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Review

Recent advances in mass spectrometry analysis of low molecular weight heparins

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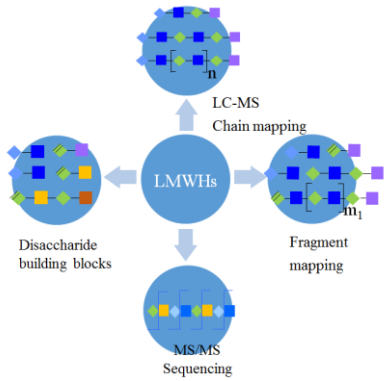
Graphical abstract



Low molecular weight heparins (LMWHs) are the most widely used anticoagulant drugs produced by chemical or enzymatic modification of parent heparin polysaccharides. The present article reviews recent advances in orthogonal and complementary mass spectrometry (MS) methodologies towards complete elucidation of natural and modified structures in LMWHs that possibly affect the drug quality, safety and efficacy.

ABSTRACT

Carbohydrates are important biomolecules and promising novel drug candidates, but their structural complexity and heterogeneity hinder their study and application. Low molecular weight heparins (LMWHs) are widely used as anticoagulants in the clinic and are considered the most successful carbohydrate-based drugs. They consist of both natural structures inherited from parent heparin and modified structures derived from depolymerization reactions. Sophisticated analytical methods are in great demand to elucidate the fine structure of LMWHs. This review summarizes recent progress in mass spectrometry (MS) that facilitates the in-depth structural characterization of LMWHs. The strategies can be sorted into top-down approaches and bottom-up approaches. In top-down approaches, the intact oligosaccharides are analyzed directly by hyphenated MS techniques to reveal their distribution and composition. Bottom-up approaches provide complementary structural information by analyzing partially enzymatically digested fragments or exhaustively degraded disaccharide building blocks of LMWHs. Advances in the MS/MS sequencing of short oligosaccharides and bioinformatics tools are also reviewed. Multidimensional analysis by MS offers a rigorous inspection of LMWHs, which is indispensable for ensuring drug quality and expanding their applications.

Keywords: LMWHs MS Structural Characterization Chain mapping Sequence

Low molecular weight heparins (LMWHs) are a class of widely used anticoagulant drugs derived from the chemical or enzymatic depolymerization of porcine intestine heparin [1]. As carbohydrate-based drugs, LMWHs exhibit structural features distinct from conventional small molecule drugs and modern protein-based biomacromolecule drugs. They are comprised of numerous oligosaccharide chains with different chain lengths, monosaccharide compositions, sulfo and *N*-acetyl substitutions, and sequences. The importance of the structural characterization of heparin and LMWHs was underestimated for a long period until the global heparin contamination crisis occurred in 2008 [2,3]. The lessons learned are that traditional analytical tests, such as molecular weight (MW) analysis by gel permeation chromatography, monosaccharide composition analysis and bioactivity analysis, are not sufficient to reflect the fine structure of a polysaccharide drug and ensure its safety and efficacy [4,5].

Heparin is constructed of repeating disaccharide units of a glucuronic acid residue (GlcA) or iduronic acid residue (IdoA) 1,4-linked to a glucosamine residue (GlcN), with various substitution patterns of sulfation at the 2-*O*-position of the hexuronic acid residue (HexA), the 3-*O*, the 6-*O* and/or the *N*- position of GlcN, and *N*-acetylation at GlcN [6]. A unique pentasaccharide sequence of –GlcNS