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Detection of saccharides by reactive desorption electrospray ionization (DESI) using modified phenylboronic acids

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ABSTRACT

We have reported previously a method for the detection of sugars via *in-situ* derivatization with phenylboronic acid $PhB(OH)_2$ using reactive desorption electrospray ionization (DESI, Chen et al., Chem. Commun. (2006) 597–599). The present study describes an improved method that employs modified phenylboronic acids including 3-nitrophenylboronic acid and *N*-methyl-4-pyridineboronic acid iodide. In contrast to using $PhB(OH)_2$, enhanced sensitivity of using 3-nitrophenylboronic acid was observed due to the stabilization of the resulting boronate ester anion by the electron-withdrawing nitro group and the limit of detections (LODs) for glucose in water using 3-nitrophenylbornic acid and phenylboronic acid were determined to be 0.11 mM and 0.40 mM, respectively. In the case of *N*-methyl-4-pyridineboronic acid iodide, the corresponding LOD is 6.9 μM and the higher sensitivity obtained is attributed to the efficient ionization of both the reactive DESI reagent and reaction product since the precursor acid with a quaternary ammonium group is pre-charged. In this case, additional important features are found: (i) unlike using phenylboronic acid or 3-nitrophenylbornic acid, the experiment, performed in the positive ion mode, is applicable to neutral and acidic saccharide solutions, facilitating the analysis of biological fluids without the need to adjust pH; (ii) simply by changing the spray solvent from water to acetonitrile, the method can be used for direct glucose analyses of both urine and serum samples via online desalting, due to the low solubility of salts of these biofluids in the sprayed organic solvent; (iii) in comparison with other sugar derivatizing reagents such as the Girard's reagent T, the *N*-methyl-4-pyridineboronic acid iodide shows higher reactivity in the reactive DESI; and (iv) the ions of saccharide DESI reaction products undergo extensive ring or glycosidic bond cleavage upon CID, a feature that might be useful in the structure elucidation of saccharides. In addition, a variant sample introduction protocol using pipette tips for saccharide solutions was also demonstrated in the reactive DESI experiments, allowing the analysis of multiple samples of a small volume (e.g., 200 nL) in a short period of time (e.g., five samples in 4 min). The method reported in this study with improved sensitivity and high selectivity along with online desalting and high throughput capability could find useful applications in saccharide analysis in complicated biological samples.

1. Introduction

Sugars are essential biological molecules which play fundamental roles in various fields. First, saccharides, either alone or as constituents of glycoproteins, proteoglycans and glycolipids, are critical to cellular events such as recognition, proliferation and signal transduction [1,2]. Second, saccharides are also key intermediates in bio-fuel processing in which bio-mass is converted into sugars and then into ethanol or methanol [3]. In addition, many body tissues depend on glucose as a primary source of energy. Due to their significant properties, the detection of saccharides is of great importance. Also, because they usually occur in complex biological matrices, the detection method must have high selectivity and sensitivity. A number of different methods have been developed for detecting saccharides, including electrochemical enzyme-based approaches [4,5], fluorescence detection [6,7], potentiometric detection [8]. Mass spectrometry (MS) is also commonly used for carbohydrate analysis due to its inherent sensitivity and capability for providing molecular structure information [9,10]. However, intact saccharides are poor analytes for MS and derivatization is often employed prior to the analysis [2]. For instance, the detection sensitivity was shown to be enhanced considerably via the derivatization using various chemical reagents such as the Girard's reagent T [11–13], 1-phenyl-3-methyl-5-pyrazolone [14], and trimethyl-(*p*-aminophenyl) ammonium derivatives [15].

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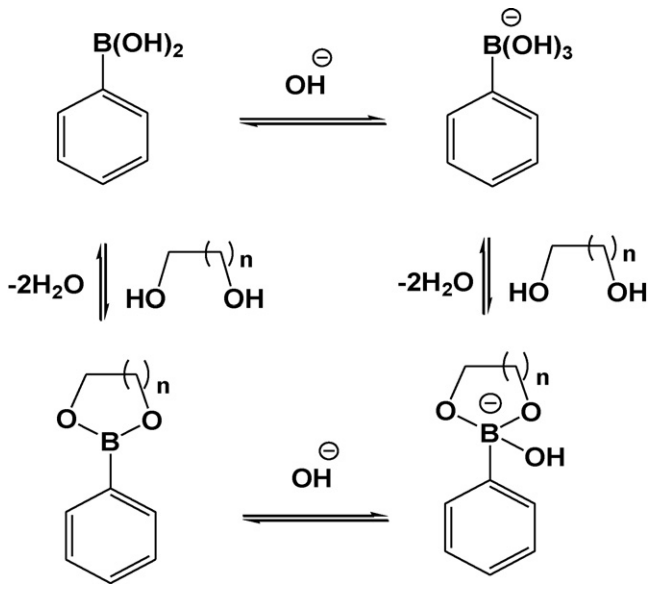
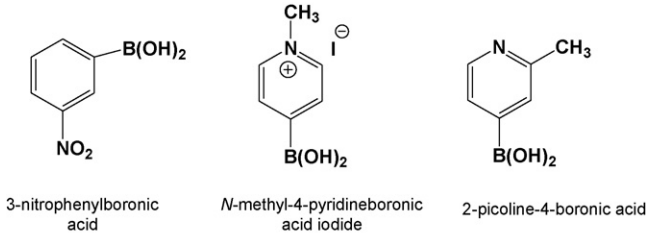


Scheme 1.

Ambient mass spectrometry [16,17] has recently been introduced to provide direct ionization of analytes with little or no sample preparation. Desorption electrospray ionization (DESI) developed by Cooks and co-workers [18] and direct analysis in real time (DART) developed by Cody and et al. [19] were the first two of this new family of technologies. It has been shown that DESI is of great value in the fast analysis of a variety of different analytes ranging from pharmaceuticals to tissue imaging [20–28]. In DESI, ionization occurs via the interaction of charged microdroplets generated in a pneumatically assisted electrospray of an appropriate solvent with solid phase samples on surfaces. In addition to being used regularly for solid sample analysis from surfaces, DESI has been recently extended to allow the direct analysis of liquid samples [29–34]. Reactive DESI [35–40] is a further development in DESI that exploits the potential for coupling specific ion/molecule reactions [41–49] with the ionization event and so greatly improves the selectivity and efficiency with which compounds with specific functionalities are detected. It involves the use of a spray solution that contains specific reagents intended to allow particular ionic reactions during the sampling process. Compared with traditional methods [13] employing solution phase derivatization followed by electrospray ionization (ESI), the online derivation in reactive DESI is much faster (typically taking seconds for one sample analysis), thereby speeding up the analytical process.

It is well known that boronic acids react with *cis*-diol containing compounds in basic aqueous medium through reversible ester formation (Scheme 1 shows the equilibria between phenylboronic acids and diols) [50–52]. Such strong binding allows boronic acids to be used as the recognition moiety in the construction of sensors for saccharides [6,53], as nucleotide and carbohydrate transporters [54], and as affinity ligands for the separation of carbohydrates and glycoproteins [55]. In a previous communication [38], we reported a reactive DESI method for rapid and selective detection of *cis*-diols employing the boronic acid chemistry. It was shown that monosaccharides like glucose and fructose on surfaces can be desorbed and ionized via a heterogeneous ion/molecule reaction with phenylboronate anions $PhB(OH)_3^-$ to form esters under ambient conditions. The experiment is unusual in that the product ions resulting from the selective reaction between phenylboronic acid anions and diol compounds were directly detected by mass spectrometry following the reactive interaction event. However, no quantitative analysis was performed in the previous preliminary



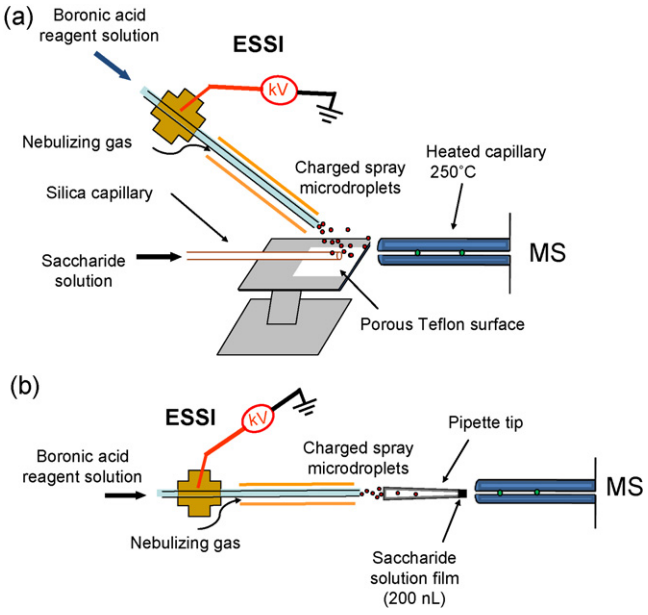
Scheme 2.

study. In the present study, we improved and extended the method, focusing on reactions employing two modified phenylboronic acids, 3-nitrophenylboronic acid and *N*-methyl-4-pyridineboronic acid iodide (structures shown in Scheme 2) in reactive DESI, in an attempt to develop a highly selective and sensitive method for rapid saccharide detection in complicated biological matrices. The purpose of using modified phenylboronic acids instead of using the parent phenylboronic acid [38] is to improve the boronic acid reactivity, to enhance the abundance of the reactant ions (thus the reaction yield), or to enable the reaction to occur under neutral or acidic physiological environments [56]. This study was also driven by our interest in the fundamental ion chemistry at atmospheric pressure and in mechanistic studies of DESI experiments.

2. Material and methods

Phenylboronic acid was purchased from Fluka (Ronkonkoma, NY), 3-nitrophenylboronic acid, glucose, fructose, galactose, *N*-acetyl-D-glucosamine, maltose, cellobiose, maltoheptaose and human serum (type AB male, from clotted male whole blood) were purchased from Sigma–Aldrich (St. Louis, MO). *N*-methyl-4-pyridineboronic acid was purchased from Wako Pure Chemical Industries, Ltd. (Japan). All solvents used were of HPLC grade purity.

The apparatus (shown in Scheme 3a) designed for liquid sample DESI [33] was first employed for the reactive DESI, in which an electrosonic spray ionization (ESSI) [57] source was used to generate charged microdroplets containing the modified phenylboronic acid reagent and analyte samples of saccharide solutions



Scheme 3.

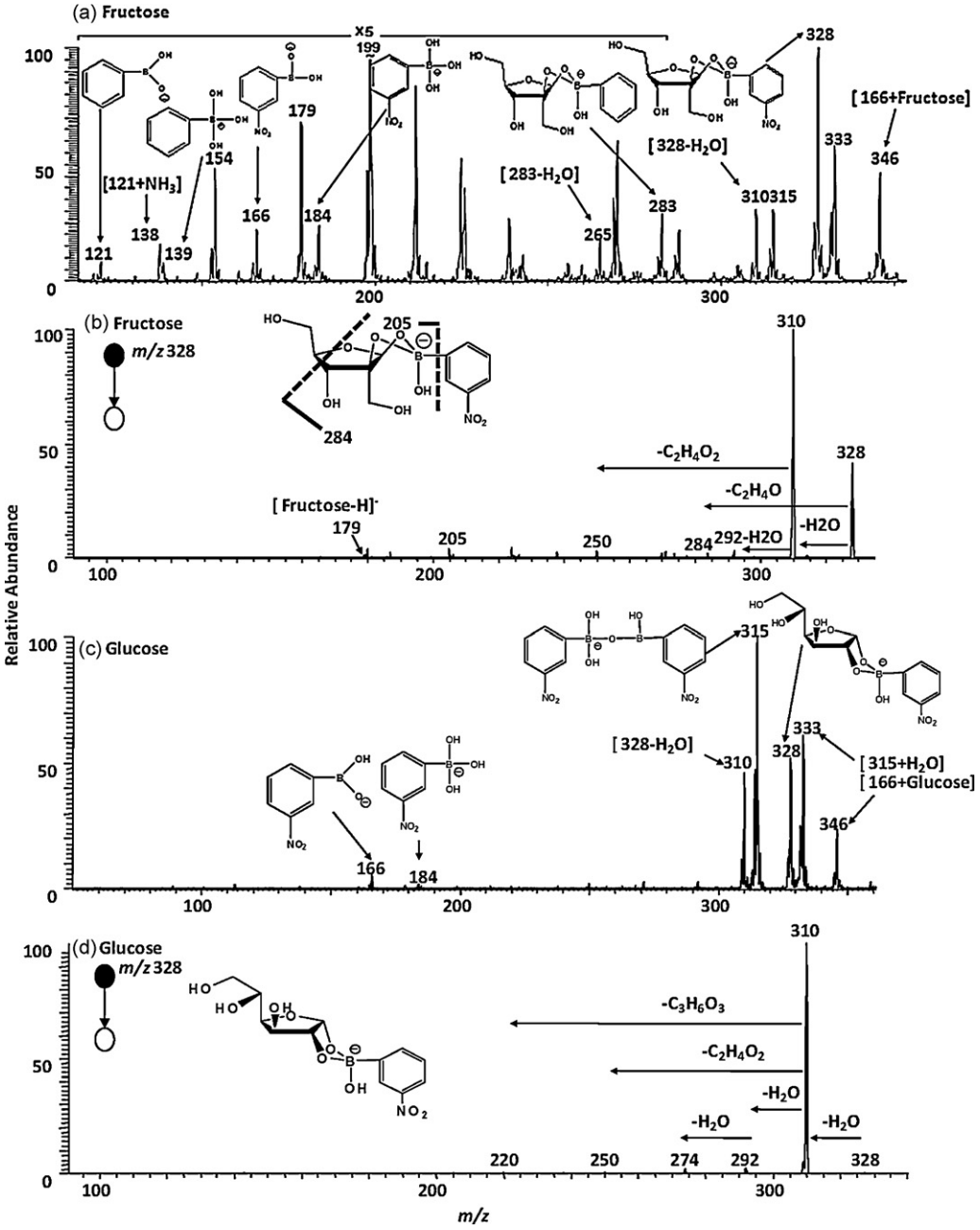
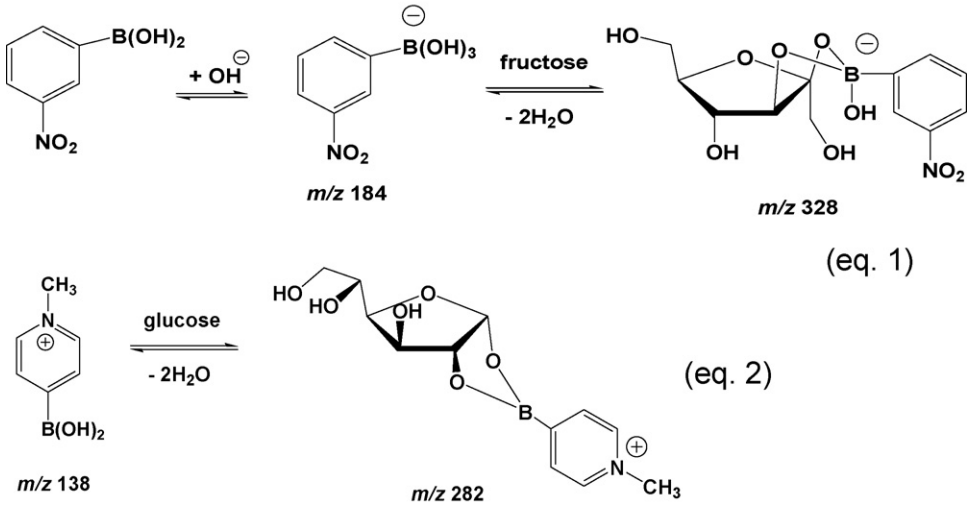


Fig. 1. (a) Reactive DESI mass spectrum showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of a mixture of phenylboronic acid and 3-nitrophenylboronic acid (0.2 mM each in H_2O with pH 9 adjusted by NH_4OH) with fructose (10 mM in aqueous solution); (b) CID MS^2 spectrum of the ion of the fructose DESI reaction product (m/z 328); (c) reactive DESI mass spectrum showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of 3-nitrophenylboronic acid (0.2 mM in water with pH 9 adjusted by NH_4OH) with glucose (5 mM in aqueous solution); (d) CID MS^2 spectrum of the ion of the glucose DESI reaction product (m/z 328).

were introduced via a fused-silica capillary by flow injection onto the DESI surface for desorption and ionization (i.e., the saccharides in solution underwent homogenous phase reactions with the modified phenylboronic acid introduced in charged microdroplets generated by ESSI). The spray voltage was set at +5 kV for the positive ion mode and −5 kV for the negative ion mode. The optimized heated transfer capillary tube temperatures was 250 °C. The nebulizing gas (N_2) pressure for ESSI was 170 psi. The ESSI spray solvent used for 3-nitrophenylboronic acid was water (pH was adjusted by adding NH_4OH) and the solvent for *N*-methyl-4-pyridineboronic acid iodide was either water or acetonitrile. ESSI spray solutions were injected at a flow rate of 3–5 μL/min by a syringe pump. Saccharide samples were dissolved in water, urine or human serum and then introduced onto a Teflon surface at a flow rate of 1–5 μL/min by a syringe pump for DESI analysis. In the case of serum samples, in order to prevent the mass spectrometer from being blocked by the desorbed serum sample, a piece of filter paper (not shown in Scheme 3a) was used to cover the inlet of the sample transfer silica capillary so that the serum can be filtered prior to be desorbed and ionized by DESI. The derivatized sugar product ions were collected and detected using either a Thermo Finnigan LCQ DECA Mass Spectrometer (San Jose, CA) or a Thermo Finnigan LCQ DECA MAX Mass Spectrometer (San Jose, CA). The limit of detections (LODs), defined as three times of standard deviations of the blanks, were calculated using the calibration curve slopes and the standard deviations of the blanks. Collision-induced dissocia-



Scheme 4.

tion (CID) was also used for further structural confirmation of the product ions.

In an alternative apparatus (shown in Scheme 3b), a small volume of saccharide solutions (200 nL) was introduced as a thin layer of liquid film contained in a pipette tip (VWR pipette tips, 1.5 cm long), which was analyzed directly without air drying. The spray-generated microdroplets containing the chemical reagent of *N*-methyl-4-pyridineboronic acid iodide were allowed to pass through the saccharide liquid film for ionization. The angle between the electrospray tip and saccharide sample and the angle between the sample and capillary inlet to the mass spectrometer were both set to 0°. This DESI geometry is similar to the transmission mode of DESI reported by Brodbelt's group [32,34]. Furthermore, the distances between the ESSI source and the pipette tip and the distance between the pipette tip and the heated capillary inlet were approximately 2 mm, and the DESI spray solution was injected at a flow rate of 5 μL/min.

3. Results and discussion

3.1. Phenylboronic acids with electron-withdrawing groups (e.g., 3-nitrophenylboronic acid)

In this experiment, 3-nitrophenylboronic acid was the first modified phenylboronic acid chosen for the reactive DESI study. We compared the reactivity of parent phenylboronic acid and 3-nitrophenylboronic acid in reactive DESI. Fig. 1a displays the reactive DESI mass spectrum showing interaction of the sprayed charge droplets containing both phenylboronic acid and 3-nitrophenylboronic acid (0.2 mM each in H_2O with pH 9 adjusted using NH_4OH) with fructose (10 mM in aqueous solution). The ion of the DESI reaction product (*m*/*z* 328) resulting from the reaction of 3-nitrophenylbornonate anion (*m*/*z* 184) and fructose by loss of two molecules of water (Eq. (1), Scheme 4) is observed as the base peak in the spectrum. As shown in its CID spectrum (Fig. 1b), water loss (the formation of *m*/*z* 310), sugar ring cleavage (the formation of *m*/*z* 284 and 250) and loss of nitrobenzene (the formation of *m*/*z* 205) were seen, confirming the product ion structure. Similarly, in Fig. 1a, the ion of the DESI reaction product (*m*/*z* 283) from the unmodified phenylboronic acid is generated via loss of two water molecules from the complexation of phenylboronate $PhB(OH)_3^-$ (*m*/*z* 139) and fructose. Thus, by comparing the abundance of two major product ions (*m*/*z* 328 vs. *m*/*z* 283 is about 16:1), it can be clearly seen that 3-nitrophenylboronic acid is much more reactive than phenylboronic acid, in the competitive reaction with fructose. This comparison is further confirmed by running the reactive DESI experiments using the two DESI reagents separately under the same experimental conditions and the intensity of the product ion from 3-nitrophenylboronic acid is about 15 times higher than that from phenylboronic acid (see spectra shown in Fig. 1-S, Supporting information). This higher reactivity of 3-nitrophenylboronic acid than phenylboronic acid is because the introduction of an electron-withdrawing nitro group into the ring of a phenylboronic acid stabilizes the boronate form of the acid, favoring the boronate ester formation [58] (e.g., the binding constants of phenylboronic acid and 3-nitrophenylboronic acid with catechol dye, an aromatic diol compound at a pH 7.5 buffer are 1500 M^{-1} and 6110 M^{-1}, respectively [59]). In Fig. 1a, *m*/*z* 310 and 265 are produced from water loss of the product ions of *m*/*z* 328 and 283, respectively. The deprotonated 3-nitrophenylboronic acid (*m*/*z* 166) and deprotonated phenylboronic acid (*m*/*z* 121) are also observed. The ion of *m*/*z* 346 corresponds to the adduct of deprotonated 3-nitrophenylboronic acid (*m*/*z* 166) and fructose. One major peak at *m*/*z* 315, corresponds to deprotonated 3-nitrophenylboronic anhydride (commercially available 3-nitrophenylboronic acid contains various amount of anhydride) and *m*/*z* 333, a deprotonated dimer ion of 3-nitrophenylboronic acid. Furthermore, the peaks at *m*/*z* 154 ([phenylboronic acid + $O_2^{-\bullet}$]) and *m*/*z* 199 ([3-nitrophenylboronic acid + $O_2^{-\bullet}$]) come from the dissolved oxygen in the aqueous solution, as evidenced by the observation that these peaks disappeared if degassed spray solutions were used.

Reactive DESI using 3-nitrophenylboronic acid as the chemical reagent can be also used to ionize glucose directly from aqueous solutions. Fig. 1c illustrates the mass spectrum showing interaction of the sprayed charged droplets containing 3-nitrophenylboronic acid anions (0.2 mM in water with pH 9 adjusted by NH_4OH) with glucose (5 mM in aqueous solution). In Fig. 1c, the ion of the DESI reaction product, shown at *m*/*z* 328, was generated via loss of two molecules of water from the reaction of 3-nitrophenylboronate (*m*/*z* 184) and glucose. Also, the deprotonated anhydride peak is observed (*m*/*z* 315) and *m*/*z* 346 is the simple adduct of deprotonated 3-nitrophenylboronic acid (*m*/*z* 166) and glucose. The CID spectrum of *m*/*z* 328 resulting from glucose (Fig. 1d) shows the consecutive loss of water (the formation of *m*/*z* 310, 292 and 274) and the further losses of $C_2H_4O_2$ and $C_3H_6O_2$ via ring cleavage from the

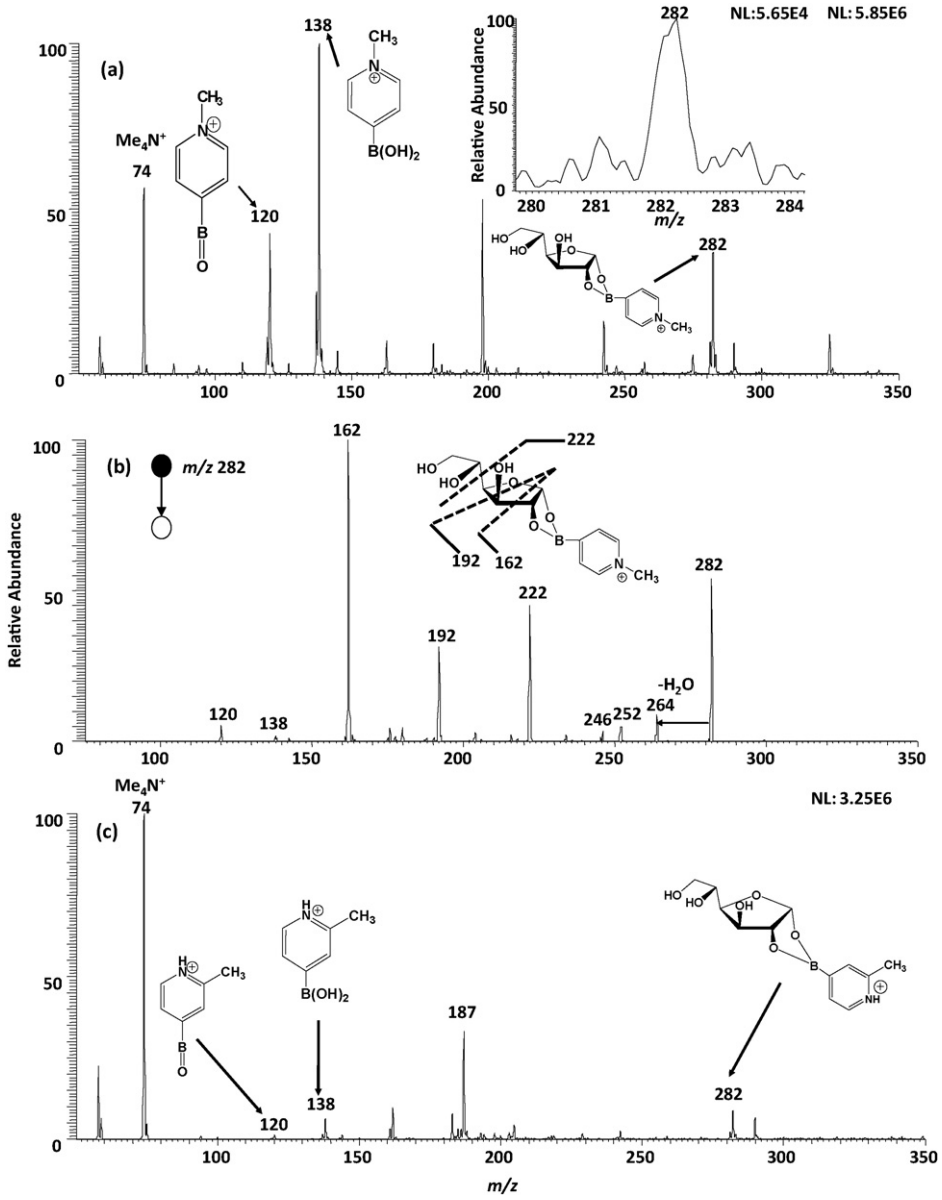


Fig. 2. (a) Reactive DESI mass spectrum showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of aqueous solution containing *N*-methyl-4-pyridineboronic acid iodide (0.2 mM) and tetramethylammonium chloride (0.2 mM, served as an internal standard) with glucose (5 mM in water). The inset shows the detection of the ion of the DESI reaction product (*m*/*z* 282) in the analysis of 10 μM of glucose in water; (b) CID MS^2 spectrum of the ion of the glucose DESI reaction product (*m*/*z* 282); (c) reactive DESI mass spectrum showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of aqueous solution containing 2-picoline-4-boronic acid (0.2 mM) and tetramethylammonium chloride (0.2 mM, severed as an internal standard) with glucose (5 mM in water).

fragment ion *m*/*z* 310 give rise to *m*/*z* 250 and 220, respectively. In this study, the limit of detection (LOD) for glucose in water using 3-nitrophenylboronic acid was determined to be 0.11 mM (S/N = 3, SIM scan mode was used to monitor the ion of *m*/*z* 328), lower than the LOD for glucose in water using phenylboronic acid (0.40 mM, S/N = 3, SIM scan mode was used to monitor the ion of *m*/*z* 283). The linear dynamic ranges observed for 3-nitrophenylboronic acid and phenylboronic acid are 0–2.0 mM (linear regression coefficient $R^2 = 0.9641$) and 0–20.0 mM ($R^2 = 0.9904$), respectively.

3.2. Phenylboronic acids with permanent positive charge tags (e.g., N-methyl-4-pyridineboronic acid iodide)

In addition to using negative reactant ions such as the 3-nitrophenylboronate (*m*/*z* 184) mentioned above, we also employed positive charge-labeled phenylboronic acid ions generated from the ESSI spray of *N*-methyl-4-pyridineboronic acid iodide. The purpose of using permanent charge-labeled compounds is to improve the sensitivity of the method by increasing the ionization efficiency of both the reactive DESI reagents and reaction products. The charge-labeling strategy has been widely used in previous mass spectrometry studies [60–64]; for example, the cationic derivatization of oligosaccharides with Girard's reagent T via hydrazone formation gave significant increase in detection sensitivity as compared with the underivatized oligosaccharides when analyzed by matrix-assisted laser desorption/ionization (MALDI) or ESI mass spectrometry [12,13]; also, betaine aldehyde was recently used as a reactive DESI reagent for rapid and selective analysis of cholesterols in serum and tissues [64]. Fig. 2a shows the ion of the DESI reaction product (*m*/*z* 282) arising from the interaction of charged microdroplets generated by ESSI of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) and tetramethylammonium chloride (0.2 mM, served as an internal standard to measure reactive DESI product ion intensity for reactivity comparison as discussed later

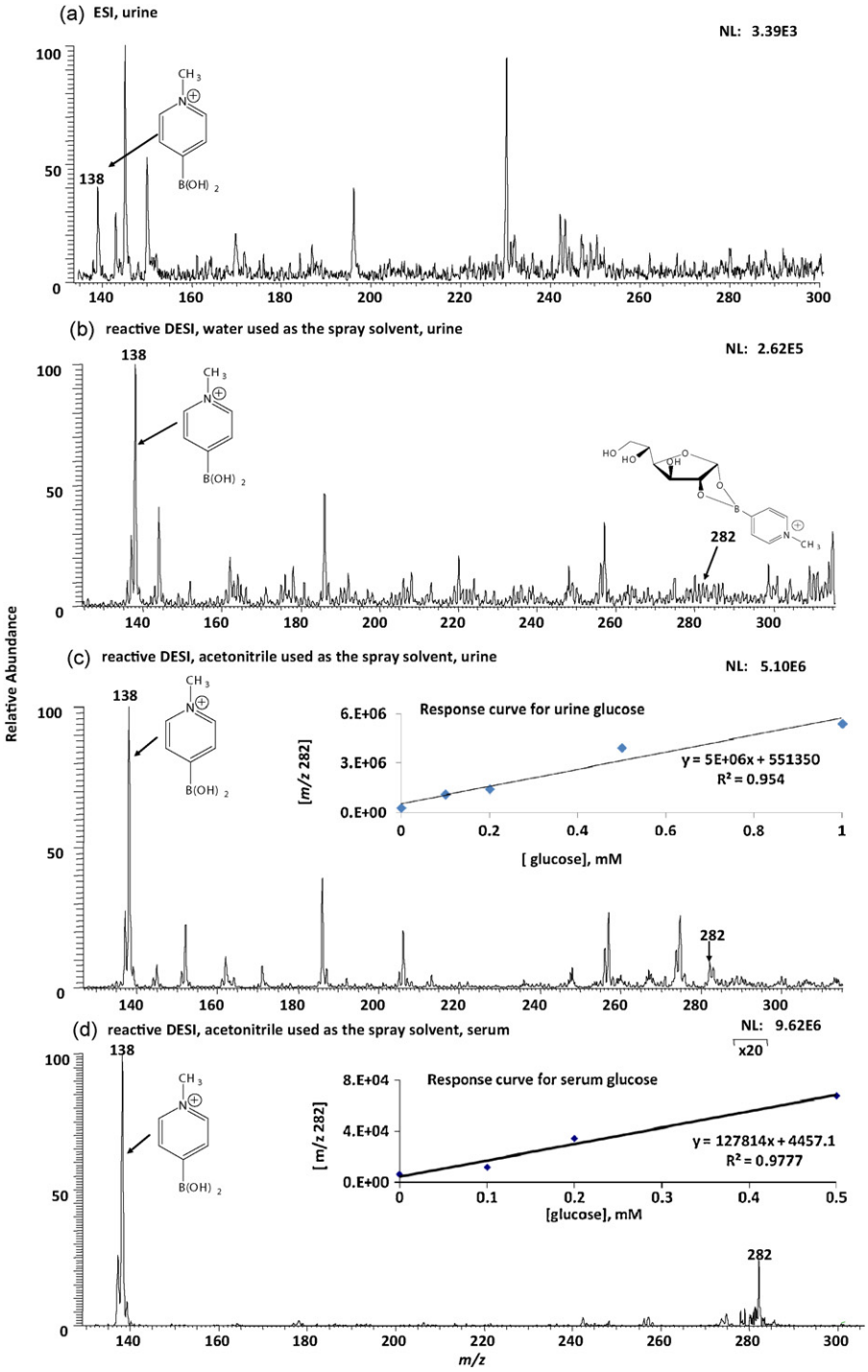


Fig. 3. (a) ESI mass spectrum of a mixed solution of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) with glucose (0.1 mM in urine), and the mixing ratio is 3:1 by volume; (b) reactive DESI mass spectrum showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) with glucose (0.1 mM in urine); reactive DESI mass spectra showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in acetonitrile) with (c) glucose (0.1 mM in urine) and (d) glucose (0.5 mM in human serum). The insets in (c) and (d) show the instrument responses vs. the glucose concentrations in the urine and serum samples, respectively.

Table 1
Results of saccharides examined by reactive DESI results using *N*-methyl-4-pyridineboronic acid iodide as the chemical reagent (0.2 mM in water).

Saccharides	Formula weight (Da)	Ions of DESI reaction product (*m*/*z*)	Fragment ions observed in MS/MS (*m*/*z*)
Glucose	180.16	282	264, 252, 246, 222, 204, 192, 180, 176, 162, 138, 120
Galactose	180.16	282	264, 252, 246, 222, 206, 204, 192, 162, 180, 176, 138, 120
Mannose	180.16	282	264, 252, 222, 204, 192, 162, 120
N-acetyl-D-glucosamine	221.21	323	305, 287, 257, 245, 222, 204, 192,162, 138, 120
Maltose	360.32	444	426, 384, 354, 324, 282
Cellobiose	342.30	444	426, 384, 354, 324, 284, 282
Maltoheptaose	1153.02	1254	1236, 1188, 1092, 1074, 930, 912, 768, 750, 606, 444

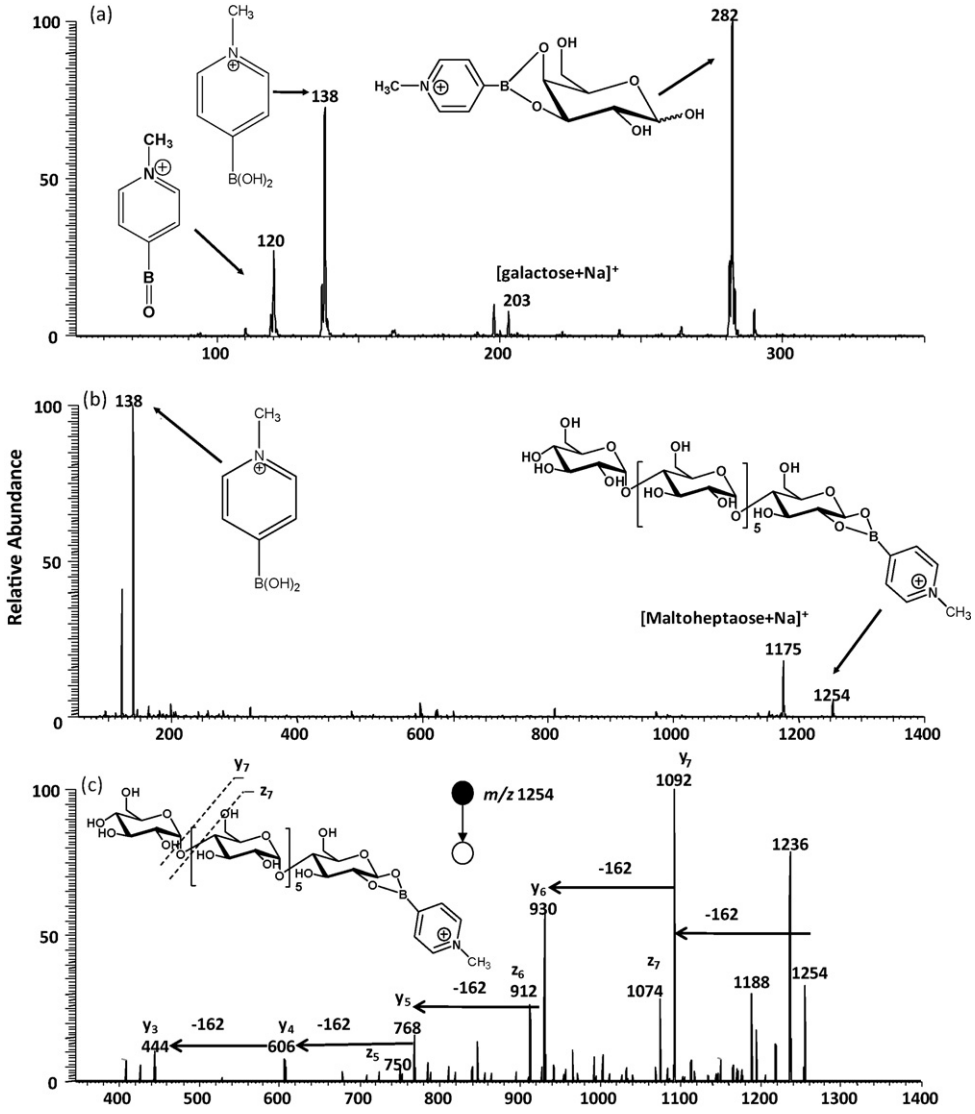


Fig. 4. Reactive DESI mass spectra showing the ionic species produced via the interaction of charged microdroplets generated by ESSI of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) with (a) galactose (5.2 mM in water) and (b) maltoheptaose (5 mM in water); (c) CID MS^2 spectrum of the ion of the DESI reaction product from maltoheptaose (*m*/*z* 1254).

in the text) with glucose (5 mM in H_2O). The reaction equation is shown in Eq. (2), Scheme 4. Upon CID (Fig. 2b), water losses give rise to the fragment ions of *m*/*z* 264 and 246. In addition, the fragment ions of *m*/*z* 252 and 222 arise from the losses of HCHO and $C_2H_4O_2$ and the formation of *m*/*z* 192 and 162 is a result of ring cleavage from *m*/*z* 282 by losses of $C_3H_6O_3$ and $C_4H_8O_4$, respectively, consistent with the proposed ion structure shown in Eq. (2), Scheme 4. In Fig. 2a, the peak of *m*/*z* 120 is produced from the dehydration of the reactant ions of *m*/*z* 138, due to the high temperature of the heated ion transfer capillary of the mass spectrometer used for ion desolvation in the experiment.

The LOD for glucose using *N*-methyl-4-pyridineboronic acid iodide was determined to be 6.9 μM (S/N = 3, SIM scan mode was used to monitor the ion of *m*/*z* 282), which is much lower than that of using phenylboronic acid, probably due to the favorable effect of charge-labeling on the ionization efficiency, as mentioned above. Fig. 2a inset further shows the obtained spectrum for the analysis of 10 μM of glucose in water using the reactive DESI and the linear dynamic range from 0 μM to 500 μM was observed for this method (R^2 = 0.9987). To further confirm this charge-labeling effect, another reactive DESI experiment using 2-picoline-4-boronic acid (Scheme 2), a neutral compound structurally similar to the *N*-methyl-4-pyridineboronic acid iodide, was also carried out. Fig. 2c shows the ionic species produced via the interaction of charged microdroplets generated by ESSI of containing 2-picoline-4-boronic acid (0.2 mM) and tetramethylammonium chloride (0.2 mM, severed as an internal standard) with glucose (5 mM in water). It has been shown that the ratio of the intensity of the ion of the DESI reaction product (*m*/*z* 282) in the case of *N*-methyl-4-pyridineboronic acid iodide (Fig. 2a) to the intensity of the internal standard ion, Me_4N^+ (*m*/*z* 74), is 0.65, which is much higher than the corresponding ratio of 0.1 in the case of 2-picoline-4-boronic acid (Fig. 2c), in agreement with the hypothesis that the sensitivity enhancement for *N*-methyl-4-pyridineboronic acid iodide does stem from the charge-labeling effect.

We further compared the reactivity of the *N*-methyl-4-pyridineboronic acid iodide with other cationic sugar derivatizing reagents like the Girard's reagent T in this reactive DESI experiment. When an aqueous solution containing both *N*-methyl-4-pyridineboronic acid iodide (0.2 mM) and Girard's reagent T (0.2 mM) was sprayed and the glucose (5 mM in water) was chosen

as sample, it was found that the intensity of the ion of m/z 282 from *N*-methyl-4-pyridineboronic acid iodide is ca. 12 times higher than that of the ion of the DESI reaction product (m/z 294) from Girard's reagent T (see spectrum in Fig. 2-S, Supporting information), suggesting that the former reagent has higher reactivity in the reactive DESI experiments. This result also indicates that the formation of boronic acid ester is easier than the formation of hydrozone for saccharides in the DESI conditions.

Unlike phenylboronic acid or 3-nitrophenylboronic acid, *N*-methyl-4-pyridineboronic acid iodide can be used to detect glucose in acidic conditions. It has been found that the ion of the DESI reaction product (m/z 282) was readily observed even when the glucose solution was acidified by acetic acid to pH 2. This feature of reactive DESI will facilitate the direct sugar detection in both neutral and acidic physiological conditions.

To test the analytical utility of the reactive DESI using *N*-methyl-4-pyridineboronic acid iodide as reagent, a urine sample (pH 6) was chosen to be examined. Urine glucose monitoring is a potentially valuable noninvasive way for clinical diagnosis of diseases such as renal defects or diabetes [65,66]. First we simply tested direct electrospray ionization of glucose-containing urine (0.1 mM glucose) mixed with *N*-methyl-4-pyridineboronic acid iodide (0.2 mM) aqueous solution (the mixing volume ratio of 1:3). As shown in the ESI mass spectrum (Fig. 3a), we failed to detect the product signal at m/z 282 and there is a high noise background in the spectrum. This is probably because of the well-known suppression of ion signals in electrospray ionization in the presence of abundant salts from urine. When reactive DESI was performed to examine the urine sample containing 0.1 mM of glucose by spraying *N*-methyl-4-pyridineboronic acid iodide in water (the injection flow rates of urine sample and spray solution are 1 μL/min and 3 μL/min, respectively, analogous to the mixing of two individual solutions in the ESI experiment mentioned above; the concentration of 0.1 mM was selected for testing because it is lower than the LOD of 1.3 mM for glucose oxidase-based sensors [67] and the LOD of 2 mM for dipsticks [68] used for urine glucose monitoring), the ion of m/z 282 is detected and the detection was confirmed with CID of the mass-selected m/z 282 that shows the correct fragmentation pattern as described above (c.f. Fig. 2b), emphasizing the known salt tolerance of DESI [33,69]. This is probably because DESI ionization does not involve in the direct spray of salt-containing urine, thus decreasing the complexity caused by salts. However, the signal of m/z 282 is weak (2.5×10^4 accounts, Fig. 3b) and background noise level is still high, probably because the merged secondary charged droplet still contains salts as the spray solution and urine are completely miscible. Interestingly, when the spray solvent of water was replaced by acetonitrile, the intensity of m/z 282 was considerably enhanced (with intensity of 7.8×10^5 accounts, Fig. 3c) and much lower level of noise was observed. Again, upon CID, the product ion of m/z 282 undergoes dehydration and ring cleavage (c.f. Fig. 2b), confirming its assignment. It is likely that the pronounced solvent effect is due to the low solubility of urine salts in sprayed organic solvent of acetonitrile. Thus, as the charged acetonitrile microdroplets containing *N*-methyl-4-pyridineboronic acid iodide pick up the sugar in urine on the surface, salts in urine were being excluded so that the resulting secondary charged droplets contain low amounts of salts. Indeed, in a separate experiment, when 900 μL of acetonitrile was added to 300 μL of the saturated sodium chloride solution (in the ratio of 3:1 by volume), we found that there was salt precipitate formed in the mixed solution, in agreement with our hypothesis. The phenomenon of salt exclusion in this reactive DESI experiment (i.e., the online desalting using organic spray solvent for DESI ionization to gain enhanced sensitivity) is analogous to that previously observed in the fused-droplet ESI (FD-ESI) experiments [70] in which ultrasonically nebulized sample aerosols (e.g., peptides and proteins) are ionized via fusion with charged methanol droplets. In our study, samples were injected directly without the nebulization process and the urine analysis did not involve any sample pretreatment processes such as extraction or drying, enabling both online desalting and online derivatization and thus suggesting a novel and fast protocol to the trace analysis of complicated biological fluids with high salt content. It is also found that a linear dynamic range of 0–1 mM of glucose in urine can be achieved ($R^2 = 0.954$, see the calibration curve in the inset of Fig. 3c).

In addition to the urine glucose test, reactive DESI was also performed to examine glucose-containing human serum samples using *N*-methyl-4-pyridineboronic acid iodide as the chemical reagent and acetonitrile as the spray solvent. Fig. 3d shows the mass spectrum obtained from the direct detection of serum containing 0.5 mM of glucose in serum using the reactive DESI and the ion of the DESI reaction product (m/z 282) can be seen clearly. The inset of Fig. 3d further displays the linear dynamic range of 0–0.5 mM ($R^2 = 0.9777$) for the analysis.

3.3. Reactions with other saccharides

Different mono-, di- and oligosaccharides, including galactose, mannose, *N*-acetyl-D-glucosamine, maltose, cellobiose and maltoheptaose, were also tested by the reactive DESI using *N*-methyl-4-pyridineboronic acid iodide as chemical reagent. In all cases, the ions of the DESI reaction products were observed and their MS/MS data are summarized in Table 1. Fig. 4a and b display the reactive DESI mass spectra of the ionic species produced via the interaction of charged microdroplets generated by ESSI of *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) with galactose (5.2 mM in water) and with maltoheptaose (5 mM in water), respectively. In the case of galactose, the ion of the DESI reaction product (m/z 282) appears as the base peak and its high intensity is probably because one favored conformer of galactose in water, galactopyranose, contains *cis*-diol functionality in the ring carbons 3 and 4. Besides the ion of m/z 282, sodium adduct ion of galactose (m/z 203) is also observed as sodium ion is ubiquitous and has high binding affinity toward carbohydrate compounds [71,72]. In the case of oligosaccharides of maltoheptaose, CID MS^2 spectrum of the ion of the DESI reaction product (m/z 1254) shows the consecutive glycosidic cleavages to produce a series of *y* and *z* ions (Fig. 4c), probably involving a charge-remote cleavage mechanism [73] (see the proposed structure and cleavages of the product ion in the inset of Fig. 4c). The formation of a series of *y* and *z* ions could be useful in the structure elucidation of oligosaccharides [74–77].

3.4. High throughput analysis using tip-sampling

One intrinsic advantage of DESI is the capability for high throughput analysis [31,78,79]. In this study of reactive DESI experiments, a variant sample introduction protocol using pipette tips was also demonstrated, allowing analyzing multiple samples of a small volume in a short period of time. As illustrated in Scheme 3b, a small volume (200 nL) of saccharide solutions was introduced as a thin layer of liquid film contained in a pipette tip. The spray-generated microdroplets containing the chemical reagent of *N*-methyl-4-pyridineboronic acid iodide were allowed to pass through the saccharide liquid film for ionization. The angle between the electrospray tip and saccharide sample and the angle between the sample and capillary inlet to the mass spectrometer were both set to 0° (such a linear arrangement is similar to that reported previously in the transmission mode DESI experiments [32], which is helpful to overcome the geometry problem for enhanced DESI sensitivity). Fig. 5 shows the extracted ion current chromatogram of the glucose DESI reaction product (m/z 282) for five glucose samples (200 nL, 5 mM in water) sequentially analyzed by reactive DESI (in the experiment, another pipette with a new sample was man-

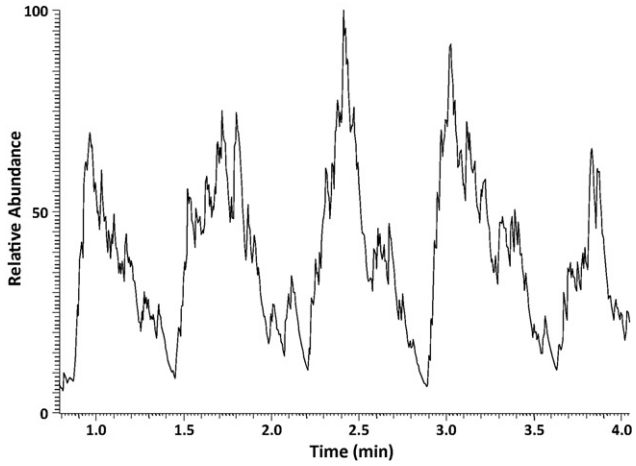


Fig. 5. Extracted ion current chromatogram of the ion of the DESI reaction product (m/z 282) for five glucose samples (200 nL, 5 mM in water) sequentially analyzed by reactive DESI using *N*-methyl-4-pyridineboronic acid iodide (0.2 mM in water) as the chemical reagent.

ually placed in between the EESI source and MS inlet for sample replacement once the analysis of last sample was done). As each sample was ionized, the ion signal of m/z 282 arose and it took 4 min to complete the analysis of the five glucose samples. This demonstration on the ionization of small volume of samples in high throughput manner might be of value in dealing with the analysis of precious biological samples in small volumes.

4. Conclusions

In summary, in comparison to the prototype reactive DESI of using phenylboronic acid, the employment of its derivatives with electron-withdrawing and especially permanently charged groups significantly enhances the sensitivity of the method and expands the application scope to acidic solution samples. The secondary ionization nature of DESI allows one to have freedom to select favorable spray solvents and chemical reagents, which allows both online desalting and online derivatization for analysis of high salt-containing biofluid samples. Given the high selectivity, fast analysis speed, salt tolerance and high throughput capability of the reactive DESI as well as the significance of saccharides, this method reported would be valuable in saccharide analysis in many complex biological samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2009.09.015.

References

[1] A. Helenius, M. Aebi, Science 291 (2001) 2364.
[2] F.N. Lamari, R. Kuhn, N.K. Karamanos, J. Chromatogr. B 793 (2003) 15–36.
[3] S. Vaidyanathan, G. Macaloney, J. Vaughan, B. McNeil, L.M. Harvey, Crit. Rev. Biotechnol. 19 (1999) 277–316.
[4] L. Clark, C. Lyons, Ann. N. Y. Aca. Sci. 102 (1962) 29.
[5] L. Clark, C. Lyons, Diabetes 44 (1995) 1005–1009.
[6] T.D. James, K.R.A.S. Sandanayake, S. Shinkai, Nature 374 (1995) 345–347.
[7] R. Ozawa, T. Hashimoto, A. Yamauchi, I. Suzuki, B.D. Smith, T. Hayashita, Anal. Sci. 24 (2008) 207–212.
[8] E. Shoji, M.S. Freund, J. Am. Chem. Soc. 124 (2002) 12486–12493.
[9] J. Zaia, Mass Spectrom. Rev. 23 (2004) 161–227.
[10] D.J. Harvey, Mass Spectrom. Rev. 25 (2006) 595–662.
[11] C.C. Lai, C.H. Tsai, F.J. Tsai, C.C. Lee, W.D. Lin, Rapid Commun. Mass Spectrom. 15 (2001) 2145–2151.
[12] H. Hong, Y. Wang, Anal. Chem. 79 (2007) 322–326.
[13] T.J.P. Naven, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 829–834.
[14] S. Suzuki, K. Kakehi, S. Honda, Anal. Chem 68 (1996) 2073–2083.
[15] M. Okamoto, T.K.-I.T. Doi, Rapid Commun. Mass Spectrom. 9 (1995) 641–643.
[16] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Science 311 (2006) 1566–1570.
[17] A. Venter, M. Nefliu, R.G. Cooks, Trends Anal. Chem. 27 (2008) 284–290.
[18] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Science 306 (2004) 471–473.
[19] R.B. Cody, J.A. Laramee, H.D. Durst, Anal. Chem. 77 (2005) 2297–2302.
[20] J.M. Wiseman, D.R. Ifa, Q. Song, R.G. Cooks, Angew. Chem. Int. Ed. 45 (2006) 7188–7192.
[21] Y.S. Shin, B. Drolet, R. Mayer, K. Dolence, F. Basile, Anal. Chem 79 (2007) 3514–3518.
[22] M.S. Bereman, T.I. Williams, D.C. Muddiman, Anal. Chem. 79 (2007) 8812–8815.
[23] G.J. Van Berkel, M.J. Ford, M.A. Deibel, Anal. Chem. 77 (2005) 1207–1215.
[24] S. Yang, J. Han, Y. Huan, Y. Cui, X. Zhang, H. Chen, H. Gu, Anal. Chem. 81 (2009) 6070–6079.
[25] A. Lane, L. Nyadong, A.S. Galhena, T.L. Shearer, E.P. Stout, R.M. Parry, M. Kwasnik, M.D. Wang, M.E. Hay, F.M. Fernandez, J. Kubanek, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 7314–7319.
[26] J. Denes, M. Katona, A. Hosszu, N. Czuczy, Z. Takats, Anal. Chem. 81 (2009) 1669–1675.
[27] M.A. Meetani, Y.-S. Shin, S. Zhang, R. Mayer, F. Basile, J. Mass Spectrom. 42 (2007) 1186–1193.
[28] M. Haapala, J. Pol, V. Saarela, V. Arvola, T. Kotiaho, R.A. Ketola, S. Franssila, T.J. Kauppila, R. Kostiainen, Anal. Chem. 79 (2007) 7867–7872.
[29] C.C. Mulligan, D.K. MacMillan, R.J. Noll, R.G. Cooks, Rapid Commun. Mass Spectrom. 21 (2007) 3729–3736.
[30] Z. Miao, H. Chen, Proceedings of the 56th Annual American Society for Mass Spectrometry Conference on Mass Spectrometry, Denver, CO, June 1–5, 2008.
[31] X. Ma, M. Zhao, Z. Lin, S. Zhang, C. Yang, X. Zhang, Anal. Chem. 80 (2008) 6131–6136.
[32] J.E. Chipuk, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 19 (2008) 1612–1620.
[33] Z. Miao, H. Chen, J. Am. Soc. Mass Spectrom. 20 (2009) 10–19.
[34] J.E. Chipuk, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 20 (2009) 584–592.
[35] H. Chen, N.N. Talaty, Z. Takats, R.G. Cooks, Anal. Chem. 77 (2005) 6915–6927.
[36] I. Cotte-Rodriguez, H. Chen, R.G. Cooks, Chem. Commun. (2006) 953–955.
[37] I. Cotte-Rodriguez, H. Hernandez-Soto, H. Chen, R.G. Cooks, Anal. Chem. 80 (2008) 1512–1519.
[38] H. Chen, I. Cotte-Rodriguez, R.G. Cooks, Chem. Commun. (2006) 597–599.
[39] G. Huang, H. Chen, X. Zhang, R.G. Cooks, Z. Ouyang, Anal. Chem. 79 (2007) 8327–8332.
[40] L. Nyadong, M.D. Green, V.R. De Jesus, P.N. Newton, F.M. Fernandez, Anal. Chem. 79 (2007) 2150–2157.
[41] R.A.J. O'Hair, Chem. Commun. (2006) 1469–1481.
[42] D. Schroder, H. Schwarz, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 18114–18119.
[43] N.M.M. Nibbering, Acc. Chem. Res. 23 (1990) 279–285.
[44] S. Gronert, Chem. Rev. 101 (2001) 329–360.
[45] R.G. Cooks, H. Chen, M.N. Eberlin, X. Zheng, W.A. Tao, Chem. Rev. 106 (2006) 188–211.
[46] M.T. Bowers, A.G. Marshall, F.W. McLafferty, J. Phys. Chem. 100 (1996) 12897–12910.
[47] C.H. DePuy, J.J. Grabowski, V.M. Bierbaum, Science 218 (1982) 955–960.
[48] H.I. Kenttamaa, R.G. Cooks, J. Am. Chem. Soc. 111 (1989) 4122–4123.
[49] M.N. Eberlin, Int. J. Mass Spectrom. 235 (2004) 263–278.
[50] H.G. Kuivila, A.H. Keough, E.J. Soboczenski, J. Org. Chem. 19 (1954) 780.
[51] J.P. Lorand, J.O. Edwards, J. Org. Chem. 24 (1959) 769.
[52] G. Springsteen, B. Wang, Tetrahedron 58 (2002) 5291–5300.
[53] H. Eggert, J. Frederiksen, C. Morin, J.C. Norrid, J. Org. Chem. 64 (1999) 3846–3852.
[54] P.R. Westmark, S.J. Gardiner, B.D. Smith, J. Am. Chem. Soc. 118 (1996) 11093–11100.
[55] V. Adamek, X.-C. Liu, Y.A. Zhang, K. Adamkova, W.H. Scouten, J. Chromatogr. 625 (1992) 91–99.
[56] S. Boduroglu, J.M.E. Khoury, D.V. Reddy, P.L. Rinaldi, J. Hu, Bioorg. Med. Chem. Lett. 15 (2005) 3974–3977.
[57] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, Anal. Chem. 76 (2004) 4050–4058.
[58] H.R. Mulla, N.J. Agard, A. Basu, Bioorg. Med. Chem. Lett. 14 (2004) 25–27.
[59] G. Springsteen, B. Wang, Chem. Commun. (2001) 1608.
[60] Y. Shinohara, J. Furukawa, K. Niikura, N. Miura, S. Nishimura, Anal. Chem. 76 (2004) 6989–6997.
[61] R. Ullmer, A. Plematl, A. Rizzi, Rapid Commun. Mass Spectrom. 20 (2006) 1469–1479.
[62] G.E. Reid, K.D. Roberts, R.J. Simpson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 16 (2005) 1131–1150.
[63] H. Han, D.J. Pappin, P.L. Ross, S.A. McLuckey, J. Proteome Res. 7 (2008) 3643–3648.

[64] C. Wu, D.R. Ifa, N.E. Manicke, R.G. Cooks, Anal. Chem. 81 (2009) 7618–7624.
[65] B. Koch, Can. J. Diabetes 28 (2004) 238–245.
[66] N. Ito, Mater. Integr. 21 (2008) 324–328.
[67] X. Gao, W. Yang, P. Pang, S. Liao, Q. Cai, K. Zeng, C.A. Grimes, Sens. Actuators B 128 (2007) 161–167.
[68] J. Dyerberg, L. Pedersen, O. Aagaard, Clin. Chem. 22 (1976) 205–210.
[69] A.U. Jackson, N. Talaty, R.G. Cooks, G.J.V. Berkel, J. Am. Soc. Mass Spectrom. 18 (2007) 2218–2225.
[70] D.-Y. Chang, C.-C. Lee, J. Shiea, Anal. Chem. 74 (2002) 2465–2469.
[71] R. Orlando, C.A. Bush, C. Fenselau, Biol. Mass Spectrom. 19 (2005) 747–754.
[72] T.J. Kauppila, N. Talaty, A.U. Jackson, T. Kotiaho, R. Kostiainen, R.G. Cooks, Chem. Commun. 23 (2008) 2674–2676.
[73] M. Gross, Int. J. Mass Spectrom. 200 (2000) 611–624.
[74] D.J. Harvey, Mass Spectrom. Rev. 18 (1999) 349–451.
[75] M.T. Cancilla, A.W. Wong, L.R. Voss, C.B. Lebrilla, Anal. Chem. 71 (1999) 3206–3218.
[76] Y.H. Ahn, J.S. Yoo, Rapid Commun. Mass Spectrom. 13 (1999) 1985–1990.
[77] J. Zaia, C.E. Costello, Anal. Chem 75 (2003) 2445–2455.
[78] A. Venter, R.G. Cooks, Anal. Chem. 79 (2007) 6368–6403.
[79] Z. Takats, J.M. Wiseman, R.G. Cooks, J. Mass Spectrom. 40 (2005) 1261–1275.