# Rapid Enantiomeric Separation of Basic Drugs and Metabolites: A Universal Strategy for Ultra-Fast Method Development.

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## 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 (2). 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 tetrasulfonate (PTS), 10 mM in water:
2 µL added to each sample.

**Instrument:**
P/ACE™ 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 Karat™ 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 20 cm) were used in all separations. The columns are rinsed daily with 25 mM lithium acetate containing 0.4% Polyethylene Oxide (PEO,MW 300,000) and 10% ethylene glycol, adjusted to pH 4.75 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 µamps for 50 µm I.D. columns.

**Temperatures:**
Capillary and sample storage = 22ºC

**Sample introduction:**
Pressure injection of 4 s at 0.3 psi or
Voltage injection of 10 s at 10 kV.

## Results

1. Of the 692 compounds in a library used in forensic drugs screening (2), 101 were known to be racemic compounds and were used in this study.

2. At a concentration of 5% HSCD, 73 of the compounds were resolved at a resolution of 2 or higher.

3. Twenty-eight (28) of the 101 compounds were selected for additional runs at a concentration of 2.5 and 7.5% of each HSCD to further improve resolution. As a result of these runs, a total of 94% of the 101 compounds were resolved at 2 or greater.

4. Examples of the excellent resolution that can be achieved are shown in Figures 1 and 2.

| Drug or Metabolite | Resolution | System* |
|---|---|---|
| Acebutolol | 2.4 | Gamma |
| Adrenaline, Nor- (Norepinephrine) | 3.5 | Gamma 2.5 |
| Aminorex, Cis-4-methyl- | 4.5 | Gamma |
| Amphetamine | 33.2 | Gamma |
| Amphetamine, 2,3-Dimethoxy- | 16.6 | Gamma |
| Amphetamine, 2,4-Dimethoxy- | 22.5 | Gamma |
| Amphetamine, 2,5-Dimethoxy- | 17.2 | Gamma |
| Amphetamine, 2,5-Dimethoxy-4-bromo- | 9.9 | Beta |
| Amphetamine, 2,5-Dimethoxy-4-ethyl- | 11.6 | Gamma |
| Amphetamine, 2,5-Dimethoxy-4-methyl- | 13.8 | Gamma |
| Amphetamine, 2,5-Dimethoxy-4-propyl- | 6.0 | Gamma |
| Amphetamine, 2,6-Dimethoxy- | 4.5 | Gamma |
| Amphetamine, 3,4-Dimethoxy- | 14.4 | Gamma |
| Amphetamine, 3,5-Dimethoxy- | 4.6 | Gamma |
| Amphetamine, 3-Methoxy-4,5-methylenedioxy- | 4.6 | Gamma |
| Amphetamine, N-Ethyl- | 14.7 | Gamma |
| Amphetamine, 4-Methylthio- (4-MTA) | 13.4 | Gamma |
| Amphetamine, Hydroxy- | 30.6 | Gamma |
| Atenolol | 3.4 | Gamma |
| Bisoprolol | 2.2 | Gamma 2.5 |
| Brompheniramine, Dinor- | 0.8 | Beta 7.5 |
| Brompheniramine, Nor- | 1.0 | Beta |
| Bupropion | 10.1 | Alpha |
| Bupropion, Erythroamino- | 7.5 | Gamma |
| Bupropion, Hydroxy- | 6.3 | Beta |
| Bupropion, Threoamino- | 7.6 | Alpha |
| Butriptyline, N-desmethyl- | 1.1 | Alpha |
| Chloroquine, N,N,-Didesethyl- | 1.9 | Gamma 7.5 |
| Chloroquine, N-Desethyl- | 1.1 | Alpha |
| Chlorpheniramine | 2.5 | Beta 7.5 |
| Chlorpheniramine, Dinor- | 1.0 | Beta 7.5 |
| Chlorpheniramine, Nor- | 1.1 | Beta 7.5 |
| Citalopram | 11.1 | Gamma |
| Citalopram N-Oxide | 11.0 | Gamma |
| Citalopram, Dinor- | 8.0 | Gamma |
| Citalopram, Nor- | 8.0 | Gamma |
| Cyclazocine | 3.8 | Beta |
| Cyclobenzaprine | 1.9 | Gamma 7.5 |
| Cyclobenzaprine, N-Desmethyl- | 5.4 | Alpha |
| Desloratadine | 6.4 | Gamma |
| Dihydro-N,N-dimethyl-5-methylene- ** | 3.2 | Alpha |
| Disopyramide, p-Cl | 4.7 | Beta |
| Disopyramide, N-Dealkylated- | 4.6 | Alpha |
| Doxapram | 7.2 | Gamma |
| Doxylamine | 2.2 | Gamma 7.5 |
| EDDP (Methadone Mtb.) | 3.0 | Beta 7.5 |
| EMDP (Methadone Mtb.) | 7.5 | Gamma |
| Ephedrine | 6.1 | Alpha |
| Ephedrine, Hydroxy | 15.6 | Gamma |
| Esmolol | 3.1 | Gamma |
| Fluoxetine | 12.0 | Alpha 2.5 |

| Drug or Metabolite | Resolution | System* |
|---|---|---|
| Fluoxetine, Nor- | 9.8 | Gamma |
| Glutethimide | 11.8 | Gamma |
| Ketamine | 2.2 | Gamma |
| Ketamine, Nor- | 4.5 | Alpha |
| Labetolol | 5.1/2.9/2.1*** | Gamma 2.5 |
| MBDB | 7.5 | Gamma |
| MDA, 2,3- | 16.7 | Gamma |
| MDA, 3,4- | 14.6 | Gamma |
| MDEA, 3,4- | 7.1 | Gamma |
| MDMA, 2,3- | 9.7 | Gamma |
| MDMA, 3,4- | 7.8 | Gamma |
| Methadone | 26.6 | Beta |
| Methamphetamine | 11.8 | Gamma |
| Methoxamine | 44.4 | Gamma |
| Methylphenidate | 14.5 | Gamma |
| Metoprolol | 4.2 | Alpha 2.5 |
| Mexiltiline | 2.7 | Gamma 7.5 |
| Midazolam | 3.1 | Gamma |
| Mirtazapine | 5.4 | Gamma |
| Mirtazepine, N-Desmethyl | 7.5 | Gamma |
| Nadolol | 2.6/1.3/split*** | Gamma |
| Nefopam | 7.6 | Alpha |
| Oxprenolol | 10.0 | Beta |
| Pentazocine | 7.2 | Gamma 7.5 |
| Pheniramine | 2.8 | Beta 7.5 |
| Phenmetrazine | 19.5 | Alpha |
| Phenylephrine, N-Desmethyl- | 3.6 | Alpha |
| Phenylpropanolamine | 42.6 | Gamma |
| Pindolol | 2.0 | Beta 7.5 |
| PMA (p-Methoxyamphetamine) | 25.3 | Gamma |
| PMMA (p-Methoxymethamphetamine) | 13.1 | Gamma |
| Propranolol | 4.3 | Alpha 2.5 |
| Pronethalol | 2.5 | Alpha 2.5 |
| Propoxyphene | 7.3 | Alpha |
| Pseudoephedrine | 5.2 | Gamma |
| Quetiapine | 4.4 | Gamma |
| Quinidine | 2.3 | Alpha |
| Salbutamol | 8.3 | Beta |
| Tetramisole | 3.6 | Gamma |
| Tramadol | 13.3 | Gamma |
| Tramadol, Nor- | 10.3 | Gamma |
| Trimipramine | 7.3 | Alpha |
| Trimipramine, Nor- | 2.0 | Alpha |
| Venlafaxine | 5.5 | Gamma |
| Venlafaxine, O-Desmethyl- | 4.3 | Gamma |
| Verapamil | 7.1 | Alpha |
| Verapamil, Nor- | 6.9 | Alpha |
| Zopiclone | 24.0 | Gamma |
| Zopiclone N-Oxide | 23.6 | Gamma |
| Zopiclone, Nor- | 31.6 | Gamma |

* All systems 5% HSCD unless indicated otherwise ** Dihydro-N,N-dimethyl-5-methylene-5H-dibenzocycloheptene-10-ethanamine, 10,11-
*** Compound with 2 chiral centers

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

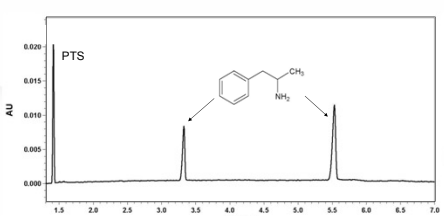



Figure 1: Amphetamine in 5% Gamma-HSCD

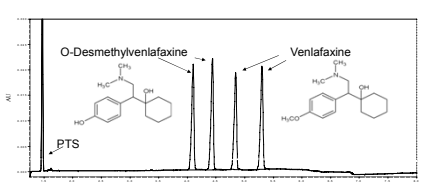



Figure 2: Venlafaxine and Metabolite in 5% Gamma-HSCD

5. This strategy was applied to a very complex racemic drug containing four chiral centers. Prior to this, the existing method used a chromatographic approach where the enantiomers were poorly resolved. To fully resolve the Active Pharmaceutical Ingredient (API) and 3 enantiomers, this systematic process was applied as follows and is illustrated in Figure 3.

Refer to steps A to E in Figure 3 (below):

A. Pressure injection of the three enantiomers) at 10% of the API run in each HSCD at 5% was performed. The starting concentation of the API was 400 nanograms per microlitre in water.

B. The best resolution between 5% systems, was obtained in Beta-HSCD. Improved resolution was obtained in 7.5% Beta-HSCD.

C. The capillary length was increased from 20 to 30 cm to the detector (40 cm total).

D. Voltage injection from the 10% solution gave excellent sensitivity, resolution and peak shape and the sample had to be diluted 20 to 1 for the final purity determination.

E. Low level purity determination result was made possible with HSCDs and voltage injection from a solution containing 400 ng/µL of the API and 0.4 ng/µL of each enantiomer.

Method development was completed in less than 3 days.

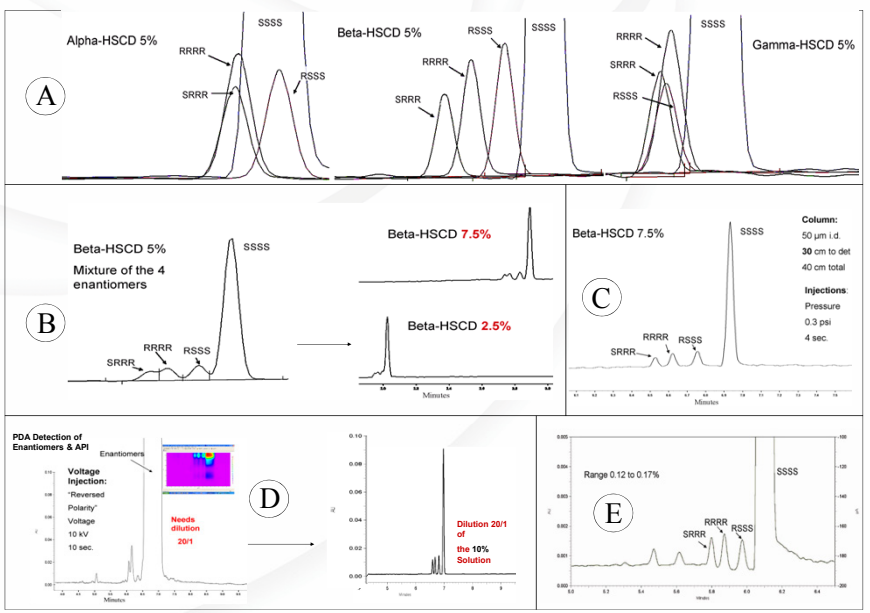



Figure 3: Rapid Method Development for Purity Analysis Using Highly Sulfated Cyclodextrins

## 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

1. Chapman, J.D and A-F. Chen, LCGC Europe, January 33 - 37 (2001)

2. Hudson, J.C. *et al.*, Can. Soc. Forens. Sci., 31(1) 1 - 29 (1998)

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