Rapid Separation and Characterization of Chiral Small Molecule Drugs with timsTOF

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Introduction

It is of crucial importance to rapidly analyze chiral molecules throughout drug discovery and development process to ensure synthesis quality and drug safety[1]. Chiral HPLC using a chiral stationary phase is a popular method to analyze chiral molecules, however, it is time consuming and costive to select proper chiral column and optimize conditions. Ion-mobility spectrometry – mass spectrometry (IMS-MS), an analytical chemistry method that separates gas phase ions based on their interaction with a collision gas and their mass, has been an important technology to reveal structural diversity of isomers and used for rapid analysis of chiral molecules by a variety of means, involving a reaction with a chiral selector to form diastereomers or complexes[2]. High resolving power of IMS MS could make it possible to separate enantiomers of small drug and drug-like molecules without any chiral selector[3], therefore, has been a focal point for MS vendors and researchers. In this study, the separation power of the trapped ion mobility spectrometry (TIMS) is explored for the rapid separation of racemic drugs Thalidomide, Oxazepam, and Verapamil solely based on the differences of collisional cross section (CCS) of enantiomers.

Methods

Thalidomide, Oxazepam, and Verapamil and solvents were purchased from Sigma. 1.0 mg/mL stock solution of each analyte was prepared in methanol; working solutions at 50 µg/mL and 1.0 µg/mL were used for direct infusion and flow-injection analysis with LCMS acquisitions performed by Elute UHPLC and timsTOF Pro 2 with TIMS enabled in ESI positive mode. Data processing was conducted in DataAnalysis 5.3 software (Bruker). A previously established 4D-Metabolomics method with m/z 20-1300 and mobility range of $1/K_0$ 0.45 – 1.45 V.s/cm^2 was used and further optimized by adjusting ion mobility resolution mode, accumulation time, duty cycle, ramp time and ion mobility range. Both mass and CCS calibration were performed prior to data analysis.

Results and Discussions

Thalidomide racemate separation and characterization

Thalidomide is the first chiral drug to study because it is infamous for causing more than 10,000 children born with a range of severe deformities and thousands of miscarriages in late 1950s and early 1960s. Almost 20 years later, Blaschke et al[4]. discovered that only the S-enantiomer is responsible for causing birth defects, while R-enantiomer is good for morning sickness in expecting woman.

Figure 1 shows the structures of enantiomers of Thalidomide, Oxazepam, and Verapamil.

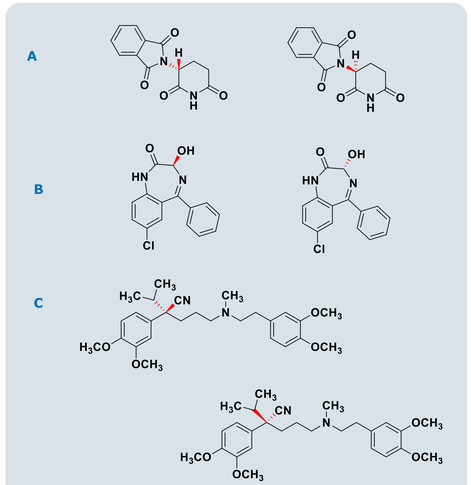

Figure 1. Structures of enantiomers of Thalidomide (A), Oxazepam (B), and Verapamil (C). Left side is R, right side is S.

Thalidomide mass spectrum, EIM and CCS

Pure enantiomer of R, S, and racemate of Thalidomide were introduced into timsTOF Pro by direct infusion using a syringe pump, also by flow injection analysis LC-MS. Its major sodium adduct ion of 281.0532 $[M+Na]^+$ were observed. The full MS spectra from EIC and EIM of Thalidomide are shown in Figure 2.

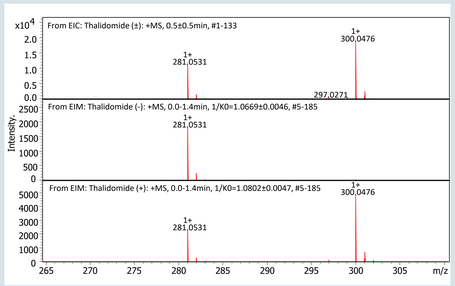

Figure 2. Mass spectrum of thalidomide

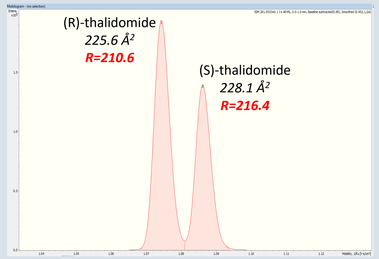

Fig 3. EIM of racemate of Thalidomide (±)

In book chapter by Philips et al[3] that the drift time profiles of R and S thalidomide are identical and have more than one peak in both positive and negative ion modes, therefore, could not be separated. We could only speculate that the drift tube IMS might not be optimal in design with high enough ion mobility separation resolution nor in proper operation.

With timsTOF, both R and S of Thalidomide are baseline separation, and each shows only one peak with great symmetry. By spiking and testing different ratio of Thalidomide (+) and Thalidomide (-), it was identified the first EIM peak belongs to Thalidomide (-). Since TIMS has two TIMS analyzers as one is for ion accumulation, the second one is to release ions, which offers great ion mobility resolving power (resolution Rs ~200) by design and make chiral separation possible based on analyte's shape and size.

Oxazepam and Verapamil ion mobility separation

A mixture of racemate of Oxazepam and Verapamil were investigated similarly. With optimized timsTOF Pro experimental condition, well baseline ion mobility separation Verapamil m/z = 455.2904 $[M+H]^+$ and

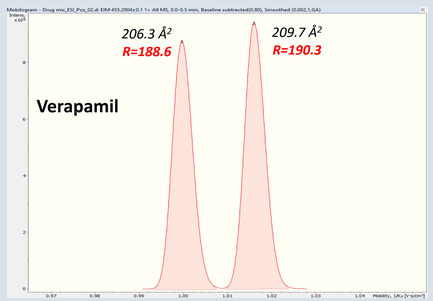

Figure 4. EIM of Verapamil

Name	Enantiomers	Trace [M+H]+	Mobility, 1/K0	Measured CCS [Å²]	Measured IM Resolution
Thalidomide	+	EIM 281.0533±0.1 + All MS	1.074	210.6	225.6
	-	EIM 281.0533±0.1 + All MS	1.086	216.4	228.1
Verapamil	I	EIM 455.2904±0.1 + All MS	1.000	206.3	188.6
	II	EIM 455.2904±0.1 + All MS	1.016	209.7	190.3
Oxyzepam	I	EIM 287.0582±0.1 + All MS	0.775	162.6	183.3
	II	EIM 287.0582±0.1 + All MS	0.783	164.3	170.4

Table 1. Ion mobility resolution and CCS of Thalidomide, Oxazepam and Verapamil

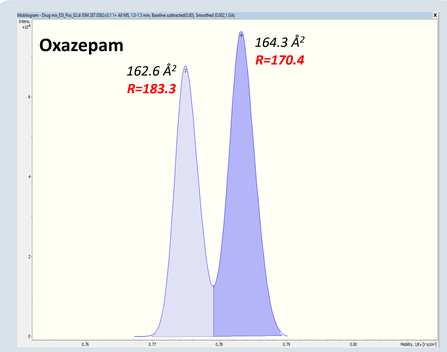

Figure 5. EIM of racemate of Oxazepam

partial ion mobility of Oxazepam m/z=287.0582 $[M+H]^+$ were achieved which are listed in Figures 4 and 5.

Flow-injection sample injection approach for the ion mobility mass spectrometry analysis of chiral drugs was also performed achieving similar results with much fast sample analysis throughput, which could be an effective way for screening chiral drugs. As ultra-high resolution mode is typically applied to achieve high ion mobility resolution to differentiate enantiomers, both mass calibration (TOF) and ion mobility (TIMS) calibration prior to chiral drug separation are required to ensure timsTOF achieving accurate and reliable data quality. With the optimized ion mobility separation conditions, this method could be applied for LC-MS to quantify enantiomer component. Please notice this workflow is mainly used as targeted workflow. For non-targeted chiral compound ion mobility separation, a survey scan is required in order to detect analytes eventually modify the TIMS experimental parameters to achieve high resolution ion mobility separation of chiral drug. Enable PASEF would assist to characterize the enantiomer chemical structure. This is a fast and cost-effective method for phrenetical analysis.

In summary, timsTOF Pro offers an ion mobility separation resolution of 200, capable to separate some chiral drugs without a chiral selector. The CCS of enantiomers measured can be used to differentiate chiral drug molecules as the 4th dimensional parameter.

References

(1) Nguyen *et al.*; International Journal of Biomedical Science, 2006 : 85-100.

(2) Zhang, *et al.*; Comprehensive Analytical Chemistry, 2019, 83: 51-81.

(3) Philips *et al.*; Chapter in *Methods in molecular biology,* Jan 2019, vol. 1939, 161-178.

(4) Blaschke *et al.*;Arzneim.-Forsch, 1979, 29: 1640-1642.

Conclusions

- Chiral drugs were successfully separated using direct infusion and flow injection method with timsTOF Pro WITHOUT A CHIRAL SELECTOR.
- CCS values of enantiomer should be used to characterize and differentiate chiral drug and drug-like molecules.
- timsTOF technologies, with two tims design, offers 200 resolution in ion mobility separation, which can significantly accelerate drug discovery processes.

Ion mobility for chiral drug separation