Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

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REVIEW

Analytical Strategies for Natural Mono- and Disaccharide Isomer Differentiation: Techniques and Applications

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Abstract: The precise structural differentiation of natural mono- and disaccharide isomers is fundamental for understanding carbohydrate bioactivity. However, this task remains challenging due to their minimal structural variances, high polarity, and lack of chromophores. This review traces the evolution of analytical technologies designed to overcome these hurdles. Early derivatization - GC-MS methods provided the foundation for isomer separation. Modern liquid chromatography-mass spectrometry (LC-MS), particularly with advanced chiral columns, now delivers high-resolution isomer profiling. Ion mobility spectrometry (IM-MS) further distinguishes conformers by their collision cross-section (CCS) values. Recent breakthroughs in non-derivatization strategies like UHPLC, coupled with the definitive structural validation offered by 2D-NMR, have revolutionized the field. We critically evaluate these methods' detection limits and throughput for practical applications in food chemistry, clinical glycomics, and pharmaceutical analysis. Looking forward, emerging directions such as AI-assisted spectral interpretation and integrated microfluidic systems promise to propel glycan analysis toward rapid, precise, and intelligent diagnostics. This review provides a practical roadmap for selecting and advancing analytical techniques for real-world isomer characterization.

Keywords: monosaccharide, disaccharide, isomer, identification methods, derivatization, non-derivatization

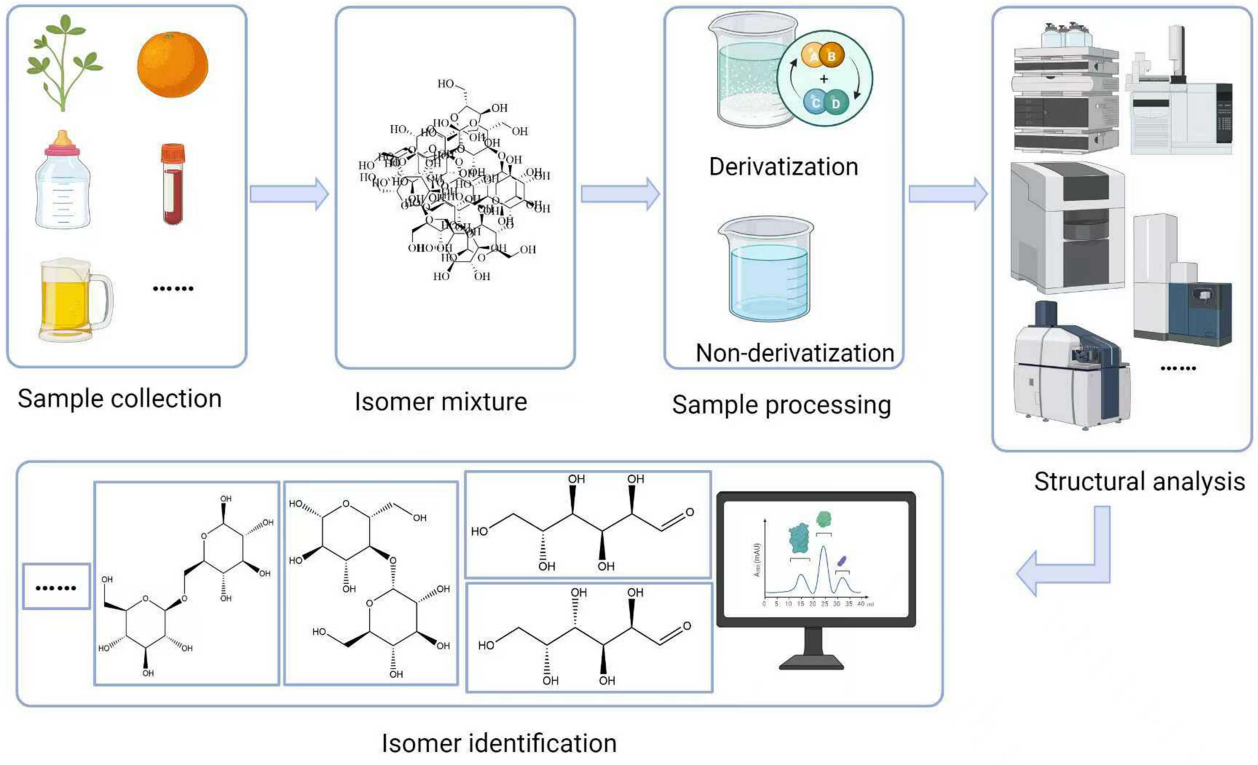
Introduction

Carbohydrates, recognized as one of the four essential biomacromolecules, act as vital mediators in critical biological processes such as cellular recognition and signal transduction through their unique stereochemical features, playing an irreplaceable regulatory role in organismal growth and development including human-related glycobiology, plant and microbial systems.[1] Their functions are encrypted in their unique stereochemical features, often termed the "sugar code." However, deciphering this code has been a long-standing challenge in glycobiology. Monosaccharides, the fundamental units of all complex carbohydrates, exist in dynamic tautomeric equilibria (eg, α/β anomers, furanose/pyranose forms). This structural complexity has historically hindered precise analysis, creating a critical knowledge gap that limits their application in biomarker discovery, biopharmaceutical development, and functional food design.[2,3] The development of high-throughput carbohydrate sequencing technologies lags significantly behind those for DNA and proteins, largely due to the profound stereochemical and structural complexity inherent to sugars.[4] These analytical advancements are paving the way for transformative applications in healthcare, from early disease diagnosis through glycan biomarkers to the rational design of glycoconjugate vaccines and targeted therapeutics.

The discrimination of monosaccharide isomers presents a fundamental challenge in glycomics, arising from subtle stereochemical variations that underlie significantly distinct biological activities. While isomers share identical molecular formulas and molar masses, minimal structural differences—such as the axial versus equatorial orientation of a single

Received: 15 August 2025
Accepted: 20 November 2025
Published: 12 December 2025

Graphical Abstract



hydroxyl group (eg, C4 in D-glucose vs D-galactose)—dictate their unique metabolic fates and receptor interactions. For instance, the C4 epimerization that distinguishes glucose from galactose is critical for cellular recognition processes, as demonstrated by their divergent roles in glycan-mediated cell adhesion and signaling pathways.[5,6] Analytically, these compounds exhibit high polarity, which often leads to poor retention in conventional reversed-phase chromatography and excessive retention in normal-phase systems, complicating their separation. Furthermore, the general lack of intrinsic chromophores or fluorophores severely limits the sensitivity of optical detection methods, collectively creating a persistent bottleneck in glycomics research.[6]

To overcome these analytical hurdles, multidimensional frameworks have been developed. Chromatographic techniques remain central to these efforts. Gas chromatography (GC), for example, often requires pre-column derivatization (eg, trimethylsilylation) to confer sufficient volatility for analysis. In the liquid phase, non-derivatized approaches have gained prominence. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) leverages the inherent weak acidity of carbohydrates for highly sensitive separations, while hydrophilic interaction liquid chromatography (HILIC) and graphitized carbon chromatography offer orthogonal retention mechanisms tailored to polar analytes.[7] Beyond chromatography, ion mobility spectrometry-mass spectrometry (IM-MS) has emerged as a powerful tool for separating isomeric species based on their distinct collision cross-section (CCS) values in the gas phase, providing insights into conformational differences that are inaccessible by mass alone.[8] For definitive structural elucidation, nuclear magnetic resonance (NMR) spectroscopy remains the gold standard, enabling absolute configurational assignment through characteristic chemical shift fingerprints and coupling constants.[9] The field is currently advancing along two complementary trajectories: derivatization strategies that enhance detectability by introducing

chromophores or fluorophores, and non-derivatized approaches that leverage the superior resolution of ultra-high-performance liquid chromatography (UHPLC) and multidimensional separations.

Despite these technological advances, a critical gap persists between the established biological significance of oligosaccharide isomers and the practical capacity for their rapid, high-resolution characterization in complex matrices. Many existing methods represent a trade-off: while techniques like 2D-NMR provide unparalleled structural detail, they are low-throughput and require substantial sample amounts. Conversely, high-throughput LC-MS methods often struggle to resolve isomers without prior derivatization or extended run times, limiting their application in dynamic biological or biopharmaceutical contexts. Furthermore, the lack of integrated platforms that combine rapid separation, sensitive detection, and automated data interpretation hinders the transition from research tools to routine analytical or diagnostic workflows.

This review aims to bridge this gap by systematically evaluating the evolution of analytical technologies, with a focus on their real-world applicability in resolving mono- and disaccharide isomers. We critically assess how sequential breakthroughs—from hyphenated chromatography-mass spectrometry to emerging microfluidic chip systems—address the long-standing challenges in fields like biopharmaceutical development and clinical diagnostics. By benchmarking performance through key metrics such as detection limits, resolution, and analytical throughput, we provide a practical roadmap to guide method selection for specific applications. Our synthesis not only captures the historical progression of natural mono- and disaccharide isomers analysis but also aims to propel the field toward an intelligent, precision-driven future, offering actionable insights for both fundamental researches.

High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) has emerged as the cornerstone technique for oligosaccharide isomer analysis, distinguished by its exceptional separation efficiency and detection sensitivity,[10] making it the preferred method for glycan structural elucidation. The commonly analytical technologies of natural monosaccharide and disaccharide isomers were listed in Table 1. The separation mechanism of HPLC separation mechanism relies on selective adsorption interactions between the stationary phase and organic solvent-based mobile phase. Optimization of column packing materials (eg, amino-modified, graphitized carbon phases) and gradient elution protocols enables precise resolution of polar oligosaccharide isomers with subtle structural differences. Detector technologies are categorized into derivatization and non-derivatization systems. Derivatization strategies, employing chromogenic/fluorogenic tags (eg, PMP, 2-AB), achieve pmol-level sensitivity in ultraviolet-visible spectroscopy/fluorescence detectors but may alter glycan ring conformations. Non-derivatized systems utilize universal detectors such as evaporative light scattering detector (ELSD) and refractive index detector (RID), while the hyphenated high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) achieves 10 fmol-level sensitivity through pH-modulated charge-state manipulation and electrochemical detection.[11,12]

The groundbreaking integration of HPLC with mass spectrometry (HPLC-MS/MS) has ushered glycomics into a high-throughput era. High-resolution mass spectrometry (HRMS) facilitates simultaneous acquisition of oligosaccharide molecular weights, monosaccharide compositions, and linkage positions via precise mass measurement (mass error < 5 ppm) and multistage fragmentation (eg, collision-induced dissociation [CID], electron transfer dissociation [ETD]). Detectable monosaccharides and disaccharides can not only be derived from plant extracts but also from biological samples.[13,14] Representative applications include HILIC coupled with Q-TOF MS for resolving 72 serum IgG glycoform isomers and graphitized carbon column-based IM-MS for distinguishing sialic acid linkage isomers via CCS differences.[15,37] Despite these advancements, challenges persist in ultra-trace sample analysis (eg, single-cell glycomics), integration of chromatographic-mass spectrometric with NMR data, and development of intelligent spectral annotation algorithms. Addressing these limitations requires innovative solutions such as miniaturized chromatographic columns and artificial intelligence-assisted data interpretation to propel glycoanalytical technologies toward enhanced precision and intelligence.

Non-Derivatization Methods

Non-derivatized HPLC strategies for oligosaccharide separation and detection avoid the complexity of derivatization but present inherent challenges. Key non-derivatization approaches (Table 2) include high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), HILIC, tandem mass spectrometry (MS/MS), and porous

Table 1 Comparison of Different Structural Analytical Technologies of Natural Monosaccharide and Disaccharide Isomers

Method	Sample Preparation	Sample	Applications	Detector	Ref.
HPLC	Underivatized	Potato and strawberry extracts	Measuring glucose, fructose, and sucrose in plant tissues	ESI-MS	[12]
	PMP derivatization	Fecal samples from infant	Rapid throughput analysis of monosaccharides in biological samples	ESI-MS	[13]
	Underivatized	Human serum samples	Analysis of serum samples and certified reference materials for overall monosaccharide composition of human serum	ESI-MS	[14]
	PMP derivatization	*Osmanthus fragrans Lour*	Determination of monosaccharides in different varieties of *Osmanthus fragrans Lour*	ESI-MS	[15]
	Underivatized	Monosaccharides (anomers) and disaccharides (regioisomers) standards	Online separation and identification of isomers	MS-IRMPD	[16]
GC	Silylation derivatisation	Cabernet Sauvignon wine	Analyses 2 monosaccharides, 8 organic acids and 13 amino acids in wine	EI-MS	[17]
	Silylation derivatisation	Hyacinth and mulberry extracts	Determination of the complex mixtures of low molecular weight carbohydrate	Quadrupole mass detector	[18]
	Trimethylsilyl-dithioacetal (TMSD) derivatization	*A. asphodeloides* and *G. ganoderma* polysaccharides	Analyses free carbohydrates in water extracts of *A. asphodeloides* roots and monosaccharides in *G. ganoderma* polysaccharides	EI-MS	[19]
	Two-step derivatization with MOA and MSTFA	Standard mixture containing 22 TMS-methoxime carbohydrate derivatives	Absolute quantitation of 22 carbohydrates	EI-MS	[20]
	Derivatization with n-propylamine and acetic anhydride	Neutral monosaccharides	Separated and detected the derivatives of seven neutral monosaccharides and two uronic acids	FID	[21]
CE	PMP derivatization	*Radix Asparagi* polysaccharide	Analysis of *Radix Asparagi* polysaccharide monosaccharide composition	UV	[22]
	Underivatized	Cellodextrin oligosaccharides	Separation of underivatized cellodextrin oligomers up to DP7 along with eight other carbohydrates	UV	[23]
	Sialic acid derivatization	Glycopeptide, N-glycan, Monosaccharide sample	Analysis of monosaccharides, oligosaccharides and glycopeptides to enable comprehensive glycoprotein analysis	MS	[24]
	PMP derivatization	Fungus polysaccharides	Simultaneous separation and identification of the monosaccharide composition in TAPs and polysaccharides PGPs	UV	[25]
	Underivatized	Coffee adulteration with soybean and corn after acid hydrolysis	Evaluate interrelationships between the monosaccharide profile and the coffee adulteration with different proportions of soybean and corn	MS	[26]

https://doi.org/10.2147/DDDT.S561012

IM-MS	Underivatized	Mature breastmilk	Analysis of human milk oligosaccharide isomers	ESI-MS	[27]
	3-carboxy-5-nitrophenylboronic acid derivatization	Carbohydrate derivatization	Separations of eight disaccharides and four monosaccharides	TOF-MS	[28, 29]
	Underivatized	Sixteen glucose isomers	Analysis of 16 glucose isomers	TOF-MS	[30]
	Underivatized	Carbohydrate containing monosaccharides, disaccharides and trisaccharides	Characterization of carbohydrate isomeric ions	TOF-MS	[31]
NMR	Deuterium labeling	Solution of honey	Discrimination in honey of as many as 22 sugars	Highly selective chemical shift filters followed by TOCSY	[32]
	Deuterium labeling	Mixtures of monosaccharides	Estimation and quantification of complex mixtures of monosaccharides	2D NMR Q-HSQC	[33]
	Deuterium labeling	Human milk oligosaccharide lacto-N-difucohexaose I	Structural determination of carbohydrates	3D HSQC-TOCSY spectra	[34]
	Deuterium labeling	Honey samples	Determination of 13 metabolites in honeys, including acids, amino acids, sugars, ethanol and hydroxymethylfurfural	[1]H NMR spectra	[35]
	Deuterium labeling	Natural polysaccharides	Develop a relevant algorithm of the automated structure elucidation	2D NMR spectra	[36]

Abbreviations: PMP, 1-phenyl-3-methyl-5-pyrazolone; TMSD, trimethylsilyl-dithioacetal; MOA, methoxyamine hydrochloride; MSTFA, N-methyl-N-trimethylsilyltrifluoroacetamide; DAD, Diode Array Detector; TAPs, *Termitomyces albuminosus* polysaccharides; PGPs, *Panus giganteus* polysaccharides; TOCSY, total correlation spectroscopy.

Table 2 Non-Derivatization Methods for Oligosaccharide Analysis

Method	Column and Flow Rate	Solvents	Samples	Separation	Detector	Ionization Mode	Ref.
HPAEC-PAD	Carbopac PA1 analytical column (4 × 250 mm, Dionex), 1 mL/min	15 mM NaOH and NaOAc	Monosaccharide standard in the mixtures	GalA, GlcA, GulA, ManA, IdoA, Neu5Ac, and Neu5Gc	PAD		[38]
	CarboPac PA200 analytical column (3 × 250 mm), 0.5 mL/min	NaOH and NaOAc	Two Galacto-oligosaccharides products (syrup and high-purity power)	Isomeric oligosaccharides	PAD and ESI-MS	–	[39]
	Carbo PAC^{TM} PA10 column (2.0 mm × 250 mm), 0.25 mL/min	12.5 mM NaOH	*C. paliurus* polysaccharide sample	Six monosaccharides including rhamnose, arabinose, galactose, glucose, mannose and xylose	PAD	N.A	[40]
	CarboPac PA20 anion-exchange column (3 × 150 mm), 0.45 mL/min	Water, NaOH and NaOAc	Hydrolysis solution of *Spirulina platensis*	13 carbohydrates	PAD and ESI-MS	+ and –	[41]
	CarboPac PA200 (3 mm × 250 mm), 0.3 mL/min	10 mM sodium hydroxide, mixture of 200 mM sodium hydroxide and 125 mM sodium acetate	Solution of wheat bran	16 standards of monosaccharides, xylo-oligosaccharides, arabinoxylo-oligosaccharides and uronic acids	PAD	N.A	[42]
HILIC	Stainless steel column slurry-packed with PA (150 mm × 4.6 mm), Acetonitrile/water: 1.0 mL/min, ethanol/water: 0.5 mL/min, methanol/water: 0.8 mL/min	Water, 200 mM ammonium formate solution (pH 3.0), acetonitrile or ethanol or methanol	Galactooligosaccharide Solution and saponins solution of *Paris polyphylla*	Fructooligosaccharide and chitooligosaccharides	UV and ELSD	N.A	[43]
	DAICEL DC-pak PTZ (4.6 × 150 mm, 5 μm), 1.0 mL/min	Acidic conditions: ACN with 0.1% FA, milli-Q water with 0.1% FA; Neutral conditions: ACN/water with 2 mM NH4OAc, milli-Q water with 2 mM NH4OAc	Mixture of 14 carbohydrates	Isomeric hexoses, pentoses and disaccharides	ESI-MS	–	[44]
	Atlantis HILIC, (150 × 4.6 mm, 3μm), TSK gel Amide-80 (150 × 4.6 mm, 5μm), Inertsil Diol (150 × 4.6 mm, 5μm), XA-mide (150 × 4.6 mm, 5μm), Click TE-Cys, Click b-CD, and Click Maltose (150 × 4.6 mm, 5μm), 1.0 mL/min	H_2O, ACN, ammonium buffer	Complex mixtures of oligosaccharide	Sucrose, turanose, cellobiose, maltose, trehalose, lactose, and melibiose	ELSD	N.A	[45]
	XBridge BEH Amide column (2.1 × 150 mm, 3.5 μm), 0.2 mL/min	Methanol/acetonitrile 60:40, 30 mM ammonium formate (pH 3.3) or 30 mM ammonium acetate (pH 4.5) buffer	Solution of labeled oligosaccharide	Labeled isomeric trisaccharides (maltotriose, panose, and isomaltotriose)	UV-ESI-MS	+	[46]
	BEH X-Bridge amide column (4.6 × 150mm, 3.5 μm), 0.4 mL/min	Water with 0.1% of ammonium hydroxide, acetonitrile with 0.1% of ammonium hydroxide, pH 10	Fifty-six cocoa bean samples from different origins and status of fermentation	Mono-, di-, tri- and tetra- saccharides, sugar alcohols and iminosugars	ESI-TOF-MS	+ and –	[47]

GCC	PGC HT column (Hypercarb, 1.0 mm × 150 mm, 3 μm), 50 μL/min	0.1% formic acid/H_2O, acetonitrile/ water (80:20) (0.1% formic acid) or isopropanol/water (80:20) (0.1% formic acid)	Synthetic Lewis antigen isomers	Four isomers	CID-MS	+	[48]
	Hypercarb column (100 mm × 2.1 mm, 5μm), 0.5 mL/ min	0.1 v/v% formic acid solution, methanol	Human milk	Lacto-N-biose (LNB) and N-acetyllactosamine (LacNAc)	ESI-MS	+	[49]
	Trap column: Hypercarb PGC (320 μm × 3 cm), 7 μL/ min; analytical nano-column: Hypercarb Kappa (75 μm × 10 cm), 0.9 μL/min	10 mM ammonium bicarbonate, acetonitrile	Pooled glycan	N-and O-glycan	ESI-MS	–	[50]
	Hypercarb column (100 mm × 4.6 mm, 5 μm), 0.3 mL/ min	20 mM ammonium bicarbonate, 80% acetonitrile with 20 mM ammonium bicarbonate, water	Heparan sulfate oligosaccharides	Two dp4 isomers, four dp6 isomers, a dp3 oligosaccharide	ESI-MS	–	[51]
Non-Derivatized MS	N.A	N.A	Solution of Medicago leaf	Maltose and sucrose	ESI-TOF-MS	–	[52]
	N.A	N.A	Monosaccharides	All 24 aldohexose and 2-ketohexose isomers	ESI-MS	–	[53]
	N.A	N.A	Oligosaccharides	Trisaccharide glycan isomers	Ultraviolet photodissociation (UVPD) and radical-directed dissociation (RDD)	N.A	[54]
	N.A	N.A	Dairy beverages	Two disaccharide isomers (lactose and sucrose)	ESI-TOF-MS	–	[55]
	N.A	N.A	Sugarcane juice	Aldohexose and ketohexose disaccharides	CID-MS/HCD-MS	+	[56]

Abbreviations: IRMPD, infrared multiple photon dissociation; ELSD, evaporative light scattering detector; ESI-TOF-MS, electrospray ionization-time of flight-mass spectrometry; dp, polymerization.

https://doi.org/10.2147/DDDT.S561012

graphitized carbon (PGC) chromatography. For instance, Duarte-Delgado et al developed an HPLC-RID method for qualitative and quantitative analysis of sucrose, glucose, and fructose in potatoes.[12] Emerging technologies, such as HPLC-MS coupled with infrared multiphoton dissociation spectroscopy, demonstrate promising applications in omics by enabling online separation and identification of disaccharide and monosaccharide isomers.[16] The monosaccharides and disaccharides used in the literature were summarized, and their chemical structural formulas are shown in Figures 1 and 2.

High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

Since its inception in the 1990s, HPAEC-PAD has become a vital tool for carbohydrate analysis due to its high sensitivity and non-derivatization advantages.[38] This method exploits the deprotonation of hydroxyl groups under highly alkaline conditions to generate oxyanions, enabling anion-exchange separation via pKa differences (neutral monosaccharides: pKa 12–14) between hydroxyl groups and positively charged stationary phases. Uronic acids require higher NaOH concentrations for elution.[39] The CarboPac PA10 column, with its narrow internal diameter, enhances separation efficiency, where hydroxyl group-dependent elution governs chromatographic optimization (eg, NaOH concentration, temperature, and elution mode).[40] For example, Zhang et al utilized isocratic elution for acidic sugars and gradient

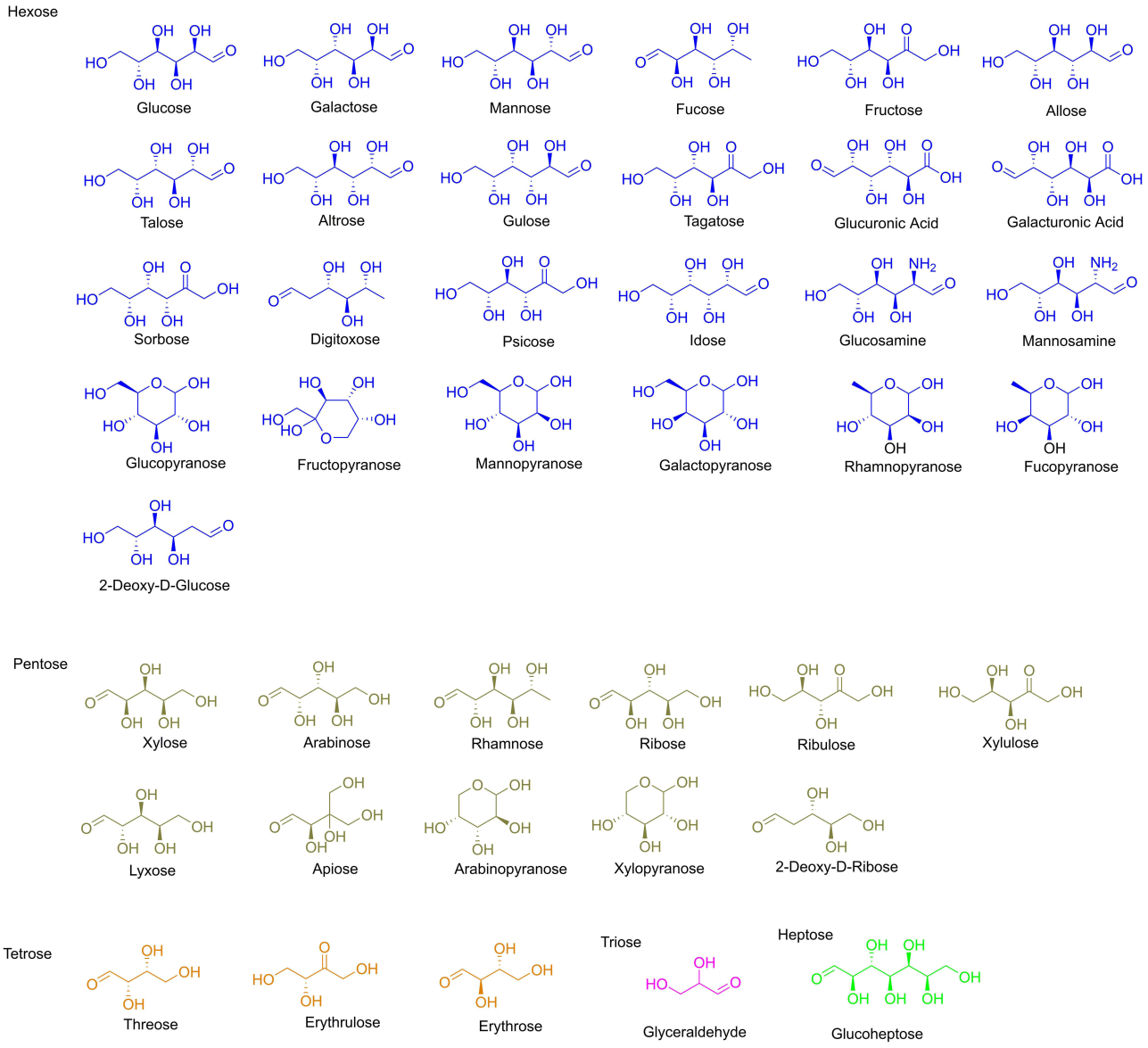


Figure 1 The structure of monosaccharide isomers.

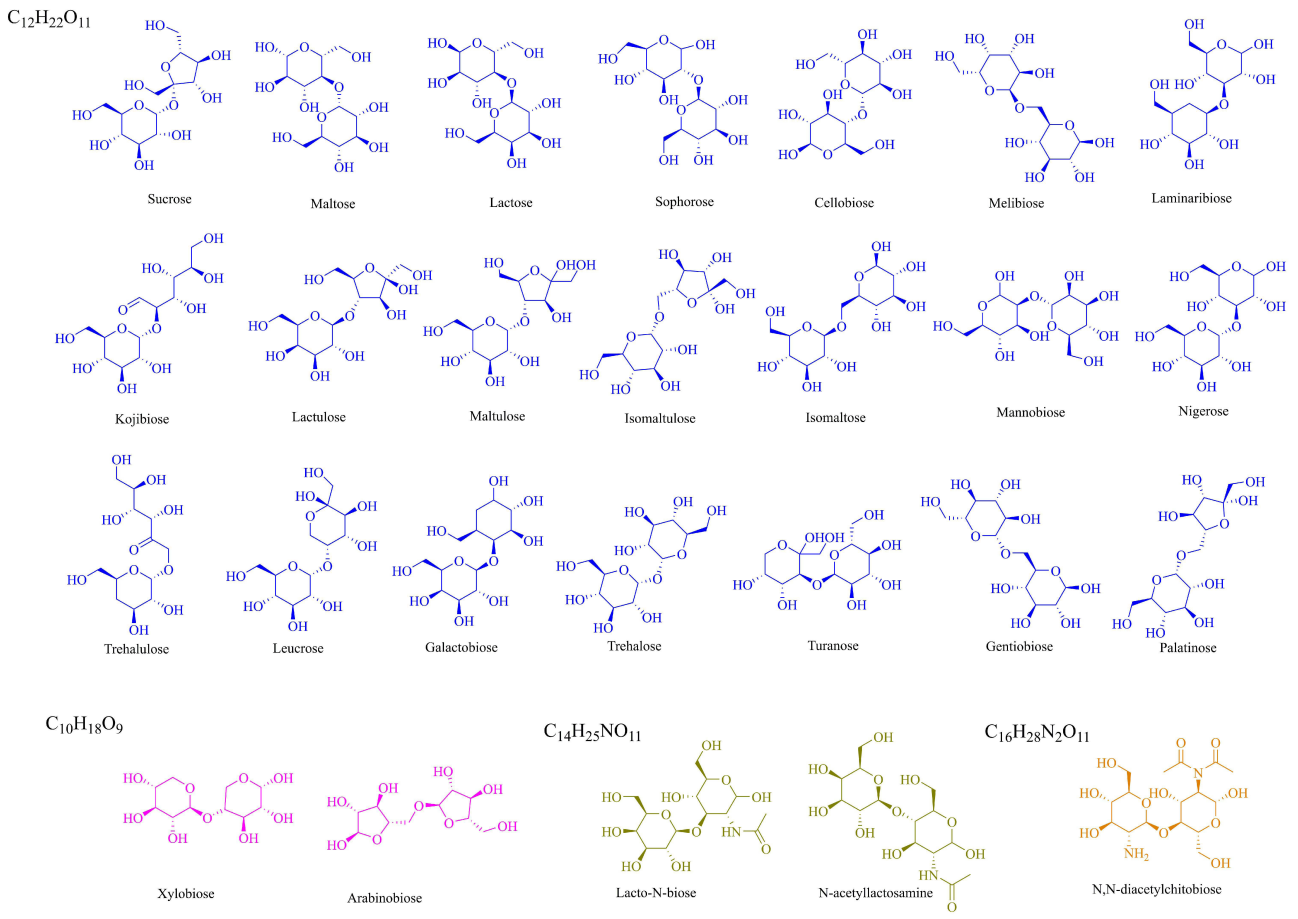


Figure 2 The structure of disaccharide isomers.

elution for neutral/alkaline sugars.[38,41] Alyassin M et al further validated that alkaline aqueous gradient elution improves oligosaccharide resolution.[42] Polysaccharide monosaccharide composition analysis typically involves acid hydrolysis pretreatment, with microwave-assisted HCl hydrolysis widely adopted for rapid monosaccharide profiling.[57]

HPAEC-PAD has established a robust system for neutral monosaccharide analysis. Xie et al determined the molar ratio (1.00:1.85:3.26:3.12:0.85:0.29) of six neutral monosaccharides (eg, rhamnose, arabinose) in Cyclocarya paliurus polysaccharides, confirming the method's high accuracy and precision.[40] Concurrently, advancements in acidic monosaccharide analysis include Wang et al's baseline separation of nine acidic/neutral monosaccharides (eg, arabinuronic acid, galacturonic acid) within 65 minutes.[58] He et al expanded its applicability to simultaneous detection of neutral, acidic, and alkaline sugars.[57] Despite its high resolution and sensitivity, limitations persist, including instrument corrosion under high alkalinity, nonlinear detector response-induced baseline drift, and quantification inaccuracies due to co-eluting components.[59,60] To address these, HPAEC-MS hyphenation achieves mass spectrometry compatibility via online desalting (eg, suppressor-based desalting) and parallel PAD-MS detection, as demonstrated by Yi et al in dextran analysis.[61] Coulier et al successfully applied this approach to lignocellulosic hydrolysate oligosaccharides.[62] Technological innovations, such as Chen et al's electrolytic eluent generation system (KMSA/KOH), enhance precision through gradient control, offering a streamlined solution for oligosaccharide analysis.[63] In summary, HPAEC-PAD excels in complex polysaccharide monosaccharide profiling through high sensitivity, derivatization-free operation, and broad-spectrum separation of neutral/acidic/alkaline sugars. However, challenges like alkaline-induced instrument corrosion, baseline instability, and co-elution limitations necessitate complementary strategies such as MS hyphenation and advanced eluent systems to overcome current technical barriers.

https://doi.org/10.2147/DDDT.S561012

Hydrophilic Interaction Liquid Chromatography (HILIC)

Since its introduction by Alpert in 1990, HILIC has continuously expanded its applications in carbohydrate analysis due to its exceptional separation performance for polar compounds.[64] This technique operates via a partitioning mechanism between a water-enriched stationary phase and a high-organic mobile phase (primarily acetonitrile/water), effectively resolving oligosaccharide isomers while enhancing electrospray ionization (ESI) efficiency through MS-compatible mobile phases. HILIC has thus become indispensable in pharmaceutical analysis, metabolomics, and glycomics research.[9,65,66] Early limitations of aminopropyl silica columns, such as poor mobile phase stability, have been mitigated by novel bonded stationary phases (eg, amide, zwitterionic, glycoside-modified phases).[66] For instance, Cai et al fabricated silica columns via polyacrylamide immobilization, achieving eco-friendly separation using methanol as an acetonitrile alternative.[43] Fu et al employed a poly-N-(1H-tetrazol-5-yl)-methacrylamide-bonded phase (DCPak PTZ column) to significantly enhance isomer resolution,[44] while Guo et al developed an N-(3-aminopropyl)imidazole-bonded phase that strengthens monosaccharide retention through specific hydrophilic interactions.[64]

During HILIC separation, α/β anomeric isomers often exhibit dual peaks due to tautomeric rate disparities, which can be eliminated by elevating column temperature (accelerating equilibration) or adding buffer salts. Separation selectivity is governed by multiple factors, with Fu et al confirming the superior performance of amide columns for monosaccharide isomer resolution after systematic evaluation of seven stationary phases.[45] Typical elution follows a polarity gradient: pentoses < hexoses < oligosaccharides, while glycans/glycopeptides exhibit stronger retention with increasing molecular weight.[67,68] Although acetonitrile remains the dominant solvent due to its low viscosity and UV transparency, its toxicity and cost have spurred exploration of alternatives. Ethanol/isopropanol systems and subcritical MeOH:H_2O:CO_2 mixtures have demonstrated comparable separation efficiency with reduced analysis time.[46,69,70]

Advances in non-derivatized detection strategies further broaden HILIC applications. Yan et al established a HILIC-charged aerosol detector (CAD) method to resolve stachyose and *Eclipta prostrata* polysaccharide monosaccharides while eliminating chloride interference.[9] Fu et al achieved baseline separation of three monosaccharides and seven disaccharide standards using an amide column-HILIC-CAD system.[45] Hyphenated HILIC-MS platforms provide dual validation through retention time alignment and multistage MS fragmentation. For example, Megías-Pérez et al quantified cocoa bean carbohydrates via HILIC-ESI-TOF-MS,[47] while Martín-Ortiz et al distinguished 23 disaccharide isomers by combining retention times with MS²fragmentation patterns.[71]

In summary, HILIC has solidified its position as a cornerstone technique in carbohydrate analysis, offering high-resolution separation of polar oligosaccharide isomers, enhanced ionization compatibility with MS detectors, and environmentally friendly stationary phases (eg, methanol substitution). However, its limitations include dependence on toxic/costly mobile phases (eg, acetonitrile), complex optimization requirements to address α/β anomer-induced peak splitting, and weak retention of low-molecular-weight sugars, necessitating multidimensional separation strategies to improve throughput.

Graphitized Carbon Chromatography (GCC)

Graphitized carbon chromatography (GCC) offers an innovative solution for analyzing strongly polar carbohydrate isomers, leveraging the unique properties of porous graphitized carbon (PGC) stationary phases. The uniform surface structure and broad pH tolerance of PGC facilitate efficient separation of polar glycans through induced dipole-dispersion interactions, with retention governed by molecular polarity and spatial configuration. This enables not only the discrimination of oligosaccharide isomers but also the resolution of α/β anomeric configurations, where glycan reduction strategies eliminate peak splitting caused by anomeric differences.[48,49,72] Compared to conventional alkyl-based reversed-phase chromatography, GCC demonstrates superior performance in separating polar sugars and accommodates both derivatization (eg, methylation, fluorescent labeling) and non-derivatization analytical strategies.[50,73] The compatibility of volatile mobile phases (eg, acetonitrile systems) with mass spectrometry further extends its utility. PGC-LC-MS hyphenation, integrating chromatographic separation with MS fragmentation, has successfully resolved polymerization degrees in heparan sulfate oligosaccharides.[51,74] Additionally, GCC coupled with aerosol detectors (eg, charged aerosol detection) enables precise identification of complex components such as heparin oligosaccharides and amino sugars.[75,76] However, challenges persist, including limited commercialization, incomplete understanding of separation mechanisms, and high costs, necessitating further optimization of stationary phase design and theoretical modeling of separation dynamics.

Non-Derivatized Mass Spectrometry

Mass spectrometry (MS) has become a cornerstone technique in glycan analysis, offering high sensitivity, rapid analysis, and rich structural insights through fragment ion characterization. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) dominate as primary ion sources, with atmospheric pressure chemical ionization (APCI) also proving effective.[75] Tandem MS configurations—including triple quadrupole (QqQ), quadrupole-time-of-flight (QTOF), Fourier-transform ion cyclotron resonance (FT-ICR), and ion trap-Orbitrap systems—enable tailored qualitative and quantitative studies of oligosaccharides through optimized ionization sources and mass analyzers. Liquid chromatography-MS (LC-MS) exploits secondary or multistage fragment ion patterns for isomer discrimination: Zhu et al differentiated hexose and hexosamine isomers using APCI-QTOF-MS via diagnostic ions ($[M+NH_4]^+$ and $[M–H]^–$) and intensity ratios,[77] while Xia et al employed ESI-ion trap MS to resolve pentoses, hexoses, and deoxy sugars using $[2M + Na–H_2O]^+$ and $[M+Na]^+$ adduct-specific fragmentation.[78] Zhan et al achieved linkage-specific disaccharide characterization through MALDI-TOF/TOF-generated $[M+Cl]^–$ adducts and their fragmentation fingerprints.[52] By leveraging chloride adduction on a novel LC-cESI-MS/MS platform, researchers achieved nanomolar-level sensitivity and orthogonal analysis for disaccharide isomers, revealing turanose as a major isomer in honey with superior performance over conventional sodium adduction methods.[79] A novel CMTSES strategy based on LC-QQQ-MS was developed, which integrates composition-dependent MRM transitions with structure-indicative LC elution segments to comprehensively profile disaccharide units and effectively differentiate their linkage, composition, and configuration isomers in real samples.[80]

Innovative strategies further enhance isomer resolution: Fixed-ligand kinetic methods enabled chiral discrimination of all 24 hexose isomers via unique fragment ion ratios,[53] while radical-directed dissociation (RDD) and ultraviolet photodissociation (UVPD) outperformed collision-based activation in distinguishing glycan isomers.[54] The CMTSES-LC-QQQ-MS strategy integrated composition-dependent MRM transitions and elution profiles to resolve mixed homo-/heterogeneous disaccharide isomers.[80] Copper-ligand complex ions (eg, $[(Cu^{2+})(A)(L\text{-His})\text{-}H]^+$), when mapped into 3D fragmentation vectors, enabled complete differentiation of 14 disaccharide isomers.[55] Chiral recognition studies using protonated tryptophan and pentose enantiomers (ribose/arabinose) revealed UVPD-based S1-S0 transition intensity differences for enantiomer quantification.[81] Glc-Fru isomers were successfully discriminated through PCA models based on ESI-MS/MS fragmentation patterns of lithium ([M+Li]+) and sodium ([M+Na]+) adducts under CID and HCD collision modes, with glycosidic bond decomposition mechanisms elucidated via deuterium-labeling experiments and DFT calculations, revealing that HCD-MS/MS was optimized for lithium adduct differentiation, while CID-MS/MS proved superior for sodium adducts.[56] Fixed-ligand kinetic workflows were extended to 12 pentose isomers (including D/L enantiomers), enabling microgram-scale absolute configuration determination.[82] Time-of-flight secondary ion mass spectrometry (TOF-SIMS) combined with PCA discriminated seven monosaccharide isomers via validated fragmentation pathways,[83] while HILIC-Q/TOF-MS optimized retention times, fragment abundance ratios (RAR), and collision energy-dependent profiles for non-derivatized mono-/disaccharide analysis in complex matrices.[6]

MS excels in unparalleled sensitivity, multidimensional fragmentation data acquisition, and versatile ion source/analyzer configurations, enabling precise isomer discrimination through advanced adduct chemistry, radical-based dissociation, and AI-driven pattern recognition. However, challenges persist, including reliance on complex data interpretation algorithms, high instrumentation costs, and limited throughput in high-complexity samples. Rigorous optimization of adduct stability and matrix effects remains critical for robust analytical applications.

Derivatization Methods for Liquid Chromatography-Mass Spectrometry (LC-MS)

The inherent absence of chromophores/fluorophores and low ionization efficiency of monosaccharides impose sensitivity limitations on direct HPLC-MS/MS analysis, necessitating derivatization strategies—primarily pre-column approaches due to lower instrumental complexity compared to post-column methods. Widely adopted reagents include anthranilic acid (AA), 2-amino-5-bromopyridine (ABP), 4-aminobenzoic acid butyl ester (ABBE), 4-aminobenzoic acid methyl ester (ABME), 1-phenyl-3-methyl-5-pyrazolone (PMP), and phenylhydrazine, with PMP emerging as the gold standard for its mild reaction conditions (no acid catalysis required), preservation of acetyl groups/stereochemistry, and strong UV

absorption at 245 nm.[56] Optimized derivatization protocols enable rapid, accurate monosaccharide profiling in polysaccharides: Fan et al characterized Osmanthus polysaccharides via pre-column PMP-HPLC-MS/MS, identifying rhamnose, xylose, ribose, glucose, mannose, trehalose, galactose, and galacturonic acid,[14] while Jiang et al quantified monosaccharides in Astragalus polysaccharides using retention time-based isomer discrimination and peak area calculations.[84] LC-MS/MS leverages adduct-specific fragmentation for structural elucidation, exemplified by Wu et al's ESI-MS analysis of Sargassum fusiforme monosaccharides[53] and Guo et al's quantitative profiling of Poria cocos polysaccharides.[85] Sun et al further established PMP-HPLC fingerprinting for Ganoderma lucidum polysaccharide quality control.[86] Despite these advances, conventional derivatization fails for ketoses (eg, fructose), driving innovations like PMP-labeled disaccharide isomer discrimination via ESI-MS/MS cross-ring/glycosidic bond cleavage patterns and statistical RIR (relative intensity ratio) analysis of three ion pairs (16 isomers resolved).[87] Comparative derivatization workflows (HACl/BSTFA vs EtOx/BSTFA) enabled GC/LC separation of 15 aldoses and complex mixture deconvolution,[88] while PMP-ESI-MSn achieved glycosidic linkage/anomer determination via diagnostic C–C bond cleavage ions.[89] Trace sample analysis (<1 mg) was revolutionized by UPLC-DAD-Q-TOF/MS with one-pot derivatization, resolving eight aldose enantiomers and rare sugars via retention time/fragment abundance ratios.[90] Derivatization significantly enhances sensitivity and enables isomer quantification, with PMP offering robust reproducibility and stereochemical integrity. Emerging strategies (eg, RIR-based MS/MS, enantioselective protocols) address ketose limitations and chiral discrimination. Multi-step derivatization increases workflow complexity; ketose incompatibility persists in conventional methods; matrix interference and adduct stability require stringent optimization.

Gas Chromatography and Gas Chromatography-Mass Spectrometry Coupling Method

GC excels in separating volatile compounds with high resolution and sensitivity, yet requires derivatization to enhance volatility and stability for carbohydrate analysis due to their inherent polarity and high boiling points.[17] Gas chromatography coupled with flame ionization detection (GC-FID) and GC-MS is widely adopted, with the latter dominating quantitative applications.[18] Derivatization strategies encompass methyl ethers, acetates, oximation, sugar alcohol acetates, saccharide acetates, and dithioacetals.[19,92] Main types of derivatives used for GC and GC-MS analysis of carbohydrates showed in Table 3.[91] Single-step derivatization (eg, acetylation/silylation) substitutes polar groups to improve volatility, though silylation risks multi-peak formation and peak overlap.[20] Two-step approaches (reduction/oximation followed by acetylation/silylation) minimize isomer complexity: reduction with NaBH4 converts aldoses to alditols but excludes ketoses, while oximation (hydroxylamine/methoxyamine HCl) eliminates furanose/pyranose isomerism, reducing chromatogram complexity via E/Z oxime formation applicable to both aldoses and ketoses.[2] Common reagents include trimethylchlorosilane (TMCS), hexamethyl-disilazane (HMDS), trifluoroacetamide (BSTFA) (silylation), and acid anhydrides (acetylation).[20] Method optimization focuses on simplifying workflows: Becker et al resolved 46 carbohydrates via ethoxyamination-silylation, enhancing peak identification;[2] Wang et al achieved stable derivatives for seven neutral monosaccharides and two uronic acids using n-propylamine/acetic anhydride.[21] Li et al developed a single-peak acetylated derivative method via Me_2SO/1-MeIm-mediated acetylation, enabling GC-MS analysis of aldoses, ketoses, and alditols in plant/fruit samples.[93] Acetal-trifluoroacetyl (Acetal-TFA) derivatives coupled with chiral/non-chiral columns enabled enantiomer resolution of C3-C6 aldoses.[94] Ketose-compatible innovations include Faraco's boronic acid derivatization (single-peak quantification of aldoses/ketoses)[95] and Xia's trimethylsilyl-dithioacetal (TSMD)-based GC-MS method for simultaneous analysis of aldoses, uronic acids, ketoses, and amino sugars.[19] GC-MS offers unparalleled resolution and sensitivity for carbohydrate profiling, with advanced derivatization (eg, oxime/acetal-TFA) minimizing isomer complexity and enabling enantiomer discrimination. Multi-step derivatization increases procedural complexity; ketose analysis requires specialized protocols. Silylation-induced peak multiplicity may compromise quantification accuracy.

Capillary Electrophoresis

Capillary electrophoresis (CE) has emerged as a versatile analytical tool for carbohydrate separation, leveraging differences in charge-to-mass ratio, molecular size, and shape under electrophoretic migration.[96] Capillary zone

Table 3 Main Types of Derivatives Used for GC and GC–MS Analysis of Carbohydrates[91]

Derivatives	Anomeric Centre	Derivatization Reaction	Advantages	Drawbacks
Methyl ethers	Non-modified (Multiple peaks)	$ROH+CH_3\text{-}X \rightarrow R\text{-}O\text{-}CH_3 + HX$	Suitable for low-MW carbohydrates and polysaccharide structural analysis	Complex chromatograms; time-consuming; thermal degradation; poor GC resolution
Acetates	Non-modified (Multiple peaks)	$ROH+Ac\text{-}X \rightarrow R\text{-}O\text{-}Ac+HX$	Suitable for low-MW carbohydrates; chemically and thermally stable	Complex chromatograms; low volatility vs TMS ethers
Trifluoroacetate	Non-modified (Multiple peaks)	$ROH+CF_3CO\text{-}X \rightarrow R\text{-}O\text{-}COCF_3 + HX$	High volatility; wide MW range; lower analysis temperature	Complex chromatograms; difficult quantitation
Trimethylsilyl ethers	Non-modified (Multiple peaks)	$ROH+(CH_3)_3Si\text{-}X \rightarrow R\text{-}O\text{-}Si(CH_3)_3 + HX$	High volatility, thermal stability; rapid mild reaction; widely used	Complex chromatograms; requires dry samples; moisture-sensitive reagents
Trimethylsilyl oximes	Modified (Two peaks)	(1) $R\text{-}CHOH\text{-}CHO+NH_2OH \rightarrow R\text{-}CHOH\text{-}C{=}NOH+H_2O$ (2) $R\text{-}CHOH\text{-}C{=}NOH+(CH_3)_3Si\text{-}X \rightarrow R\text{-}CHOSi(CH_3)_3\text{-}C{=}NO\text{-}Si(CH_3)_3 + HX$	Reduces chromatographic peaks to E/Z isomers; applicable to aldoses/ketoses	Requires dry samples; derivatives stable long-term
Trimethylsilyl alkyl oximes	Modified (Two peaks)	(1) $R\text{-}CHOH\text{-}CHO+NH_2OR' \rightarrow R\text{-}CHOH\text{-}C{=}NOR'+H_2O$ (2) $R\text{-}CHOH\text{-}C{=}NOR'+(CH_3)_3Si\text{-}X \rightarrow R\text{-}CHOSi(CH_3)_3\text{-}C{=}NO\text{-}R'+HX$	Reduces chromatographic peaks to E/Z isomers; applicable to aldoses/ketoses	Requires dry samples
Alditol acetates	Modified (One peak)	(1) $R\text{-}CHOH\text{-}CHO+NaBH_4 \rightarrow R\text{-}CHOH\text{-}CH_2OH$ (2) $R\text{-}CHOH\text{-}CH_2OH+Ac\text{-}X \rightarrow R\text{-}CHOAc\text{-}CH_2O\text{-}Ac+HX$	Single peak per aldose; stable; used for composition analysis	Loss of stereochemistry; ketoses yield mixtures; lengthy process; moisture sensitivity
Aldononitrile acetates	Modified (One peak)	(1) $R\text{-}CHOH\text{-}CHO+NH_2OH \rightarrow R\text{-}CHOH\text{-}C{=}NOH+H_2O$ (2) $R\text{-}CHOH\text{-}C{=}NOH+Ac\text{-}X \rightarrow R\text{-}CHOAc\text{-}C{\equiv}N+HX$	Single product per aldose; water-tolerant	Not suitable for ketoses; inappropriate for mixed aldose/ketose samples
Dialkyl dithioacetals	Modified (One peak)	(1) $R\text{-}CHOH\text{-}CHO+R'\text{-}SH \rightarrow R\text{-}CHOH\text{-}CH(S\text{-}R')_2 + H_2O$ (2) $R\text{-}CHOH\text{-}CH(S\text{-}R')_2+(CH_3)_3Si\text{-}X \rightarrow R\text{-}CHOSi(CH_3)_3\text{-}CH(S\text{-}R')_2$	Single peak per aldose; stable products	Difficult preparation; variable yields; byproduct formation

https://doi.org/10.2147/DDDT.S561012

electrophoresis (CZE) stands out for its simplicity, rapid analysis, minimal sample consumption, and eco-friendliness (low organic solvent usage), making it ideal for carbohydrate profiling.[22] Detection strategies include direct/indirect UV (eg, photo-oxidation at 270 nm,[97] photochemical-induced UV for oligosaccharides[23]), contactless conductivity (C4D), and fluorescence-based methods. While native CE struggles with unmodified monosaccharide detection due to lacking chromophores/fluorophores, derivatization reagents like APTS (8-aminopyrene-1, 3, 6-trisulfonate) and PMP (1-phenyl-3-methyl-5-pyrazolone) enable pre-column labeling for laser-induced fluorescence (LIF) or UV detection. CE-MS hyphenation enhances specificity and sensitivity: Khatri integrated microfluidic CE-MS with TMT tagging for multiplexed glycan quantitation.[24] For derivatization-based separations, Hu et al demonstrated PMP derivatization coupled with borate complexation under alkaline conditions to separate 10 aldoses/uronic acids,[25] while Lu et al extended this to 11 aldoses in honey.[97] Daniel et al further utilized CE-MS to identify coffee adulteration via monosaccharide profiling.[26] Comparative studies highlight CE's superior resolution and throughput over LC/GC methods, exemplified by Zhao et al's CZE-MS analysis of plant-derived mono-/di-/trisaccharides[96] and Oliver et al's cellulose analysis without sample pretreatment.[98] CE offers unmatched resolution, rapid analysis (<10 min), and minimal sample/reagent consumption, with derivatization-LIF achieving sub-picomolar sensitivity. Derivatization complexity (eg, APTS labeling), matrix-dependent migration reproducibility, and limited commercial CE-MS interfaces hinder routine adoption.

Ion Mobility Mass Spectrometry

IM-MS has emerged as a high-throughput structural elucidation tool for carbohydrate isomers, leveraging gas-phase ion separation based on CCS, charge, mass, and ion-neutral interactions.[27,99] By resolving ions in weak electric fields with inert buffer gases (N_2/He), IM-MS complements traditional MS by distinguishing isobaric isomers (eg, positional/stereoisomers) with lower sample requirements and faster analysis than NMR or LC.[28] Key parameters include arrival time distributions (ATDs) and instrument-independent CCS values, which reflect ion conformation and drift gas effects.[99] Torano et al pioneered conformer distribution (CD) fingerprinting via ATDs to identify glycan isomers,[100] while Gaye et al differentiated 16 glucose isomers using CCS-guided diastereomer complexes with metals/ligands.[30] Gas composition optimization (N_2 for maximal CCS reproducibility[101]) and derivatization strategies (eg, PMP labeling,[8] 3C5NBA[29]) enhance CCS disparity, enabling monosaccharide/disaccharide isomer resolution. IM-MS/MS workflows (eg, IMS-CID-IMS-MS[31]) exploit precursor/fragment ion CCS correlations for structural confirmation. By employing 4-(3-methyl-5-oxo-pyrazolin-1-yl) benzoic acid (CPMP) derivatization coupled with IMS analysis, researchers successfully distinguished disaccharide isomers, achieving near-baseline separation with a high resolution of 1.484 for the [M+2CPMP+H]+ ion species.[3]

Integration with chromatographic systems expands applicability: Antonius et al coupled HILIC with traveling-wave IM-MS (3-AQ-HILIC-TWIMMS) for pectin oligomer isomer quantification,[102] while Reymond et al implemented 2D-LC-IM-MS for lignocellulosic biomass analysis.[103] CCS databases (eg, Glycobase 43[104]) accelerate isomer identification via standardized references. Technological advancements include collision-induced fingerprinting (CIF) for energy-resolved isomer differentiation,[105] cyclic IM (cIM) separators for high-resolution MS/MS,[106,107] and ambient ionization with in situ methylation for rapid glycosidic linkage analysis in complex matrices. Ion mobility methods on a cIMS instrument were used to build a database of HR-IMS fingerprints for various underivatized monosaccharide stereoisomers. The conditions were fully compatible with MS/MS fragmentation approaches, and verified that these fingerprints afford the identification of monosaccharidic fragments released upon collisional fragmentation of oligosaccharides.[108] An ambient ionization tandem mass spectrometry approach coupled with in situ methylation using tetramethylammonium hydroxide was used to rapidly differentiate disaccharide isomers by generating characteristic methylated marker ions, enabling precise identification of glycosidic linkages, compositions, and configurations without chromatographic separation. The method's practicality is demonstrated through direct analysis of disaccharide isomers in complex commercial products such as honey, wine, and milk, offering a high-throughput solution for real-world saccharide characterization with minimal sample pretreatment.[109] Derivatization-enhanced strategies, such as CPMP-IMS for lactose/maltose quantification in beer/milk,[3] demonstrate method versatility. IM-MS achieves rapid, high-resolution isomer discrimination with minimal sample consumption, complemented by CCS database interoperability and compatibility with hybrid chromatographic systems. The reproducibility of CCS measurements is influenced by drift

gas and ionization settings, while the limited database coverage for rare isomers restricts identification capability, further compounded by the high cost of advanced systems like cIM-MS.

Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy serves as a cornerstone technique for carbohydrate structural elucidation, combining non-destructive analysis, high precision, and reproducibility. While 1D $^{1}H/^{13}C$ NMR provides foundational monosaccharide composition data, peak overlap necessitates advanced 2D/multidimensional methods (eg, ^{1}H-^{1}H COSY, HSQC, HMBC) to resolve glycosidic linkages and stereoisomers.[106,107] Innovative protocols enhance utility: Inagaki et al established L-cysteine methyl ester derivatization for absolute configuration determination (D/L) via ^{1}H NMR chemical shifts in ginsenoside monosaccharides,[5] while Sassaki et al leveraged JC-H coupling (155 Hz) in Q-HSQC for α/β anomer discrimination.[33] Multidimensional approaches (eg, Qi Shi's 3D HSQC-TOCSY) streamline structural assignments without derivatization.[34] High-throughput applications include Campo's ^{1}H NMR quantification of honey carbohydrates[35] and Schievano's CSSF-TOCSY method for simultaneous identification/quantitation of 22 saccharides in aqueous honey extracts.[110] Computational tools address spectral complexity: Florbela Pereira's 1D ^{13}C-NMR simulations predict oligosaccharide linkages,[32] and Kapaev's GODESS software models 2D NMR spectra for unresolved peaks.[36] Sensitivity limitations (sample quantity/purity) are mitigated by cryoprobes, ultrahigh-field magnets, and hyperpolarization techniques, expanding NMR's scope to nanoscale carbohydrate analysis. NMR offers non-destructive, absolute structural determination with atomic-level resolution, excelling in stereochemistry and linkage analysis without chromatographic separation. High sample purity/quantity requirements and spectral complexity demand advanced instrumentation/computational aids; throughput lags behind MS-based methods. Emerging hyperpolarization technologies promise enhanced sensitivity for trace analysis.

Other Methods

A hetero-octameric Mycobacterium smegmatis porin A (MspA) nanopore engineered with phenylboronic acid (PBA) adapters achieved precise disaccharide isomer discrimination (sucrose, lactose, maltose, and six α-D-glucopyranosyl-D-fructose variants) at 99% accuracy via machine learning-assisted current signature analysis, marking the first nanopore-based detection of glycosidic linkage diversity. Practical validation through direct identification of isomaltulose additives in commercial sugar-free yogurt underscores its potential for real-time saccharide monitoring and nanopore sequencing.[-111] Separately, a homochiral organic photoelectrochemical transistor (OPECT) integrated Au nanoparticle (AuNP)-decorated TiO_2 nanotube arrays (TNT) with a chiral Cu(II)-MOF (c-CuMOF) gate electrode for enantioselective glucose sensing. Stereospecific catalytic oxidation of captured D/L-glucose to H_2O_2 triggered catalytic precipitation, amplifying drain current (I_D) responses with ultra-low LODs (0.05 μM for D-Glu, 0.07 μM for L-Glu), while elucidating biomimetic stereoselectivity mechanisms.[112] Both methods offer breakthrough sensitivity and specificity—nanopores enable label-free, real-time isomer discrimination; OPECT achieves enantiomer detection at sub-μM levels with mechanistic insights. Nanopore accuracy depends on adapter stability and machine learning training data scope; OPECT requires complex photoelectrode fabrication and lacks multiplexing capability for complex matrices. By achieving rapid cryogenic IR spectral acquisition within 10 seconds, researchers have successfully directly coupled LC with IR spectroscopy, providing a real-time, orthogonal dimension for molecular fingerprinting that identifies oligosaccharide isomers and impurities simultaneously during a standard analytical run.[113] Recently, a novel label-free oligosaccharide sensing platform based on the OmpF nanopore was developed, which leverages intrinsic electroosmotic flow to detect neutral oligosaccharides at concentrations as low as 6.4 μM and, combined with machine learning, achieves 99.9% accuracy in distinguishing tetrasaccharide isomers based on glycosidic linkage differences directly in complex biological matrices.[114] By employing a synthesized reactive matrix (TMNTA) and an internal standard, a MALDI-TOF/TOF MS/MS method achieves absolute quantification of glucose and fructose isomers through diagnostic ion ratios, offering a simple and rapid solution for complex sample analysis.[115] A novel computational technique that combines quantum tunneling with artificial intelligence has been reported, demonstrating excellent sensitivity in distinguishing carbohydrate anomers and stereoisomers and opening a route for integration with next-generation sequencing.[4] A hetero-octameric Mycobacterium smegmatis porin A nanopore equipped with a phenylboronic acid adapter has been demonstrated to directly discriminate

disaccharide isomers, successfully differentiating not only common disaccharides like sucrose and lactose but also resolving six α-D-glucopyranosyl-D-fructose linkage isomers with 99% accuracy when assisted by machine learning.[111]

Conclusions and Perspectives

This review has charted the technological evolution in carbohydrate analysis, moving from conventional separations to intelligent, multi-dimensional platforms. Our contribution lies in synthesizing this progress into a practical evaluation framework that directly discusses the historic trade-offs between resolution, throughput, and structural certainty, thereby empowering researchers to select optimal strategies for discriminating natural monosaccharide and disaccharide isomers in applications such as biologics quality control, clinical biomarker discovery, or food authentication.

The shift toward streamlined, non-derivatization workflows and hyphenated systems like HILIC-IM-MS represents more than a technical advance. It fundamentally enhances our ability to probe structure–function relationships in glycans, thereby translating analytical precision into meaningful biological insight. Looking ahead, emerging technologies such as functionalized nanopores, biomimetic sensors, and chip-integrated platforms hold transformative potential. When integrated with advances in machine learning and quantum-assisted modeling, these tools are poised to enable real-time, mono- and disaccharide isomers profiling. This convergence will pave the way for a new era of precision glycobiology, one that seamlessly connects molecular structure to biological mechanism and function.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the Natural Science Foundation of China (No. 82574698), Natural Science Foundation of Hunan Province (No. 2023JJ60523), Natural Science Foundation of Changsha City (No. kq2403043), Shandong Provincial Natural Science Foundation of China (No.ZR2023LZY011), and China's Foundation for Youth Entrepreneurship and Employment (No.17, fifth phase).

Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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