Introduction

Description

Carbohydrates, in the form of mono-, oligo-, poly-saccharides, glycopeptides, glycolipids, glycosaminoglycans, proteoglycans, or other glycoconjugates, constitute the most abundant and complex family of bio(macro)molecules. They have long been known to participate in many biological processes.
From their simplest to their most complex forms, they are divided into several categories, covered by the generic term of glycans. Such a division distinguishes (1) monosaccharides, (2) oligosaccharides, (3) polysaccharides and (4) substances derived from monosaccharides. Monosaccharides are the single building blocks, i.e. the monomeric constituents of glycans chains. Oligosaccharides are defined as carbohydrates that comprise 2-10 monosaccharides, linked together in a linear or branched way. The term “polysaccharides” refers to glycan chains built up from more than 10 monosaccharides, also linked together in a linear or branched way. The border line between oligosaccharides and polysaccharides is not drawn strictly. The collective term glycoconjugates covers a large family of substances the main types being glycoproteins, glycopeptides, peptidoglycans, proteoglycans, glycolipids and lipopolysaccharides. Glycoproteins are proteins where the covalently attached glycan chains result from a co-translational or post-translational modification. In N-glycosylation,glycan occurs at the amide nitrogen on the side-chain of the asparagine whereas in O-glycosylation, glycan chain is on the hydroxyl oxygen on the side-chain of hydroxylysine, hydroxyproline, serine or threonine. Glycolipids are glycosyl derivatives of lipids such as acylglycerols, ceramides and prenols.
Determination of the three-dimensional (3D) structural and dynamical features of complex carbohydrates, carbohydrate polymers, and glycoconjugates, along with the understanding of the molecular basis of their associations and interactions, represents the main challenges in structural glycoscience. Nevertheless, a prerequisite to attempt establishing such three-dimensional features, is the determination of the chemical structure, also referred to as primary structure, i.e. the nature of the constituting monosaccharide units, along with the nature of the linkage to the neighboring units. This is far from trivial due to the plethora of monosaccharides that occur in nature. An exhaustive coverage of this aspect can be found at the e-chapter “The Symbolic Representation of Monosaccharides in the Age of Glycobiology” at http://glycopedia.eu.
The determination of the primary structure of glycans aims at defining the following items :

- Monosaccharide composition : nature of each constituting monosaccharide along with absolute configuration.
- Linkage patterns : monosaccharide positions involved in the glycosidic linkages. Determination of possible branching points.
- Ring size : distinction between furanosidic and pyranosidic rings.
- Anomeric configuration : ?- or ? -configuration at the glycosidic linkage.
- Sequences of monosaccharide residues and, if the glycan has a regular structure, determination of the repeating unit.
- Identification, localization and distribution of possible substituents on hydroxyl groups.
- Molecular size.

The aim of this chapter is to offer a general background to those researchers who need to analyse the constituting monosaccharides of a complex glycan. The chapter will not cover all the existing methodologies, but will focus on the use of Gas Chromatography-Mass Spectrometry (GC-MS) which provides answers to the first three items of the above mentioned list. The main rules needed for the interpretation of the obtained results are clearly presented, along with a library of GC-MS spectra to be used as reference.