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Qualitative and quantitative analysis of enantiomers by mass spectrometry: Application of a simple chiral chloride probe via rapid in-situ reaction

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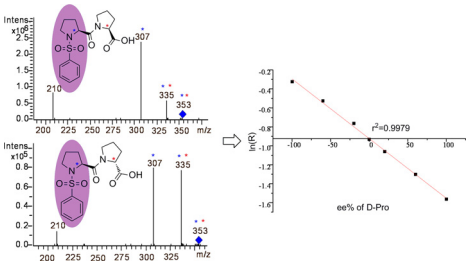
HIGHLIGHTS

- A convenient method based on a simple chiral probe for rapid enantiomer determination in mass spectrometry was developed.
- This method is applied for enantiomer determination both qualitatively and quantitatively.
- The method has great potential for chirality screening in both chemical solvent and biological solution.

GRAPHICAL ABSTRACT



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ABSTRACT

A tandem mass spectrometry method for high-sensitivity qualitative and quantitative discrimination of chiral amino compounds is conducted. The method is based on a chemical derivation process that uses a simple reagent, L-1-(phenylsulfonyl)pyrrolidine-carbonyl chloride, as the probe. The method is applicable in both organic solutions and biological conditions. Twenty-one pairs of enantiomer containing amino acids, amino alcohols, and amines are used to produce diastereomers using the probe via in situ reaction for 20 s at room temperature. The resulting diastereomers are successfully recognized based on the relative peak intensities of their fragments in positive mode, with the chiral recognition ability values ranging from 0.35 to 3.83. The L/D ratio of Pro spiked at different concentrations (enantiomeric excess) in both acetonitrile and dog plasma is determined by establishing calibration curves. This method achieves a lower limit of quantification of 50 pmol in analyzing amino acids using an extract ion chromatograph. The relative standard deviation for both qualitative and quantitative results is <5%. Thus, the present method is demonstrated as a new and practical technique of rapidly and sensitively determining enantiomers of amino compounds.

1. Introduction

Chiral compounds and their biological activities have important functions in chemistry and life sciences. Qualitative and quantitative analyses of chiral compounds are crucial in organic synthesis, pharmacological research, and pathological mechanism discovery. Numerous approaches, including polarimetry, circular dichroism, chromatography, nuclear magnetic resonance and capillary electrophoresis, have been developed for the recognition of chiral

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compounds [1–4]. Mass spectrometry, traditionally regarded as a "blind" technique of enantiomeric analysis, has become a popular technology of chiral determination because of the following advantages: (1) high sensitivity and short analysis time, (2) capacity to explain intrinsic chiral mechanism by isolating it from the solvent in gas phase, (3) allowance of mixture analysis via tandem mass spectrometry, and (4) potential for high-throughput screening as a result of its feasible combination with chromatography [5].

Enantiomers exhibit the same characteristics in mass spectrometry because of their identical physical and chemical properties. Therefore, the most effective way to analyze enantiomers using the MS technique is to introduce a diastereomeric environment before or during the analysis by adding additives, such as supramolecules, biological macromolecules and metal complexes [6]. In 1995, Sawada et al. [7] reported an enantiomer-labeled guest method using fast atom bombardment mass spectrometry by comparing the different intensities of the diastereomeric host–guest complex ions. This method obtained significant results in analyzing amino acid esters. Lebrilla et al. [8–10] described a host–guest exchange reaction method of analyzing amino acids by detecting the differences of exchange rate constants between the chiral selector and enantiomers using Fourier transform ion cyclotron resonance mass spectrometry. Cooks et al. [11–14] identified and quantified chiral amino acids by studying the different dissociation kinetic energies of metal-bound trimeric cluster ions and proton bond dimeric cluster ions using electrospray ionization (ESI). They also applied the kinetic method in determining the *ee* value of amino acids in mixtures. Moreover, it has been demonstrated that chiral analysis can be accomplished by examining the different reactivities (RA) of diastereomers, including gas phase ion–molecule reaction and proton transfer reaction [15–21].

In addition to the methods mentioned above in which diastereomeric noncovalent complex ions are formed, the measurement of enantiomeric composition using covalent diastereomers via derivatization is also possible [22,23]. However, only partially successful results have been reported using the derivatization method because of the complicated process and long derivation time associated with it. To our knowledge, chirality determination in biological condition using ESI mass spectrometry has not been explored. To apply the chemical derivatization method in rapidly analyzing chirality via mass spectrometry, looking for a proper chiral probes (CP) is the key step.

Acyl chloride is widely used as a pre-column derivatization reagent in the quantification of drugs and metabolomics using liquid chromatography–mass spectrometry (LC–MS) [24,25]. The acyl chloride probe noticeably enhances ESI sensitivity, facilitates MS-based quantification, and identifies amine- and phenol-containing samples [26–28]. Therefore, in this study, a rapid and sensitive in situ chemical derivatization method was developed to qualify and quantify chiral amino compounds in organic solutions and biological conditions using mass spectrometry with L-(1-(phenylsulfonyl)pyrrolidine-2-carbonyl chloride (L-PSPCC), which was used in asymmetric synthesis and can be made commercially available [29,30].

2. Experimental

2.1. Chemicals and reagents

All D- and L-amino acids (optical purity > 99%) were purchased from Aladdin and used without further purification. L-PSPCC probe was synthesized. The procedures and characterization data for the probe were included in the Supporting Information. HPLC-grade methanol and acetonitrile were obtained from Tedia Company (USA), and water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA).

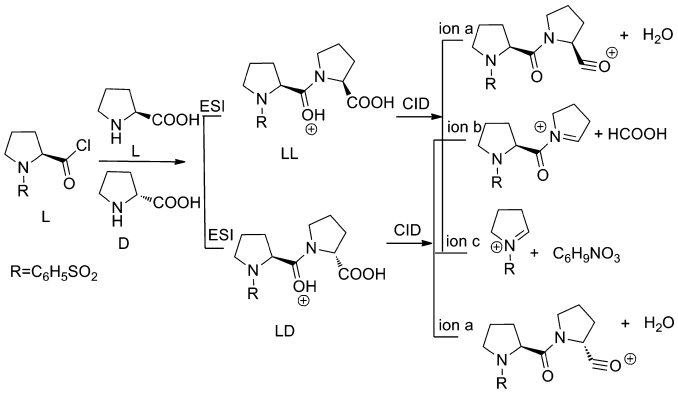

Fig. 1. Derivatization reaction procedure of Pro with L-PSPCC and the proposed mechanism for fragmentation of produced diastereomer ions.

2.2. ESI–MS/MS experiment procedure

The mass spectrometry experiments were carried out using a Bruker Esquire ^{3000}plus ion trap mass spectrometer (Brucker-Franzen Analytik GmbH, Bremen, Germany) equipped with an electrospray ionization (ESI) interface in positive ion mode. Nitrogen was used as the nebulizing gas at a pressure of 10 psi and the drying gas at a flow rate of 5 L min^{-1}. The drying gas temperature was set at 250 °C and the capillary voltage was set at 4000 V with a scan speed of 20,000 *m*/*z* units per second. Solutions containing analytes were pumped into the mass spectrometer at a flow rate of 6 μL min^{-1}. The collision induced dissociation mass spectra were obtained with helium as the collision gas at appropriate collision energy after isolation of the desired precursor ion. The mass window for precursor ion selection was at 1 *m*/*z*. The measurements for each spectrum data were repeated three times.

2.3. In-situ reaction for chiral analytes with the probe

A 0.05 mmol mL^{-1} solution of analytes in H_2O was prepared as the stock solution and the probe was kept in acetonitrile at 0.5 mmol mL^{-1}. The working solutions were prepared by sequential dilutions with acetonitrile. Anhydrous acetonitrile was used as the solvent in order to avoid the hydrolysis or alcoholysis of acyl chloride probe. Fig. 1 shows the in-situ reaction scheme for derivatization of amino-containing compounds. The reaction was allowed to proceed at room temperature with shaking 20 s. Then the mixtures were ready to be injected into a mass spectrometer.

2.4. Determine the reactivity of analytes with the probe

The HPLC analysis was performed on an Agilent 1100 series LC system (Aglient Technologies, Palo Alto, CA, USA). C18 column (hypersil ODS2, 4.6 × 250 mm, 5 μm) was used for the separation at room temperature. Mobile phase consisted of water (1% CH_3COOH) and acetonitrile (5:95, v/v) at a flow rate of 1 mL min^{-1} was used with the detection wavelength of 220 nm.

2.5. Blood sampling

Animals were provided by College of Pharmaceutical Sciences in Zhejiang University (Zhejiang, China). They were kept in an environmentally controlled breeding room at 22 °C and had free access to food and water until 18 h prior to experiments. All procedures involving animals were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China. A blood sample (0.4 mL) was collected from the suborbital vein into