Presentation Outline

♦ Capillary Zone Electrophoresis
♦ Basic Drug Screening
♦ Confirmation with CE/MS/MS
♦ Chiral Analysis – Crystal Meth
♦ Ion Analysis - GHB
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♦ Capillary Zone Electrophoresis
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Capillary Zone Electrophoresis
Drug Screening

- Forensic Toxicology
- Drug Development (Clinical Trials)
- Illicit (Street) Drugs

More analyte available
Basic Drug Screening

- Basic drugs in acidic solution are Cations.
CZE + PDA detection = Strength of methodology

- CZE gives better resolution - peak capacity
- Voltage injection - cleanup
- Process of Elimination with reproducible mobility data (< 0.3% RSD).
- Voltage injection - cleanup
- UV Spectra Library with modern search algorithms.
Injection Methods

• Amount of sample injected
CZE + PDA detection
= Strength of methodology

- CZE gives better resolution - peak capacity
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- Process of Elimination with reproducible mobility data (< 0.3% RSD).
- UV Spectra Library with modern search algorithms.
# Mobility with Spectral Confirmation

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Spectral Library Search
Mobility with Spectral Confirmation

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Spectral Library Search

Trazodone confirmed
Mobility

• … is the best parameter used for method validation
• … allows superior reproducibility and accuracy compared to use of migration times.
• … is hardware and capillary independent,
• … allows for easy cross-platform validation of CE methods.
CZE + PDA detection = Strength of methodology

- CZE gives better resolution - peak capacity
- Voltage injection - cleanup
- Process of Elimination with reproducible mobility data (< 0.3% RSD).
- UV Spectra Library with modern search algorithms.
Peak Search using Spectral Library
Mobility

60cm x 50um fused-silica capillary, 100mM phosphate pH 2.380, 25kV, 200nm UV detection
Presentation Outline

- Capillary Zone Electrophoresis
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- Ion Analysis - GHB
Confirmation by CE/MS/MS
Confirmation by CE/MS/MS

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Whole Blood Case Extract

- IS
- ES
- ?

Mobility Search

UV Library Search

Trazodone Detected
Confirmation by CE/MS/MS

Whole Blood Case Extract

Mobility Search

UV Library Search

CE/MS/MS

Trazodone Detected

Drug Mobility

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CE/MS/MS

Select Ion Monitoring 572-155

Trazodone Detected

MS/MS

C<sub>9</sub>H<sub>11</sub>N<sub>4</sub>O<sub>5</sub> (416)

Trazodone Confirmed
Confirmation by CE/MS/MS

P/ACE™ MDQ System

Waters* Micromass* Quattro Premier XE* Tandem Quadrupole Mass Spectrometer

*All trademarks are property of their respective owners.
2001 Impaired Driving Case

Cotinine

Cocaine

Cocaethylene

Nicotinamide

0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002

0
10
20
30
40
50
60
70
80
90
100

5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Minutes

AU

PDA - 200nm
2001-8669 Exh. 1 Blood CZE 2.38
2001 Impaired Driving Case

IS

Cocaine MRMs

12.83 14.019

Cocaethylene MRMs

12.83 16.284

13.04 23.067

2001-8669 cocaine

Oct 05, 2001

Copy Detected Peaks

M RM of 6 Channels ESI+

212.9 = 193.7

492

Area

MRM of 6 Channels ESI+

304.2 = 181.9

492

Area

MRM of 6 Channels ESI+

316.3 = 195.3

492

Area

MRM of 6 Channels ESI+

316.3 = 195.3

492

Area
Cocaine MRM
304.2 → 81.8
Cyclodextrins and CE/MS
CE/MS/MS – Calibration Mixture in 1.2% β-CD CE/MS

PDA - Beckman MDQ

Legend:

1 - MEGX (Lidocaine Metabolite)
2 - Lidocaine
3 - Methoxamine IS
4 - Norketamine
5 - Ketamine
6 - 3,4-MDA
7 - 3,4-MDMA
8 - 3,4- MDEA
9 - Diphenhydramine
CE/MS/MS – Calibration Mixture in 1.2% β-CD CE/MS

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Cyclodextrins

• Static Cyclodextrins

• Charged Cyclodextrins
Static Cyclodextrin (_indent)Environment
Static Cyclodextrin (_ENVIRONMENT)
Cyclodextrins

- **Static Cyclodextrins**
  - stationary in the capillary
  - interact with the enantiomers
  - form complexes with non-chiral drugs
  - additional screening information
  - poor resolutions
Static Cyclodextrins

2.38

1.2 β-CD

Lidocaine
MEGX
IS
Nor-K
Ketamine (K)
MDA
MDMA
MDEA
DPH

Minutes

AU

0.00
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0.040

0.00
0.01
0.02
0.03
0.04

14
16
18
20
22
24
26
Static Cyclodextrins

Lidocaine

MEGX

IS

Nor-K

MDA

MDMA

Ketamine (K)

MDEA

DPH

1.2 β-CD

2.38
Static Cyclodextrins

- Lidocaine
- MEGX
- IS
- Nor-K
- MDA
- Ketamine (K)
- MDMA
- MDEA
- DPH

1.2 β-CD

2.38
Cyclodextrins

- Static Cyclodextrins
- Charged Cyclodextrins
Charged Cyclodextrins’ Environment
Cyclodextrins

- Static Cyclodextrins
  - stationary in the capillary
  - interact with the enantiomers
  - form complexes with non-chiral drugs
  - additional screening information
  - poor resolutions

- Charged Cyclodextrins
  - highly sulfated, alpha, beta and gamma
  - negatively charged – anions
  - give increased resolution
  - high resolution -> short-end injections
Figure 1: Venlafaxine and Metabolite in 5% Gamma-HSCD

John C. Hudson, Beckman Coulter, Inc., Fullerton, CA, USA

Introduction

This work describes the use of charged cyclodextrins in a continuing search for a universal strategy for separation of enantiomeric drug substances. The use of highly sulfated cyclodextrins (HSCDs), originally proposed by Chapman and Chen (1), is a well-established and efficient approach used in many laboratories worldwide. In this study, a group of compounds was selected from a set of drugs and metabolites of pharmaceutical and forensic interest. This group of compounds was challenging because it included many closely related metabolites of drug substances in addition to the parent drugs. The results confirmed those of the earlier study (1) and allowed the current strategy to be used as a model for ultra-fast methods development. The strategy has been applied in the determination of drug purity for the pharmaceutical industry. An example of purity analysis illustrates the steps and practical considerations required for a successful outcome.

Material and Methods

Chemicals:
Solutions of alpha-, beta-, and gamma - HSCD at a concentration of 20% w/v and all other reagents were purchased from Beckman Coulter, Fullerton, CA, USA. The reagents were prepared as per the enclosed product documentation.

Drug and Metabolite Standards:
Standards were purchased from Cerilliant Corporation, Round Rock, TX, USA or were obtained as a gift from the Royal Canadian Mounted Police; Forensic Laboratory, Winnipeg, MB, Canada or Dr. Robert Meatherall, St. Boniface Hospital, Winnipeg, MB, Canada.

Solutions of these drug and metabolite standards were purchased or prepared at a concentration of 1 mg/mL and diluted to 25 ppm (25 ng/µL) in distilled and deionized water.

Reference Marker:
1,3,6,8-Pyrene tetracarboxylate (PTS), 10 mM in water; 2 µL added to each sample.

Instrument:
PIACETM MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA) equipped with a Photodiode Array Detector (PDAD) with detection at 200 nm (scanning 190-350) and 32 Karlion Version 7 Software.

Run Buffers: All chiral separations were performed in 5% HSCD in 25 mM triethylammonium phosphate pH 2.5 unless otherwise noted as either run in 2.5% or 7.5% of the HSCD.

Capillaries and Conditioning:
Fused-silica capillaries, 50 µm I.D. x 30 cm (effective length 25 cm) were used in all separations. The columns were rinsed daily with 25 mM lithium acetate containing 0.4% Polyethylene Oxide (PEO, MW 300,000) and 10% ethylene glycol, adjusted to pH 4.70 to speed up column equilibration.

Applied Voltage:
The voltage was set at 15 kV (500 V/cm) resulting in running currents of 140 to 180 nAmp for 50 µm I.D. columns.

Temperatures:
Capillary and sample storage = 22°C

Table 1: Resolution and HSCD Systems for 101 Basic Drugs and Metabolites

For Research Use Only. Not for use in diagnostic procedures.

Conclusion

Finding a universal method for chiral separations has been a challenge for many years. An important group of drugs and metabolites, expected to be frequently detected because of widespread use by the general population, was used to evaluate the proposed universal strategy. These 101 racemic drugs and metabolites are of interest to both the pharmaceutical and forensic communities. The compounds were rapidly screened and resolved with resolutions of 2 or greater for over 94% of the group.

References


Cyclodextrins

• Rapid Method Development

• Roche Example
Roche – New Drug Substance

- 4 Chiral Centers.
- 4 Enantiomeric Standards Available.
- SSSS Configuration is Active Pharmaceutical Ingredient (API).
- Major Enantiomer – RRRR.
- Two other impurities – RSSS and SRRR.
- Molecular Weight 377 as free base.
- Available as HCl salt – very water soluble.
- All injections from water.
Beta – Best HSCD Candidate

Beta-HSCD 5%

PTS
Beta – Best HSCD Candidate

Beta-HSCD 5%

RRRR

RSSS

SSSS

AU

Minutes
Mixture – Pressure Injection

Beta-HSCD 5%

Mixture of the 4 enantiomers
Mixture in 7.5% Beta-HSCD

Beta-HSCD 7.5%

Column:
50 µm i.d.
30 cm to det
40 cm total

Injections:
Pressure
0.3 psi
4 sec.
Mixture – Voltage Injection

Voltage Injection:
“Reversed Polarity”
Voltage
10 kV
10 sec.
**PDA Shows Mixture Components**

**Voltage Injection:**

“Reversed Polarity”

Voltage

10 kV

10 sec.

**Needs dilution 20/1**
After Dilution – Sharp Peaks

Voltage
Injection:
“Reversed Polarity”
Voltage
10 kV
10 sec.

Dilution 20/1 of the 10% solution
Dilution - 0.1% Purity Possible

Dilution 10/1 of the 1% solution
Purity Study – 0.1% Results

Range 0.12 to 0.17%
Methamphetamine (Crystal Meth)

- Methamphetamine and its metabolite, amphetamine

- HSCD discriminating power

- Migration time reversals

- Fast Method Development
Meth(M), Amp(A) and IS in Alpha 5%
Meth/Amp/IS in Alpha or Beta 5%
Meth/Amp/IS in Alpha or Beta 5%
Meth/Amp/IS in Alpha, Beta & Gamma 5%
Meth/Amp/IS in Alpha, Beta & Gamma 5%
Meth/Amp/IS in Alpha, Beta & Gamma 5%
Fast Method Development - Amp/Meth

Comparison between normal and fast methods for the analysis of methamphetamine and amphetamine.
Forensic Applications
Ion Analysis

- Clandestine Labs - Chemicals
- Poisoning cases
- Tampering cases – HCl in juice
- KCl in syringe
- Environmental Cases
- DFSA (GHB)
Cations Test Mixture
(Designed for Acidic Drug Counterions)
Inorganic Anions Test Mixture

1 = Chloride
2 = Nitrate
3 = Sulfate
4 = Azide
5 = Fluoride
6 = Phosphate
Organic Anions Test Mixture

UV 230nm

Chloride | Formate | Succinate | Acetate | Propionate | Butyrate | Valerate | Caproate | Octanoate

AU

Minutes

2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0
Organic Anions Test Mixture

UV 230nm

Molecules:
- Chloride
- Azide
- Formate
- Succinate
- Acetate
- Propionate
- Butyrate
- Valerate
- Caproate
- Octanate

GHB
GHB Standard 100 ng/µL in Water

GHB

\[ \text{HOCH}_2\text{CH}_2\text{CH}_2\text{COO}^- \]

IS

AU

Minutes

UV - 230nm
GHB in Extracts of 0.1 mL of Blood

Graph showing the presence of GHB, Butryate, Blank, and IS in test mixes.
GHB in Extracts of 0.1 mL of Blood

- Test Mix
- Butryate
- IS
- GHB
- Blank

Minutes

AU
Acknowledgements

RCMP Forensic Laboratory – Winnipeg, Canada
Cam Lyttle
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Marco Girod
Maurice Notter

Beckman Coulter, Fullerton CA
Jeff Chapman
Hans Dewald

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Recent Advances in Analysis of Small Molecules for Forensic and Pharmaceutical Scientists

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Fullerton, CA, USA