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The analysis of 80 simple saccharides by ion chromatography mass spectrometry

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Sugars or saccharides exist as structural and stereo- isomers and can be difficult to analyse, due to insufficient analyte separation. By coupling chromatography with mass spectrometry, the reliability of the analysis can be improved, but there is still potential for erroneous results due to the vast array of saccharide isomers present in nature. In this study, we present a method for the analysis of 80 saccharides analysed using an ion chromatograph coupled to a mass spectrometer. The method successfully separated, identified and quantitated 21 monosaccharides, 28 disaccharides, 15 trisaccharides, 7 tetrasaccharides, and 9 sugar alcohols. The feasibility of this method was demonstrated through the analysis of commercially available rare and common sugar samples, as well as honeys. While the method showed promising results for these compounds, further validation will be required to determine its applicability in more stringent fields such as forensics and clinical analysis.

1 Introduction

Whilst commonly known sugars such as glucose, fructose, sucrose are abundant in nature, they represent only a small fraction of the diversity of sugars that exist.[1] Hundreds of other, rare or atypical sugars are present in biological systems.[1] While generally these atypical sugars are minor and often overlooked components, some are significant, for example, stingless bee honey which contains trehalulose as the major disaccharide.[2,3]

Some of these rare sugars have been reported as being healthier than sucrose with properties including: glucose absorbance regulating, infection healing, acariogenic, antiaging, low-calorific sweeteners, prebiotic, antibacterial and antioxidant activities.[1,4–7] As such, a retail market aimed at selling healthy sugars or carbohydrates has developed, and was reported to be worth USD $1.65 billion in 2023.[8] The standard method for the routine analysis of sugar, (liquid chromatography by AOAC methods, such as AOAC 984.22) does not cover these analytes and new methodology is required.[9,10]

The analysis of sugars requires unequivocal accuracy *i.e.* no misidentification. This is important not only for food analysis but for biological, metabolomics, industrial, medical, legislative, cultural and economic reasons.[1,11–16] Inaccurate analysis (not measuring the true value), can lead to detrimental outcomes. For example trehalulose in stingless bee honey was historically mis-reported as maltose.[2] The reported health effects of these two sugars are completely contrasting.[2]

Despite being simple organic molecules, sugars or saccharides have been difficult to analyse.[1] Sugars lack chromophores/fluorophores and are poorly ionised by mass spectrometry (MS), without derivatization.[11,17] Multiple possible structures, including stereoisomers, exist for any given molecular formula, with distinct glycosidic linkages possible between the anomeric carbon of one sugar monosaccharide (see Fig. 1), and any of the hydroxyl groups of another.[6] Additional complexities include different ring structures (furanose or pyranose), anomers (α &/or β) and degree of polymerisation (DP),[18] each derived from the parent monosaccharides parent aldoses or ketoses (shown in Fig. 1 in their acyclic forms). Hence a vast array of simple saccharides exist, each with their own physical, chemical and implied properties, such as glycaemic and sweetness indexes.[19,20]

Various methods for sugar analysis have been developed, each with their own strengths and weaknesses.[9] Chromatographic methods include gas chromatography (GC), high performance liquid chromatography (HPLC) and ion chromatography (IC), all of which can be coupled to mass spectrometers as a 'universal detector'.[11,21] The advantage of MS is that the compound's molecular mass can be used as an additional factor to verify the identity of the compounds present. Since most saccharides are isomers, the use of MS is, only effective if co-elution is minimised and good isomer separation is achieved.[1] An alternative analytical strategy relies on tandem mass spectrometry (MS/MS) to give characteristic fragment ions to differentiate sugar isomers. This can manifest as subtle differences in relative intensities of fragment ions, rather than

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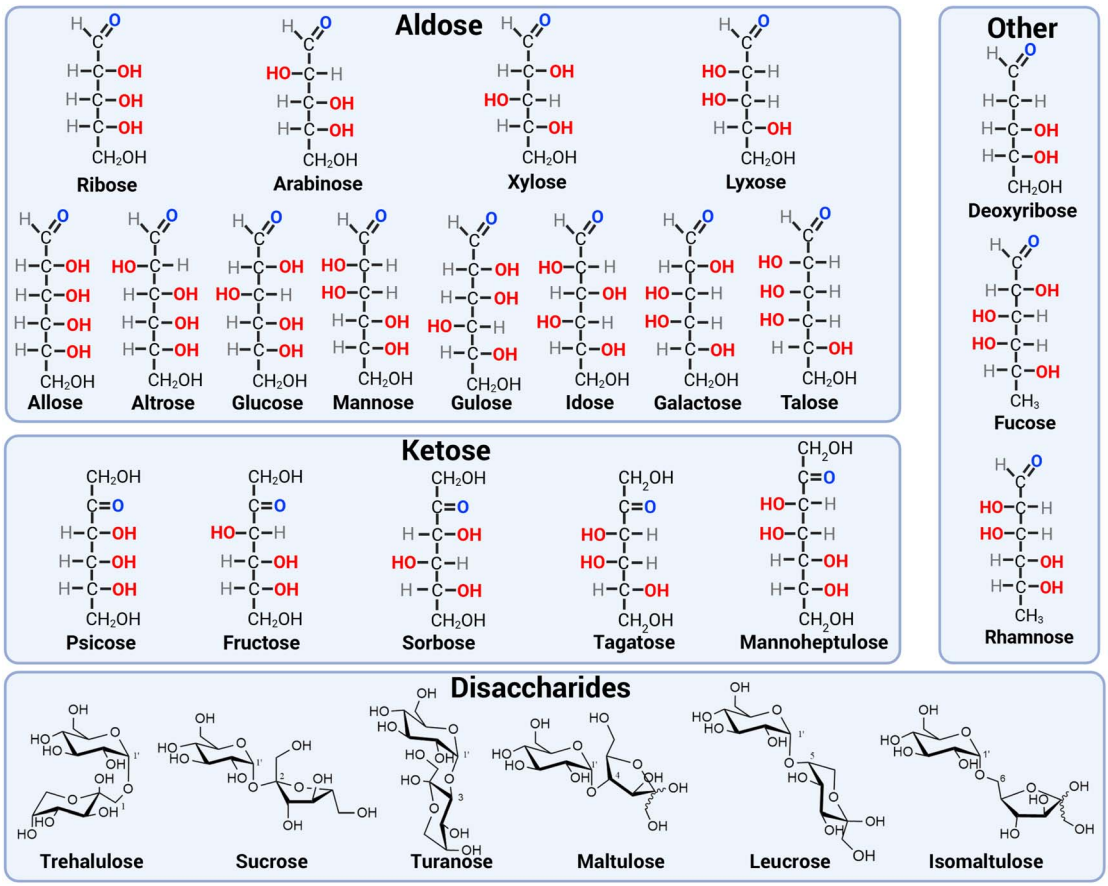

Fig. 1 Structures and names of some mono- and disaccharides used in the IC-MS method. For monosaccharides, hydroxyl groups (red) and carbonyl groups (blue) are coloured to highlight the differences between structures. For glucose–fructose disaccharides, the carbons atoms involved in the glycosidic bond are indicated to highlight structural differences. Only D isomers shown, with the L configuration is some instances, being the naturally occurring sugar[18] (created in https://BioRender.com, disaccharides from ChemDraw v23.1.1.3).

unique patterns.[22] Nuclear magnetic resonance spectroscopy can provide superior characterisation of pure compounds, but this becomes more challenging and time consuming, when samples are not pure.

Of the three chromatography systems, IC has been described as providing the best selectivity for the separation of sugars.[21] Meyer *et al.*, 2022 compared the number of sugars that could be separated by HPLC-MS and GC-MS in biological samples and found that 14 neutral sugars could be analysed by GC-MS without co-elution, while HPLC-MS was only able to analyse nine.[11] In comparison, a recent paper reported an IC-MS method which identified 21 sugars, in honey.[23] Apart from the increased number of sugars detectable by IC-MS, there are two other advantages IC has over the other two instruments.

The first advantage is that no sample derivatisation is required, which simplifies sample preparation, reduces lab costs, time, and effort. Another advantage is that no chromatographic anomer separation occurs (with resultant multiple chromatographic peaks) due to the highly caustic mobile phase, which also reduces time required for data analysis. IC-MS instrumentation is not as commonplace as GC-MS or HPLC-MS and there has been a relative lack of methods developed for sugar analysis using the IC-MS. Most of the applications of IC to saccharide analysis have employed highly sensitive pulsed amperometric detection (IC-PAD).[24] The combination of IC separation and MS enables differentiation based on mass, such that chromatographic separation is only required for isobaric saccharides.[23]

To build on existing analysis methods, we have developed an IC-MS method for the analysis of 80 mono-, di-, tri-, tetrasaccharides (DP 1–4) and a small number of sugar alcohols (DP 1–2). The method has been validated for both the qualitative and quantitative analysis of these sugars and has been used to confirm the contents of a number of commercially available dietary supplements claiming to be rare or major sugars as well as two honeys.

2 Experimental method

2.1 Chemical and materials

2.1.1 Chemicals and reagents. Maltulose monohydrate (99%) was sourced from Chem-Impex (Wood Dale, USA). Arabinobiose (90%), cellotriose (96%), inulotriose (88%), laminarbiose (95%), levanbiose (90%), levantriose (90%), and mannotriose (99%) were sourced by Megazyme (Wicklow, Ireland). Erlose (98%), isomaltose (97%), maltotetraose (97%) and

panose (97%), were sourced from Nagase Viita (Okayama, Japan). Leucrose (95%), and 3-fucosyllactose (95%) were sourced from Combi-blocks (San Diego, USA). Trehalulose (95%) was sourced from carbosynth (Compton, UK). Allolactose (98%), epicellobiose (95%), inulobiose (98%), and neokestose (98%) were sourced from BOC Sciences (Shirely, USA). Idose and 6-glucopyranose maltotriose (98%) were sourced from Toronto Research Chemicals (Toronto, Canada) and stachyose (99%) was obtained from Tokyo Chemical Industry (Tokyo, Japan).

The remaining (58) sugars and sugar alcohols (90% or greater purity) as well as lithium chloride and ammonium chloride (<99.9%) were purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile was sourced from ThermoFisher (Waltham, USA). Ultrapure water (18.2 MΩ) was prepared onsite from a Merck Milli-Q Advantage A10 water purification system coupled to a Q-Pod Element dispenser (Darmstadt, Germany).

The 80 sugar reference materials were prepared as nine stock solutions, each containing sugars at 100 mg L^{-1} (apart from idose). Individual reference material calibration curves were made by serial dilution of the nine stock solutions to cover a 0.25 to 25 mg L^{-1} range in ultrapure water. Only linear concentration ranges were used (see Table S1 of the SI, as ranges are different per analyte). Typical R^2 values ranged from 0.990 to 0.999.

2.1.2 Preparation of dietary supplement samples. To determine the compliance with labelling requirements 18 samples claiming to be single-ingredient sugars were purchased during Jul–Sep 2024. These included sugars sold as food additives and/or as dietary supplements. All samples were purchased from internet suppliers, which included three samples described as 100% allulose (psicose), three as (90 to 100%) dextrose (glucose), two as fructose (100%), two as palatinose (purity unspecified or 100%), two as trehalose (90% or 100%). Two samples of xylitol (100%) and one sample each of erythritol (100%), lactose (100%), maltose (30%) and ribose (100%) were also acquired.

Each sample (approximately 0.3 g) was weighed into a 50 mL centrifuge tube and 30 mL of ultrapure water added. Samples were shaken at 2000 rpm, in a lab mixer (Heathrow Scientific, UK) until dissolved. Samples were analysed as further 10-, 100- and 1000-fold dilutions. All solutions were filtered through 0.2 μm regenerated cellulose membrane filters (Merck, Germany) into amber chromatography vials for analysis.

2.1.3 Preparation of honey samples. Two samples of honey were purchased during Mar–May 2025 from a local supermarket. To determine the accuracy of the method, a control material of honey was sourced in June 2025 from a European proficiency trial company (Honey 90-0146 from BIPEA, France). The material was reported to contain three mono-, six di- and five trisaccharides.

Samples were prepared as above, with the addition of centrifugation in a Hettich Rotanta 460R centrifuge (Kirchlengern, Germany) at 10 000×*g* for 10 min.

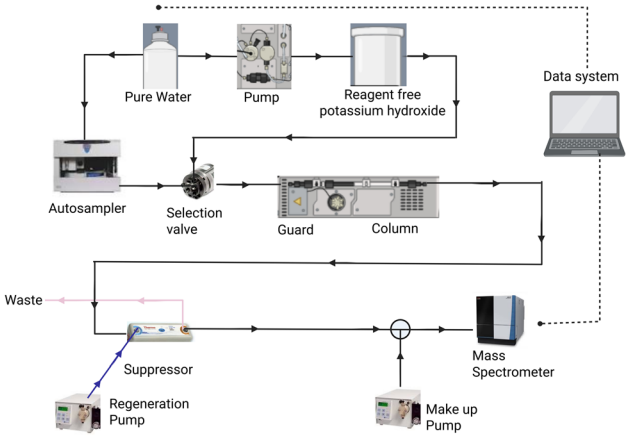

Fig. 2 Schematic illustration of IC-MS components. Arrows denote direction of mobile phase flow (created in https://BioRender.com).

2.2 Instrument details

The analyses were carried out using a ThermoFisher Dionex ICS-6000 Ion Chromatograph (Sunnyvale, USA) with a 2 mm potassium hydroxide "reagent-free" channel with an AS-AP autosampler. Sample extracts were chilled on the autosample at 16 °C during analysis. Prepared samples and standards (2 μL each) were injected onto a CarboPac PA210™ column (Thermo Scientific, 2 × 150 mm) with a CarboPac PA210™ guard column (2 × 30 mm). The potassium hydroxide (KOH) mobile phase was produced by a "reagent-free" eluent generation cartridge. The gradient elution conditions were as follows: 0.01–33.90 min, 12 mM KOH; 34.00–74.90 min, 100 mM KOH; 75.00–89.00 min, 12 mM KOH.

MS data were acquired with a single quadrupole mass spectrometer (ISQ EC, ThermoFisher Scientific™, Singapore), coupled to the ion chromatograph. The spectra were collected in negative ion full scan mode between 120 and 750 *m*/*z*. The instrument was controlled and data analysed using ThermoFisher Chromeleon® software (version 7.3.2).

As the KOH mobile phase used for separation was not compatible with MS, two additional AXP pumps were employed. The first pump was used for continuous supply of ultrapure water at 0.25 mL min^{-1} to a ThermoFisher Dionex ADRS 600 (2 mm) suppressor running at 40 mA, which removed the KOH from the mobile phase. The second pump provided a stream of 0.05 mM lithium chloride or ammonium chloride *via* a tee piece placed before the MS to promote the ionisation of the sugars (make-up phase). A simplified schematic of the instrument is shown in Fig. 2.

3 Results & discussion

3.1 Mass spectrometer optimisation

MS parameters optimised for saccharide analysis, included ionisation mode and adduct types, as well as make-up phase and ionisation voltage. A 10 mg L^{-1} solution of nine mixed mono-, di-, tri- and tetra-saccharides (galactose, glucose,

fructose, sucrose, lactose, maltose, melezitose, raffinose, and stachyose) was used for optimisation.

Sugars are inherently poorly ionised by electrospray ionisation-mass spectrometry (ESI-MS) forming pseudo-molecular ions $[M + H]^+$ in positive-ion mode or $[M - H]^-$ in negative-ion mode.[9] Ionisation can be enhanced by the post-column addition of salts *via* a tee piece, first described in 2005 by Bruggink *et al.*, with the use of 0.5 mM lithium chloride in water.[25] The resulting lithium adducts were analysed in positive ion mode using a single quadrupole mass spectrometer coupled to an ion chromatograph.[25] Lithium salts are not volatile and may cause formation of deposits within the mass spectrometer, resulting to reduced performance over time.[26,27]

The use of chloride adducts detected in negative ion mode is another option that has been reported in numerous MS methods for classical saccharide analysis.[28] Recent studies have reported using halide adducts (Cl^- and Br^-) to distinguish five isomers of sucrose and four isomers of maltose, *via* tandem mass spectrometry-generated diagnostic fragment ions.[1] This method was able to detect structural isomers in complex matrices such as raw honey and was also found to be applicable to selected mono and oligosaccharides.[22] Acetonitrile has been reported in HPLC-MS applications, where is it used as mobile phase.[22] Acetonitrile has a lower desolvation temperature than water, which is useful if analytes of interest are temperature sensitive.

In this study, lithium, ammonium and chloride adducts were compared to determine the best additive for saccharide analysis by IC-MS. The peak intensities of both lithium and ammonium adducts using positive ion mode, and chloride adducts using negative ion mode, in both water and acetonitrile make-up phase, are summarised in Fig. 3A. Individual responses for each of the adducts in acetonitrile, are shown in Fig. S1–S3 of the SI.

For all saccharides tested, chloride adducts were found consistently to provide the greatest peak areas. It was also observed that formation of ammonium $[M + NH_4]^+$ adducts resulted in peak broadening which compromised chromatographic performance to such an extent that it was impossible to separate galactose from glucose, which elute close together. Peak broadening also resulted in lower average peak sizes for monosaccharide ammonium adducts. Based on these results, the chloride adduct $[M + {}^{35}Cl]^-$ was selected as the optimum. Acetonitrile in the make-up phase was found to provide better signal response compared with water in the make-up phase. This finding was consistent with previous studies, such as Tedesco *et al.*, 2020 who found that organic phases afforded a better response than a purely aqueous make-up phase.[23]

Once the adduct type and ionisation mode were selected, other parameters, such as spray temperature, make-up phase flow rate, ion transfer tube temperature and ESI source voltage were optimised. Results of spray temperature ramping determined that 250 °C was the optimum spray temperature for chloride adducts of all four saccharide types in the solutions tested (see Fig. S3 of SI). This was complemented by 0.1 mL min^{-1} as the optimal flow rate (see Fig. 3B), 350 °C as optimal ion transfer tube temperature (see Fig. 3C) and 2.5 kV (see Fig. 3D) as the optimal ion source voltage. An example extracted ion chromatogram for the five common sugars, is shown in Fig. 4A.

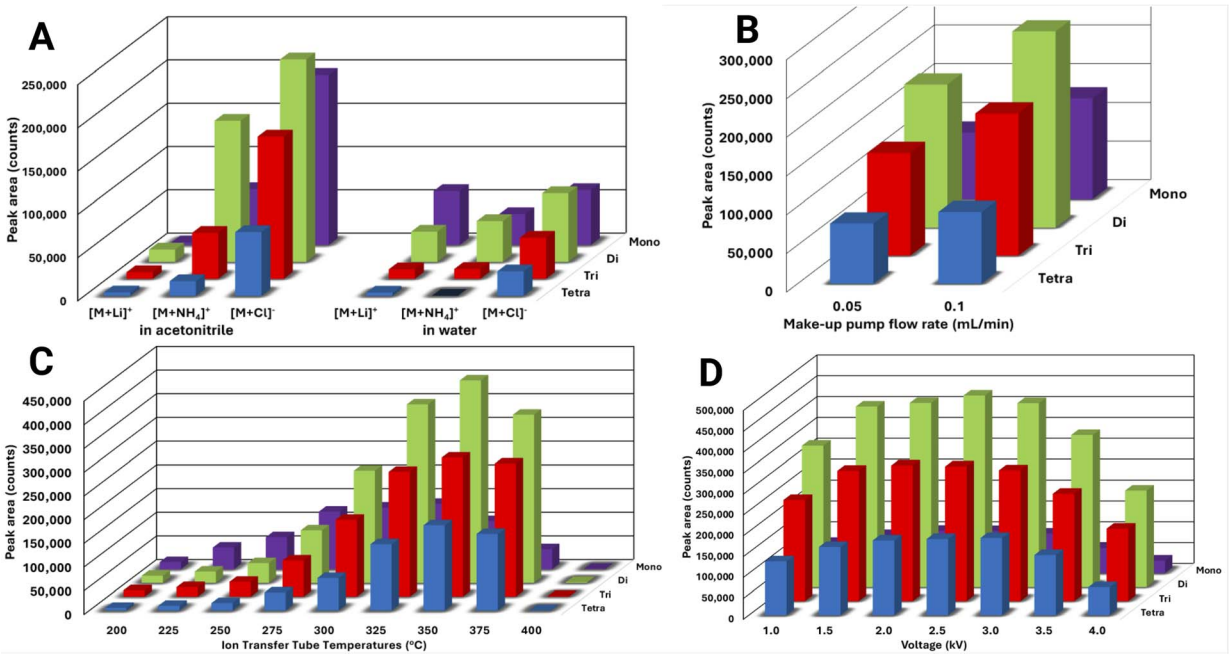

Fig. 3 MS method development parameters. (A) Peak sizes of saccharide adducts with regards to $[M + Li]^+$ from lithium chloride, as well as both $[M + NH_4]^+$ and $[M + {}^{35}Cl]^-$ arising from ammonium chloride, in both water and acetonitrile make-up phase solutions. (B) Peak areas of $[M + {}^{35}Cl]^-$ saccharide adducts with regards to make-up pump phase flow rate. (C) Peak areas of $[M + {}^{35}Cl]^-$ saccharide adducts with regards to ion transfer tube temperature. (D) Peak areas of $[M + {}^{35}Cl]^-$ saccharide adducts with regards to source voltage.

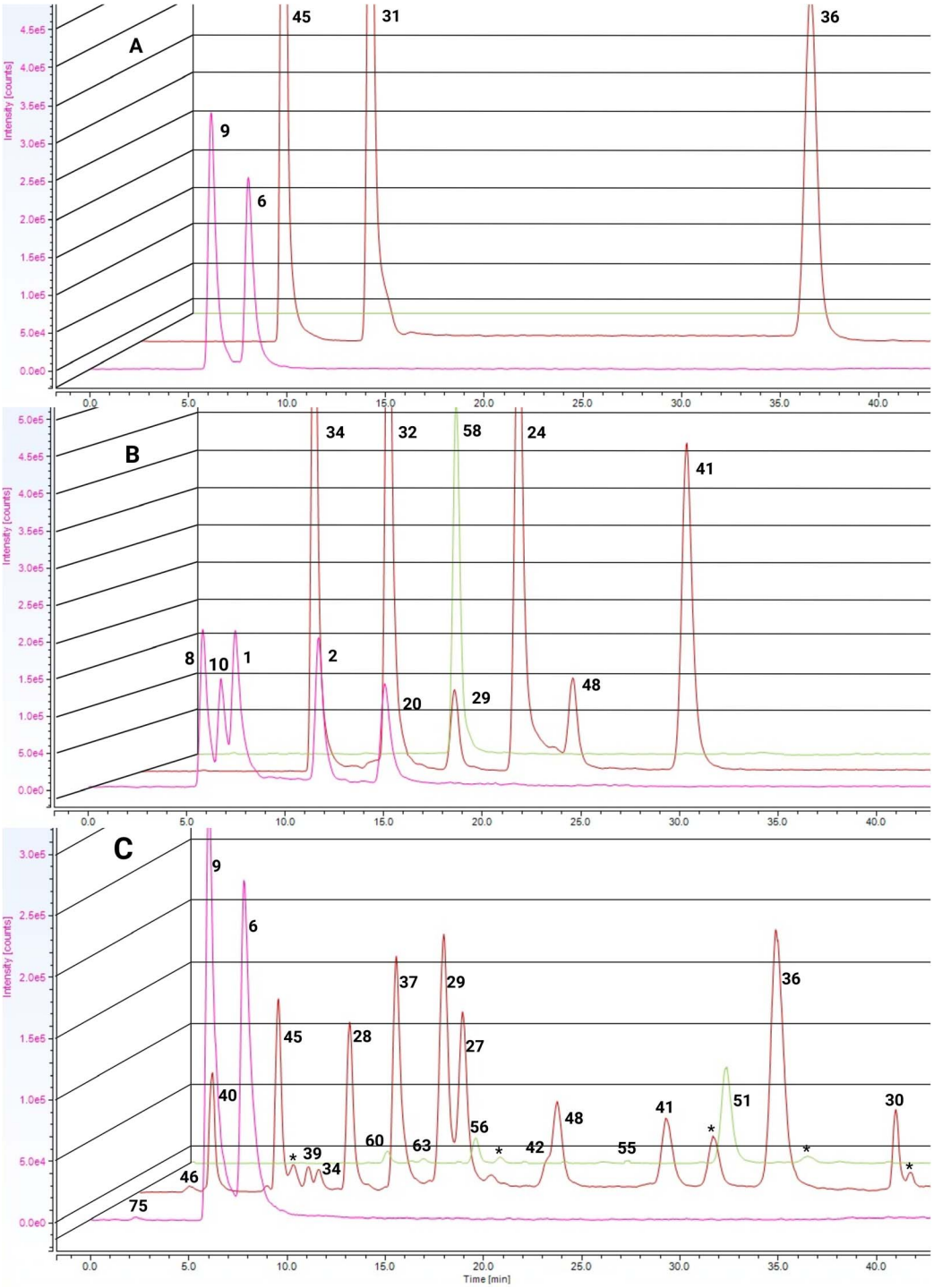

Fig. 4 3D Extracted ion chromatograms from a mixture of standards of monosaccharides-(pink), disaccharides (brown) & trisaccharides (green) in: (A) a standard of 5 common sugars: glucose (9), fructose (6), sucrose (45), lactose (31) and maltose (36). (B) Standard mix 2: galactose (8), gulose (10), allose (1), leucrose (34), altrose (2), lactulose (32), talose (20), maltotriose (58), kojibiose (29), cellobiose (24), turanose (48), nigerose (41). (C) Red gum honey: inositol (75), trehalose (46), glucose (9), neotrehalose (40), fructose (6), sucrose (45), melibiose (39), leucrose (34), isomaltose (28), melezitose (60), maltulose (37), raffinose (63), kojibiose (29), gentibiose (27), 1-kestose (56), palatinose (42), turanose (48), isomaltotriose (55), nigerose (41), erlose (51), maltose (36), inulobiose (30). Unknowns denoted with *. Compound numbering from Tables S1 and S2 in SI (adapted in https://BioRender.com).

3.2 Method validation

The elution order of the individual sugar reference materials was determined using the optimised MS parameters. At this point, co-elution of several sugar alcohols of the same molecular mass was observed. These included the co-elution of xylitol with ribitol, as well as mannitol with galactitol and sorbitol. Specific IC columns for the separation of sugar alcohols exist and can be used as an alternative if selectivity of these co-eluting sugar alcohols is required.[29] Since this study employed mainly aldoses and ketoses, method development used the CarboPac PA210™ column. The remaining 80 sugar reference materials were divided into nine stock solutions each containing sugars at 100 mg L^{-1} (apart from idose). The combinations for each solution were selected in a way that the identity of the respective compounds could not be confused if there was a slight shift in retention times. As an example, the extracted ion chromatograms for standard mix 2 is shown in Fig. 4B. The results of the method validation of the 80 sugar reference materials are shown in Table S1 of the SI.

Chlorine has two stable isotopes, ^{35}Cl and ^{37}Cl (m/z) that results in two adduct peaks, being observed for all analytes in an approximate 3 : 1 ratio. An example of the peaks observed for levanbiose, is shown in Fig. 5. This diagnostic ratio, $[M + {}^{35}Cl]^-$ to $[M + {}^{37}Cl]^-$ can be used for confirmation, without having to check the mass spectrum for each adduct (see Fig. 5).[22] Since sugar alcohols are two m/z heavier than the corresponding sugars, there is a possibility of misidentifying sugar alcohols, if their retention times are close. An example is maltose (m/z 377/379) and maltitol (m/z 379/381), where both maltose and maltitol are also observed when using the (m/z 379) mass channel. Hence, whilst both $[M + {}^{35}Cl]^-$ and $[M + {}^{37}Cl]^-$ can be used for qualitative assessment, only the $[M + {}^{35}Cl]^-$ ion was used for quantitation.

3.3 Comparison with existing methodology

Our optimised and validated method enabled the ready separation and quantitation of 80 sugars within 70 min. As the column used provides excellent analyte selectivity and the analysis uses full scan, any of the other reported 'hundreds of rare sugars' could be observed, assuming no co-elution.[1] This is a significant improvement on previous reported studies.

3.3.1 Other ion chromatography methods. A review of IC-MS methods for saccharide analysis revealed that the greatest number of neutral saccharides quantified in a single study was 18 mono-, 11 di-, two tri-saccharides, and 12 sugar alcohols across two methods using a triple quadrupole mass spectrometer.[30] Multiple studies have also attempted coupling IC to different mass spectrometers to improve the number of saccharides that can be analysed in different sample matrices. Tedesco *et al.*, 2020 used a single quadrupole mass spectrometer to detect seven mono-, eight di-, four tri- and one tetrasaccharide in honey, while Sanz Rodríguez *et al.*, 2022 used a triple quadrupole mass spectrometer to detect 25 xylooligosaccharides of varying DP lengths from wood pulp.[23,31] Use of high-resolution mass spectrometers paired with IC was reported in two papers, with Panseri *et al.*, 2021 using an Orbitrap™ to quantify four saccharides (glucose, lactose, galactose, lactulose) in cheese, and Zhao *et al.*, 2020 using an ion trap time-of-flight (IT-TOF) mass spectrometer to analyse eight monosaccharides in sludge.[15,32]

The LODs of the saccharides determined in the present study range from 10 to 1000 μg L^{-1} (refer to SI Table S1). These results

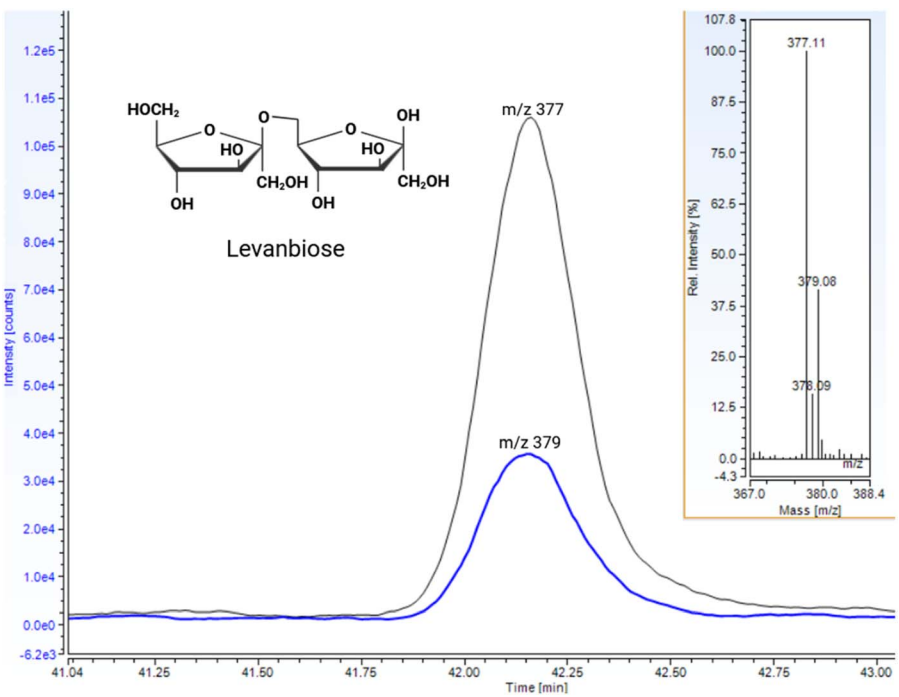

Fig. 5 Example of chlorine-35 $[M + {}^{35}Cl]^-$ and confirmation chlorine-37 $[M + {}^{37}Cl]^-$ extracted ion chromatograms (3 : 1) for the disaccharide levanbiose with mass spectrum inset (adapted in https://BioRender.com).

are similar to Tedesco (5–100 μg L^{-1}). This was found to be better than required, as sugars are typically present in 'percent levels' in foods.[23] Therefore, a method-based limit of detection (MOD) is also reported for the current method in Table S1. These MODs of 0.1 to 1 mg L^{-1} are comparable to the LODs reported by Zhao *et al.*, 2020 (0.37–2.15 mg L^{-1}), Sanz Rodríguez *et al.*, 2022 (0.003–0.3 mg L^{-1}), and Panseri *et al.*, 2021 (1–10 mg L^{-1}) and the MOD reported by Tedesco *et al.*, 2020 (0.05–4 mg L^{-1}).[15,23,31,32] Relative standard deviations in the present study range from 0.47 to 6.87%, which are also within what was reported by Zhao *et al.*, 2020 (0.54–3.76%), Tedesco *et al.*, 2020 (1–29%), Panseri *et al.*, 2021 (6–7%), and Sanz Rodríguez *et al.*, 2022 (2.27–5.27%).[15,23,31,32]

3.3.2 Gas and liquid chromatography methods. As simple sugars are readily soluble in aqueous media, numerous methods have been developed using liquid chromatography (LC). This technique has, however, been described as providing the poorest selectivity for sugars when compared to GC and IC.[21] Hydrophilic interaction liquid chromatography (HILIC) columns are the usual column choice for separation due to the polar nature of sugars. A recent study had quantified nine monosaccharides and three sugar alcohols from five different plant parts using triple quadrupole MS with LOD ranges from 0.02 to 0.25 mg L^{-1} and repeatability of 0.48–1.94%.[17] The greatest number of saccharides previously detected was using LC-MS,[7] although this reported many different types of sugar analytes that were not directly comparable to this study. To aid this comparison only neutral sugars and sugar alcohols from that study are compared. Therefore this includes quantitation of 28 sugars (14 mono-, four di-, four tri-, three tetra-saccharides, and three sugar alcohols) across two methods using a LC triple quadrupole mass spectrometer.[7] An LOD of 0.016 pmol or 3 pg L^{-1} and CV of 7.2% was reported for the monosaccharide analysis method while an LOD of 0.013 mg L^{-1} was reported for the alcohol-soluble carbohydrates.[7]

In comparison with LC methodology, GC-MS offers higher sensitivity for more complex samples but with less repeatability for quantitative analysis.[33] GC-MS has two main drawbacks compared to LC-MS: (a) a mandatory derivatization step due to the non-volatile nature of sugars, (b) oximation-based derivatization generates two chromatographic peaks for every sugar, with the oximes formed at the anomeric carbon (generating *E* or *Z* isomers), with different retention times. Even with derivatisation, GC-MS methods still encounter several co-eluting saccharides.[34] An example of this co-elution in GC-MS methods is a study by Sanz, Sanz and Martinez-Castro, 2004, which reported the separation of 26 di- and tri-saccharides in honey using two methods.[35] Only 11 of the sugars in the study did not exhibit co-elution for either *E* or *Z* isomer. A common solution, is to use multiple columns per sample or multiple temperature programs on the same column, to separate the analytes for elucidation of the coincident sugars.[34,35] The LOQ range for the honey samples in this study was reported to be 0.01 to 2.61 g/100 g of honey.[35]

4 Analysis of samples

4.1 Analysis of commercial products by IC-MS

The suitability and reliability of this IC-MS method was demonstrated using commercial sugar samples sold as food additives or dietary supplements. Using the optimised IC-MS method, analysis revealed that most of the commercial sugar

Table 1 Results of commercially available sugar samples analysed by IC-MS

No.	Claimed component	Molecular weight (g mol^{-1})	Saccharides detected (% as g/100 g)
Sugar alcohols			
1	100% Erythritol	122.12	100% Erythritol
2	100% Xylitol	152.15	100% Xylitol
3	100% Xylitol	152.15	100% Xylitol
Monosaccharides			
4	100% Ribose	150.13	100% Ribose
5	100% Allulose (psicose)	180.16	100% Allulose (psicose)
6	100% Allulose (psicose)	180.16	100% Allulose (psicose)
7	Allulose (psicose)[a]	180.16	100% Allulose (psicose)
9	90% Dextrose (glucose)	180.16	100% Dextrose (glucose)
10	90% Dextrose (glucose)	180.16	100% Dextrose (glucose)
11	100% Dextrose (glucose)	180.16	100% Dextrose (glucose)
12	100% Fructose	180.16	100% Fructose
13	100% Fructose	180.16	100% Fructose
Disaccharides			
14	100% Lactose	342.30	100% Lactose
15	30% Maltose	342.30	30% Maltose, 30% maltotriose 0.3% glucose, 0.1% maltulose
16	100% Palatinose	342.30	99.99% Palatinose, 0.01% trehalulose
17	Palatinose[a]	342.30	99.99% Palatinose, 0.01% trehalulose
18	100% Trehalose	342.30	100% Trehalose
19	90% Trehalose	342.30	100% Trehalose

[a] Purity not claimed.

samples contained the saccharides stated in the ingredients lists, as shown in Table 1. No additional compounds with matching molecular masses but absent from the 80-reference materials, was observed. This suggests that the products' purity was high and production processes were well controlled. This is considered a result of enzymatic conversion processes of creating rare sugars, for example use of fructosyl-transferases, which can generate multiple rare saccharides, not just the preferred target.[36]

Only three sugar samples deviated from their stated compositions. Notably, two of these sugar samples, #16 and #17, which claimed to contain "100% palatinose" and "palatinose" (purity unspecified), respectively, were found to contain detectable amounts of trehalulose (0.01 g/100 g sample). This finding suggests that the manufacturers of these sugar samples may have used immobilised enzymes from a bacterial (*Erwinia rhapontici* or *Protoaminobacter rubrum*) source to convert sucrose to palatinose, a processes known to produce trehalulose as a minor by-product.[37]

Retail sample #15, labelled as containing "30% maltose", was also found to contain 30% maltotriose (30 g/100 g sample), along with detectable amounts of glucose (0.3%), and maltulose (0.1%). This is consistent with the composition of maltose produced *via* enzymatic hydrolysis of starch amylose using α-amylase. It is possible that the small amount of maltulose detected is the result of a Lobry de Bruyn–Alberda van Ekenstein transformation of maltose, which can be triggered by heating the sample during production.[38]

4.2 Honeys

Honey is a complex matrix of carbohydrates with various studies reporting differing compositions. For example, it has been reported that honey can contain up to three mono-, 13 di-, and 11 trisaccharides.[39] In this study, the two honey samples were found to contain two mono-, 12 di-, eight trisaccharides (see Fig. 4C and Table S2 of the SI). Three of the previously reported trisaccharides, theanderose, laminaritriose or isopanose were not covered by the current method, therefore their presence/absence could not be confirmed. In addition to the sugars previously reported, three sugar alcohols, three di- and two tetrasaccharides were also found in the two samples by this method. A small number of di- and trisaccharides that did not match the 80 reference materials available, were also observed (see Fig. 4C).

The proficiency trial sample (covering 14 saccharides) was analysed, and the results compared to those generated by participants in the trial. It was found that for each of the analytes listed, the laboratory would have received a Z score within ±1 (*i.e.* excellent accuracy and method reliability) for every statistically valid analyte.

5 Conclusion

As saccharides have many possible structural isomers, attention must be paid to their analysis by MS, as insufficient separation can lead to erroneous results. As sugar analysis is vital for several industries and sciences, these errors can have flow-on consequences. Given the rising health concerns around the consumption of both detrimental (carcinogenic, intolerance, metabolism) and beneficial (prebiotic, anticaries, low-calorific) sugars, detailed saccharide analysis will have direct health implications.

IC has been stated to have the best separation of saccharides of the three main chromatographic techniques. Supporting this claim, we have demonstrated that the method described here can identify and quantify 80 mono-, di-, tri- and tetrasaccharides as well as a select group of sugar alcohols. The repeatability and LODs of the method were consistent with contemporary methods for these analyte types. As far as the authors are aware, this demonstrates the largest number of saccharides, quantifiable by a single chromatographic method at this time.

The effectiveness of the method was demonstrated through the analysis of a number of single-component sugars, of retail purity. The analytical results confirmed that the samples contained the compounds claimed and no further compounds with the same molecular mass were observed. Two honey samples were analysed and a proficiency sample assessed. Further work is needed to assess the broader applicability of the method, particularly in the fields of glycomics, clinical and forensics analysis, where rigorous validation is required.

Author contributions

H. S. A. Yates: conceptualization, methodology, formal analysis, writing original, writing review and editing. J. F. Carter: supervision, writing review and editing. J. Zhang: writing original. M. T. Fletcher: supervision, writing review and editing. V. S. Santiago: writing original, formal analysis, writing review and editing. N. L. Hungerford: supervision, writing review and editing, formal analysis.

Conflicts of interest

The authors decare no conflict of interest.

Data availability

The method validation data supporting this article have been included as part of the supplementary information (SI). Supplementary information: averaged response for each adduct observed in this study for mass spectrometer optimisation Fig. S1–S3 and method validation parameters Table S1 and Honey data Table S2. See DOI: https://doi.org/10.1039/d5ay01489a.

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