**Supporting Information**

**Precise Analysis of Thyroxine enantiomers in Pharmaceutical Formulation by Mobility Difference based on cyclodextrin**

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1. **Calculation parameters used in IMOS software:**

Method used: Trajectory Method

Number of Orientations: 330

Gas molecules per orientation: 330

The TM Method used is TMLJ (4-6-12 potential)

Gas: N2

Reduction Coef: 1.000

Molecular mass of Gas: 28.00 Da

Alpha polarization: 1.70 A3

Radius of gas: 1.50 A

Temperature: 304 K

Pressure: 101325 Pa

1. **Trapped Ion Mobility Spectrometry-Mass Spectrometry**

All ion mobility analysis for the complexes was performed on a TIMS-TOF instrument from Bruker Daltonik (Bremen, Germany) equipped with electrospray ionization (ESI) source. Mass and mobility calibration for the TIMS-TOF were measured with tune mix prior to the experiment. Before the experiment, the calibration state of the TIMS-MS was to assess with tuning mix to ensure its accuracy and precision, followed by direct 500 μL sample injection through a syringe pump (Giarmata, Romania). The instrument condition was set according to the optimization for the generation of target mass peaks, and some significant parameters of the TIMS-MS are as below:

In brief, a mass scan allows all ions to be detected and the m/z 322.0212 to m/z 2121.9331 was calibrated via the calibration solution performed in positive ion mode. Simultaneously, for higher resolution IMS analysis, calibration was performed over a smaller mobility range (1/K0, 0.7-2.14) using three m/z values (622.0290, 922.0098, 1221.9906) in positive mode or negative mode. Meanwhile, the ramp voltage range was kept constant but shifted to lower or higher voltages to detect all the required calibrate ions. The main experimental conditions were similar with the previous reports (Wu et al., 2022; Liu et al., 2022), which employed as following: the ionization source conditions were end plate offset of 500 V; spray tip voltage was 3.0 kV; sample solution flow rate was 2.0 μL min-1; nebulizer gas pressure was 0.3 bar; drying gas was 2.0 L min-1 at 200 °C; The TOFMS settings were rf funnel 1 of 300 Vpp; rf funnel 2 was 400 Vpp; multipole rf was 500 Vpp; quadrupole ion energy was 5 eV at low mass of 200 m/z; transfer time was 80 μs; prepulse storage was 7 μs; ramp time was 500 ms and accumulation time was 50 ms; rolling range was 25X. The mass range was set at m/z 100-3000. The duty cycle was 2%. Herein, TIMS parameters (accumulation and ramp time) were adjusted to obtain maximum resolving power for a set of saccharides. Besides, the intensity measured in this work is the relative intensity, which defines the height of the base peak (the strongest peak) in the mass spectrum as 100%, and the remaining peaks are expressed in proportion to the base peak. And the extensive experimental data analysis and processing is performed using the DataAnalysis 5.0 software.

In TIMS, the mobility (K) is one of crucial parameter, which can be described as: (Ridgeway et al., 2018)

$K=\frac{V\_{g}}{E}=A(\frac{1}{\left(V\_{elution}-V\_{out}\right)})$ (1)

Where the $V\_{g}$ is gas velocity and E is the electric field under the ion elutes, A is the calibration constant and associated with calibration solution, $V\_{elution}$ is elution voltage and $V\_{out}$ is the voltage of the last electrode in the analyzer.

Another key parameter is collision cross section (CCS), which is calculated by K0, The CCS is defined as the equation below: (Ridgeway et al., 2018)

$CCS=\frac{\sqrt{18π}}{16}\frac{q}{\sqrt{kT}}\sqrt{\left(\frac{1}{M}+\frac{1}{m}\right)}\frac{1}{N}\left(\frac{1}{K\_{0}}\right)$ (2)

Where the q is the charge number of ions, k is the Boltzman’s constant, T is the gas temperature, M and m are the molecular weight of the ions and buffer gas, and N is the number density of the buffer gas.

1. **The energy of the complexes by Chemical Theoretical Calculation**

Nine possible structures for the complexes of [β-CD+D-T4+H]+ and [β-CD+L-T4+H]+ were first optimized with a AutoDock software, the affinity for the nine possible modes for the complex as displayed in Table S1, and the mode with lower relative energies were marked in red and selected further optimized to obtain the final structure by Gaussian. The affinity for the optimized structures by Gaussian was shown in Table S2, and their CCS value was calculated using IMoS program and the trajectory method. And the modes marked in red have a good CCS agreement with the experimental, then those structure were selecsoted as the models for the structure measurement of [β-CD+D/L-T4+Ca]2+. Similar, nine possible structures for [β-CD+D-T4+Ca]2+ and [β-CD+L-T4+Ca]2+ were first optimized with a AutoDock software, and the complexes with relatively low energy were further optimized by Gaussian, then the favored complexes were CCS calculated and compared with the experimental results.

 **Table S1** The possible mode and the affinity for the complexes by AutoDock software

|  |
| --- |
| Affinity (kcal mol-1) |
| Mode | [β-CD+D-T4+H]+ | [β-CD+L-T4+H]+ | [β-CD+D-T4+Ca]2+ | [β-CD+L-T4+Ca]2+ |
| 1 | -4.9 | -5.3 | -0.971 | -1.035 |
| 2 | -4.9 | -5.2 | -0.937 | -1.027 |
| 3 | -4.8 | -5.0 | -0.882 | -0.941 |
| 4 | -4.8 | -5.0 | -0.763 | -0.862 |
| 5 | -4.8 | -5.0 | -0.746 | -0.858 |
| 6 | -4.7 | -5.0 | -0.744 | -0.857 |
| 7 | -4.6 | -4.9 | -0.738 | -0.825 |
| 8 | -4.6 | -4.9 | -0.730 | -0.813 |
| 9 | -4.5 | -4.9 | -0.727 | -0.769 |

 **Table S2** The affinity for the complexes by Gaussian

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| --- |
| Affinity (106 kcal/mol) |
| Mode | [β-CD+D-T4+H]+ | [β-CD+L-T4+H]+ | [β-CD+D-T4+Ca]2+ | [β-CD+L-T4+Ca]2+ |
| 1 | -3.251 | -3.252 | -3.27289 | -3.27409 |
| 2 | -3.233 | -3.034 | -3.27287 | -3.27407 |
| 3 | -3.230 | -3.031 | -3.27225 | -3.27365 |
| 4 | -3.226 | -3.029 | -3.27204 | -3.27315 |
| 5 | -3.220 | -3.029 | - | - |

1. **A comparison of the proposed method with the prior methods**

**Table S3** A comparison of the proposed method with the prior methods

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Matrix | Separation method | Detected method | Time | LOD | Reference |
| serum | using a chiral mobile phase additive and a silica column | HPLC | 12 min | 0.1 μg ml-1 | (Wang et al., 2003) |
| pharmaceuticals | crown ether type chiral stationary phase | HPLC | chiral stationary phase preparation needs 1 mouth | -- | (Jeon, et al., 2010) |
| water, human serum and commercial tablet | chiral multifarene [3,2,1] and g-C3N4 quantum dots | electrochemical | >2 h | 67 pM ≈ 52 ng mL-1  | (Zhao et al., 2021) |
| plasma specimen | Chiral Crown Ether Column | UPLC-ESI-MS/MS | 8 min | 0.5 ng mL-1 | (Thapa et al., 2020) |
| pharmaceutical formulations | Chiral Crown Ether Derived Chiral Stationary Phase | UPLC-ESI-MS/MS | -- | 0.01 μg mL−1 | (Lee et al., 2022) |
| pharmaceutical tablet | Simple complex with β-CD and metal ion | IMS-MS | <5 min | 0.11 ng mL-1 | Proposed method |

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