In Search of a Generic Chiral Strategy: 101 Separations With One Method

In any approach to drug discovery, the challenges presented to the analytical chemist are compounded when a product contains one or more chiral centres. Enantiomers are stereoisomers that display chirality, having one or more asymmetric carbon centres, allowing them to exist as non-superimposable mirror images of one another. These isomers are difficult to analyse as they are both physically and chemically identical and differ only in the way they bend plane-polarised light and in their behaviour in a chiral environment.

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The key to separating enantiomers is to first create diastereomers from these enantiomers. Diastereomers may be created through chemical derivatisation with a 'chiral' reagent, or they may be formed transiently through interactions with chiral selectors. The latter, of course, is usually the most desirable as it is the easiest to employ. These chiral selectors have historically been introduced in the form of chromatographic media using HPLC, SFC or GC as the separation technique. Although chromatography has been a very effective methodology for chiral separations, the process of developing the methodology tends to be very expensive as development time is long, column lifetimes are short, and costs of chiral reagents are high.

Capillary electrophoresis has proven the most ideal analytical tool for this purpose, as it is simple to construct and modify a chiral environment within a capillary. The use of cyclodextrins for differential host-guest complexation of enantiomers is by far the most common 'solution-based' chiral selector and is the basis of the chiral separation strategy that we propose. The primary strategy [1] focuses on the use of highly sulfated cyclodextrins (HSCDs) which are a family of three chiral reagents [2]. This strategy first involves screening the compound for separation using all three (α, β and γ) HSCDs and then optimising the reagent which yields the best resolution.

The objective of the current study was to investigate the use of charged cyclodextrins in the on-going search for a generic strategy for the separation of enantiomeric drug substances. A group of compounds selected from a set of drugs and metabolites of pharmaceutical and forensic interest was separated using HSCDs. This was a challenging group because it included many closely related metabolites of drug substances, in addition to the parent drugs. For simplicity, the screening strategy was designed to separate the enantiomers of individual compounds, although we present examples of separation of both drugs and their metabolites.

MATERIALS AND METHODS

Chemicals: Solutions of alpha-, beta and gamma-HSCD at a concentration of 20% w/v and all other reagents were obtained from Beckman Coulter, Inc, Fullerton, CA, USA. These reagents were prepared per the following table:

Concentration of HSCD	Volume and Concentration of HSCD	Volume of 50mM Phosphate Buffer	Volume of Water*	Final Volumes
7.50%	2.75 mL of 20%	3 mL	0.25 mL	6 mL
5%	1.5 mL of 20%	3 mL	1. 5mL	6 mL
2.50%	0.75 mL of 20%	3 mL	2.25 mL	6 mL

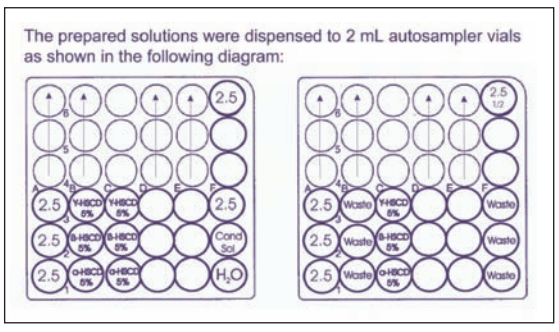

Figure 1. Tray location: 2.5 = 25 mM Buffer Cond Sol = Conditioning Solution

The prepared solutions were dispensed to 2ml autosampler vials as shown in *Figure 1*.

Drug and Metabolite Standards: Standards were purchased from Cerilliant Corporation, Round Rock, TX, USA, or obtained as a gift from either 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 1mg/mL and diluted to 25ppm (25ng/µL) in water.

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

Instrument: P/ACE™ MDQ Capillary Electrophoresis System (Beckman Coulter, Inc.) equipped with a Photodiode Array Detector (PDAD) with detection at 200nm (scanning190-350) and 32 Karat™ Version 7 Software.

Run Buffers: All chiral separations were performed in 5% HSCD in 25mM triethylammonium phosphate pH2.5, unless otherwise noted as either run in 2.5% or 7.5% of the HSCD.

Capillaries and Conditioning: Fused-silica capillaries, 50µm I.D. x30cm (effective length 20cm) were used in all separations. The columns are rinsed daily with 0.4% PEO (MW 300,000), 10% ethyleneglycol and adjusted to pH 4.75 to speed up column equilibration.

Applied Voltage: The voltage was set at 15kv (500v/cm), which resulted in running currents of 140 to180µamps for 50µm I.D. columns.

Temperatures: Capillary and sample storage =22°C.

Sample Introduction: Pressure injection of 4 seconds at 0.3psi.

System Suitability: The instrument was checked daily with test mixtures designed to assess the performance of both the reagents and the instrument. Example runs are shown in *Figure 2*.

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

1. All drugs were basic drugs and a subset of a large group (692) included in a screen developed by Hudson et al. [3].
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 cyclodextrinin 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.
2. This was necessary because many of the metabolites were rare compounds and were not available as accurately known quantitative standards.
3. The group of 101 compounds was run in the 5% solution of each HSCD.
4. Results are shown in *Table 1* and include the resolution and the corresponding HSCD System.
5. Two examples of HSCD separations of venlafaxine, citalopram and their metabolites in Gamma-HSCD are shown in *Figures 3 and 4*.
6. Of the 101 compounds, 28 were selected for additional runs at a concentration of 2.5 and 7.5% of each HSCD.
7. Ninety-five (95) of the 101 compounds (94%) had a resolution of 2.0 or greater.
8. A resolution of 1.0 or greater was determined using a USP model for 100 of the 101 compounds.

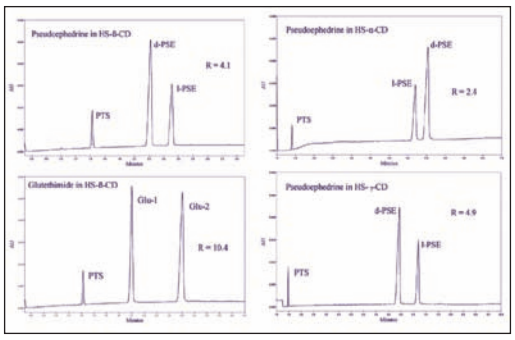

Figure 2. System Suitability Test Mixtures

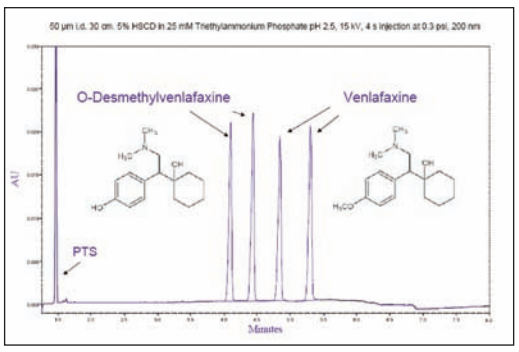

Figure 3. Venlafaxine and Metabolite in 5% Gamma-HSCD

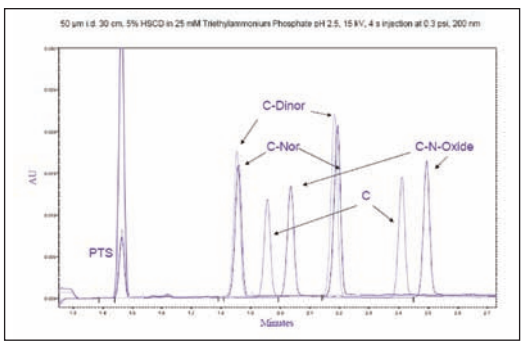

Figure 4. Citalopram (C) and Metabolites in 5% Gamma-HSCD

CONCLUSION

Capillary electrophoresis offers the advantages of high efficiency separations [4], short analysis times, short equilibrium times when changing selector and low selector consumption [5-9].

CE is also low risk in that there are no expensive HPLC columns that can be damaged and it offers a "green" alternative to HPLC with minimal solvent waste production. These factors combine to make chiral method development by CE rapid and cost effective when compared with other technologies.

Transfer of chiral CE methodologies to quality control highlights the need for reproducibility in separation and assay robustness. Historically, cyclodextrins used for chiral analysis have been notorious for lot-to-lot variability [10-12]. The reason was due primarily to the manufacturing intent of these reagents, typically produced in large scale for applications other than chiral analysis.

Sulfated cyclodextrins with more than one-half of the possible sites sulfated are referred to as 'highly sulfated.' Highly sulfated cyclodextrins with average degrees of sulfation of [11, 12, 13] for α-, β-, γ-CD, respectively, with a narrow range of heterogeneity and low batch-to-batch variations, were synthesized and used in CE by Evangelista and Chen [13].

These reagents are manufactured by the supplier using a controlled sulfation reaction and formulated to produce consistent lots. All HSCDs are developed using strict quality control procedures which test the reagents' consistency in functional assays.

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 after 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 baseline resolution was achieved for over 94% of the group without method optimisation. The result confirms the usefulness of this rapid solution to complex enantiomeric separations.

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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