

Chiral Mass Spectrometry: An Overview

Dong-Qi Han^{1,2}, Zhong-Ping Yao^{1,2*}

¹State Key Laboratory of Chemical Biology and Drug Discovery, Food Safety and Technology Research Centre and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China

²State Key Laboratory of Chinese Medicine and Molecular Pharmacology (Incubation) and Shenzhen Key Laboratory of Food Biological Safety Control, Shenzhen Research Institute of Hong Kong Polytechnic University, Shenzhen 518057, China

*Author to whom correspondence should be addressed:

Dr. Zhong-Ping Yao

Department of Applied Biology and Chemical Technology

The Hong Kong Polytechnic University

Hung Hom, Kowloon

Hong Kong

Tel: (852) 34008792. Fax: (852) 23649932

E-mail: zhongping.yao@polyu.edu.hk

List of abbreviations:

CCS: Collision cross section

CID: Collision-induced dissociation

CR: Chiral recognition ratio

CS: Chiral selector

DFT: Density functional theory

DMS: Differential ion-mobility spectrometry

DOPA: Dihydroxyphenylalanine

DTIM-MS: Drift tube ion mobility mass spectrometry

ee: Enantiomeric excess

FAIM-MS: Field asymmetric waveform ion mobility mass spectrometry

FTICR: Fourier transform ion cyclotron resonance

IM-MS: ion mobility mass spectrometry

IR: Infrared

IRMPD: Infrared multiphoton dissociation

IRPD: Infrared photodissociation

KM: Kinetic method

M: Metal ion

MALDI: Matrix-assisted laser desorption/ionization

MS: Mass spectrometry

MS/MS: Tandem mass spectrometry

PD: Photodissociation

Ref: Reference compound

TIMS: Trapped ion mobility spectrometry

TWIM-MS: Travelling wave ion mobility mass spectrometry

UV: Ultraviolet

UVPD: Ultraviolet photodissociation

Abstract

Chiral analysis by mass spectrometry has attracted much attention due to its advantages in speed, sensitivity and specificity. In this review, recent advances in chiral analysis by mass spectrometry are summarized, with the methods based on tandem mass spectrometry (e.g., kinetic method, chiral recognition ratio method and photodissociation mass spectrometry method) and ion mobility mass spectrometry highlighted. Different methods are compared, and the limitations of the current studies and the prospects of chiral mass spectrometry are discussed.

Keywords

Chiral recognition; mass spectrometry; kinetic method; chiral recognition ratio method; photodissociation mass spectrometry; ion mobility mass spectrometry.

1. Introduction

Many basic biomolecules such as amino acids, saccharides and nucleotides are chiral and are building blocks of important macromolecules for various biological functions [1]. The two enantiomers of chiral drugs can have different biochemical pathways which lead to very different metabolic rates and biological effects, e.g., one enantiomer may have desired beneficial effects while the other may be inactive or even have undesirable side effects. Therefore, chiral analysis is of fundamental and applied importance. Various techniques have been applied for chiral analysis, including mass spectrometry (MS), X-ray crystallography, circular dichroism, nuclear magnetic resonance and chromatography [2], among which MS shows attractive advantages such as short analysis time, low limit of detection and the ability to specifically detect analytes from complex mixtures [3]. MS can also provide a unique environment to probe chiral recognition without interference of solvents or other mediators, providing a better understanding on fundamental mechanism of the chiral recognition [4]. Therefore, there have been significant interests in developing MS-based platforms for chiral analysis.

MS is intrinsically “chiral-blind”, because two enantiomers have the same mass and typically show identical mass spectra. To distinguish enantiomers by MS, a chiral environment should normally be introduced, which is generally made by introduction of a chiral selector into the sample solution or chiral gas into the mass spectrometer. Chiral discrimination can then be observed using single-stage MS, tandem MS (MS/MS), or ion mobility MS (IM-MS) [3–6]. The signals detected by single-stage MS could be easily affected by the used solvents, the concentrations of analytes and chiral selectors, and the MS conditions, and in general, single-stage MS shows poorer

specificity, sensitivity and reproducibility than MS/MS. Chiral analysis by single-stage MS detection is thus not commonly used now. In this review, we will focus on the MS/MS approach, including the two most common methods, i.e., the kinetic method (KM) and the chiral recognition ratio (CR) method, and the promising photodissociation MS (PD-MS) method, as well as the IM-MS approach. Their developments, limitations and future prospects are highlighted and discussed as below.

2. Chiral analysis by MS/MS-based approaches

Chiral recognition in these studies is based on the different MS/MS fragmentation patterns of complexes formed from the two enantiomers. Complexes are normally formed by chiral analytes (A) and chiral selectors (or called reference compounds, CS or Ref) with or without metal ions (M). The complexes are normally detected as proton or metal-bound cluster ions and presented as $[(A)_n(Ref)_y + mH]^{m+}$ or $[M_x(A)_n(Ref)_y - mH]^{z+}$. The complexes can be dimers (e.g., $[(A)(Ref) + H]^+$) [7], trimers (e.g., $[(A)(Ref)_2 + H]^+$, $[(M^{2+})(A)(Ref)_2 - H]^+$) [8,9], tetramers (e.g., $[(M^{2+})_2(A)(Ref)_3 - 3H]^+$) [10] or even octamers [11], with or without the presence of one or more metal ions (typically divalent transition metal ions such as Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , or alkali metal ions such as Li^+ , Na^+ , K^+).

The typical method used for dissociation of complexes in chiral MS analysis is collision-induced dissociation (CID). When the complexes formed from two enantiomers are subjected to the same CID condition, they can have different dissociation rates, leading to their different spectral patterns. As shown in Figure. 1, the two diastereomeric complex ions (i. e.; $[Cu^{2+}(3,5\text{-diiodo-L-tyrosine})_2(R\text{-DOPA}) - H]^+$ and $[Cu^{2+}(3,5\text{-diiodo-L-tyrosine})_2(S\text{-DOPA}) - H]^+$), formed from the chiral selector

(i.e., 3,5-diiodo-L-tyrosine) and the two enantiomers (i.e., R-DOPA and S-DOPA) in the presence of Cu^{2+} ion, were observed to lose 3,5-diiodo-L-tyrosine with different abundances in their CID spectra [12]. Chiral recognition can then be determined by comparing the intensity ratios of the product ions (i.e., KM) or of the product ions and precursor ions (i.e., CR method) between the two enantiomers. Photodissociation (PD) is another dissociation method that has also been applied in chiral MS analysis [13]. Different from CID that relies on translational excitation of ions, in PD, ions accumulate energy for dissociation via absorption of one or more photons with wavelengths ranging from infrared (IR) to ultraviolet (UV). The complexes formed from the two enantiomers may dissociate with very different fragmentation patterns under the PD conditions, and chiral recognition may be directly determined by comparing the generated PD spectral patterns [14], in addition to calculating and comparing their intensity ratios [15].

2.1 Kinetic method (KM)

KM uses MS/MS to study competitive gas-phase fragmentation of cluster ions, which typically involves the loss of a monomeric molecule and formation of another protonated cluster ion via two competing routes [16]. Early in 1997, Shen et al. used KM to distinguish the enantiomers of 2,3-butanediol with a number of reference compounds, e.g., propyl ether [7]. The fragmentation rates of the proton-bound dimeric cluster ions $[\text{A} + \text{H} + \text{Ref}]^+$ were utilized to reveal the small differences in free energies resulted from the differences in chirality. Vekey and Czira applied KM to distinguish 4 pairs of amino acid enantiomers via fragmentation of proton-bound trimers to diastereomeric dimers, which was capable of distinguishing the energy differences between the homo- and heterochiral dimeric ions [8]. Differentiation of enantiomers was further improved by comparing the relative kinetics of competitive dissociations

rate of the metal-bound trimeric ions [9]. Dissociation of the trimeric ion $[M(A)(Ref)_2 - H]^+$ typically involved competitive loss of A or Ref to produce dimeric ions. The differences in steric interactions of the diastereomeric cluster ions $[M(A_S)(Ref) - H]^+$ and $[M(A_R)(Ref) - H]^+$ could be recognized by the measured differences in branching ratios for dissociation of the complexes. The chiral discrimination for $[M(A_S)(Ref)_n - H]^+$ (where n is the number of the reference compound) could be described using R_{chiral} which could be calculated using Equation 2.1.1 [17]. The further the R_{chiral} value is from unity, the higher the degree of the chiral discrimination. The natural log of R_{chiral} was found to be linearly related to enantiomeric excess (ee), allowing quantitative chiral analysis [18].

$$R_{chiral} = \frac{[M(A_S)(Ref)_{n-1} - H]^+ / ([M(Ref)_n - H]^+)}{[M(A_R)(Ref)_{n-1} - H]^+ / ([M(Ref)_n - H]^+)} \quad (2.1.1)$$

The fixed ligand kinetic method was then introduced to simplify the dissociation kinetics and improve the chiral recognition [19]. In this method, one of the reference compounds (Ref) in the trimeric ion $[M(A)(Ref)_2 - H]^+$ was replaced by a high metal affinity fixed ligand (L^{fixed}), which would not be lost during the two-way fragmentation and could thus ensure consistent fragment ions for the comparison [20].

By using KM, chiral discrimination of monosaccharides, amino acids, amino alcohols, α -hydroxy acids, peptides, and chiral drugs such as DOPA, ephedrine, flindokalner and isoproterenol has been achieved, with compounds such as amino acids or modified amino acids as the chiral selectors and typically at the presence of divalent transition-metal ions such as Cu^{2+} , Zn^{2+} and Ni^{2+} [5,6,18].

2.2 Chiral recognition ratio method (CR method)

In 1999, Yao et al. [21] developed and applied the CR method for chiral differentiation of amino acids by analysing the CID behaviours of mainly their trimeric ions. In this method, the difference between the two diastereomeric complex ions was determined by the abundance ratio of product ion to precursor ion. Chiral recognition of 19 common amino acids could be achieved by using *N-tert*-butoxycarbonylphenylalanine, *N-tert*-butoxycarbonylproline and *N-tert*-butoxycarbonyl-*O*-benzylserine as the chiral selectors, and the results indicated that steric hindrance, π - π interaction and hydrogen bonding played important roles in the chiral recognition of amino acids with these chiral selectors [22,23]. The CR method could also be used for chiral recognition involving metal-centred complex ions, e.g., dimeric $[M(A)(Ref) - H]^+$ [24], trimeric $[M(A)(Ref)_2 - H]^+$ [25] ions. Very recently, Yu and Yao [26] applied this method for chiral differentiation of naproxen by analysing binuclear metal bound tetrameric complexes (i.e., $[(M)_2(A)(Ref)_3 - 3H]^+$) with amino acids such as histidine. Density functional theory (DFT) calculations, together with CID and IM-MS, were employed to reveal the structures of the binuclear metal bound tetrameric complexes, and it was shown that the difference in interaction between the amino group of one histidine and the naphthyl ring of naproxen caused the dissociation efficiency difference between the two diastereomers. Chiral discrimination for $[M_m(A)(Ref)_n - H]^+$ (where *m* and *n* are the numbers of the metal ion and reference compound, respectively) could be determined with an CR value as expressed by Equation 2.2.1.

$$CR = \frac{[M_m(A_S)(Ref)_{n-1} - H]^+ / [M_m(A_S)(Ref)_n - H]^+}{[M_m(A_R)(Ref)_{n-1} - H]^+ / [M_m(A_R)(Ref)_n - H]^+} \quad (2.2.1)$$

Yao et al. [23] found that the relationship between the observed dissociation efficiency (r , i.e., the intensity ratio of the product ion to the precursor ion) and the ee was nonlinear (see Figure 2a for the curve), with the relevant equation as shown in Equation 2.2.2, where a , b and c are constants. A linear calibration plot could be obtained by plotting r versus $1/(c + ee)$ (see Figure 2b), or by plotting $1/(r - r_0)$ versus $1/ee$ (see Figure 2c), where r_0 is the r value for the racemic mixture. This methodology was successfully applied in the ee determination of amino acids with less than 2% measurement error.

$$r = a + \frac{b}{c + ee} \quad (2.2.2)$$

The CR method has been successfully applied in the chiral discrimination of amino acids, dialkyl tartrates, hydroxyl esters, peptides, naphthol and chiral drugs such as atenolol, tamslosin, zolmitriptan, borneol and naproxen with amino acids, cinchona alkaloid derivative, crown ether, cyclodextrin etc. as the chiral selectors [5,6,12,27], and the use of metal ions for increasing the differences between the diastereomeric complex ions [28].

The KM and CR methods have been compared for chiral recognition in some studies. Both the KM and CR methods offered similar good results for ee measurements and similar reproducibility for chiral recognition in the study of arginine with N-blocked arginine [25]. However, since the CR method only required one product ion for comparison, it could be used for the fragmentations with only one product ion or only a second product ion of very low intensity, which could not be measured or tended to have very high errors by the KM, and could also be used in some cases where the

results contradicted with the KM [29,30]. The KM was in general expected to provide higher accuracy with the presence of a reference dissociation channel for comparison. In differentiation of 10 chiral drugs with iodo-substituted amino acids as the chiral selectors, Karthikraj et al. [12] used the KM for analysis of 3 chiral drugs that showed two abundant product ions in the MS/MS spectra of their diastereomeric complex ions, and the CR method for analysis of 7 chiral drugs that showed only one product ions in the MS/MS spectra of their diastereomeric complex ions, indicating that the KM and CR methods complemented with each other for chiral analysis of various analytes.

2.3 Photodissociation mass spectrometry method

PD, including infrared photodissociation (IRPD, also including infrared multiphoton dissociation IRMPD) and ultraviolet photodissociation (UVPD), has been mainly implemented on Fourier transform ion cyclotron resonance (FTICR) mass spectrometers [13]. PD activates and fragments ions using photons, with the energy accumulation and dissociation pathway very different from that of CID, and can provide mass spectra or frequency (or wavelength) domain spectra which might be able to differentiate diastereomeric complexes with very small differences [31,32].

In 2012, Filippi et al. [33] reported the observation of clearly different IRPD spectra of diastereomeric noncovalent complexes formed from chiral bis(diamido)-bridged basket resorcin[4]arene with enantiomers of cytosine, cytidine, and its epimer, cytarabine. Chiral discrimination of alanine [34] and isoleucine [35] with permethylated β -cyclodextrin was observed using IRPD, and enantiomers of glutamic acid [36] showed remarkably different IRPD fragmentation of their protonated dimers. Octameric complexes were employed for chiral differentiation of serine [11], proline [14] and

threonine [37] using IRPD-MS. As shown in Figure 3A, similar fragmentation patterns were observed in the IRPD mass spectra of $[(L\text{-Ser})_7(L\text{-Pro}) + H]^+$ (Figure 3A-a) and $[(L\text{-Ser})_7(D\text{-Pro}) + H]^+$ (Figure 3A-b). However, $[(L\text{-Ser})_6(L\text{-Pro})_2 + H]^+$ (Figure 3A-c) and $[(L\text{-Ser})_6(D\text{-Pro})_2 + H]^+$ (Figure 3A-d) showed clearly different IRPD mass spectra, allowing them to be unambiguously distinguished. As shown in Figure 3B, the absorption peak at 3425 cm^{-1} was sustained for the L-Pro substituted octamer but was shifted to 3465 cm^{-1} for the D-Pro substituted one (Figure 3B-a and b), and this change became clearer when two serine were replaced (Figure 3B-c), which might be related to the stretching of the H-bonded and helpful for the structure elucidation [14].

UV photons could provide higher energy for fragmentation than IR photons, so UVPD could typically allow faster and more fragmentation than IRPD and CID. In 2010, Scuderi et al. [15] used UVPD-MS to study the fragmentation of protonated dimers formed from cinchona alkaloids with quinine and quinidine. With the excitation wavelength of 310 nm, more fragmentation than CID was obtained to enable differentiation of the enantiomers. UVPD-MS has also been successfully applied in chiral analysis of monosaccharides with tryptophan [38], and tryptophan with dialanine peptide [39].

3. Chiral analysis by ion mobility mass spectrometry (IM-MS)

In IM-MS, gaseous ions are directed through a drift tube to interact with the drift gas under an electric field, and are separated based on their size and shape, which are measured as collision cross section (CCS) [40]. Diastereomeric complex ions of enantiomers can have different CCS values and different drift time, and can thus be separated in IM-MS [41,42]. Major IM-MS techniques, including drift tube ion

mobility mass spectrometry (DTIM-MS), travelling wave ion mobility mass spectrometry (TWIM-MS), field asymmetric waveform ion mobility mass spectrometry (FAIM-MS), differential ion-mobility spectrometry (DMS) and trapped ion mobility spectrometry (TIMS), have been applied for chiral analysis.

3.1 Use of drift gas modifiers

In 2006, Dwivedi et al. [43] demonstrated the gas-phase separation and resolution of enantiomers by modifying the drift gas in the drift tube with chiral vapour of 2-butanol. The selective interactions between enantiomers and the chiral modifier allowed the individual enantiomers to have different gas-phase ion mobilities and be separated. Chiral differentiations of atenolol, serine, methionine, threonine, methyl α -glucopyranoside, glucose, penicillamine, valinol, phenylalanine and tryptophan were observed by this method. The addition of the chiral modifier to the drift gas was found to reduce the mobilities of both the enantiomers, but to different extents that enabled the separation of the two enantiomers. These results showed the potential for development of a rapid method for separation and measurement of chirality.

Nachtigall et al. [44] also used 2-butanol as the chiral modifier in the drift tube for chiral and structural characterization of enantiomers by matrix-assisted laser desorption/ionization coupled with TWIM-MS (MALDI-TWIM-MS). Piperidine enantiomers were cleaved and ionized from the resin by MALDI, and interacted with the drift gas (pure N₂ or chiral modifier: (S)-2-butanol/N₂), followed by separation and detection by IM-MS. The enantiomers showed the same drift time with pure N₂ while different drift time with the chiral modifier. Mechanistic study with DFT calculation revealed that the two complex ions [(S/R)-enantiomer + (S)-2-butanol]⁺ had different

CCSs and thus different drift time.

3.2 Use of conventional chiral selectors

In 2014, Domalain et al. [45] reported differentiation of phenylalanine, tryptophan and tyrosine enantiomers by TWIM-MS analysis of their complexes with copper (II) and chiral proline. They found that, with the increased drift time difference, the peaks that were shifted to longer drift time could have increased peak width. Similar to the definition in chromatography, as shown in equation 3.2.1, the peak-to-peak resolution R_{p-p} was suggested to describe the chiral discrimination in the IM-MS spectra, where t_1 , t_2 and W_1 , W_2 are the drift time and peak widths of the two diastereomeric complex ions, respectively. Normally, a larger CCS difference of the complex ions corresponds to a larger R_{p-p} value.

$$R_{p-p} = \frac{2(t_2 - t_1)}{W_1 + W_2} \quad (3.2.1)$$

In the study of chiral cognition of amino acids by IM-MS, Yu and Yao [10] found that tetrameric complexes with binuclear metal ions $[(M)_2(A)(Ref)_3 - 3H]^+$ could generally improve the chiral discrimination compared to the trimeric complexes with single metal ion $[M(A)(Ref)_2 - H]^+$. Their results revealed that amino acids with aromatic rings or long and active side chains tended to have larger CCS differences and thus better enantioselectivity of their enantiomers (see Figure 4). They also compared the chiral recognition by MS/MS and IM-MS and found that the two approaches might be based on very different mechanisms for chiral differentiation. The chiral recognition by MS/MS might be more dependent on the energy difference of the diastereomeric complexes and thus more sensitive to the structural rigidity and active groups in the

diastereomeric complexes, while the chiral recognition by IM-MS might be more dependent on the size and shape difference of the diastereomeric complexes and thus more sensitive to the more flexible or extended structures. The utility of IM-MS for ee determination of amino acids was also investigated in this study [10]. Similar to the CR method [22], a linear calibration curve for ee determination could be obtained by plotting r versus $1/(100 - ee)$, or plotting $1/(r - r_0)$ versus $1/ee$, where r is the peak area ratio between the two diastereomeric complex ions. An absolute error less than 1% was obtained for ee determination of most of the samples investigated.

Mie et al. [46] applied FAIM-MS for chiral separation of six pairs of amino acid enantiomers via analysis of their metal-bound trimeric complexes with a mixed N_2 and He drift gas. For the chiral analysis using DMS, Zhang et al. [47,48] used *N*-tert-butoxycarbonyl-O-benzylserine as the chiral selector to form proton-bound diastereomeric dimers to allow resolution of the amino acid enantiomers. Use of lower-molecular-weight carrier gases, e.g., 50:50 He: N_2 rather than 0:100 He: N_2) was found to significantly improve the resolution.

4. Conclusions and prospects

MS/MS has been widely used for providing information about the affinity and dissociation properties of enantiomers with chiral selectors, which could allow for chiral differentiation. Methods such as KM and CR methods have been developed for determination of chiral discrimination and ee, and PD has shown features that are different from CID for chiral analysis. These methods are complementary to each other and can be applied for analysis of various chiral analytes. Furthermore, the information such as CCS and drift time, afforded by IM-MS measurements, offers a new way to

measure and distinguish the diastereomeric complexes formed by chiral analytes and selectors or interactions between chiral analytes and chiral gas modifiers. The mechanism of chiral recognition by IM-MS might be very different from that by MS/MS, and these two methods may be combined to solve different chiral analysis problems.

Although MS has been demonstrated as a powerful and promising tool for chiral analysis, most of the studies have been limited to simple chiral molecules such as amino acids or analogues and model chiral compounds. Development of MS approaches for analysis of chiral drugs and analysis of chiral analytes in complex real-life samples is thus an important task for chiral mass spectrometry. Although many chiral selectors have been employed, obtaining deep insights into the recognition mechanisms and developing a general rule for finding new chiral selectors are still under pursuing. IM-MS has merged as a very promising tool with distinct features and advantages for chiral mass spectrometry, but the resolutions of the currently available IM-MS instruments have limited the applications of IM-MS. With the improved resolution of IM-MS instruments which has been developing in the past years, IM-MS, together with MS/MS and other mass spectrometric techniques, are expected to play increasingly important roles in chiral analysis.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 81874306), Hong Kong Research Grants Council (Nos. 153040/15P, 153348/16P, 153041/17P, C5031-14E, C4002-17G and R4005-18), and The University Research Facility in Chemical and Environmental Analysis and the University Research Facility

in Life Sciences of The Hong Kong Polytechnic University.

References

- [1] R.A. Hegstrom, D.K. Kondepudi, The Handedness of the universe, *Sci. Am.* 262 (1990) 108–115. doi:10.1038/scientificamerican0190-108.
- [2] A. Rocco, Z. Aturki, S. Fanali, Chiral separations in food analysis, *Trac-Trends Anal. Chem.* 52 (2013) 206–225. doi:10.1016/j.trac.2013.05.022.
- [3] H. Awad, A. El-Aneed, Enantioselectivity of mass spectrometry: Challenges and promises, *Mass Spectrom. Rev.* 32 (2013) 466–483. doi:10.1002/mas.21379.
- [4] L.M. Wu, F.G. Vogt, A review of recent advances in mass spectrometric methods for gas-phase chiral analysis of pharmaceutical and biological compounds, *J. Pharm. Biomed. Anal.* 69 (2012) 133–147. doi:10.1016/j.jpba.2012.04.022.
- [5] X.L. Chen, Y. Kang, S. Zeng, Analysis of stereoisomers of chiral drug by mass spectrometry, *Chirality.* 30 (2018) 609–618. doi:10.1002/chir.22833.
- [6] X.Y. Yu, Z.P. Yao, Chiral recognition and determination of enantiomeric excess by mass spectrometry: A review, *Anal. Chim. Acta.* 968 (2017) 1–20. doi:10.1016/j.aca.2017.03.021.
- [7] W.Y. Shen, P.S.H. Wong, R.G. Cooks, Stereoisomeric distinction by the kinetic method: 2,3-butanediol, *Rapid Commun. Mass Spectrom.* 11 (1997) 71–74. doi:10.1002/(sici)1097-0231(19970115)11:1<71::Aid-rcm794>3.0.Co;2-o.
- [8] K. Vekey, G. Czira, Distinction of amino acid enantiomers based on the basicity of their dimers, *Anal. Chem.* 69 (1997) 1700–1705. doi:10.1021/ac960931m.

- [9] W.A. Tao, D.X. Zhang, F. Wang, P.D. Thomas, R.G. Cooks, Kinetic resolution of D,L-amino acids based on gas-phase dissociation of copper(II) complexes, *Anal. Chem.* 71 (1999) 4427–4429. doi:10.1021/ac990530h.
- [10] X.Y. Yu, Z.P. Yao, Chiral differentiation of amino acids through binuclear copper bound tetramers by ion mobility mass spectrometry, *Anal. Chim. Acta.* 981 (2017) 62–70. doi:10.1016/j.aca.2017.05.026.
- [11] F.X. Sunahori, G.C. Yang, E.N. Kitova, J.S. Klassen, Y.J. Xu, Chirality recognition of the protonated serine dimer and octamer by infrared multiphoton dissociation spectroscopy, *Phys. Chem. Chem. Phys.* 15 (2013) 1873–1886. doi:10.1039/c2cp43296j.
- [12] R. Karthikraj, S. Prabhakar, M. Vairamani, Differentiation of enantiomeric drugs by iodo-substituted L-amino acid references under electrospray ionization mass spectrometric conditions, *Rapid Commun. Mass Spectrom.* 26 (2012) 1385–1391. doi:10.1002/rcm.6237.
- [13] J.S. Brodbelt, Photodissociation mass spectrometry: new tools for characterization of biological molecules, *Chem. Soc. Rev.* 43 (2014) 2757–2783. doi:10.1039/c3cs60444f.
- [14] G.H. Liao, Y.J. Yang, X.L. Kong, Chirality effects on proline-substituted serine octamers revealed by infrared photodissociation spectroscopy, *Phys. Chem. Chem. Phys.* 16 (2014) 1554–1558. doi:10.1039/c3cp53469c.
- [15] D. Scuderi, P. Maitre, F. Rondino, K. Le Barbu-Debus, V. Lepere, A. Zehnacker-Rentien, Chiral recognition in cinchona alkaloid protonated dimers: mass spectrometry and UV photodissociation studies, *J. Phys. Chem. A.* 114 (2010) 3306–3312. doi:10.1021/jp9094497.
- [16] R.G. Cooks, P.S.H. Wong, Kinetic method of making thermochemical

- determinations: Advances and applications, *Acc. Chem. Res.* 31 (1998) 379–386. doi:10.1021/ar960242x.
- [17] W.A. Tao, D.X. Zhang, E.N. Nikolaev, R.G. Cooks, Copper(II)-assisted enantiomeric analysis of D,L-amino acids using the kinetic method: Chiral recognition and quantification in the gas phase, *J. Am. Chem. Soc.* 122 (2000) 10598–10609. doi:10.1021/ja000127o.
- [18] W.A. Tao, F.C. Gozzo, R.G. Cooks, Mass spectrometric quantitation of chiral drugs by the kinetic method, *Anal. Chem.* 73 (2001) 1692–1698. doi:10.1021/ac001150v.
- [19] L.M. Wu, R.G. Cooks, Chiral analysis using the kinetic method with optimized fixed ligands: Applications to some antibiotics, *Anal. Chem.* 75 (2003) 678–684. doi:10.1021/ac0260948.
- [20] R. Berkecz, A.R.M. Hyrylainen, F. Fulop, A. Peter, T. Janaky, P. Vainiotalo, J.M.H. Pakarinen, Chiral discrimination of beta-3-homo-amino acids using the kinetic method, *J. Mass Spectrom.* 45 (2010) 1312–1319. doi:10.1002/jms.1844.
- [21] Z.P. Yao, T.S.M. Wan, K.P. Kwong, C.T. Che, Chiral recognition of amino acids by electrospray ionisation mass spectrometry/mass spectrometry, *Chem. Commun.* (1999) 2119–2120. doi:10.1039/a906131b.
- [22] Z.P. Yao, T.S.M. Wan, K.P. Kwong, C.T. Che, Chiral analysis by electrospray ionization mass spectrometry/mass spectrometry. 1. Chiral recognition of 19 common amino acids, *Anal. Chem.* 72 (2000) 5383–5393. doi:10.1021/ac000729q.
- [23] Z.P. Yao, T.S.M. Wan, K.P. Kwong, C.T. Che, Chiral analysis by electrospray ionization mass spectrometry/mass spectrometry. 2. Determination of

- enantiomeric excess of amino acids, *Anal. Chem.* 72 (2000) 5394–5401.
doi:10.1021/ac000730p.
- [24] H.J. Lu, Y.L. Guo, Evaluation of chiral recognition characteristics of metal and proton complexes of di-*o*-benzoyl-tartaric acid dibutyl ester and L-tryptophan in the gas phase, *J. Am. Soc. Mass Spectrom.* 14 (2003) 571–580.
doi:10.1016/s1044-0305(03)00147-8.
- [25] K.A. Schug, W. Lindner, Stereoselective discrimination and quantification of arginine and N-blocked arginine enantiomers by formation and dissociation of calcium-mediated diastereomeric trimer complexes with a chiral reference compound using electrospray ionization-ion trap tandem, *J. Am. Soc. Mass Spectrom.* 16 (2005) 825–834. doi:10.1016/j.jasms.2005.02.008.
- [26] X.Y. Yu, M.C. Chau, W.K. Tang, C.K. Siu, Z.P. Yao, Self-assembled binuclear Cu(II)-histidine complex for absolute configuration and enantiomeric excess determination of naproxen by tandem mass spectrometry, *Anal. Chem.* 90 (2018) 4089–4097. doi:10.1021/acs.analchem.7b05407.
- [27] H. Lu, Y. Guo, Chiral recognition of borneol by association with zinc(II) and L-tryptophan in the gas phase, *Anal. Chim. Acta.* 482 (2003) 1–7.
doi:10.1016/S0003-2670(03)00171-5.
- [28] Q. Yu, J. Cao, G. Lu, Z.X. Wang, The role of central ion in chiral recognition by taking phenylalanine as an example, *Sci. China Ser. B-Chemistry.* 52 (2009) 1136–1141. doi:10.1007/s11426-009-0080-5.
- [29] J.J. Park, C.S. Lee, S.Y. Han, Proton transfer accounting for anomalous collision-induced dissociation of proton-bound Hoogsteen base pair of cytosine and guanine, *J. Am. Soc. Mass Spectrom.* 29 (2018) 2368–2379.
doi:10.1007/s13361-018-2060-5.

- [30] H. B. Oh, S.-C. Jo, S.Y. Han, Y. Seong, An anomalous dissociation of protonated cluster ions of DNA guanine-cytosine base-pair, *Mass Spectrom. Lett.* 2 (2011) 73–75. doi:10.5478/MSL.2011.2.3.073.
- [31] J. Seo, S. Warnke, K. Pagel, M.T. Bowers, G. Von Helden, Infrared spectrum and structure of the homochiral serine octamer–dichloride complex, *Nat. Chem.* 9 (2017). doi:10.1038/nchem.2821.
- [32] V. Scutelnic, M.A.S. Perez, M. Marianski, S. Warnke, A. Gregor, U. Rothlisberger, M.T. Bowers, C. Baldauf, G. Von Helden, T.R. Rizzo, J. Seo, The structure of the protonated serine octamer., *J. Am. Chem. Soc.* 140 (2018) 7554–7560. doi:10.1021/jacs.8b02118.
- [33] A. Filippi, C. Fraschetti, S. Piccirillo, F. Rondino, B. Botta, I. D’Acquarica, A. Calcaterra, M. Speranza, Chirality effects on the IRMPD spectra of basket resorcinarene/nucleoside complexes, *Chem. Eur. J.* 18 (2012) 8320–8328. doi:10.1002/chem.201200614.
- [34] S.S. Lee, S. Park, Y. Hong, J.U. Lee, J.H. Kim, D. Yoon, X. Kong, S. Lee, H. B. Oh, Chiral differentiation of D- and L-alanine by permethylated beta-cyclodextrin: IRMPD spectroscopy and DFT methods, *Phys. Chem. Chem. Phys.* 19 (2017) 14729–14737. doi:10.1039/c7cp01085k.
- [35] S.S. Lee, J.U. Lee, J.H. Oh, S. Park, Y. Hong, B.K. Min, H.H.L. Lee, H.I. Kim, X.L. Kong, S. Lee, H.B. Oh, Chiral differentiation of d- and l-isoleucine using permethylated β -cyclodextrin: infrared multiple photon dissociation spectroscopy, ion-mobility mass spectrometry, and DFT calculations, *Phys. Chem. Chem. Phys.* 20 (2018) 30428–30436. doi:10.1039/c8cp05617j.
- [36] J. Klyne, A. Bouchet, S. Ishiuchi, M. Fujii, M. Schneider, C. Baldauf, O. Dopfer, Probing chirality recognition of protonated glutamic acid dimers by

- gas-phase vibrational spectroscopy and first-principles simulations, *Phys. Chem. Chem. Phys.* 20 (2018) 28452–28464. doi:10.1039/c8cp05855e.
- [37] J. Ren, Y.Y. Wang, R.X. Feng, X.L. Kong, Investigation of L/D-threonine substituted L-serine octamer ions by mass spectrometry and infrared photodissociation spectroscopy, *Chinese Chem. Lett.* 28 (2017) 537–540. doi:10.1016/j.ccllet.2016.10.032.
- [38] A. Fujihara, Y. Okawa, Chiral and molecular recognition of monosaccharides by photoexcited tryptophan in cold gas-phase noncovalent complexes as a model for chemical evolution in interstellar molecular clouds, *Anal. Bioanal. Chem.* 410 (2018) 6279–6287. doi:10.1007/s00216-018-1238-9.
- [39] A. Fujihara, N. Maeda, S. Hayakawa, Chiral recognition between l-alanine peptides and tryptophan enantiomers probed by ultraviolet photodissociation in the gas phase, *J. Mass Spectrom.* 51 (2016) 257–260. doi:10.1002/jms.3750.
- [40] G. Paglia, J.P. Williams, L. Menikarachchi, J.W. Thompson, R. Tyldesley-Worster, S. Halldorsson, O. Rolfsson, A. Moseley, D. Grant, J. Langridge, B.O. Palsson, G. Astarita, Ion mobility derived collision cross sections to support metabolomics applications, *Anal. Chem.* 86 (2014) 3985–3993. doi:10.1021/ac500405x.
- [41] M.M. Zimnicka, A. Troć, Acid-based approach for separation of peptide epimers using IM-MS, *J. Mass Spectrom.* 54 (2019) 620–628. doi:10.1002/jms.4362.
- [42] J.T. Zhang, H.Y. Wang, X. Zhang, F. Zhang, Y.L. Guo, Study of short-lived and early reaction intermediates in organocatalytic asymmetric amination reactions by ion-mobility mass spectrometry, *Catal. Sci. Technol.* 6 (2016) 6637–6643. doi:10.1039/c6cy01051b.

- [43] P. Dwivedi, C. Wu, L.M. Matz, B.H. Clowers, W.F. Siems, H.H. Hill, Gas-phase chiral separations by ion mobility spectrometry, *Anal. Chem.* 78 (2006) 8200–8206. doi:10.1021/ac0608772.
- [44] F.M. Nachtigall, M. Rojas, L.S. Santos, MALDI coupled to modified traveling wave ion mobility mass spectrometry for fast enantiomeric determination, *J. Mass Spectrom.* 53 (2018) 693–699. doi:10.1002/jms.4206.
- [45] V. Domalain, M. Hubert-Roux, V. Tognetti, L. Joubert, C.M. Lange, J. Rouden, C. Afonso, Enantiomeric differentiation of aromatic amino acids using traveling wave ion mobility-mass spectrometry, *Chem. Sci.* 5 (2014) 3234–3239. doi:10.1039/c4sc00443d.
- [46] A. Mie, M. Jornten-Karlsson, B.O. Axelsson, A. Ray, C.T. Reimann, Enantiomer separation of amino acids by complexation with chiral reference compounds and high-field asymmetric waveform ion mobility spectrometry: Preliminary results and possible limitations, *Anal. Chem.* 79 (2007) 2850–2858. doi:10.1021/ac0618627.
- [47] J.D. Zhang, K.M.M. Kabir, W.A. Donald, Metal-ion free chiral analysis of amino acids as small as proline using high-definition differential ion mobility mass spectrometry, *Anal. Chim. Acta.* 1036 (2018) 172–178. doi:10.1016/j.aca.2018.06.026.
- [48] J.D. Zhang, K.M.M. Kabir, H.E. Lee, W.A. Donald, Chiral recognition of amino acid enantiomers using high-definition differential ion mobility mass spectrometry, *Int. J. Mass Spectrom.* 428 (2018) 1–7. doi:10.1016/j.ijms.2018.02.003.

Figure captions

Figure 1. CID mass spectra of $[\text{Cu}^{2+}(\text{3,5-diiodo-L-tyrosine})_2(\text{R-DOPA}) - \text{H}]^+$ (a) and $[\text{Cu}^{2+}(\text{3,5-diiodo-L-tyrosine})_2(\text{S-DOPA}) - \text{H}]^+$ (b) (Reproduced from Ref. [12] with permission).

Figure 2. The plot of r value versus ee (a), r value as a function of $1/(ee - 170.5442)$ (b), $1/(r - r_0)$ as a function of $1/ee$ (c), for L-Histidine with *D-N-tert*-butoxycarbonyl-O-benzylserine as the chiral selector (Reproduced from Ref. [23] with permission).

Figure 3. (A) IRPD mass spectra of $[(\text{L-Ser})_7(\text{L-Pro}) + \text{H}]^+$ (a), $[(\text{L-Ser})_7(\text{D-Pro}) + \text{H}]^+$ (b), $[(\text{L-Ser})_6(\text{L-Pro})_2 + \text{H}]^+$ (c) and $[(\text{L-Ser})_6(\text{L-Pro})_2 + \text{H}]^+$ (d), obtained at a laser wavelength of 2990 cm^{-1} and irradiation time of 0.5 s; (B) IRPD spectra of $[(\text{L-Ser})_8 + \text{H}]^+$ (a), $[(\text{L-Ser})_7(\text{L-Pro}) + \text{H}]^+$ and $[(\text{L-Ser})_7(\text{D-Pro}) + \text{H}]^+$ (b), $[(\text{L-Ser})_6(\text{L-Pro})_2 + \text{H}]^+$ and $[(\text{L-Ser})_6(\text{L-Pro})_2 + \text{H}]^+$ (c). (Reproduced from Ref. [14] with permission).

Figure 4. IM-MS spectra for $[(\text{Cu}^{2+})_2(\text{X}_{\text{L/D}})(\text{Trp}_{\text{L}})_3 - 4\text{H} + \text{Na}]^+$ where X is tyrosine (A), phenylalanine (B), glutamine (C), glutamic acid (D), methionine (E) and $[(\text{Cu}^{2+})_2(\text{X}_{\text{L/D}})(\text{His}_{\text{L}})_3 - 4\text{H} + \text{Na}]^+$ where X is tryptophan (F), tyrosine (G), glutamine (H) and threonine (I). Inset in (A) is the monoisotopic peak of the diastereomeric ion extracted for the IM-MS analysis, and similar monoisotopic peaks were extracted for IM-MS analysis of other diastereomeric ions (Reproduced from Ref. [10] with permission).

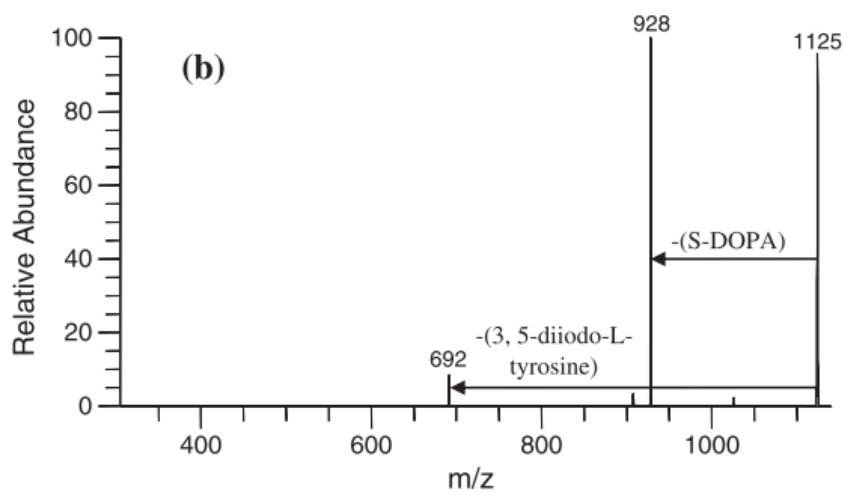
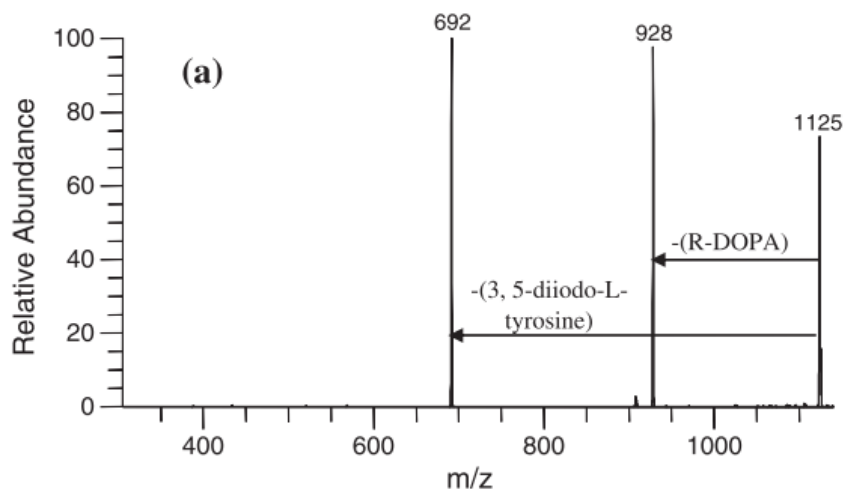


Figure 1

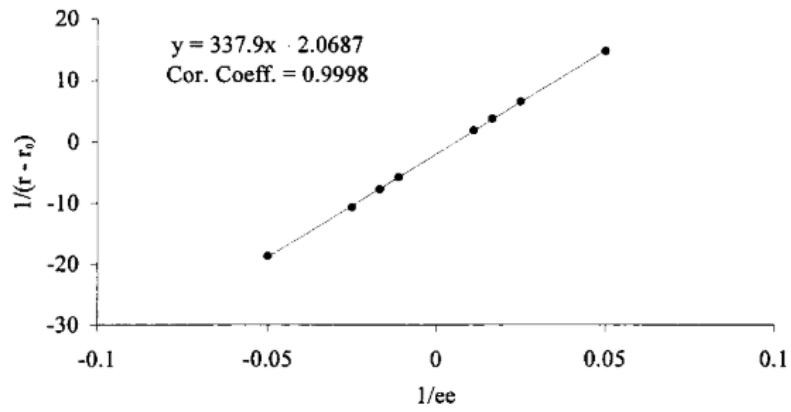
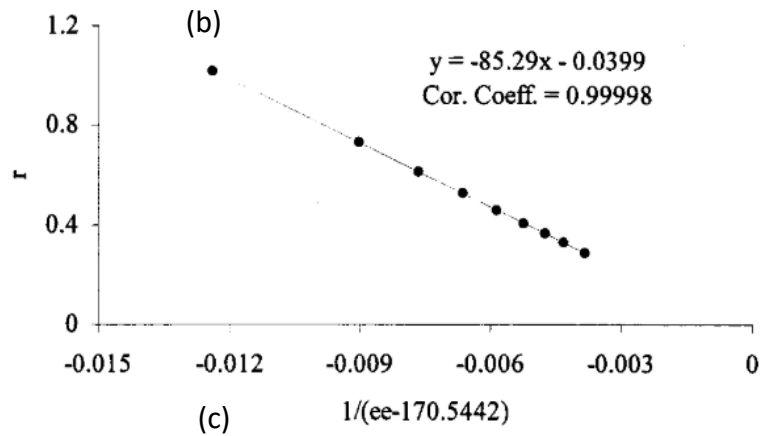
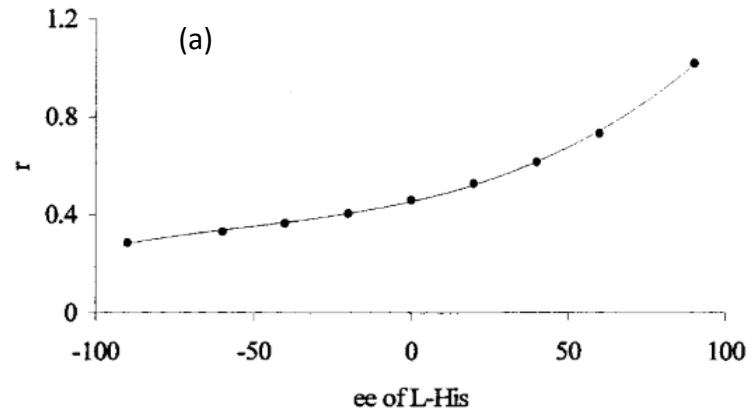


Figure 2

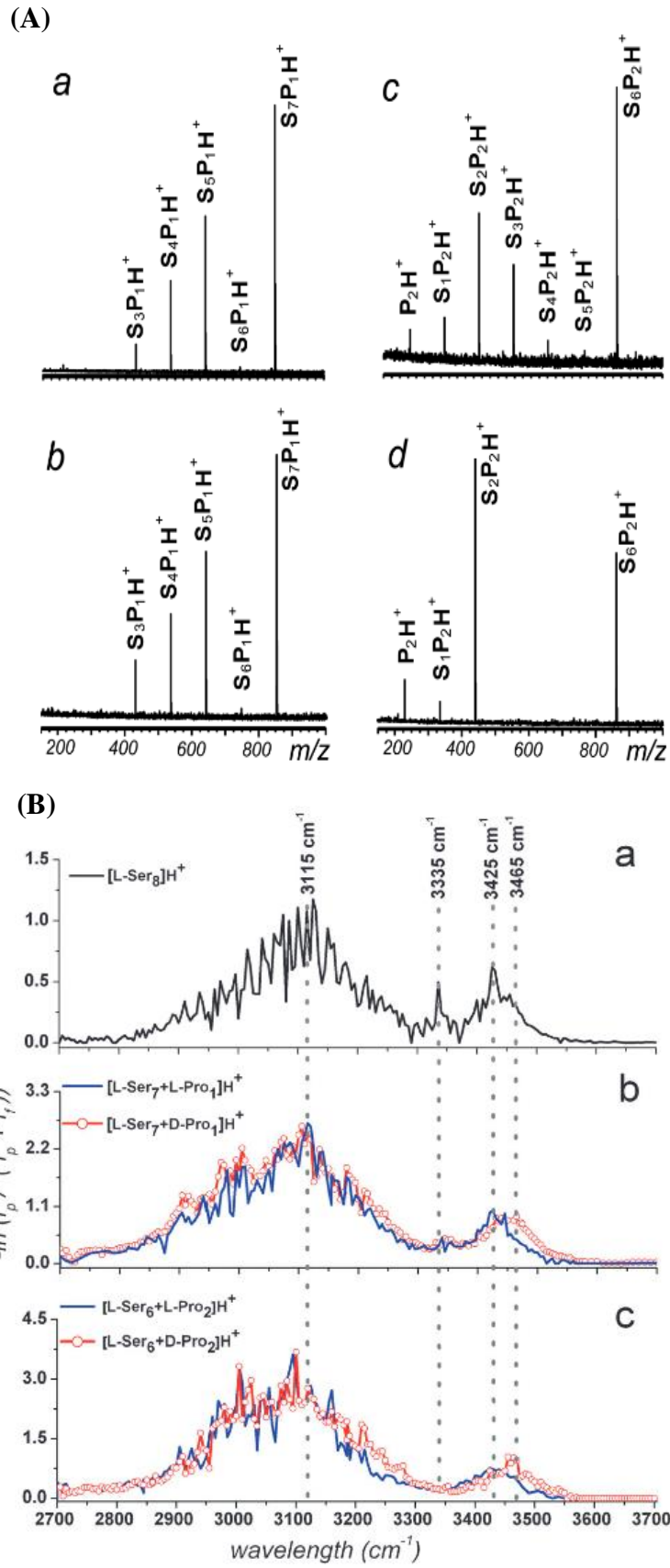


Figure 3

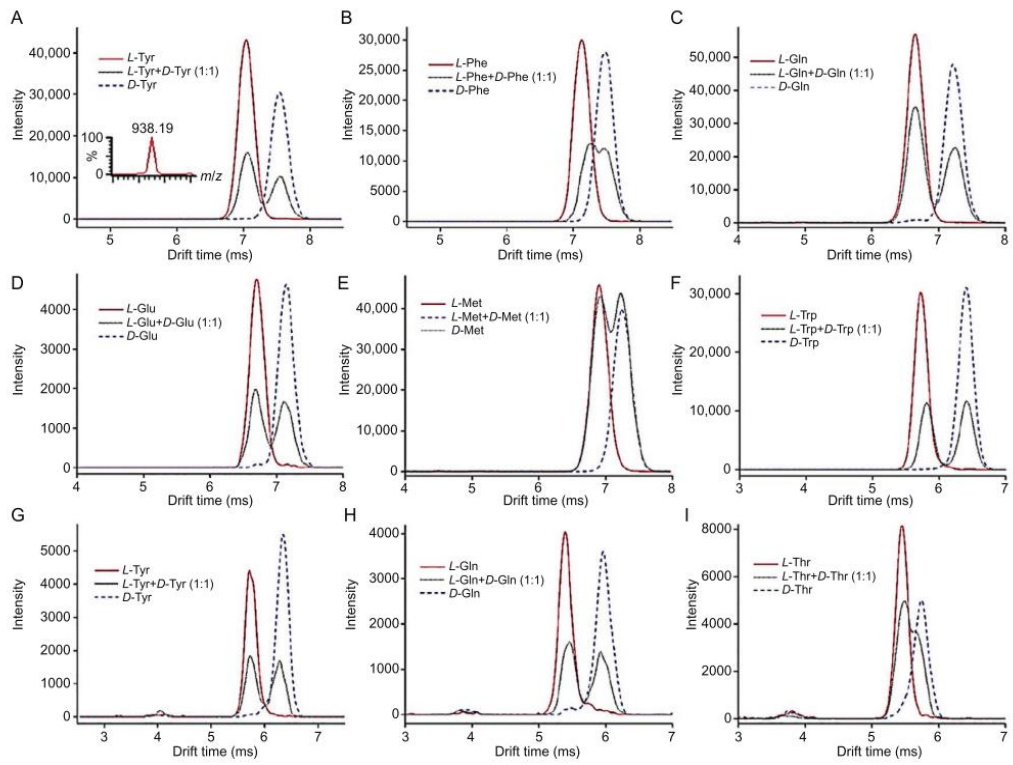


Figure 4