Fundamental Characterization of the Separation of Steroid Enantiomer Pairs by Reversed-Phase HPLC Using Polarity Models

Mst Ummul Khair, Eric Kipruto, Ph.D., David J Anderson Ph.D.

Department of Chemistry, Cleveland State University, Cleveland, OH,44115, United States

Abstract

- Steroid enantiomers such as abiraterone metabolite pairs, estradiol isomers, bile acid enantiomer pairs play a role in the diagnosis of cancer treatment, female reproductive diseases, and metabolic regulations, respectively.
- Separation of the steroid enantiomers will allow examination of the enantiomer effectiveness in clinical treatment and diagnostic efficacy.
- Due to structural chemical similarities leading to co-elution the steroid enantiomers are very difficult to separate.
- The objective of this study is to discover fundamental principles that would predict reversed phase separation.

Introduction

Enantiomers are important because only one of the enantiomer pair molecules is active to bind to an enzyme or receptor to cause its physiologic effect, while the other molecule of the enantiomer pair is inactive. This work studies the effect of the three components of polarity of the mobile phase, as well as the sum of them, on the separation enantiomers, determining which ones affect the separation. The three components of polarity are hydrogen bond donor acidity (α), hydrogen bond donor basicity (β) and dipolarity/polarizability (π*).

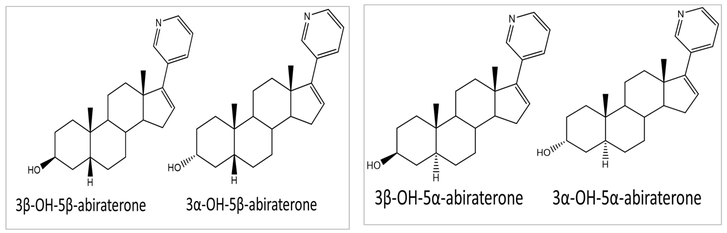


Figure 1. Abiraterone-metabolites enantiomer pair

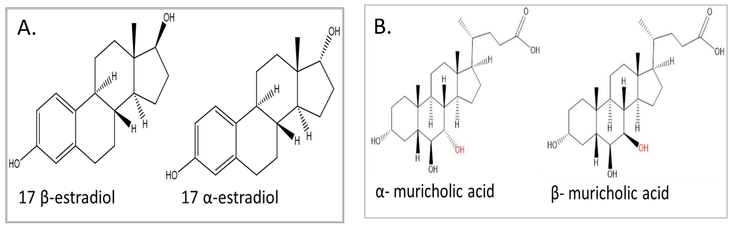


Figure 2. A. Estradiol enantiomer pair B. Bile acid enantiomer pair

Instruments and Parameters

Column: Zorbax Eclipse Plus C_{18}, 150 mm x 2.1 mm, 3.5 µm (Agilent)
Shimadzu LC-UV, Flow rate 0.25 ml/min, detection at 214 nm. Column Temp at 25^{0}C.
Shimadzu LCMS 2020, SIM monitoring mode (single quadrupole mass spectrometer)
Flow rate 0.2 ml/min, Column Temp. 40^{0}C.

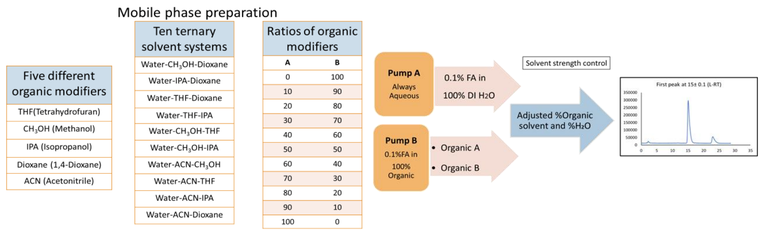
Experimental design

$$\text{Resolution, } R_S = \frac{\sqrt{N}}{4} \times \frac{(\alpha - 1)}{\alpha} \times \frac{k}{1+k}$$

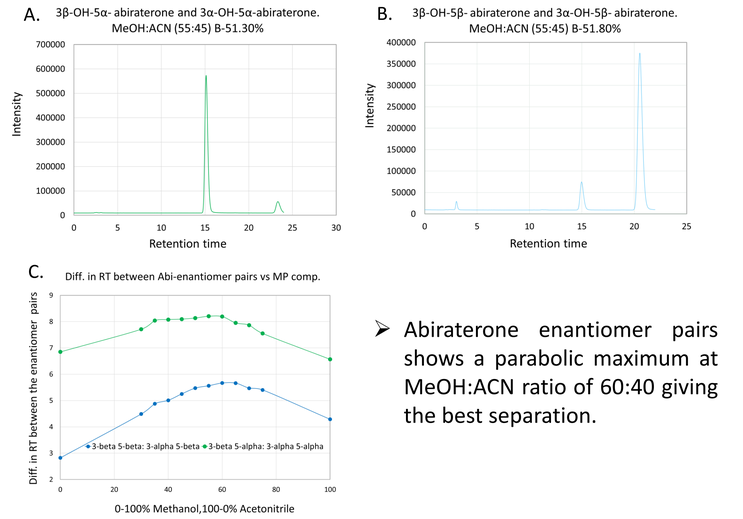
constant — Same C18 column
constant — Mobile phase solvent strength constant

Varying the mobile phase polarity:
Water : Organic A: Organic B (Ternary solvent system) (different ratios)
→ The % of water and the % of organic
→ The target retention time of the first peak at 15 minutes
→ Mobile phase solvent strength constant

The ratio of the organic modifiers are changed to vary the mobile phase polarity and the % organic and %water is adjusted to keep the k constant



Results



➢ Abiraterone enantiomer pairs shows a parabolic maximum at MeOH:ACN ratio of 60:40 giving the best separation.

Figure 3. A-B. LC Chromatograms for abiraterone enantiomer pairs and C. RT vs MP comp. plots

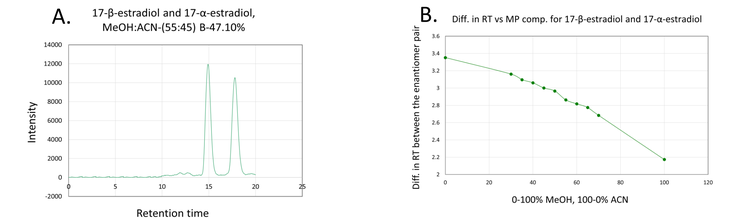


Figure 4: A. LC-Chromatogram for estradiol enantiomer pair and B. RT vs MP comp. plot

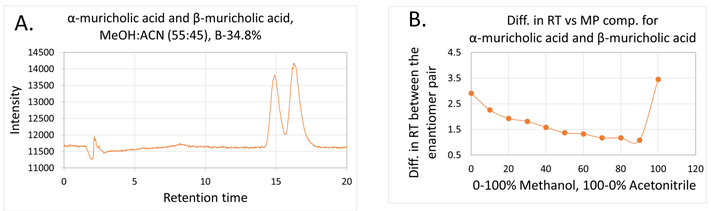


Figure 5: A. LC-Chromatogram for bile acid enantiomer pair and B. RT vs MP comp. plot

➢ The best separation is achieved for estradiol enantiomer pair with water and 100% acetonitrile and for bile acid with water and 100% methanol.

Table 1: Total polarity and Fractional polarity components literature values

Solvent	$E_T(30)$,(P')	α/Σ	β/Σ	π*/Σ
Water	63.1	0.4057	0.1698	0.4245
ACN	45.6	0.15	0.25	0.6
MeOH	55.4	0.43	0.29	0.28
Dioxane	36	0	0.4	0.6
THF	37.4	0	0.49	0.51
IPA	48.4	0.35	0.43	0.22

$E_T(30)$ – Total polarity
α - (acidity fraction of total polarity)
β - (basicity fraction of total polarity)
π*-(dipolarity/polarizability fraction of total polarity)

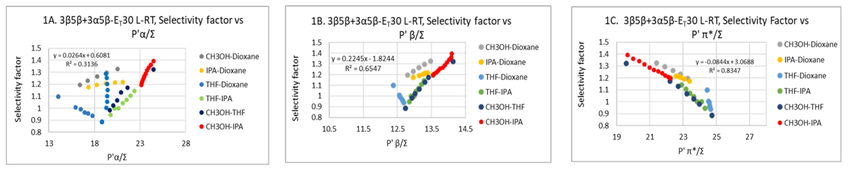
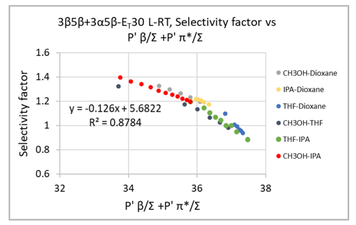


Figure 6. Individual polarity component (1A. Acidity 1B. Basicity 1C. Dipolarity) vs selectivity factor plots for abiraterone enantiomer pair.

➢ Among the three plots, dipolarity and basicity polarity components are following a trend and correlating well with selectivity factor.



➢ When the basicity and dipolarity polarity components are added up for the abiraterone enantiomer pair, it shows better correlation with selectivity factor.

Figure 7. The sum of basicity and dipolarity polarity component vs selectivity factor plots for abiraterone enantiomer pair.

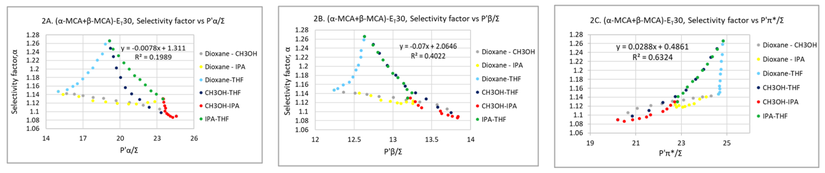
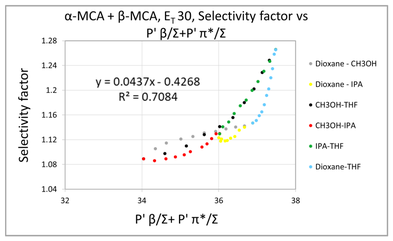


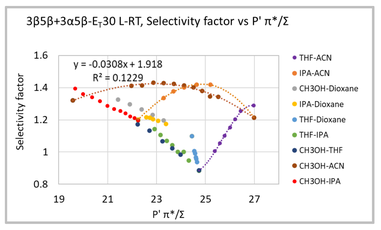
Figure 8. Individual polarity components vs selectivity factor plots for bile acid enantiomer pair.

➢ Among the three plots, dipolarity and basicity polarity components are following a trend and correlate well with selectivity factor although there is a little discrepancy observed for the blue data.



➢ When the basicity and dipolarity polarity component are added up for the abiraterone enantiomer pair, it shows better correlation with selectivity factor , even the blue data is now following the trend.

Figure 9. The sum of basicity and dipolarity polarity components vs selectivity factor plots for bile acid enantiomer pair.



➢ Three acetonitrile containing mobile phases marked as purple, orange and brown color showing parabolic shapes do not follow the trends identified for the non-ACN mobile phases.

Figure 10. Dipolarity polarity component vs selectivity factor plots for abiraterone enantiomer pair for both acetonitrile and non-acetonitrile containing mobile phases.

Conclusions

- The sum of basicity and dipolarity polarity components is the determiner of the enantiomer pair separation.
- Although acetonitrile containing mobile phases give good separation, but it does not show good correlation with other non-acetonitrile containing mobile phases.

Future work

- Investigate more enantiomer pairs and study the different interactions between solute and solvent.
- Investigate other properties besides polarity that affects the separation for acetonitrile containing mobile phases.