In-source Microdroplet Derivatization using Coaxial Contained-Electrospray Mass Spectrometry for Enhanced Sensitivity in Saccharides Analysis

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ABSTRACT: Online, droplet-based in-source chemical derivatization is accomplished using a coaxial-flow contained-electrospray ionization (contained-ESI) source to enhance sensitivity for the mass spectrometric analysis of saccharides. Derivatization is completed in microseconds by exploiting the reaction rate acceleration afforded by electrospray microdroplets. Significant improvements in method sensitivity are realized with minimal sample preparation and few resources when compared to traditional benchtop derivatizations. For this work, the formation of easily ionizable phenylboronate ester derivatives of several mono-, di-, and oligosaccharides is achieved. Various reaction parameters including concentration and pH were evaluated and a Design of Experiments approach was used to optimize ion source parameters. Signal enhancements of greater than two orders of magnitude were observed for many mono- and disaccharides using in-source phenylboronic acid derivatization, resulting in parts-per-trillion (picomolar) limits of detection. In addition, amino sugars such as glucosamine, which do not ionize in negative mode, were detected at low parts-per-billion concentrations, and isobaric sugars such as lactose and sucrose were easily distinguished. The new in-source derivatization approach can be employed to expand the utility of ESI-MS analysis for compounds that historically experience limited sensitivity and detectability, while avoiding resource-intensive, bulk-phase derivatization procedures.

Compartmentalization of reaction systems has many advantages. Microreactors consume smaller amounts of reagent and solvent than bulk reactors, allow more precise control of reaction conditions, and enable rapid mixing. Microdroplets and thin films in particular have proven to be dynamic reaction systems.[1-12] The large surface area-to-volume ratio facilitates rapid evaporation leading to significant increases in reagent concentration and surface charge density as well as extreme pH changes and fluctuations in temperature and density gradients within the microdroplet or thin film. Collectively, these effects reduce entropic barriers and create a kinetically-favorable environment capable of carrying out reactions several orders of magnitude faster than conventional bulk reaction systems.[1-4,7,13-20]

Microdroplets and thin films have been employed to carry out several different types of synthetic chemical reactions including Girard reactions,[1,7] Michael additions,[1,13] Mannich and Claisen-Schmidt condensations,[7,11,13,19] Pomeranz-Fritsch synthesis,[11] Katritzky reactions,[21,22] and even five-component spiro-pyrrolidine synthesis.[23]

For this work, we seek to leverage the rate acceleration afforded by thin films and electrospray microdroplets to carry out online, in-source chemical derivatization using a unique, coaxial flow contained-electrospray ion source (contained-ESI).[24,25] The contained-ESI source is a modified electrospray platform which allows for the introduction of liquid and/or vapor phase reagents directly into the ion source. To date, the contained electrospray ion source has been used to modify source conditions to overcome matrix suppression[25] and study protein folding.[24] Other variations of in-source, dual emitter platforms have been reported previously for use in applications ranging from polymer coating microdroplets and microemulsions[26] to studying protein structure[27] and isotopic exchange.[28,29] However, the contained-electrospray ion source is designed to facilitate both thin film and microdroplet reactions simultaneously, making it a promising approach for in-source chemical derivatization.

Chemical derivatization is a common practice for improving detectability and sensitivity for many different analytical methodologies. However, traditional benchtop derivatization procedures require time, labor, and resource commitments that ultimately limit throughput and increase analysis cost. To overcome these limitations, we employ the contained-ESI source as an online, in-source derivatization platform. The sample and reagent are coaxially introduced into the ion source via separate capillaries. Mixing and reaction occur in the ESI emitter during, or just prior to, the electrospray process. This setup allows analyte derivatization to be accomplished in high yield on the seconds to microseconds time scale[24,30] rather than hours or days as is typical for conventional derivatization protocols. Further, the online approach allows full automation and minimizes sample manipulation.

The aim of the current work is to test the merits of in-source derivatization in an empirical and quantifiable way using the coaxial contained-electrospray ion source by boosting sensitivity for a set of mono- and disaccharides. Electrospray ionization mass spectrometry (ESI-MS) is the preferred analytical technique for these compounds due to its inherent specificity. However, saccharides generally exhibit poor sensitivity in conventional ESI-MS due to the absence of an easily protonated or deprotonated moeity.[31] Analysis utilizing electrospray ionization or atmospheric pressure chemical ionization (APCI) commonly relies on the detection of alkali metal adducts in positive mode,[32,33] or halogen adducts in negative mode.[34] Adducts typically do not fragment well by collision induced dissociation (CID), limiting the value of tandem mass spectrometry (MS/MS) analysis. Augmentation of these compounds with easily protonated or deprotonated functional groups has the potential to significantly enhance sensitivity while generating specific and informative MS/MS spectra.

For this work, two different derivatization reactions were evaluated: (1) formation of sugar-Schiff bases via nucleophilic addition and (2) conversion to phenylboronate esters using

arylboronic acids. Both approaches were first evaluated using a set of common mono- and disaccharides. Method optimization was performed for the best performing reaction and sensitivity improvements afforded by in-source derivatization were quantified. More than 100-fold improvement in sensitivity was recorded for most sugars tested using the phenylboronic acid derivatization. Finally, applicability of the method to larger saccharides in a mixture was established using a set of oligosaccharides.

EXPERIMENTAL

Coaxial Contained-Electrospray Ion Source. A schematic of the coaxial contained-electrospray ion source is included in **Figure 1** and images are included in **Figure S1**. The source is constructed from a stainless-steel tee with compression fittings (Swagelok®, Solon, OH) and graphite ferrules. The sample and reagent streams are introduced coaxially via two separate fused silica capillaries of 100 µm internal diameter each (190 µm outer diameter), which extend into a second, larger fused silica capillary of 450 µm internal diameter. The sample and reagent solutions are delivered using syringe pumps at flow rates of 10 µL/min and 5 µL/min, respectively.

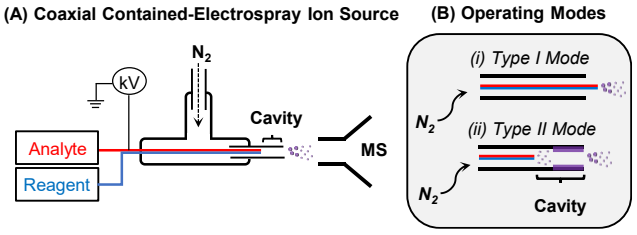


Figure 1. (**A**) The dual feed coaxial contained-electrospray ion source facilitates chemical derivatization directly within the ion source. (**B**) Two modes of operation can be employed: in Type I mode (i), reactions occur in the electrospray microdroplets, while Type II mode (ii) allows both thin film and microdroplet reactions.

The contained-electrospray ion source can be operated in two different configurations. In Type I mode (**Figure 1B,i**), which was the primary operating mode for the work described herein, the sample and reagent capillaries protrude from the outer capillary ~ 1 mm to function in a conventional electrospray format. The two inner capillaries converge at the tip to create a single Taylor cone containing both the reagent and sample. Mixing and reaction are initiated in the Taylor cone and proceed within the electrosprayed microdroplets as they travel to the mass spectrometer. In Type II mode (**Figure 1B,ii**), the two inner capillaries are retracted into the outer capillary, creating a cavity where a discontinuous thin film forms on the inside walls of the outer capillary as the two streams are fed into the source. Due to the extended reaction time afforded by the thin film, Type II mode can be used to carry out reactions requiring longer mixing times, and the added thin film is expected to supplement the enrichment effect of the electrospray droplets.

Optimization of sheath gas flow is critical whether operating in Type I or Type II mode. High flow rates (>80 psi) provide efficient desolvation and spray stabilization but will reduce mixing and reaction time. Conversely, low flow rates maximize reaction time but will result in reduced desolvation, limiting the concentration effect within the thin film and/or electrospray microdroplets.

Chemicals and Reagents. D-(+)-Glucose (99.5%), fructose (99%), galactose (99%), D-glucosamine (100%), sucrose (99.5%), α-lactose monohydrate (99%), D-(+)-raffinose pentahydrate (99%), butylamine (99.5%), benzylamine (99.5%), suberic acid (98%), tetraethylammonium bromide (98%), and 4-[(dimethylamino)-methyl]phenylboronic acid (95%) were purchased from Sigma-Aldrich (St. Louis, MO). β-cyclodextrin (99.6%), acetonitrile (HPLC grade), and TRIS-HCl pH 8.0 buffer were purchased from Fisher Scientific (Pittsburgh, PA). Glucose tetrasaccharide (>97%) and maltopentaose (99.9%) were obtained from Biosynth-Carbosynth (Itasca, IL), while 4-(carboxymethyl)phenylboronic acid (98%) was obtained from AK Scientific, Inc. (Union City, CA) and phenylboronic acid (>97%) was obtained from Strem Chemicals (Newburyport, MA).

High purity deionized water with 18.2 MΩ·cm resistivity was prepared using a Milli-Q filtration system (Merck Millipore, Burlington, MA). High-purity nitrogen was used as the sheath gas.

Mass Spectrometry Analysis and pH Measurement. Mass spectrometry analysis was conducted using a Thermo Velos Pro LTQ linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA). Optimized contained-ESI source settings for the phenylboronic acid derivatization are included in **Table S1**. Experimental parameters for Schiff base reactions were not formally optimized. Preliminary tests to assess viability were performed under the following conditions: source configuration = Type I mode, sheath gas pressure (N_2) = 10 psi, source voltage = +5 kV, capillary temperature = 200 °C, sample concentration = 20 µM, sample flow rate = 10 µL/min, reagent concentration = 2 mM, reagent flow rate = 5 µL/min.

Tandem mass spectrometry was performed using collision-induced dissociation (CID). CID settings were analyte specific and are included in **Table S2**. Data were acquired and processed using Thermo Fisher Scientific Xcalibur 2.2 SP1 software. pH measurements were obtained using a Mettler Toledo S220 pH meter (Schwerzenbach, Switzerland). The pH meter was calibrated each day using standard pH buffers.

Design of Experiments Optimization. Optimization of the contained-electrospray ion source parameters was performed using Stat-Ease® Design Expert version 12 software (Stat-Ease, Inc, Minneapolis, MN). Additional details of the experimental design are included in **Table S3**.

Preparation and Analysis of Calibration Curves. Preparation and analysis of calibration curves for the phenylboronic acid-derivatized analytes were performed as follows: a series of standards for each sugar were prepared in a mixture of 1:1 acetonitrile/water. Methanol was avoided due to the tendency to react with the reagent, forming the associated phenylboronic acid methyl esters. Each standard was fortified with the internal standard suberic acid at a concentration of 2 ppm and pH was adjusted to approximately 10 using ammonium hydroxide. The derivatization solution consisted of 4 mM phenylboronic acid in 1:1 acetonitrile/water with pH adjusted to approximately 10 using ammonium hydroxide. Analysis was performed in negative-ion mode using the optimized method parameters shown in **Tables S1 and S2**. Selected Reaction Monitoring (SRM) ion transitions for each sugar were monitored and ion counts were normalized using the response of the internal standard.

For the native analytes (i.e., without derivatization), calibration standards for analysis in negative mode were prepared as described above, including pH adjustment and addition of

internal standard. Standards for analysis in positive mode were prepared in 0.5 mM sodium formate in glass vials and fortified with the internal standard tetraethylammonium bromide at a concentration of 0.1 ppm. pH adjustment was not necessary since the sodiated adducts were being monitored. Analysis in negative-ion mode was performed using conventional electrospray conditions with a source voltage of -5.5 kV, sheath gas pressure of 80 psi, and capillary temperature of 200 °C, while positive-ion analysis was performed using a source voltage of +5 kV, sheath gas pressure of 80 psi, and capillary temperature of 200 °C. Standard solutions were infused at a rate of 10 µL/minute and data were collected in either selected ion monitoring (SIM) or SRM mode, whichever exhibited the highest sensitivity (see **Table S2**).

Data for each standard were collected in triplicate and the average normalized responses were used to construct the calibration curves. Limits of detection (LODs) were calculated using the slope of the calibration curve and the standard deviation of the blank responses.

RESULTS AND DISCUSSION

Formation of Sugar-Schiff Bases. Initial efforts to achieve signal enhancement via online derivatization using the contained-electrospray ion source focused on a two-component Schiff base formation. In this well-known reaction, a carbonyl undergoes nucleophilic addition and subsequent condensation to form an alkylimine. For this work, the aldehyde offered by the open-chain conformation of reducing sugars was reacted with butylamine, which served as the nucleophile. Subsequent loss of water generates the Schiff base derivative of the sugar, providing a highly basic functional group capable of efficiently protonating in positive mode. The resulting increase in ionization efficiency of the imine in comparison with the native, unreacted molecule is expected to afford some degree of signal enhancement.

In-source Schiff base derivatization (**Figure 2A**) was evaluated for the monosaccharide glucose and disaccharides lactose and sucrose. Both glucose and lactose are reducing sugars capable of generating an acyclic aldehyde in solution. As shown in **Figure 2B,C**, the Schiff base derivatives of these two compounds were formed in relatively high yield using the contained-electrospray platform.

Unsurprisingly, sucrose, which is a non-reducing sugar and does not undergo ring opening, did not generate the Schiff base derivative (**Figure 2D**). Attempts were made to facilitate the reaction by pre-oxidizing an alcohol group to produce the necessary carbonyl using various oxidizing agents including periodate, hypochlorite, and peroxide. Although the desired Schiff base was observed after pre-oxidation and in-source derivatization, the oxidation process proved too aggressive, forming a multitude of products including polyaldehydes and carboxylic acids, many of which generated their own Schiff bases upon in-source derivatization (**Figure S2**). When the aim is to improve sensitivity, the formation of many different derivatives is not ideal because the absolute signal is spread amongst all of the corresponding ions, ultimately limiting the degree of enhancement that can be achieved. Further, among the virtues of the in-source derivatization platform are added simplicity and efficiency. Requiring offline pre-modification steps prior to online derivatization adds unwanted complexity and requires additional time, labor, and resources.

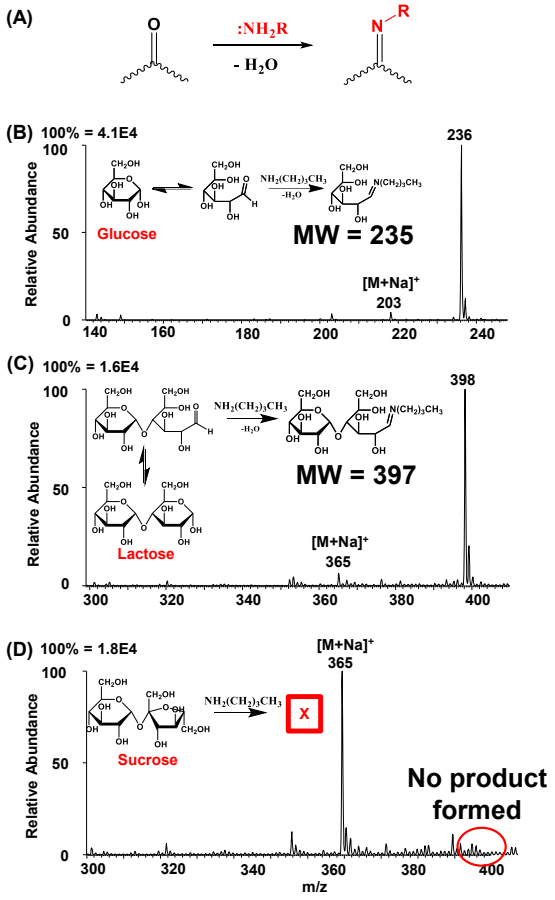
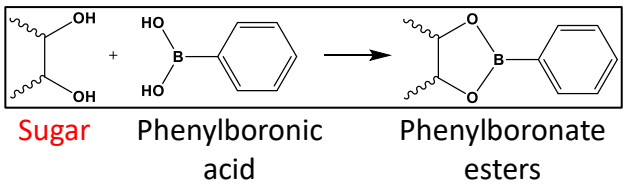


Figure 2. Schiff bases are formed by reacting the free aldehyde of the sugar with a primary amine (**A**). Positive-ion mode full-scan mass spectra reveal the products of in-source Schiff base derivatization for glucose (**B**), lactose (**C**), and sucrose (**D**). The Schiff base for sucrose was not formed due to lack of an available carbonyl.

Formation of Phenylboronate Esters. To avoid limiting our approach to only reducing sugars, other derivatization chemistries were considered. There are many ways to functionalize alcohol groups including alkylation, esterification, and carbamate formation. However, in many cases, these reactions require water labile reagents and/or extreme pH conditions, which can degrade polysaccharides by hydrolyzing the glycosidic linkage. Phenylboronic acids, on the other hand, are stable in aqueous solution and react with *vicinal* diols under relatively mild conditions to generate the corresponding phenylboronate esters (**Scheme 1**). This well-known approach to diol functionalization has been reported for several classes of compounds including steroids, catecholamines, sugars, and carbohydrates.[12,35-43]

Scheme 1. General reaction of phenylboronic acid with vic-diols.



In-source derivatization of glucose with phenylboronic acid (PBA) resulted in the formation of both the monosubstituted and disubstituted phenylboronate esters (**Figure 3A**). For monosaccharides, the binding of one boronate group at the C1/C2 position induces conformation changes that promote the binding of a second boronate group.[35] Consequently, the bis(phenylboronate) analogs of the monosaccharides tested [e.g., glucose (MW 180 Da)] were more abundant (*m/z* 351) than the monosubstituted analytes (*m/z* 265 and *m/z* 283). The disaccharides lactose and sucrose (MW 342 Da) produced primarily the monosubstituted species (*m/z* 427 and *m/z* 445).

In most cases, both the deprotonated and hydroxylated precursor ions of the derivatized species were observed. Boron is a Lewis acid, which can easily accommodate a hydroxide ion in solution to form the hydroxylated precursor ion.[35] Formation of the deprotonated molecule may arise due to the electron deficiency of boron in the phenylboronate tag, which draws electron density away from the nearest alcohol group, making it less basic and therefore more willing to deprotonate in comparison with the underivatized molecule.

Although derivatization was accomplished for sucrose, which was not possible using the Schiff base reaction, overall yield was somewhat lower than that observed for glucose and lactose. To facilitate comparison, reaction yield was defined as the ratio of the abundance of the derivatized species to that of the remaining underivatized analyte. Phenylboronic acids prefer to react with *cis*-diol moieties[35], which sucrose does not possess. Addition to *trans*-diols and 1,3-diols is less favored leading to reduced yield and sensitivity gains.

In the case of glucose, the disubstituted derivative observed at *m/z* 351 offered the largest degree of signal enhancement in comparison with the native analyte and generated a considerably more abundant and informative product ion spectrum upon collision-induced dissociation (**Figure 3B**). When employing single reaction monitoring (SRM) or multiple reaction monitoring (MRM) for analysis, more abundant product ion formation can deliver additional gains in sensitivity to supplement the gains afforded by improved ionization efficiency. Further, most of the product ions generated by the glucose-PBA derivative (with the exception of *m/z* 247 and *m/z* 229 which represent the loss of Ph-B=O and Ph-B(OH)$_2$, respectively) contain portions of both the reagent molecule and the sugar substrate, which can aid in structure elucidation.

In addition to improved sensitivity, online PBA derivatization can assist in differentiating isobaric saccharides. Lactose and sucrose have the same molecular weight, chemical makeup, and similar structures. However, they are easily distinguished using tandem mass spectrometry after in-source PBA derivatization (**Figure 3C**). In particular, product ions at *m/z* 367 and *m/z* 349 are unique to lactose and are attributed to the loss of C2H4O2 and subsequent loss of water from the linear form of the sugar, formed naturally in solution (Figure 3Ci). Since sucrose is a non-reducing sugar and does not undergo ring opening in solution, these product ions are not generated (Figure 3Cii). Small differences such as this might be used to differentiate reducing and non-reducing sugars. Similarly, PBA derivatives of the isobaric monosaccharides glucose, fructose, and galactose generally produce the same product ions but can be distinguished by the relative abundances of those product ions (**Figure S3**). Fructose, a furanose, produces *m/z* 175 as the most abundant product ion while glucose and galactose, both pyranoses, preferentially generate the product ion at *m/z* 265. Thus, although complete structure elucidation is likely not possible using PBA derivatization with CID alone, it does capture sufficient structural diversity to differentiate known isobaric analogs.

Two functionalized PBA analogs were also evaluated as derivatization reagents: 4-[(dimethylamino)-methyl]phenylboronic acid (DMAMPBA), and 4-(carboxymethyl)phenylboronic acid (CMPBA). The dimethylamino functionality was expected to afford greater ionization efficiency in positive-ion mode while the carboxylic acid analog was included to enhance ionization in negative mode.

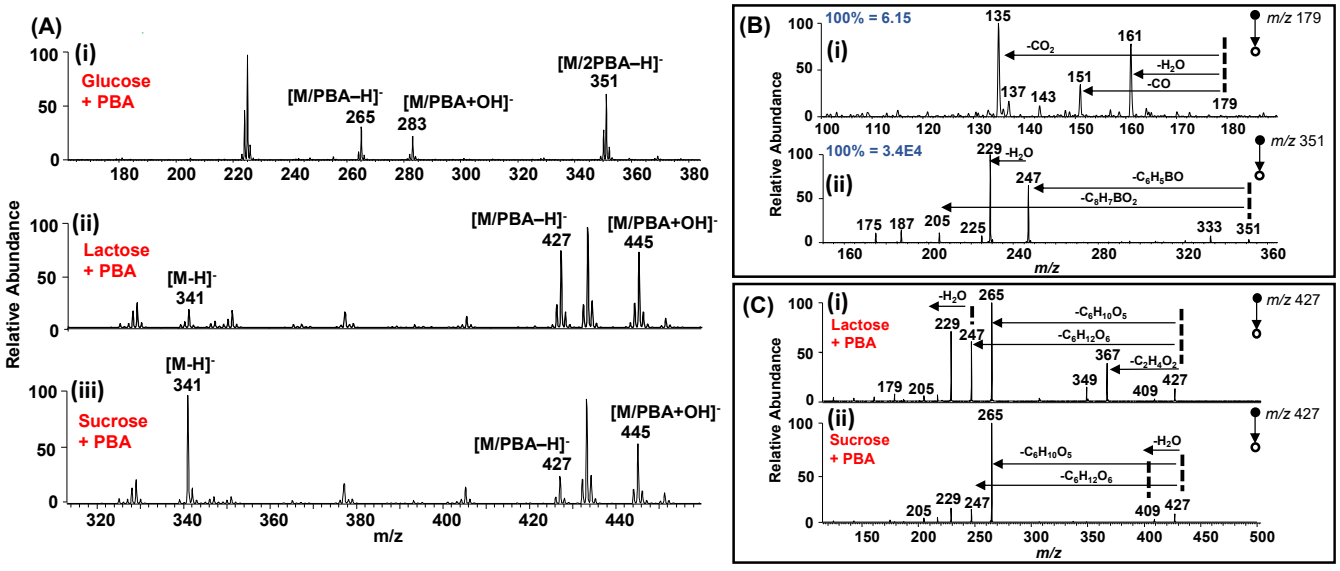


Figure 3. (**A**) Negative-ion mode full scan mass spectra acquired during online, in-source PBA derivatization of glucose (i), lactose (ii), and sucrose (iii) showing the formation of the phenylboronate esters ([M/PBA]) and bis(phenylboronate) esters ([M/2PBA]). (**B**) A comparison of product ion spectra (MS/MS) for a 5 µM glucose standard without derivatization (i) and after in-source PBA derivatization (ii) shows a significant gain in sensitivity after derivatization. (**C**) The isobaric sugars lactose (i) and sucrose (ii) are differentiated using tandem mass spectrometry after PBA derivatization. Data were acquired using the optimized settings shown in Table S1.

In-source derivatization reactions carried out using DMAMPBA and CMPBA yielded the expected monosubstituted products as well as a small amount of the disubstituted sugars (**Figures S4 and S5**). However, the products generated via DMAMPBA and CMPBA derivatization were similar in abundance to the native, underivatized analyte, suggesting these reactions did not offer significant signal enhancement. In the case of DMAMPBA, it is possible that the basic pH that is optimal for the reaction (pH~10) inhibited ionization. In addition, the product ion spectra of the DMAMPBA and CMPBA derivatives were much less informative than that observed using PBA. Both readily lose functional groups associated with the reagent rather than the substrate, hindering identification and limiting the utility of SRM or MRM for analysis. Specifically, the MS/MS spectra of the DMAMPBA and CMPBA derivatives are dominated by the loss of dimethylamine and carbon dioxide, respectively, which inform the structure of the reagent rather than the analytes.

Since PBA derivatization offered the greatest potential for signal enhancement in preliminary testing and produced the most informative product ion spectra, PBA was selected as the derivatization reagent for further optimization and assessment.

Optimization of Reaction Conditions and Source Parameters. The ultimate goal of online derivatization using the contained-ESI source is to maximize signal rather than reaction yield. Thus, it is critical to arrive at a set of optimized conditions that balance sufficient reaction yield with maximum ionization efficiency. Ion source and mass spectrometer parameters that must be optimized include operating mode (Type I or Type II), cavity size (for Type II mode), sheath gas pressure, source voltage, and MS inlet capillary temperature. In addition, the pH of the sample and reagent solutions can prove critical for both the reaction and ionization processes.

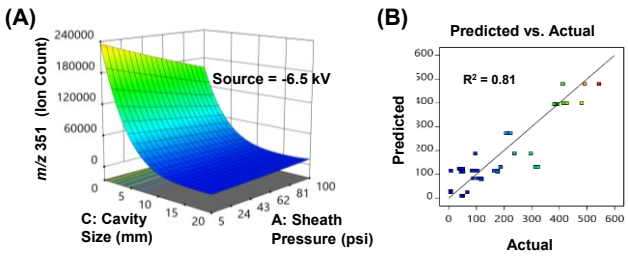
Optimal reaction pH was determined by performing a series of bulk-phase reactions, for which pH was adjusted using ammonium hydroxide or acetic acid, and measuring the abundance of the desired product using conventional ESI-MS. The optimal pH for the sugar-phenylboronic acid reaction was found to be about pH 10-11, which is consistent with previous reports.[10] Thus, all subsequent testing was carried out by adjusting the pH of both the sample solution and reagent solution to pH 10 using dilute ammonium hydroxide.

The capillary temperature of the mass spectrometer has been shown to have a significant effect on microdroplet reaction yield in ESI-MS studies.[44] For this work, four different sugars were used to estimate the optimal capillary temperature by monitoring the abundance of the reaction products using various inlet temperatures ranging from 200 °C to 440 °C. The highest responses were obtained for capillary temperatures between 300-350 °C (**Figure S6**). Thus, the MS capillary temperature was set to 325 °C for all subsequent tests.

The coaxial contained-ESI source parameters were optimized using a Design of Experiments (DoE) approach. Due to the potential interdependence between these parameters, it was important to consider not only the individual parameters themselves but also the interaction between the parameters. A Box-Behnken three-factor central composite design using response surface modeling was employed to optimize sheath gas pressure, source voltage, and cavity size for the contained-electrospray source. Glucose was used as the model substrate. In preliminary range-finding experiments, the precursor ion for the disubstituted derivative of glucose (*m/z* 351) generated the largest response of the three ions observed during the reaction, which includes the two precursor ions attributed to the monosubstituted derivative (*m/z* 265 and *m/z* 283). Thus, the ion count of the disubstituted glucose derivative at *m/z* 351 was monitored to determine optimal conditions. Details of the experimental design are included in **Table S3**.

The coded model (equation 1) and response surface plot for the DoE optimization are shown in **Figure 4A**. All factors with p-values greater than 0.1 were considered insignificant and omitted from the model. The plot of actual versus predicted values (**Figure 4B**) suggests the model is sufficiently predictive for the intended purpose. As the response surface plot illustrates, maximum response for the disubstituted glucose derivative is obtained using no cavity (Type I mode), low sheath gas pressure (0-5 psi), and high source voltage (-6.5 kV). Thus, droplet formation likely occurs via a traditional ESI mechanism resulting from the high electric field, essentially unaided by the nebulizing effect of the sheath gas. Mixing of the sample and reagent streams is initiated in the Taylor cone and continues within the resulting microdroplets during flight to the mass spectrometer. Transit times are extended due to the absence of high sheath gas flow, allowing for longer reaction times and higher response for the derivatized species.

Details of the final, optimized method conditions for in-source PBA derivatization of sugars are provided in **Table S1**.



Equation 1: coded model

$$\sqrt{Ion\ Count} = 123 - 7.65A + 50.8B - 140C + 34.9AC - 52.8BC - 62.9B^2 + 134C^2$$

A = Sheath Pressure (psi); B = Source Voltage (kV); C = Cavity Size (mm)

Figure 4. (A) DoE optimization of contained-electrospray ion source parameters for in-source PBA-derivatization of glucose, and **(B)** comparison of actual vs. predicted response values.

Signal Enhancement via In-Source Derivatization. Calibration curves for a set of six mono- and disaccharides were generated via in-source PBA derivatization using the optimized method conditions (**Figure S7**). The calibration curves were found to be linear over several orders of magnitude. In most cases, parts-per-trillion (picomolar) LODs were achieved, an improvement of greater than two orders of magnitude over the underivatized analytes. Details are included in **Table 1**.

Gains in sensitivity are the result of both improved ionization of the analytes after derivatization as well abundant product ion formation during CID, allowing for the use of more sensitive SRM analysis. With the exception of sucrose, the underivatized sugars did not generate abundant product ions (see example for glucose in **Figure 3B**). In these cases, SIM acquisition proved to be more sensitive than SRM and was used to determine LODs for comparison with the derivatized analytes (**Table S2**). LODs for the underivatized analytes were determined for both the

Table 1. Calibration curves and limits of detection achieved for various mono- and disaccharides both with and without derivatization. Significant gains in sensitivity were achieved via in-source PBA derivatization.

Analyte	PBA Derivatized			Underivatized		LOD Improvement
	Linear Range (nM)	R^2	$[M-H]^-$ LOD nM (ppb)	$[M-H]^-$ LOD nM (ppb)	$[M+Na]^+$ LOD nM (ppb)	
glucose	5 - 5000	0.997	0.82 (0.15)	180 (33)	200 (36)	220-fold
fructose	12.5 - 5000	0.995	1.2 (0.22)	150 (27)	160 (28)	125-fold
galactose	12.5 - 2000	0.992	0.66 (0.12)	160 (29)	150 (27)	225-fold
glucosamine	125-10000	0.999	33 (6.0)	N/A	N/A	-
lactose	9.5 - 2375	0.996	0.59 (0.20)	260 (88)	23 (7.9)	40-fold
sucrose	2 - 1000	0.999	0.72 (0.25)	4.5 (1.5)	5.7 (2.0)	6-fold

sodiated molecule in positive-ion mode and the deprotonated species in negative-ion mode (see **Table S2**). The mode of operation that exhibited the highest sensitivity was used for the comparison in Table 1.

Glucosamine and other amino sugars typically require analysis in positive-ion mode because they do not ionize readily in negative mode. Using in-source PBA derivatization and SRM analysis, glucosamine was detectable at low parts-per-billion concentrations in negative mode (**Table 1**), which is preferred for many other saccharides. Although amino sugars are expected to exhibit high sensitivity in positive mode, the ability to detect glucosamine and other amino sugars in negative mode allows for the analysis of different types of sugars in a single analysis. A representative product ion spectrum for the glucosamine-PBA derivative (*m/z* 264) can be found in **Figure S8**.

Notably, the improvement in sensitivity afforded by PBA derivatization was less prominent for sucrose. This is likely attributed to the absence of *cis*-diol groups on the molecule, leading to slightly reduced yield in comparison with the other sugars (see **Figure 3A**). In addition, unlike the other sugars, SRM analysis was possible for the native, underivatized analyte due to abundant fragmentation, which resulted in a lower detection limit. Consequently, less significant sensitivity gains were achieved for sucrose.

Analysis of Oligosaccharides in TRIS-HCl Buffer. In-source PBA derivatization was also evaluated for a set of higher-order saccharides in a standard buffer system. A mixture containing raffinose, glucose tetrasaccharide, maltopentaose, and β-cyclodextrin in TRIS-HCl buffer was analyzed using in-source PBA derivatization. The monosubstituted PBA derivatives of each of the oligosaccharides were evident in the full scan mass spectrum (**Figure 5,B**).

Chloride adducts were also formed in relatively high abundance due to the presence of the TRIS-HCl buffer. Adducts are commonly used for the analysis of sugars and other compounds that do not efficiently protonate or deprotonate under electrospray conditions. However, in many cases, adducts do not readily fragment by CID, prohibiting MS/MS analysis. In other cases, fragmentation results in loss of the neutral form of the adduct to form the protonated or deprotonated species, generating a relatively uninformative product ion spectrum. For example, the MS/MS spectrum of the raffinose-Cl adduct (**Figure 5Cii**) is dominated by the peak representing loss of HCl and provides little structural information, whereas the raffinose-PBA derivative produces a much more structurally informative product ion spectrum (**Figure 5Ci**). Interestingly, the PBA/β-cyclodextrin derivative was generated in low yield in comparison with the other oligosaccharides. This is partly due to the fact that β-cyclodextrin does not contain any *cis*-diol groups, which is the preferred orientation for the PBA reaction to proceed efficiently. PBA is also known to form an inclusion complex with β-cyclodextrin due to interactions between the phenyl group of the reagent and the hydrophobic inner cavity of cyclodextrin.[45] Thus, though the β-cyclodextrin phenylboronate ester can be generated via in-source derivatization (as evidenced in Figure 5), the lack of *cis*-diol groups and competing complexation limit reaction yield.

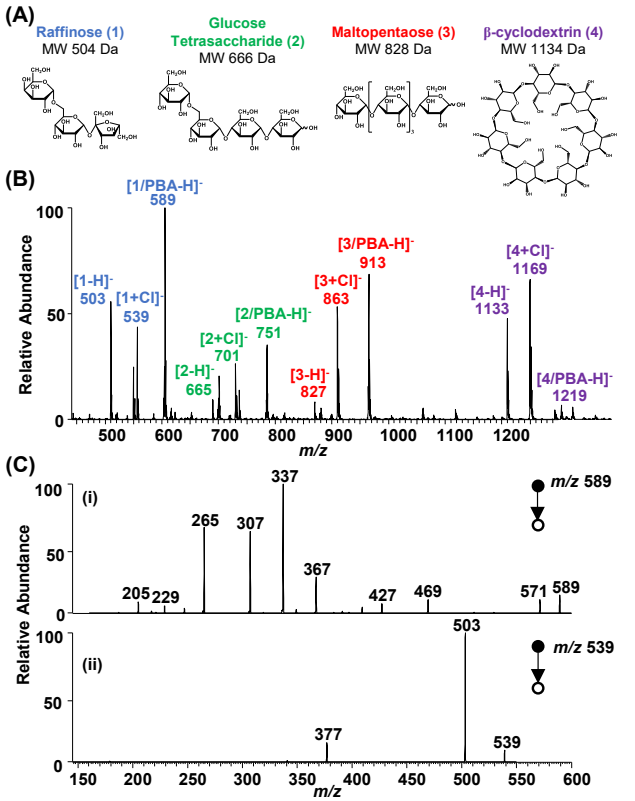


Figure 5. A 20 μM mixture of four oligosaccharides (**A**) prepared in 10 mM TRIS-HCl buffer was analyzed using in-source PBA derivatization. The full scan spectrum (**B**) shows the presence of the associated phenylboronate esters ([#/PBA] for each of the oligosaccharides. (**C**) The product ion spectrum of the PBA derivative of raffinose (i) is much richer and more informative than that of the chloride adduct of raffinose (ii). Data were acquired using the optimized settings shown in Table S1.

CONCLUSIONS

A droplet-based, in-source chemical derivatization approach has been developed and demonstrated for the analysis of sugars. The coaxial contained-electrospray platform allows for derivatization reactions to be carried out online, directly within the ion source to enhance sensitivity for saccharide analysis. In-source derivatization is a much simpler and faster alternative to traditional time, labor, and resource-intensive benchtop derivatization protocols and requires minimal user input. Picomolar limits of detection for many mono- and disaccharides were achieved using phenylboronic acid derivatization, an improvement of more than two orders of magnitude versus the native, unreacted analyte. The setup was also demonstrated to be applicable to oligosaccharides in complex matrix and allowed for the detection of amino sugars in negative-ion mode.

The in-source derivatization approach described herein can be adapted to enhance sensitivity or detectability for other classes of compounds by employing alternative reactions. Though not utilized here, simple modification of the contained-electrospray source can also be made to allow the integration of vapor phase reagents, an approach used previously to improve ionization and study pH effects on the charge state of proteins.[24,25] In addition, the co-axial configuration of the contained-ESI source is expected to be compatible with high-performance liquid chromatography (HPLC) to facilitate online derivatization of the HPLC eluent during the ionization process, adding an additional level of separation for isomer distinction and complex sample analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Images of the contained-ESI source and emitter (Figure S1); tables containing optimization details and in-source derivatization method parameters (Tables S1-S3); Schiff base formation from sucrose oxidation products (Figure S2); MS/MS spectra of glucose, fructose, and galactose after PBA derivatization (Figure S3); in-source derivatization of glucose using DMAMPBA and CMPBA (Figures S4, S5); optimization of MS inlet capillary temperature (Figure S6); calibration curves for PBA-derivatives of several sugars (Figure S7); MS/MS spectrum of glucosamine after PBA derivatization (Figure S8). (PDF)

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Notes

The authors declare no competing financial interests.

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