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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 46451-2025

**Determination of chiral amino acids - High performance liquid
chromatography tandem mass spectrometry with pre-column
derivatization**

手性氨基酸的测定 柱前衍生高效液相色谱串联质谱法

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part 1: Structure and Drafting Rules of Standardization Documents". Drafting.

Please note that some content in this document may involve patents. The publishing organization of this document assumes no responsibility for identifying patents.

This document was proposed and is under the jurisdiction of the National Technical Committee on Standardization of Biochemical Testing (SAC/TC387).

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High-efficiency pre-column derivatization for the determination of chiral amino acids Liquid chromatography-tandem mass spectrometry

1 Scope

This document describes the use of pre-column derivatization high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for the detection of D-/L-alanine, D-/L-serine, and D-/L-proline.

Acids, D-/L-valine, D-/L-threonine, D-/L-cysteine, D-/L-leucine, D-/L-isoleucine, D-/L-asparagine, D-/L-Aspartic acid, D-/L-glutamine, D-/L-glutamic acid, D-/L-histidine, D-/L-phenylalanine, D-/L-arginine, D-/L-tryptophan D-/L-Lysine, D-/L-Ornithine, D-/L-Citriline, D-/L-Hydroxyproline, D-/L-alpha-Aminoadipic acid, D-/L-Phosphosphireine
Methods for determining D-/L-kynurenine, D-/L-2-aminobutyric acid, D-/L-methionine and D-/L-tyrosine.

This document applies to the determination of the content of the above-mentioned free chiral amino acids in biochemical reagents and biological samples.

2 Normative references

GB/T 6682

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 Free chiral amino acids

A naturally occurring free amino acid that does not combine with other amino acids to form proteins or peptides.

Note 1. Includes free L-amino acids and free D-amino acids.

Note 2. This category of amino acids does not include hydrolyzed amino acids produced by chemical or enzymatic hydrolysis of proteins.

4 Principles

After the protein in the sample was precipitated with sulfosalicylic acid, the amino acids were extracted using formic acid aqueous solution, and then reacted with triethylamine under the catalysis of...

N-alpha-(5-fluoro-2,4-dinitrophenyl)-L-propanamide underwent a derivatization reaction, and the derivative was analyzed by triple quadrupole liquid chromatography-tandem mass

spectrometry.

Quantitative analysis using the internal standard method.

5 Reagents and Consumables

Unless otherwise stated, all reagents used in this method are of analytical grade, and the water is Grade I water as specified in GB/T 6682.

5.1 Reagents

5.1.1 Methanol (CH₃OH). CAS No. 95713-52-3.