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Ionization of glycans from alkali metal salt-impregnated paper

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ABSTRACT

Ambient ionization of glycans is simply and efficiently achieved by spraying from an alkali metal salt-impregnated paper surface. Monosaccharides, oligosaccharides and ring glycans easily form abundant alkali metal adduct ions, and give simple and clean high-quality mass spectra. The enhancement is specific for glycans, compared to a wide variety of non-glycan compounds present in a matrix. In addition, molecular weight of unknown glycans can be further identified based on the ion mass difference of various alkali metal adduct ions from a certain compound when using a mixed salt-impregnated paper containing five cation salts. Successful determination of glycans and glycoconjugates in plant extracts, honey, blood and urine demonstrates the practicability of this approach to complicated matrices, especially biological matrices.

1. Introduction

Ambient ionization mass spectrometry (AIMS) has been rapidly developed because of its typical advantages such as real-time, rapid as well as high-throughput for analysis of samples with no need of chromatographic separation or sample preparation, and multiple AIMS sources have been proposed [1]. To further improve AIMS performance, different strategies such as chemical derivatization, hyphenating AIMS with ion mobility spectrometry, chemical tagging, etc., have been advocated [2].

Paper spray (PS) ionization [3] is the simplest ionization mode of AIMS and a routine ionization source [4]. As the spray ionization from paper matrix, paper characteristics significantly affect the performance of PS-MS, e.g. selectivity, sensitivity, and ionization efficiency [5–12]. Recently, a Ag⁺ adduction strategy for the distinction of isomers, i.e., THC and CBD, by PS-MS was demonstrated by our group, which provided a compelling example of the idea that metal ion adduction is a powerful method to improve PS-MS performance [13].

For the application of AIMS in the life sciences, the direct ambient analysis of glycans is highly desirable. Focusing on AIMS of glycans, current PS-MS analysis does not have a good specificity, because of the

formation of multi-ions such as [Glycan + Na]⁺, [Glycan + K]⁺, [Glycan + K + O]⁺, [Glycan + K+2O]⁺, etc. [14]. Alternatively, direct analysis in real time (DART)-MS [15] and reactive desorption electrospray ionization (DESI)-MS [16] have also been tested. However, the detectable range of molecular weight for DART and selectivity to glycans for DESI, respectively, were limited [17,18]. Therefore, other AIMS strategies for the analysis of glycans are needed. Based on the adduction phenomenon, the addition of controlled amounts of salts was proposed for DESI-MS analysis of neutral carbohydrates and steroids [19]. Although the detection limits are significantly improved by the addition of low concentrations of salts to the spray solvent, the tolerance toward such salts was limited (max. 10–100 μM concentrations in the spray solution), similar to ESI. In addition, little adduct formation and high ion suppression was observed when salt was present in the analyte and not in the spray solvent in DESI [20].

To further improve PS-MS performance for glycan analysis, in the current work we first introduce a simple alkali metal salt-impregnated paper spray MS (SIPS-MS) approach to the analysis of neutral glycans, including monosaccharides, oligosaccharides, and ring glycans, such as cyclodextrins. After a stepwise introduction of the method, we outline the formation of abundant alkali metal-glycans adduct ions, and

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demonstrate its application for direct detection of glucose and glycoconjugates in plant extracts, honey, blood and urine, even in a complex biological matrix containing a series of non-glycan compounds.

2. Experimental

2.1. Chemicals and materials

Codonopsis pilosula and *Panax ginseng* extracts reference materials, standards of ribose, fructose, glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, rutin and quercetin were purchased from the National Institute of Food and Drug Control (Beijing, China). Neogaroctaose, agarononaose and neogaroodecaose were purchased from Xibao Biotechnology Co. Ltd (Shanghai, China). α -, β - and γ -Cyclodextrin were purchased from Macklin Reagent (Shanghai, China). Cytosine, adenine, cysteine, leucine, *cis*-1,2-cyclohexanediol, *trans*-1,2-cyclohexanediol and tetrahydropyran-2-methanol were purchased from J&K Chemicals. 3-Fucosyllactose and 2'-fucosyllactose were purchased from Glycosci. Honey was purchased from Shanghai Guanshengyuan Bee Products Co. Ltd. Blood and urine samples were collected from the Hunan provincial disease control and prevention center in Changsha. Ethylene glycol and glycerol were purchased from Sinopharm Chemical Reagent Co. Ltd. HPLC-grade methanol was obtained from Tedia Inc. (Fairfield, OH, U.S.A). Other reagents were of analytical grade. Water was prepared by a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA, USA). The structures of all tested compounds in this study were shown in Table S1.

2.2. Instruments and conditions

PS-MS analysis was performed using a Micromass ZQ 2000 mass spectrometer (Manchester, UK). It was equipped with Quadrupole Mass Analyzer, MasslynxTM 4.0 data system software, high voltage power supply and A XYZR moving plate. The positive ion mode was selected to obtain the MS profile. The extractor voltage was 3 V, the RF lens voltage was 0.5 V and the source temperature was 105 °C. The sample volume applied onto the paper was 15 μ L. According to the properties of the different compounds, the mass scan range, cone voltage and spray voltage were set at 100–2000 Da, 20–50 V, 3.0–3.5 kV, respectively.

A XYZR moving plate was made in-house for positioning the paper tip accurately relative to the MS cone. An isosceles triangular piece of chromatography paper was cut out to the dimensions: 10 mm (height) $\times$ 5 mm (base), and was held at a distance of 4–6 mm from the inlet of the mass spectrometer by an XYZR moving plate. The optimized high voltage was supplied directly to the paper triangles by copper clips to induce ionization. 15 μ L of the sample solution was applied to the paper triangle substrate for PS-MS chemical profile analysis.

2.3. Preparation of standard solutions

Single-standard solutions: when the tested compounds were a solid, 0.01 g of standard powder was extracted by 10 mL of methanol in an ice ultrasonic bath for 30 min, except three substances namely α -, β - and γ -cyclodextrin, which were dissolved in 50% methanol/50% water (v/v). When it was a liquid, the compound was dissolved and diluted with methanol. In this manner, we obtained a series of stock standard solutions. Mixed Glycan standard solutions were obtained by accurately measuring out the appropriate amount of ribose, fructose, and maltose to one container and mixing them.

For PS-MS analysis, the concentration of stock standard solutions was 1000 μ g mL⁻¹. Working solutions for PS-MS analysis were diluted with methanol to 5 μ g mL⁻¹. For PS-MS quantitative analysis of glucose in the urine, ribose (5.0 μ g mL⁻¹) was used as the internal standard (I.S.). Stock standard solutions of glucose were diluted with methanol,

and the concentrations for the calibration curve were 10, 20, 50, 100 and 150 μ g mL⁻¹, respectively.

2.4. Preparation of alkali metal salt-impregnated paper substrate

For the preparation of alkali metal salt-impregnated paper, Na impregnation is given as example - the others are analogous. First, an aqueous solution of 0.01 mol L⁻¹ alkali metal salt was prepared. Then the blank paper was placed into this salt solution and ultrasonication was applied for 50 min at room temperature; then the paper was removed with tweezers and dried in an oven at 100 °C for 12 h, to obtain alkali metal salt-impregnated paper.

2.5. Preparation of samples

Plant extracts: 0.5 g of dried powder (*Codonopsis pilosula* and *Panax ginseng*) were extracted by 10 mL of methanol + water (80:20, v/v) in an acoustic wave bath containing ice-water for 30 min. Blood: 2–3 μ L of frozen whole blood was gently dropped onto blank paper and alkali metal salt-impregnated paper; then the paper was placed in a ventilated place at room temperature for 3 h. Urine: method 1: 2–3 μ L of frozen whole urine were placed onto blank paper and alkali metal salt-impregnated paper, after which the paper was placed in a ventilated place at room temperature for 3 h to evaporate the water in the urine; method 2: The water in urine was removed under a gentle N₂ stream at 40 °C, and the dried residue was re-dissolved in methanol containing an internal standard. The methanol solution was dropped on Na₂SO₄-impregnated paper.

3. Results and discussion

3.1. Monosaccharides and disaccharides alkali metal adduct ions by SIPS-MS

In a typical experiment, a triangular piece of chromatography paper was dipped into an aqueous alkali salt solution (Li₂SO₄, Na₂SO₄, K₂SO₄, Rb₂SO₄, Cs₂SO₄ or a mixture thereof). As shown in Fig. S1, when the concentration of Na₂SO₄ was higher than 0.01 mol L⁻¹, interference peaks with salt cluster signals, i.e. *m/z* 165, 307, 449, 591, 733, and 875, occurred. When the salt concentration was 0.01 mol L⁻¹ or lower, the interference peak of the salt cluster signal almost disappeared. Besides, the salt concentration also affects the MS intensity of metal adduct ions; although the MS intensity of 0.005 mol L⁻¹ Na₂SO₄ was slightly higher than that of 0.01 mol L⁻¹, for K₂SO₄, Rb₂SO₄, and Cs₂SO₄, 0.01 mol L⁻¹ had a higher MS intensity than 0.005 mol L⁻¹ (not given here). Therefore, 0.01 mol L⁻¹ was selected as the concentration for the five alkali salt solutions after comprehensive consideration, which ensured overall less MS background interference as well as a relative high MS intensity. Alkali metal salt-impregnated paper was dried after its preparation (see section 2.4), and placed on a home-made XYZR moving plate for accurately positioning the paper tip relative to the MS cone. Then 15 μ L of sample solution (in methanol) was dropped onto the paper substrate, after which a voltage of +3.5 kV was applied for PS-MS analysis [21].

For monosaccharides and disaccharides we selected ribose, fructose, and maltose as model compounds (5 μ g mL⁻¹ in methanol), and a low-resolution nominal mass MS was used to most directly show proof of principle. The alkali metal adduct ions yielded the only abundant signal on different alkali metal salt-impregnated paper, i.e. [M+Li]⁺, [M+Na]⁺, [M+K]⁺, [M+Rb]⁺, and [M+Cs]⁺, i.e. *m/z*: [ribose + Li]⁺ = 157, [ribose + Na]⁺ = 173, [ribose + K]⁺ = 189, [ribose + Rb]⁺ = 235, [ribose + Cs]⁺ = 283 and [fructose + Li]⁺ = 187, etc. Using blank, untreated paper, there is no [M+H]⁺ signal and only a small [M+Na]⁺ signal, resulting from the naturally occurring, low Na⁺ content in the paper matrix. Compared with the blank paper, the signal-to-noise ratio

(S/N) increased by almost two orders of magnitude. The absolute abundance of alkali metal adduct ions generally follow the order: $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ (see Fig. 1). This suggests that stronger affinities of the alkali metal ions to glycans lead to higher signals. It is coincident with that reported in reference [22].

We also selected flavone glycosides (rutin) and quercetin, a typical polyphenol aglycone, and these were also ionized on an alkali metal salt-impregnated paper. It was found that the ionization of quercetin was very poor under these conditions (see Fig. S2 & Table S2) and almost no alkali metal adduct ions were observed; in sharp contrast, the rutin showed the same good alkali metal adduction and ionization characteristics, confirming the high structure selectivity in the ionization on the salt-impregnated substrate for glycans and glycoconjugates.

3.2. Linear oligosaccharides and cyclic glycans alkali metal adduct ions by SIPS-MS

To investigate the ionization ability of alkali metal salt-impregnated paper to oligosaccharides, we used maltose oligosaccharides (up to maltoheptaose; degree of polymerization (DP) 1–7) and neogaroibiose oligosaccharides with DP 8–10; mass range to 1549) as model compounds on such ‘five salts’-impregnated paper. For the whole series a high abundance of alkali metal adduct ions could be produced. For DP1-DP6, we obtained $[\text{M}+\text{Li}]^+$, $[\text{M}+\text{Na}]^+$, $[\text{M}+\text{K}]^+$, $[\text{M}+\text{Rb}]^+$ and $[\text{M}+\text{Cs}]^+$ (see Fig. S3-1 of taking DP-4 as an example). From DP7 onwards, also $[\text{M}+2\text{Li}]^{2+}$, $[\text{M}+2\text{Na}]^{2+}$, $[\text{M}+2\text{K}]^{2+}$, $[\text{M}+2\text{Rb}]^{2+}$ and $[\text{M}+2\text{Cs}]^{2+}$ were observed (see Fig. S3-2 of taking DP-9 as an example). This formation of multi-charged ions is very useful for the MS analysis of oligo- and polysaccharides which thus easily stay within the optimal detection range of even standard mass spectra.

SIPS-MS analysis of cyclic glycans α -, β - and γ -cyclodextrin (5 $\mu\text{g mL}^{-1}$ in 1/1 methanol/ H_2O) also shows a high abundance of alkali metal adduct ions with some clear trends (see Fig. S4 of taking β -cyclodextrin as an example). First, from the absolute abundance of $[\text{M}+\text{Li}]^+$, $[\text{M}+\text{Na}]^+$, $[\text{M}+\text{K}]^+$, $[\text{M}+\text{Rb}]^+$, $[\text{M}+\text{Cs}]^+$, α -cyclodextrin shows the strongest metal affinity (see Table S2). Second, $[\text{M}+\text{K}]^+$, $[\text{M}+\text{Rb}]^+$ and $[\text{M}+\text{Cs}]^+$ signals from single-metal impregnated paper are often accompanied by $[\text{M}+\text{Na}]^+$ signals, which are produced from low content Na^+ ions always present in the paper matrix. This $[\text{M}+\text{Na}]^+$ signal is especially intense in the spectrum of cesium-impregnated paper, which is more obvious than in linear glycans (see Figs. 1 and 2 the Cs_2SO_4 spectrum). This implies that for these cyclic structures the differences of affinities to various metals are even stronger. Third, when using a 120 V cone voltage on five mixed salt-impregnated paper, collision-induced dissociation (CID) of $[\alpha\text{-cyclodextrin} + \text{met}]^+$ were shown in Fig. 3 α -cyclodextrin continuously eliminated anhydrous

Fig. 2. Typical mass spectra for α -, β -, γ -cyclodextrin on Cs_2SO_4 -impregnated paper. M_α , M_β and M_γ are the relative molecular masses of α -cyclodextrin, β -cyclodextrin and γ -cyclodextrin respectively.

Fig. 3. Paper spray mass spectrum for α -cyclodextrin on five mixed salt-impregnated paper by employing a 120 V cone voltage. M_α is the relative molecular masses of α -cyclodextrin. I, II, III and corresponding to the sequential elimination of an anhydroglucose unit ($\Delta m/z = 162$), two anhydroglucose units ($2\Delta m/z = 324$), three anhydroglucose units ($3\Delta m/z = 486$), and four anhydroglucose units ($4\Delta m/z = 648$), respectively.

Fig. 1. Paper spray typical mass spectra for mixed glycan standard on blank and five kinds of alkali metal salt-impregnated paper (e.g. Li_2SO_4 , Na_2SO_4 , K_2SO_4 , Rb_2SO_4 and Cs_2SO_4 -impregnated paper). M_R , M_F and M_M are the relative molecular masses of ribose, fructose and maltose respectively. Note: the ion intensities (e^3) represent the absolute abundance values of the peak with the highest ion intensity.

glucose units [23] and different metal adducts also displayed analogous fragmentation modes. As a result, we can effectively obtain abundant fragment information at the same time.

3.3. Determination of molecular weight of mixed glycans by SIPS-MS

When analyzing by SIPS-MS a mixed standard solution containing known three glycans (ribose, fructose and maltose) using a 'five-salt'-impregnated paper simultaneously containing equal amounts of Li^+ , Na^+ , K^+ , Rb^+ , and Cs^+ salts in one paper, the mass spectrum displays fifteen clear main peaks, and they are easily divided into three categories (see Fig. 4). Specifically, m/z 157, 173, 189, 235, and 283, corresponds to the $[\text{M} + \text{Li}]^+$, $[\text{M} + \text{Na}]^+$, $[\text{M} + \text{K}]^+$, $[\text{M} + \text{Rb}]^+$ and $[\text{M} + \text{Cs}]^+$ peaks of ribose, respectively; m/z 187, 203, 219, 265, and 313 corresponds to $[\text{M} + \text{Li}]^+$, $[\text{M} + \text{Na}]^+$, $[\text{M} + \text{K}]^+$, $[\text{M} + \text{Rb}]^+$ and $[\text{M} + \text{Cs}]^+$ of fructose, respectively; m/z 349, 365, 381, 427, and 475 corresponds to $[\text{M} + \text{Li}]^+$, $[\text{M} + \text{Na}]^+$, $[\text{M} + \text{K}]^+$, $[\text{M} + \text{Rb}]^+$ and $[\text{M} + \text{Cs}]^+$ of maltose, respectively. The results again reflect the difference in affinity of glycans towards different alkali metal ions, and the molecular weight difference can also help in determining the molecular weight of unknown glycans.

3.4. Investigation of structural affinity domain of glycans towards alkali metal ions

From the analyses of linear and cyclic glycans, it is thus found that the alkali metal ion adduction is highly sensitive towards the detection of glycan structures. In order to investigate the structural basis of the affinity of glycans with alkali metal ions on salt-impregnated paper, we investigated different compounds with *trans*-pinacol, *cis*-pinacol and 2-hydroxymethyl-tetrahydropyran structure domains. As characteristic examples ethylene glycol, glycerol, *cis*-1,2-cyclohexanediol, *trans*-1,2-cyclohexanediol and tetrahydropyran-2-methanol were used for SIPS-MS. The results showed that there was almost no alkali metal adduction to *trans*-pinacol, however, ethylene glycol, glycerol, *cis*-pinacol and 2-hydroxymethyl-tetrahydropyran structure domains displayed a high affinity to alkali metal ions (see Fig. S5 & Table S2).

In addition, such structure-dependent differences were also observed in more complex glycans, like 3-fucosyllactose and 2'-fucosyllactose (see Fig. 5a and b & Table S2), two typical human milk oligosaccharides. Surprisingly, 3-fucosyllactose formed abundant $[\text{M} + \text{Li}]^+$, $[\text{M} + \text{Na}]^+$, $[\text{M} + \text{K}]^+$, $[\text{M} + \text{Rb}]^+$, $[\text{M} + \text{Cs}]^+$ adducts, while 2'-fucosyllactose did not. To investigate the difference, we calculated the affinity of 3-fucosyllactose and 2'-fucosyllactose to Na^+ by B3LYP and wB97XD density

functional theory calculations 6-311 + G(d) basis set with basis set superposition error correction (All computations were performed with the Gaussian16 suite of programs, with the B3LYP and wB97XD functionals as implemented in there; a 6-311 + G (d,p) basis set was used throughout, and complexation energies were corrected using a basis set superposition error, which typically was on the order of 1 kcal/mol. The electrostatic potential map was generated using Gaussview 6). These results were showed in Fig. 5c and d, the electrostatic potential surface of both sugars was calculated and then the Na^+ ion was placed at several positions of high negative potential, after which the [glycan + Na^+] adduct structures were optimized. Independent of the method, [3-fucosyllactose + Na^+] was found to be ~ 7 kcal/mol more stable than [2'-fucosyllactose + Na^+]. Detailed investigation shows this may be due to subtle differences in the Na^+ binding; for example, the three shortest Na...O distances in [3-fucosyllactose + Na^+] average out to 2.258 ± 0.042 Å, while in [2'-fucosyllactose + Na^+] this is 2.339 ± 0.080 Å. This computational result agrees with the experimental finding that 3-fucosyllactose can easily form Na^+ adducts, while 2'-fucosyllactose cannot, and highlights the structural sensitivity of SIPS-MS.

3.5. Application of SIPS-MS to analysis of glycans in complicated matrices

In order to investigate the specificity and anti-interference ability of glycan and glycoconjugate ionization by SIPS-MS, complicated matrices including *Panax ginseng* extracts, *Codonopsis pilosula* extracts, honey, dry blood spots and urine were tested.

3.5.1. Application to plant extracts

Glycans and glycoconjugates are normal natural and bio-active components in plants. *Codonopsis pilosula* is a perennial species of flowering plant in the bellflower family, and an extract was obtained via ultrasonication in methanol/water (80/20). There are six MS peaks visible upon ionization from blank paper: m/z 203, 219, 381, 399, 543, and 705 (see Fig. 6). Only based on these results, it is difficult to ascertain the molecular weight of any component. However, from different salt-impregnated papers, there are 5 strong response peaks corresponding to the $\text{Li}^+ \sim \text{Cs}^+$ adduction ions (see Fig. 6). Therefore, the molecular weight can be quickly and accurately calculated to 180, 342, 360, 504 and 666, respectively which corresponding to glucose (or fructose), maltose (or sucrose), D-mannose, raffinose and stachyose.

Panax ginseng extract contains a variety of ginsenosides. The PS-MS profile of ginsenosides in *Panax ginseng* extract from blank and salt-impregnated papers is given in Fig. S6. From blank paper, the abundance of ginsenosides is very low, and background signals almost submerge the ginsenosides responses. However, the abundance of alkali metal adduction ions significantly increases on the salt-impregnated papers, with a S/N increase by almost 2 orders of magnitude. Depending on the regularities of the mass differences between the various alkali metal ions used, the molecular weight of the ginsenosides can be easily calculated, i.e. 639, 771, 785, 801, 947 and 1079, respectively. These molecular masses are consistent with the values of ginsenosides determined in Refs. [24,25].

3.5.2. Application in honey adulteration

Honey gets its sweetness from the monosaccharides fructose and glucose, and has about the same relative sweetness as sucrose. Since the latter is much cheaper to be obtained, adulteration of honey is nearly always caused by addition of sucrose. Quality control of honey thus involves the monitoring of fructose, glucose, and sucrose. Our SIPS-MS approach can rapidly analyze the sugar in honey samples. A typical mass spectrum of salt-impregnated paper spray MS of sucrose-adulterated honey is given in Fig. 7a. The $[\text{M} + \text{Na}]^+$ of fructose and glucose (m/z 203) is clearly displayed on NaCl-impregnated paper spray MS, and the added sucrose can be observed from the [sucrose + Na^+] peak (m/z

Fig. 4. Paper spray mass spectrum for glycans-mixed standard (ribose, fructose and maltose) on five mixed salt-impregnated paper. Note: Red, blue, and green labelled correspond to the five characteristic peaks (Li^+ , Na^+ , K^+ , Rb^+ , and Cs^+) of ribose, fructose and maltose, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5. Paper spray mass spectra on blank and Li_2SO_4 -impregnated paper for 3-fucosyllactose (a) and 2'-fucosyllactose (b). Structure, electrostatic potential energy surface and possible Na^+ -coordinated conformers of 3-fucosyllactose (c) and 2'-fucosyllactose (d). M_{3-F} and $\text{M}_{2'-F}$ are the relative molecular masses of 3-fucosyllactose (m/z 488) and 2'-fucosyllactose (m/z 488), respectively.

Fig. 6. Paper spray mass spectra for *Codonopsis Pilosula* extracts on blank and five kinds of alkali metal salt-impregnated paper.

365). Unadulterated honey does not display such sucrose-derived peaks in SIPS-MS.

3.5.3. Application in blood and urine samples

The levels of blood sugars, primarily glucose, are important indicators for the diagnosis of diabetes, and are in healthy individuals kept within a narrow range. For salt-impregnated paper spray MS analysis,

Fig. 7. Paper spray mass spectra for honey (a) and dry blood spots (b) on blank and Na_2SO_4 -impregnated paper. $M_{G/F}$, M_S and M_G are the relative molecular masses of glucose/fructose, sucrose and glucose, respectively.

the glucose ionization ($[\text{glucose} + \text{Na}]^+$, m/z 203) is effective although there is high content of protein and salt in samples (see Fig. 7b). In standard MS analyses the existence of multi ion forms such as $[\text{M}+\text{H}]^+$, $[\text{M}+\text{Na}]^+$, $[\text{M}+\text{K}]^+$ etc. is very unfavorable for the accurate quantitative analysis. However, when glycans and glycoconjugates are analyzed by SIPS-MS, a single alkali metal adduction ion (especially on Na impregnated paper) is present, because of high content of salt in the paper matrix, which is very beneficial for accurate quantitative analysis.

To this end, we used Na_2SO_4 -impregnated paper to quantitatively analyze glucose in undiluted urine samples (see method 1 of preparation of urine samples). When directly dropping urine on blank or Na_2SO_4 -impregnated paper to be analyzed, primarily a large number of water cluster ions was observed that have a mass difference of 18 (e.g. m/z 221, 239, 257, 275, et., see Fig. S7a). Therefore, a simple pretreatment was employed, i.e. the water in urine was removed under a gentle N_2 stream at 40°C , and the dried residue was re-dissolved in methanol containing an internal standard (see method 2 of preparation of urine samples), which presented a clean mass characterized peak $[\text{glucose} + \text{Na}]^+$, m/z 203 (see Fig. S7b). The methanol solution was dropped on Na_2SO_4 -impregnated paper, and analyzed by SIPS-MS. Using ribose as internal standard, a calibration curve was constructed

through the abundance ratios of $[\text{glucose} + \text{Na}]^+$ to $[\text{ribose} + \text{Na}]^+$ vs. concentration of glucose. The linearity range was $10\text{--}150\text{ }\mu\text{g mL}^{-1}$ ($R^2 = 0.9995$) (see Fig. S8). The limit of detection (LOD) and limit of quantification (LOQ) were $1.0\text{ }\mu\text{g mL}^{-1}$ and $3.0\text{ }\mu\text{g mL}^{-1}$, respectively; the RSD is 4.2–7.5% and the recovery of glucose was 98.2, 101.4, 105.3% at 10, 50, 120 $\mu\text{g mL}^{-1}$, respectively. These results show SIPS-MS displays both good linearity and reproducibility. This is in stark contrast with paper spray analysis of glucose in urine using blank paper, which is basically impossible because of small signals and highly complicated background signals. Furthermore, limits of detection (LODs, $S/N = 3$) and limits of quantification (LOQs, $S/N = 10$) of target compounds in five different matrices, such as *Panax ginseng* extracts, *Codonopsis pilosula* extracts, honey, dry blood spots and urine by SIPS-MS (using Na_2SO_4 -impregnated paper) and PS-MS (using blank paper) (see Table 1). Compared with the PS-MS of using blank paper, the LODs and LOQs of most target compounds increased almost one orders of magnitude by SIPS-MS of using Na_2SO_4 -impregnated paper. These results indicate the strong potential of SIPS-MS towards the analysis of real biological samples and tradition Chinese medicine extracts.

Table 1

Limits of detection (LODs, $S/N = 3$) and limits of quantification (LOQs, $S/N = 10$) of target compounds in five different matrices, such as *Panax ginseng* extracts, *Codonopsis pilosula* extracts, honey, dry blood spots and urine by SIPS-MS (using Na_2SO_4 -impregnated paper) and PS-MS (using blank paper).

Matrices	Target compounds	SIPS-MS			PS-MS		
		Characteristic m/z values	LODs ($\mu\text{g mL}^{-1}$)	LOQs ($\mu\text{g mL}^{-1}$)	Characteristic m/z values	LODs ($\mu\text{g mL}^{-1}$)	LOQs ($\mu\text{g mL}^{-1}$)
Plant extracts (<i>Panax ginseng</i> extracts)	Rh ₁	662	1.0	3.0	662	10	30
	F ₃	794	2.0	5.0	794	15	45
	^a Rg ₂ /Rg ₃	808	3.0	10.0	ND	ND	ND
	^a Rg ₁ /R _f	824	0.8	2.5	ND	ND	ND
	^a Re/Rd	970	0.5	1.5	970	10	30
	^a Rc/Rb ₂ /Rb ₃	1102	1.0	3.0	1102	10	30
	^a Glucose/	203	0.1	0.5	203	20	55
Plant extracts (<i>Codonopsis pilosula</i> extracts)	Fructose						
	^a Maltose/	365	0.3	1.0	381	15	40
	Sucrose						
	D-mannose	383	1.0	4.0	399	20	60
	Raffinose	527	0.5	1.5	ND	ND	ND
	Stachyose	689	0.8	2.0	ND	ND	ND
	Sucrose	365	0.2	1.0	365	20	50
Honey	Glucose	203	2.5	8.0	203	25	80
Dry blood spots	Glucose	203	1.0	5.0	203	20	60

Note: ^a refers to isomers. ND, not detected by PS-MS method.

4. Conclusions

In summary, the trivial impregnation of filter paper with alkali metal salts allows for the highly sensitive and specific detection of glycans by paper spray MS. The principle behind this salt-impregnated paper spray MS is the efficient formation of [Glycan + Met]⁺ adducts. Since larger oligosaccharides display adducts with multiple charges, this will also allow the analysis of large and complex oligosaccharides. Compared with other ambient MS methods, SIPS-MS is both trivial in its use, extremely low cost and much more sensitive towards glycans analysis. These results were already evident with a low-end mass spectrometer, which demonstrates proof of principle. As a result, this indicates great potential for the detection of trace glycans, especially when combined with ion mobility MS (to distinguish isomers) [26] and multi-stage mass spectrometry.

Credit author statement

Ping Guo: Methodology, Investigation, Writing-Original draft preparation, Ke Min: Data curation, Software, Wei Luo & Si Huang: Funding acquisition, Visualization, Zihui Yang & Ming Ma: Supervision administration, Shubin Liu & Zhengfa Fang: Validation, Bo Chen: Writing-Reviewing and Editing, Conceptualization, Han Zuilhof: Term, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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