# HILIC on Poly-Hydroxyl Stationary Phases in Protein-Rich-Food Supplements

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Monosaccharides, disaccharides, oligosaccharides, and polysaccharides are essential sources of dietary energy. In the food industry, certain monosaccharides like glucose, galactose, and fructose, and disaccharides like lactose, sucrose, and maltose, are known for their sweet taste. With the increasing prevalence of public health issues such as obesity and diabetes, it is essential to increase consumer awareness about sugar consumption and monitor the intake of processed foods.

Keywords: HILIC ; ELSD ; saccharides ; sugar alcohols ; isomaltulose (Palatinose)

## 1. Introduction

Monosaccharides, disaccharides, oligosaccharides, and polysaccharides are essential sources of dietary energy. In the food industry, certain monosaccharides like glucose, galactose, and fructose, and disaccharides like lactose, sucrose, and maltose, are known for their sweet taste [1]. Additionally, nutritive sweeteners like sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, and erythritol, which are sugar alcohols, are used.

The amount of total sugars consumed can vary based on age group, with infants consuming up to 38% of their overall energy intake and adults consuming around 13% [2]. However, consuming excessive or frequent amounts of fructose, glucose, and sucrose can contribute to various health issues. Multiple studies have indicated a close relationship between the consumption of both glucose and fructose and the emergence of type 2 diabetes over the past few decades [3][4]. Additionally, high sugar intake can lead to weight gain, obesity [5], negative effects on oral health such as dental caries [6], cardiovascular diseases [7], some cancers [8], and an increased risk of Alzheimer's disease [9]. Sugar alcohols can also have negative effects on individuals with irritable bowel syndrome (IBS) [10]. To address these risks, health organizations have published recommendations for reducing added dietary sugar intake, with the World Health Organization (WHO) recommending that free sugars make up less than 10% of total caloric consumption [11][12].

With the increasing prevalence of public health issues such as obesity and diabetes, it is essential to increase consumer awareness about sugar consumption and monitor the intake of processed foods. Various regulatory authorities, such as the European Union (EU), Food and Drug Administration (FDA), and Food Safety and Standards Authority of India (FSSAI), have made it mandatory to declare the sugar content on product labels. According to the definition in EU Regulation, saccharides are every saccharide metabolized by a human, including polyols; sugars are mono- and disaccharides, excluding polyols. FDA's new policy states that an analysis is necessary when the sugar content in foods exceeds 1% [13].

Therefore, it is necessary to assess the carbohydrate composition of the relevant foods and drinks and thereby reduce the consumption of foods with unknown carbohydrate composition and sugar alcohols. Carbohydrate analysis is prescribed by EU law so that customers can assess the nutritional value of foodstuff. As follows from the paragraphs above, although carbohydrates and sugar alcohols (polyols) are Generally Recognized as Safe (GRAS), their amount should be continuously monitored by manufacturers and legislation agencies to ensure customer safety and information regarding the potential health concerns of certain ingredients.

## 2. Isomaltulose in Food Supplements

Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect that are marketed in dose form (e.g., pills, tablets, capsules, liquids in measured doses). A wide range of nutrients and other ingredients might be present in food supplements, including carbohydrates, vitamins, minerals, amino acids, essential

fatty acids, fiber, and various herbal extracts. There are reviews on food supplements, e.g., [14], and also on herbal food supplements for body weight reduction [15].

Food and dietary supplements may contain a variety of sugar alcohols, monosaccharides, disaccharides, and oligosaccharides. Some of the commercially available food supplements contain rapidly metabolized carbohydrates like glucose and fructose for “quick energy”, while maltodextrins may serve as a sustained energy source. In 2008, the FDA (Food and Drug Administration) included isomaltulose in substances eligible for health claims, and subsequently, the European Food Safety Authority (EFSA) also affirmed its positive health impact. Nowadays, isomaltulose can be found in the market as a sugar substitute in tooth-friendly chewing gum, instant teas designed to prevent tooth decay, and lifestyle nutrition products. Isomaltulose has also become popular as a part of “healthy” or “complete food” [16]. The use of isomaltulose (Palatinose) has also led to clinical trials exploring its potential benefits in dietary supplements not only for enhancing physical performance [17], but also in pre-diabetes and diabetes treatment [18][19][20].

## 3. Methods for Sugar Determination

Today, determining mono- and disaccharides stands out as one of the most requested tests in food analysis laboratories. The comprehensive analysis of glucose, fructose, sucrose, lactose, and maltose is essential for determining the overall sugar content in diverse food products. The evolving landscape of complex food matrices and product innovations underscores the necessity of scrutinizing sugar content in a wide array of foods, including cereals, dairy products, sweets, beverages, and sauces [1].

Sugar analysis proves valuable for monitoring claims in low-calorie foods, assessing energy content, checking fruit juice quality including adulteration, determining lactose levels in milk, and measuring lactose in low-lactose or lactose-free foods [21][22], monosaccharides from starch-based glucose or sucrose hydrolysis [23], or horticultural sugars [24].

The main path to analyze carbohydrates is either gas chromatography after derivatization or HPLC (mainly reversed-phase HPLC on C18-columns), ion exchange chromatography on cation exchange columns in, typically, $K^+$, $Ca^{2+}$, or $Pb^{2+}$ cycle, or by high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD), hydrophobic interaction chromatography, and size exclusion chromatography or capillary electrophoresis.

## 4. HILIC-ELSD

Hydrophilic interaction liquid chromatography (HILIC) is popular for separation of polar analytes on polar columns in aqueous–organic mobile phases rich in organic solvents (typically acetonitrile) [25][26][27][28], although the term HILIC was not coined until 1990 by Alpert [29]. Overview of methods for natural carbohydrates can be found in [28]. A recent review on HILIC application in bioanalytical chemistry is available in [30] or specifically for glycopeptide analysis in [31].

Since 1975 [32], the amine-bonded silica stationary phase has been widely used for the separation of saccharides and polyols, and is still recommended, although disadvantages of amine-bonded silica columns are (i) a short life-time due to the formation of glycosamides between the stationary phase amines and reducing sugars (column deactivation) and (ii) also bleeding of the aminopropyl ligand. Recently, thanks to advances in HPLC stationary phase technology, several amino phases are available on the market that overcome these drawbacks: either replacing silica gel with a polymer support or by using a carbamoyl- or an amide- groups (BEH, ethylene bridged hybrid) as stationary phases [33]. Stationary phases for HILIC have been reviewed by Guarducci et al. [34]. In the last decade, HILIC on poly-hydroxyl stationary phases was used for analysis, separation, and determination of saccharides [35][36][37][38][39].

Carbohydrates do not contain suitable chromophores for common UV detection, so that, apart from derivation, other types of detection principles must be applied. Polarimetry or universal refractive index detector (RID) are still in use; recently, Tiwari et al. validated a method on an amino column (mobile phase acetonitrile–water) with RID [27]. Evaporative light-scattering detector (ELSD) is today very popular for the detection of poly- or oligo-saccharides after hydrolysis of various products [40][41][42]. A review on carbohydrate analysis with ELSD can be found in [43]. Extreme selectivity of poly-hydroxyl stationary phases with ELSD was utilized for separation and identification of glucose, fructose, and rhamnose after hydrolysis of glycosides [36].

## 5. Protein-Rich Sample Preparation

Analyzing protein-rich aqueous samples, such as milk, plasma, and food supplements, directly with certain techniques is challenging due to the presence of various interferences and incompatibility with instrumental conditions. Consequently,

effective sample preparation steps are essential for protein-rich aqueous samples before conducting LC or GC analysis [44]. Various methods, including liquid–liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), and magnetic solid-phase extraction (MSPE), have been developed for this purpose [45]. Among these methods, LLE stands out as one of the oldest and most widely employed techniques for preparing protein-rich aqueous samples. It offers advantages in terms of simplicity and cost-effectiveness, making it a popular choice in scientific research and routine applications [46]. Efforts have been devoted to enhancing the traditional LLE technique, aiming for a faster, simpler, and more efficient methodology [47].

The presence of a high amount of proteins requires a denaturation step which is very often performed in biology (denaturation of peptides) [48] or proteomics [49]. However, denaturation protocols may differ depending on sample type, experimental goals, and the analytical method used. Many factors are considered when designing sample preparation strategies, including source, type, physical properties, abundance, and complexity of the proteins. Compared to, e.g., cell samples, special food supplements with added pure proteins represent a relatively simple matrix, so thermal denaturation and routine deproteinization with acetonitrile may be sufficient.

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