Rapid Enantiomeric Separation of 101 Basic Drugs and Metabolites: A Generic Strategy Using Highly Sulfated Cyclodextrins

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Introduction

This work describes the investigation of the use of charged cyclodextrins in the on-going search for a generic strategy for separation of enantiomeric drug substances. The use of highly sulfated cyclodextrins (HSCDs) is a well established and efficient approach at work in many laboratories worldwide. Chapman and Chen (1) proposed this generic strategy to quickly allow researchers to assess whether the use of HSCDs is the appropriate approach to solving individual separation problems. In that study, a set of basic, neutral and acidic compounds was used to evaluate the efficiency of the HSCD protocol. It was determined that in the large group of compounds analyzed (160 compounds), separations with resolution of 1.0 or greater could be achieved for 156 of the 160 compounds analyzed. What was even more remarkable was that this was achieved with no further optimization of the initial conditions. In this present study a group of compounds selected from a set of drugs and metabolites of pharmaceutical and forensic interest became available for study. This group of compounds was a challenging set because it included many closely related metabolites of drug substances in addition to the parent drugs.

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 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 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 0.4% PEO (MW 300,000), 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) which resulted 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

Selection of Racemic Compounds:
Racemic compounds were selected for study on the following basis:

1. All drugs were basic drugs and were a subset of a large group (692) included in a screen developed by Hudson *et al.* (2).

2. From the initial group of 692 compounds, a smaller subset of 299 compounds which had been run in 1.2% ß-Cyclodextrin and 0.5% hydroxypropyl-ß-cyclodextrin in pH 2.38 phosphate buffers, was evaluated.

3. Evaluation of the data from these systems and examination of their chemical structures helped to identify which compounds were available as racemates.

4. A final group of 101 racemic compounds was selected for the HSCD study.

Results

1. Each drug or metabolite standard was run multiple times in order to adjust the concentration of the solution and determine the resolution at the appropriate concentration. This was necessary because many of the metabolites were rare compounds and were not available as accurately known quantitative standards.

2. The group of 101 compounds was run in the 5% solution of each HSCD.

3. Results are shown in Table 1 and include the resolution and the corresponding HSCD System.

4. Two examples of HSCD separations of venlafaxine, citalopram and their metabolites in Gamma-HSCD are shown in Figures 1 and 2.

5. Of the 101 compounds, 28 were selected for additional runs at a concentration of 2.5 and 7.5% of each HSCD.

6. Ninety-five (95) of the 101 compounds (94%)had resolutions of 2.0 or greater.

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

Drug or Metabolite	Resolution	System*	Drug or Metabolite	Resolution	System*
Acebutolol	2.4	Gamma	Fluoxetine, Nor-	9.8	Gamma
Adrenaline, Nor- (Norepinephrine)	3.5	Gamma 2.5	Glutethimide	11.8	Gamma
Aminorex, Cis-4-methyl-	4.5	Gamma	Ketamine	2.2	Gamma
Amphetamine	33.2	Gamma	Ketamine, Nor-	4.5	Alpha
Amphetamine, 2,3-Dimethoxy-	16.6	Gamma	Labetolol	5.1/2.9/2.1***	Gamma 2.5
Amphetamine, 2,4-Dimethoxy-	22.5	Gamma	MBDB	7.5	Gamma
Amphetamine, 2,5-Dimethoxy-	17.2	Gamma	MDA, 2,3-	16.7	Gamma
Amphetamine, 2,5-Dimethoxy-4-bromo-	9.9	Beta	MDA, 3,4-	14.6	Gamma
Amphetamine, 2,5-Dimethoxy-4-ethyl-	11.6	Gamma	MDEA, 3,4-	7.1	Gamma
Amphetamine, 2,5-Dimethoxy-4-methyl-	13.8	Gamma	MDMA, 2,3-	9.7	Gamma
Amphetamine, 2,5-Dimethoxy-4-propyl-	6.0	Gamma	MDMA, 3,4-	7.8	Gamma
Amphetamine, 2,6-Dimethoxy-	4.5	Gamma	Methadone	26.6	Beta
Amphetamine, 3,4-Dimethoxy-	14.4	Gamma	Methamphetamine	11.8	Gamma
Amphetamine, 3,5-Dimethoxy-	4.6	Gamma	Methoxamine	44.4	Gamma
Amphetamine, 3-Methoxy-4,5-methylenedioxy-	4.6	Gamma	Methylphenidate	14.5	Gamma
Amphetamine, N-Ethyl-	14.7	Gamma	Metoprolol	4.2	Alpha 2.5
Amphetamine, 4-Methylthio- (4-MTA)	13.4	Gamma	Mexilitine	2.7	Gamma 7.5
Amphetamine, Hydroxy-	30.6	Gamma	Midazolam	3.1	Gamma
Atenolol	3.4	Gamma	Mirtazapine	5.4	Gamma
Bisoprolol	2.2	Gamma 2.5	Mirtazepine, N-Desmethyl	7.5	Gamma
Brompheniramine, Dinor-	0.8	Beta 7.5	Nadolol	2.6/1.3/split***	Gamma
Brompheniramine, Nor-	1.0	Beta	Nefopam	7.6	Alpha
Bupropion	10.1	Alpha	Oxprenolol	10.0	Beta
Bupropion, Erythroamino-	7.5	Gamma	Pentazocine	7.2	Gamma 7.5
Bupropion, Hydroxy-	6.3	Beta	Pheniramine	2.8	Beta 7.5
Bupropion, Threoamino-	7.6	Alpha	Phenmetrazine	19.5	Alpha
Butriptyline, N-desmethyl-	1.1	Alpha	Phenylephrine, N-Desmethyl-	3.6	Alpha
Chloroquine, N,N,-Didesethyl-	1.9	Gamma 7.5	Phenylpropanolamine	42.6	Gamma
Chloroquine, N-Desethyl-	1.1	Alpha	Pindolol	2.0	Beta 7.5
Chlorpheniramine	2.5	Beta 7.5	PMA (p-Methoxyamphetamine)	25.3	Gamma
Chlorpheniramine, Dinor-	1.0	Beta 7.5	PMMA (p-Methoxymethamphetamine)	13.1	Gamma
Chlorpheniramine, Nor-	1.1	Beta 7.5	Propranolol	4.3	Alpha 2.5
Citalopram	11.1	Gamma	Pronethalol	2.5	Alpha 2.5
Citalopram N-Oxide	11.0	Gamma	Propoxyphene	7.3	Alpha
Citalopram, Dinor-	8.0	Gamma	Pseudoephedrine	5.2	Gamma
Citalopram, Nor-	8.0	Gamma	Quetiapine	4.4	Gamma
Cyclazocine	3.8	Beta	Quinidine	2.3	Alpha
Cyclobenzaprine	1.9	Gamma 7.5	Salbutamol	8.3	Beta
Cyclobenzaprine, N-Desmethyl-	5.4	Alpha	Tetramisole	3.6	Gamma
Desloratadine	6.4	Gamma	Tramadol	13.3	Gamma
Dihydro-N,N-dimethyl-5-methylene- **	3.2	Alpha	Tramadol, Nor-	10.3	Gamma
Disopyramide, p-Cl	4.7	Beta	Trimipramine	7.3	Alpha
Disopyramide, N-Dealkylated-	4.6	Alpha	Trimipramine, Nor-	2.0	Alpha
Doxapram	7.2	Gamma	Venlafaxine	5.5	Gamma
Doxylamine	2.2	Gamma 7.5	Venlafaxine, O-Desmethyl-	4.3	Gamma
EDDP (Methadone Mtb.)	3.0	Beta 7.5	Verapamil	7.1	Alpha
EMDP (Methadone Mtb.)	7.5	Gamma	Verapamil, Nor-	6.9	Alpha
Ephedrine	6.1	Alpha	Zopiclone	24.0	Gamma
Ephedrine, Hydroxy	15.6	Gamma	Zopiclone N-Oxide	23.6	Gamma
Esmolol	3.1	Gamma	Zopiclone, Nor-	31.6	Gamma
Fluoxetine	12.0	Alpha 2.5			

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

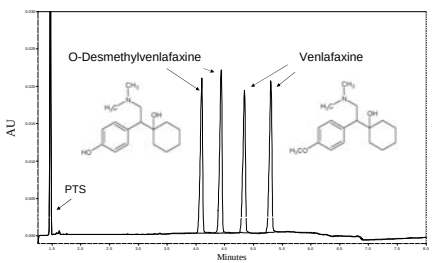

Figure 1: Venlafaxine and Metabolite in 5% Gamma-HSCD

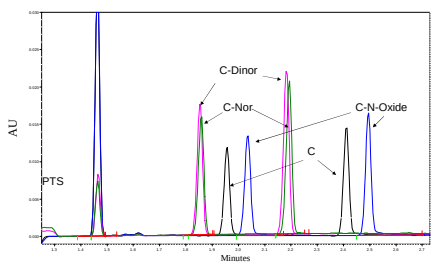

Figure 2: Citalopram (C) and Metabolites in 5% Gamma-HSCD

Conclusion

Finding a generic method for chiral separations has been a challenge for many years. A unique group of drugs and metabolites, expected to be found because of wide spread administration to the general population, was used to evaluate the highly sulfated cyclodextrin strategy. These 101 racemic drugs and metabolites are of interest to both the pharmaceutical and forensic communities. The compounds were screened and base-line resolution was achieved for over 94% of the group without method optimization. The result confirms the usefulness of this rapid solution to complex enantiomeric separations.

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)