **192 nm** 25 Kv

CElixir™ pH 6.2

4. Methods and Applications

In capillary Electrophoresis analytes migrate as a function of the ratio of their net charge (given by the pH of the buffer) to their hydrodynamic size.

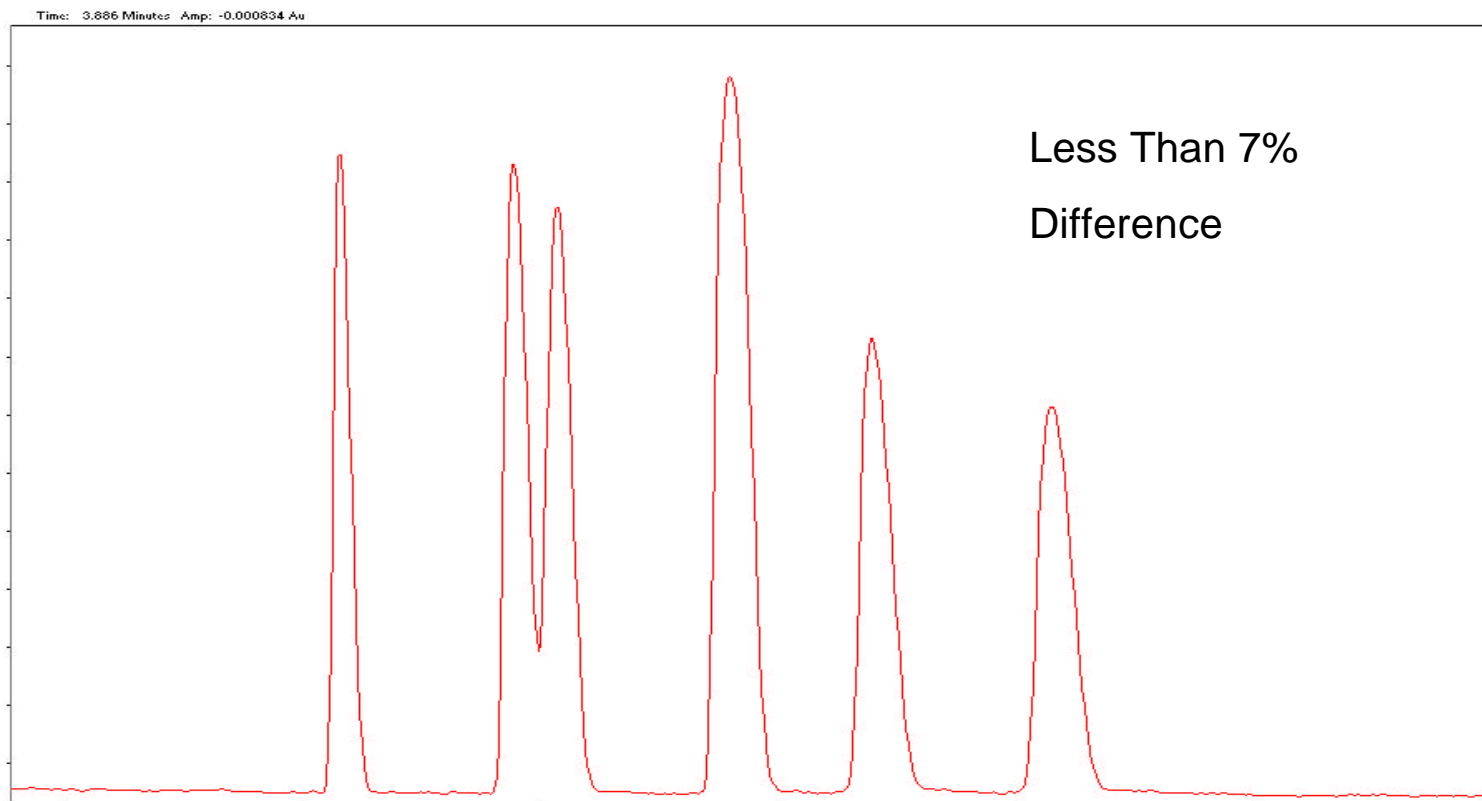
Separate analytes of same charge; if your analytes differ by less than 7% in charge to mass ratio or for positional isomers, to achieve the separation one can:

c. Add amphoteric surfactants such as SB12 which allows you to separate analytes of similar charge:

See e-gram of Imipramine, Desipramine, Amitriptyline, Trimipramine, Norclomipramine, (Tricyclic Antidepressants) at pH 2.5. Notice that the peaks have no “tailing” as you would find with traditional CZE or HPLC at this pH level.

4. Methods and Applications

Tricyclic Antidepressants



Capillary: 50 μ m x 40 cm

Injection 5 sec 192 nm 25 Kv

CElixir™ pH 2.5 with 0.8% SB12

7 min

4. Methods and Applications

Amphoteric Substances can be difficult to reproducibly separate in CZE.

Separate Amphoteric Substances; if your analytes are amphoteric peptides, amino acids, proteins or antibiotics, you can find the optimal pH for by adjusting CElixir's pH. At the right pH separation will occur. See Synthetic Peptide separation.

In the separation of Synthetic Peptide. A separation similar to one you would achieve with HPLC is quickly achieved at pH 2.5

Using CElixir at pH 4.27 the main peak migrates after the EO Flow. This indicates that the pK is around 4.0 – 4.2.

By “bracketing the pH and then using pH 3.26, the main peak is separated into two peaks.

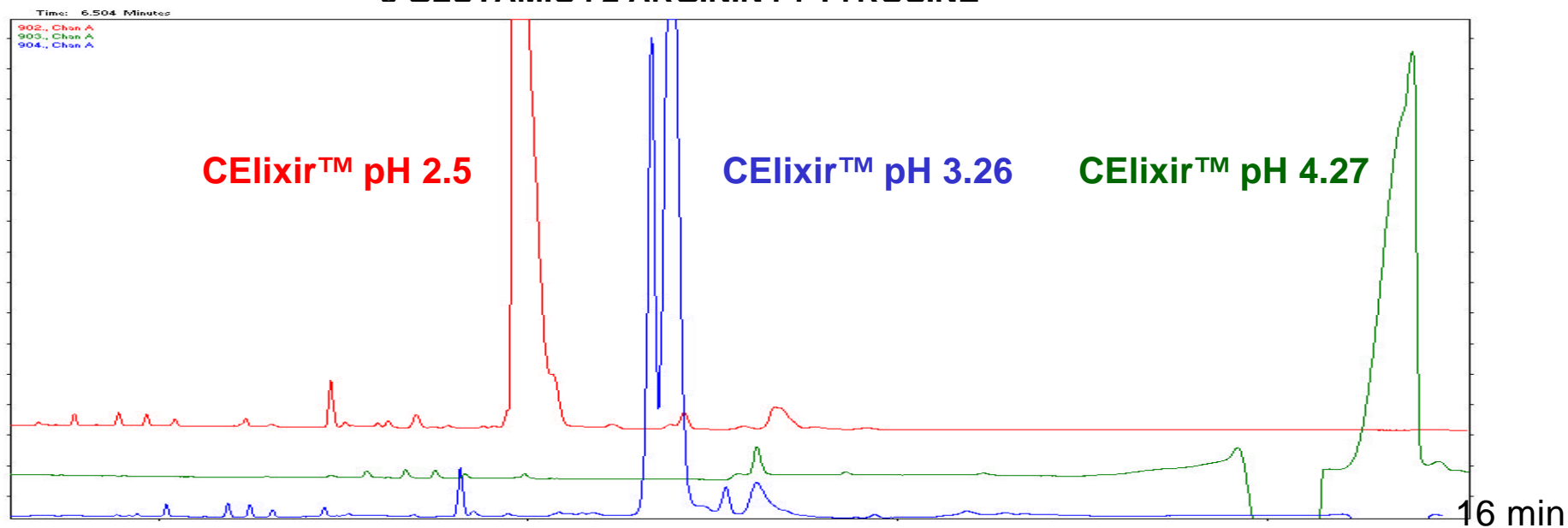
4. Methods and Applications

SYNTHETIC PEPTIDE

5 GLUTAMIC . 2 ARGININ . 1 TYROSINE

Amphoteric

Peptide



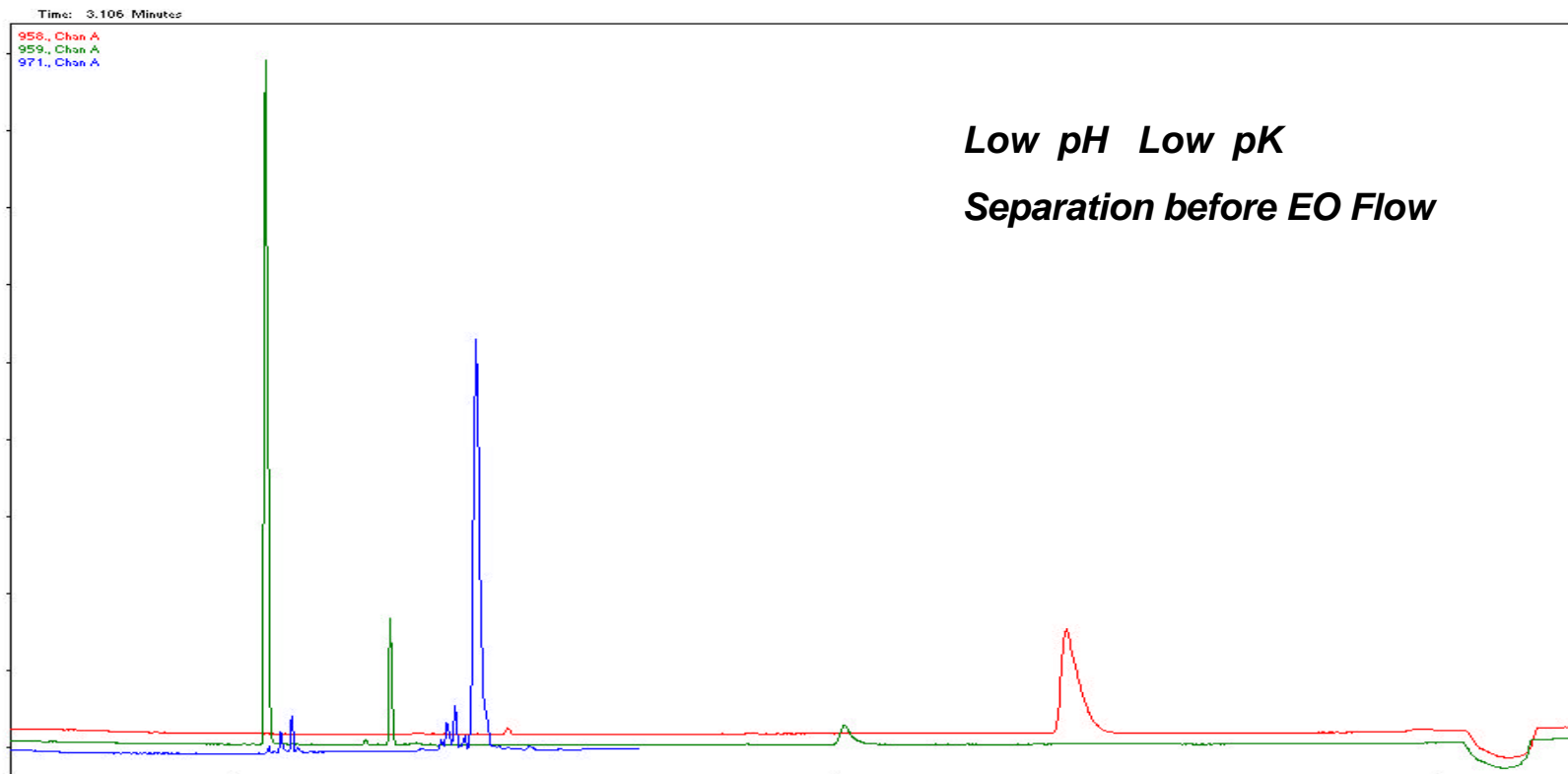
4. Methods and Applications

In CZE, many basic compounds are separated at higher pH due to the lack of control over the EO Flow.

Separate Basic Compounds before the EO Flow; with CElixir's very fast EO Flow at low pH, you can get compounds with very low pK to migrate before the EO Flow. See Diazepene e-gram.

4. Methods and Applications

CLOZAPIN , FLURAZEPAM and CLONAZEPAM



Capillary 75 μ m x 60 cm

Injection 5 sec 25 Kv

CElixir™ pH 2.5

16 min

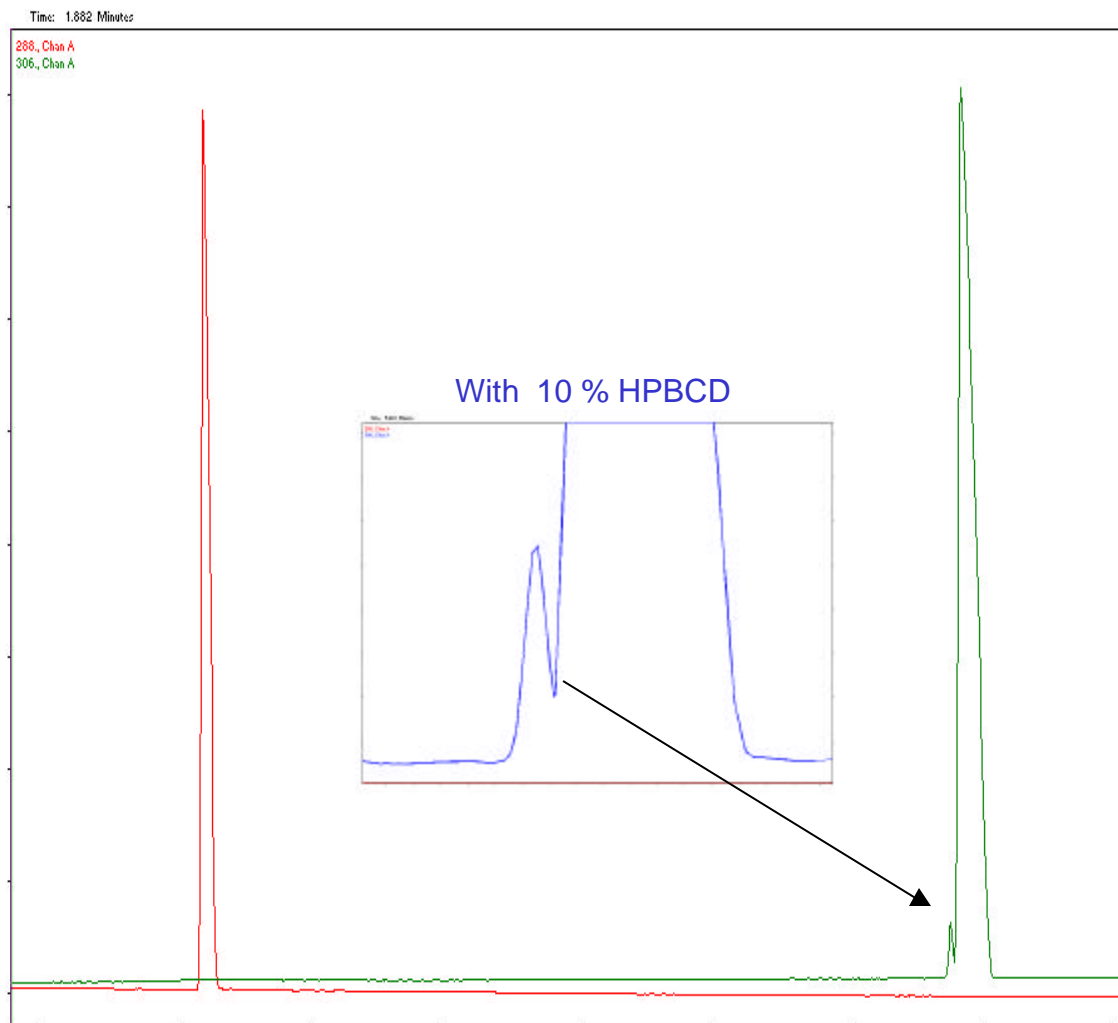
4. Methods and Applications

In CZE, many neutral compounds are not separated by charge to mass ratio and migrate with the EO Flow.

Separate Neutral Compounds by Adding Cyclodextrins; Due to the higher solubility with CElixir, neutral Cyclodextrins can be added to CElixir in higher concentrations allowing for the detection of neutral impurities. See MEPHENTERMINE

4. Methods and Applications

MEPHENTERMINE



*Neutral
Impurities*

Cap 50 μ m x 40 cm
Injection 5 sec 192 nm 25 Kv
CElixir™ pH 2.5

4. Methods and Applications

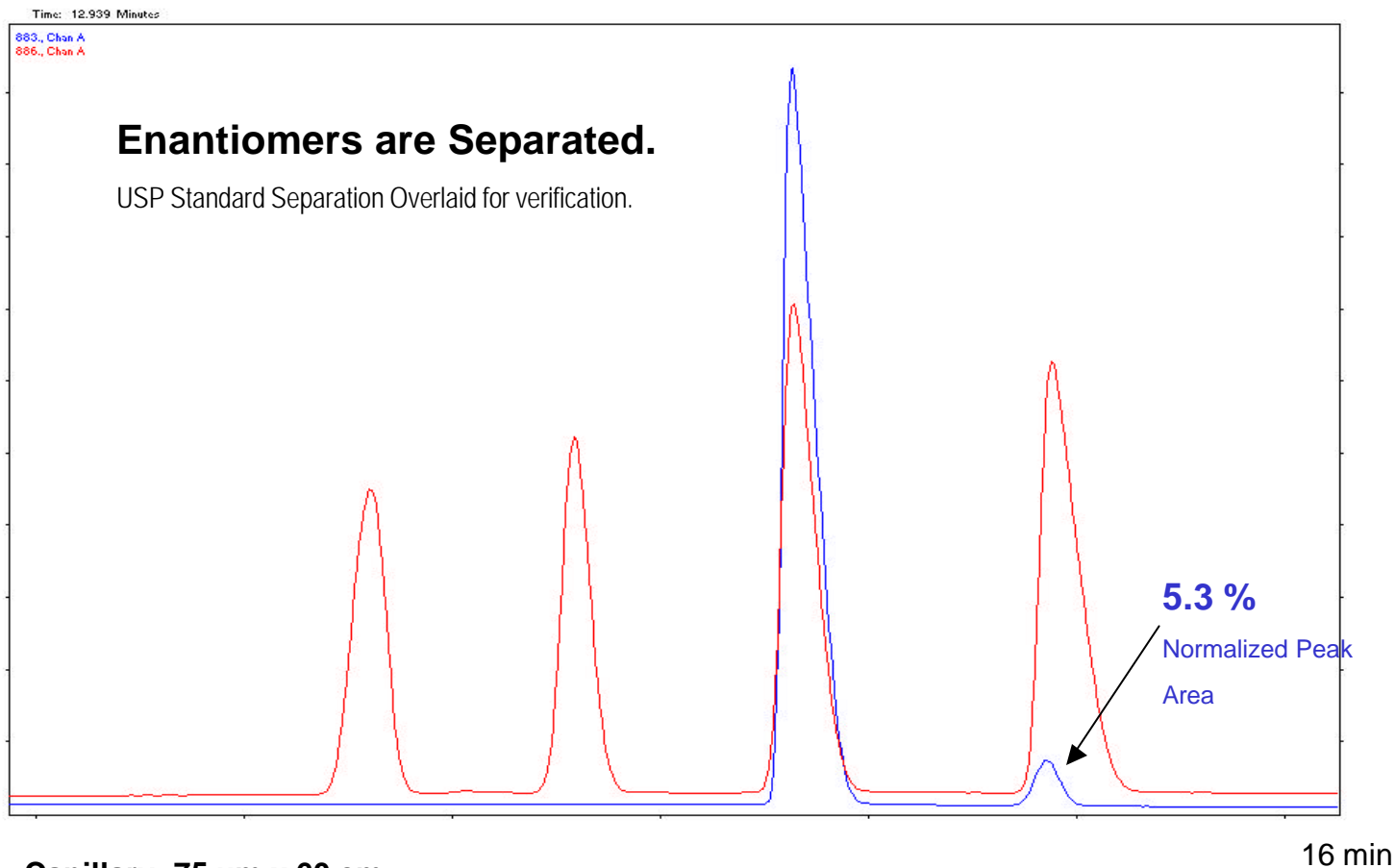
In CZE, many neutral compounds are not separated by charge to mass ratio and migrate with the EO Flow.

Separate Isomers: With the higher solubility of neutral Cyclodextrins with CElixir, enantiomers and cis-trans isomers can reliably and quickly be separated. CElixir can be prepared to contain up to 16% Cyclodextrin.

4. Methods and Applications

D/L EPINEPHRINE + D/L NOREPINEPHRINE

L-EPINEPHRINE USP > 97 %, by HPLC



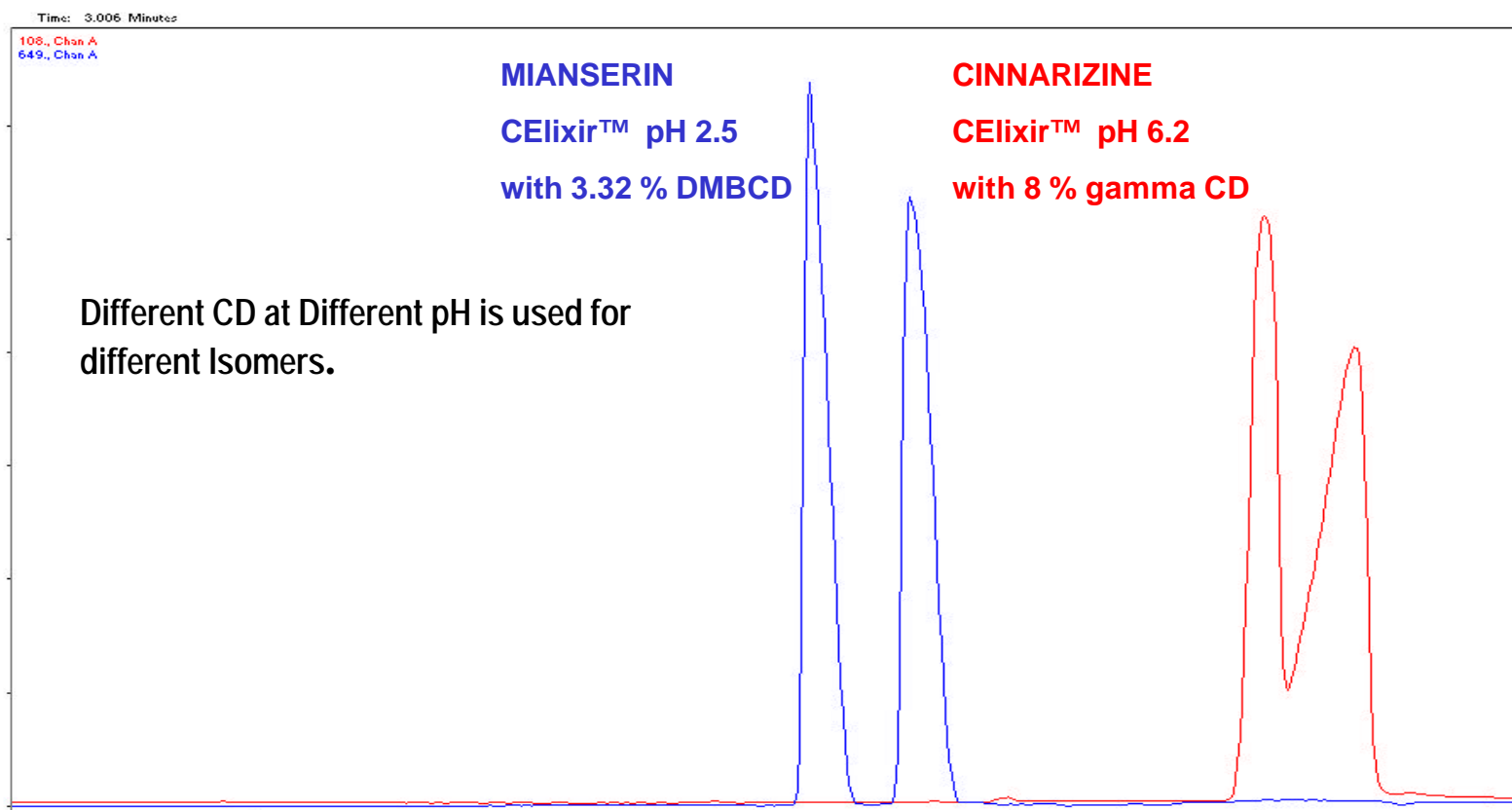
Capillary: 75 μ m x 60 cm

Injection 5 sec 25 Kv

CElixir™ pH 2.5 with 13 % BDMCD

4. Methods and Applications

Separation of Cis/Trans Isomers



Capillary: 50 μ m x 40 cm

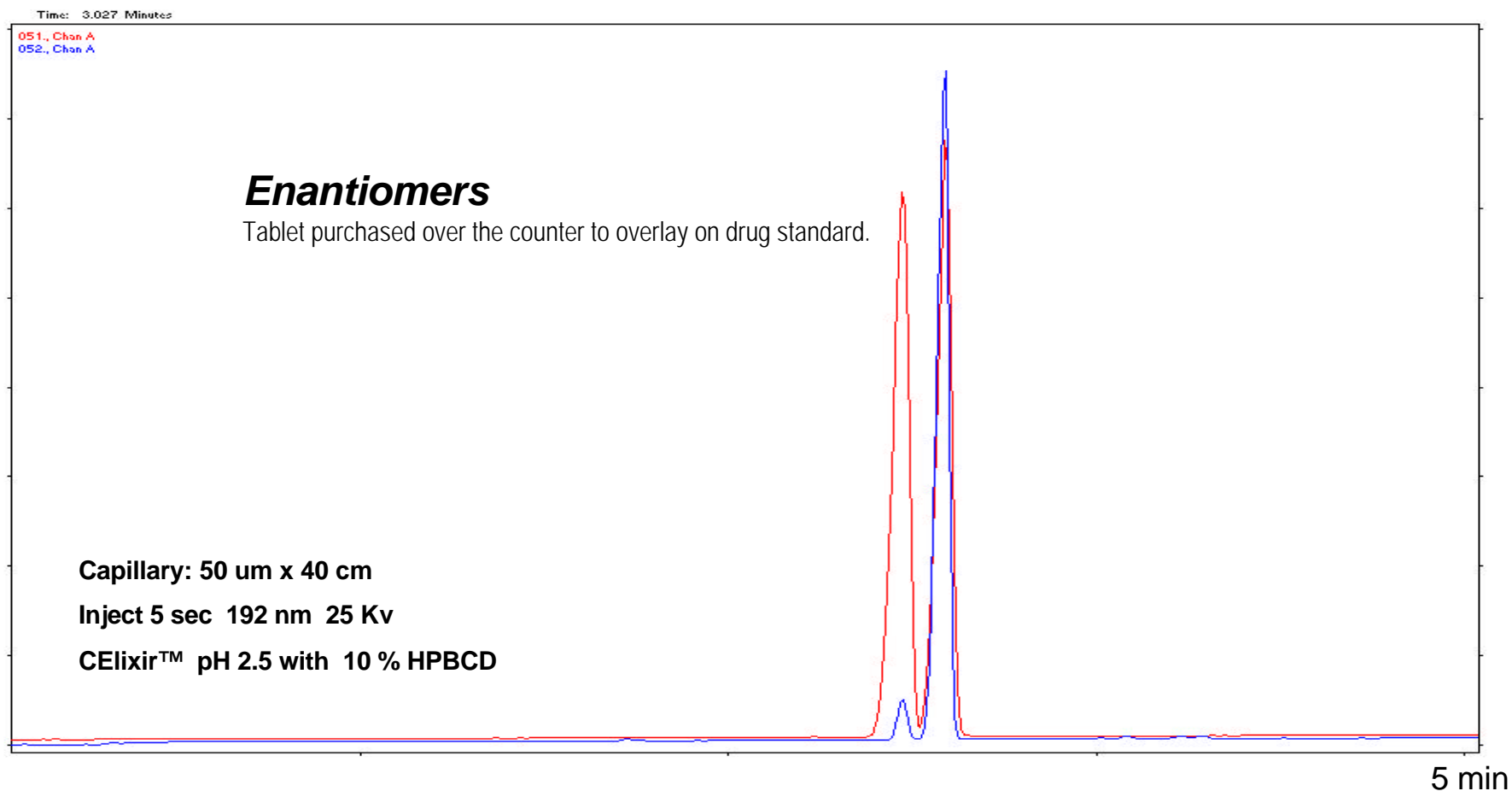
6 min

Injection 5 sec **192 nm** 25 Kv

4. Methods and Applications

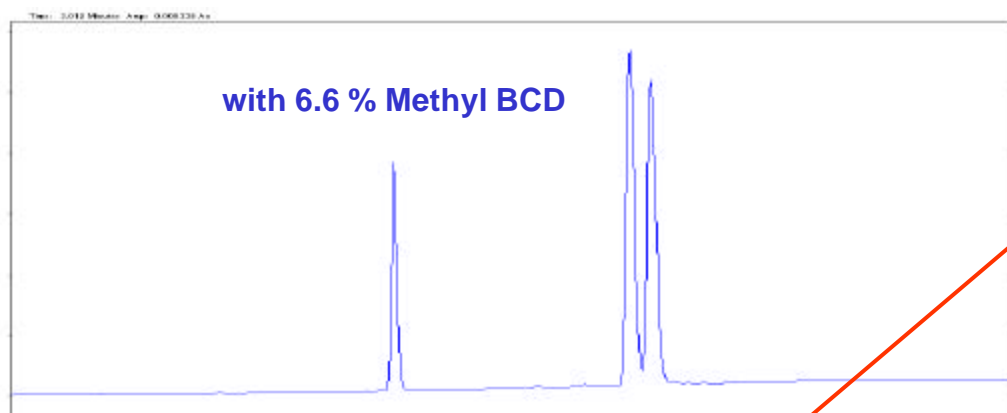
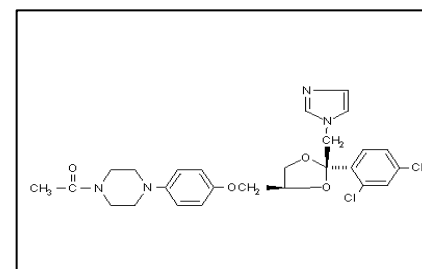
DL CHLORPHENIRAMINE

DEXTROCHLORPHENIRAMINE (TABLET)



4. Methods and Applications

KETOCONAZOLE



7 min.



10 min.

Four Chiral Centers Separated
High Concentration of CD with CELixir makes this possible.

Cap 50 μ m x 40 cm
Injection 5 sec 192 nm 25 Kv
CELixir™ pH 2.5

5. Conclusion

The CZE method using dynamic buffers with polycation and polyanion is reproducible, reliable and robust assuring constant migration times from day to day and from capillary to capillary. This gives the user confidence in reliability and robustness needed for **Pharmaceutical** laboratories.

When using **CElixir™** and a new capillary the target values are immediately obtained after a 1 minute rinse with NaOH. This gives the user much more instrument up-time. **More lab through-put.**

Method development is made easy due to short analysis time and absence of interaction with the capillary wall between runs.

MicroSolv R&D Laboratory Activities

When you expect more from electrophoresis.

For more than 8 years, Eutech Scientific Services along side of MicroSolv Technology Corporation has worked in Capillary Electrophoresis.

As a result of our long experience in the development of HPCE, we are now able to provide patented and marketed innovations for capillary electrophoresis.



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