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Profiling Analysis of Low Molecular Weight Heparins by Multiple Heart-Cutting Two Dimensional Chromatography with Quadruple Time-of-Flight Mass Spectrometry

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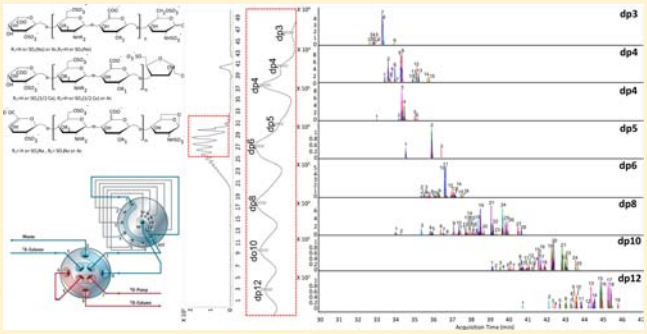
Supporting Information

ABSTRACT: Low molecular weight heparins (LMWHs) are polydisperse and microheterogenous mixtures of polysaccharides used as anticoagulant drugs. Profiling analysis is important for obtaining deeper insights into the structure of LMWHs. Previous oligosaccharide mapping methods are relatively low resolution and are unable to show an entire picture of the structural complexity of LMWHs. In the current study a profiling method was developed relying on multiple heart-cutting, two-dimensional, ultrahigh performance liquid chromatography with quadruple time-of-flight mass spectrometry. This represents an efficient, automated, and robust approach for profiling LMWHs. Using size-exclusion chromatography and ion-pairing reversed-phase chromatography in a two-dimensional separation, LMW components of different sizes and LMW components of the same size but with different charges and polarities can be resolved, providing a more complete picture of a LMWH. Structural information on each component was then obtained with quadrupole time-of-flight mass spectrometry. More than 80 and 120 oligosaccharides were observed and unambiguously assigned from the LMWHs, nadroparin and enoxaparin, respectively. This method might be useful for quality control of LMWHs and as a powerful tool for heparin-related glycomics.



Heparin is one of the oldest drugs still used as a clinical anticoagulant, one of the few carbohydrate drugs, and one of the most complex drugs.[1,2] Heparin is a mixture of sulfated linear polysaccharides with the most common repeating disaccharide unit corresponding to 2-*O*-sulfo-α-L-iduronic acid (IdoA2S) 1 → 4-linked to 6-*O*-sulfo, *N*-sulfo-α-D-glucosamine (GlcNS6S), and variable disaccharide units being β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA) 1 → 4-linked to 6-*O*-sulfo and/or 3-*O*-sulfo and/or *N*-sulfo (GlcNS) or *N*-acetylated (GlcNAc) α-D-glucosamine (Figure S1).[1,2] A rare pentasaccharide sequence comprises the antithrombin III (AT)-binding site, GlcNAc/NS6S (1-4) GlcA (1-4) GlcNS3S,6S (1-4) IdoA2S (1-4) GlcNS6S, and is important for heparin's anticoagulant activity.[3,4] Like all other natural polysaccharides, heparin has dispersity of molecular weight (MW) and a microheterogenous structural composition with multiple sequences and the structures of its many different polysaccharide chains can depend on extraction sources and production processes.[5,6] Different compositions, sequences, and structures of heparin chains can lead to different activities and, thus, quality control issues. The sulfo group location, number, and the placement of IdoA and GlcA are critically important for the specificity of heparin binding to different proteins.[7] In 2007−2008, a rapid onset, acute side effect associated with heparin was reported, which was believed to be caused by a contaminant, oversulfated chondroitin sulfate (OSCS), leading to hypotension and resulting in nearly 100 deaths.[8−10] This crisis initially went undetected as pharmacopeial methods were insufficient to monitor heparin structure or activity. For many years, analysts have been challenged to understand the complicated structures and sequences of heparin, limiting heparin's quality control and its use in new apllications.

In the 1990s, low molecular weight heparins (LMWHs) were introduced to reduce bleeding side effects in patients needing anticoagulation.[11] Different strategies have been applied to

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produce LMWHs from heparin, including enzymatic digestion, chemical β-elimination, and degradation by nitrous acid.[12] The relatively shorter polysaccharide chains in LMWHs retain their anticoagulant domain, corresponding to their AT-binding site, but these chains are too short to form heparin-AT-thrombin ternary complexes, which had been associated with heparin's bleeding side effects.[13,14] However, conversion of heparin to LMWH increases structural complexity. For example, nadroparin is produced by nitrous acid degradation of heparin, introducing a 2,5-anhydromannitol residue into most of the reducing-ends of the nadroparin chains,[12,15,16,17] and enoxaparin is produced from heparin by chemical β-elimination, introducing an unsaturated uronic acid residue at most of the nonreducing ends of the enoxaparin chains and occasionally introducing a 1,6-anhydroglucosamine residue at the reducing-end of the enoxaparin chains (Figure S1).[12,15,16,17] Therefore, structural elucidation and quality control are often more complicated for LMWHs than for heparin. Unfortunately, structural analysis and quality control of these LMWHs have relied primarily on the determination of molecular weight polydispersity, sulfation level, terminal residue compositional analysis, disaccharide compositional analysis, and most recently oligosaccharide mapping. Oligosaccharide mapping represents the only way to monitor the structure of intact components in LMWHs. However, current methods for oligosaccharide mapping, such as strong anion exchange chromatography (SAX),[18] ion-pairing reverse phase chromatography (IPRP),[19,20] reverse phase with precolumn derivatization,[21] hydrophilic interaction chromatography (HILIC),[22,23] and size-exclusion chromatography (SEC),[24] are often unable to separate many of the components present in LMWHs. The oligosaccharide components detected in a LMWH, using conventional separation methods, are limited and often do not provide accurate quantification because of their poor separation. Although analysis by mass spectrometry (MS) often follows these chromatographic separations, the types of oligosaccharides detected with MS in each LMWH may only represent some of the chains present in these complex mixtures.[25−27] Moreover, the sequences of all the possible oligosaccharides in a LMWH, predicted using software like GlycoResoft 2.0, is often greater than those observed in oligosaccharide mapping.[28−30] Thus, there still is no comprehensive method to profile the chains present in a LMWH, and the full characterization of LMWH remains an unmet challenge.[31]

In this article, we developed a loop-based, multiple heart-cutting (MHC) two-dimensional liquid chromatography (2D LC) separation method with quadrupole time-of-flight (Q/TOF) MS detection to obtain a more complete picture of LMWH. Nadroparin and enoxaparin were used as the model LMWHs for development of this method. MHC-2D LC provides an efficient, automated, and robust method, in which the oligosaccharides of LMWHs with different degree of polymerization (dp) are separated in a first dimension (^{1}D) using SEC and, then, the oligosaccharides of the same size but with different sequences are separated in a second dimension (^{2}D) by IPRP chromatography. Each oligosaccharide is then identified using Q/TOF MS analysis, providing a deeper insight into the LMWH structure.

MATERIALS AND EXPERIMENTS

Materials. Nadroparin was obtained from the European Pharmacopeia (EP, Strasbourg, France). Enoxaparin was obtained from United State Pharmacopeia (USP, Rockville, MD). Other chemical reagents were all LC−MS grade. The nadroparin octasaccharide mixture was prepared using size exclusion chromatography (Supporting Information).

Experiments. *Two-Dimensional Liquid Chromatography.* The experiments were performed on an Agilent two-dimensional UHPLC system with a Q/TOF MS. A size exclusion column was used in the ^{1}D chromatography. The sample solution (20 mg/mL, 3 μL) was eluted at 30 °C and detected at 210 nm (nadroparin) or 232 nm (enoxaparin). A C18 column was applied in the ^{2}D chromatography with IPRP mode, and details are provided in the Supporting Information, Table S1.

The two dimensions of the system were interconnected by a new MHC interface.[32] This interface incorporates a 2-pos/4-port valve to which a selector valve was coupled. The valve electronically controlled by external drives. The selector valve bears a cluster of six sampling loops. This makes a parking deck with 6 loop positions. The deck permits sampling/parking of targeted aliquots of the effluent from the ^{1}D column. The parked aliquots were automatically injected to the ^{2}D column. (Figure 1) The ^{2}D column was equilibrated for 20 min with 5% mobile phase B between two peak aliquot injections.

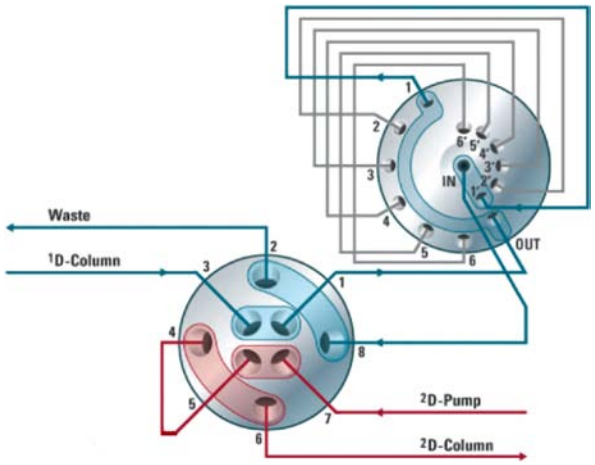

Figure 1. Schematic of the loop-based multiple heart-cutting two-dimensional chromatography system.

MS Parameters and Data Processing. The detailed MS parameters and data processing is provided in the Supporting Information. Extracted compound chromatography (ECC) was used to process all the MS data. Each peak in the ECC includes all MS signals corresponding to one oligosaccharide, such as ions with different charge states, different PTA adducts, and fragments associated with in-source sulfo group loss. All peaks were assigned using GlycoResoft 2.0 software, and these assignments were confirmed before being listed in the Supporting Information, Tables S2 and S3.

RESULTS

SEC and IPRP chromatography are two major separation techniques used for oligosaccharide mapping.[19,24] This study is designed based on these two techniques with SEC being applied as the ^{1}D chromatography. The SEC method was developed in 2012 to provide a stable method to profile LMWH.[24] IPRP was used as the ^{2}D chromatography with an optimized ion-pairing reagent reported in 2009.[19] The pore size of the C18 column used in ^{2}D was optimized at 300 Å,

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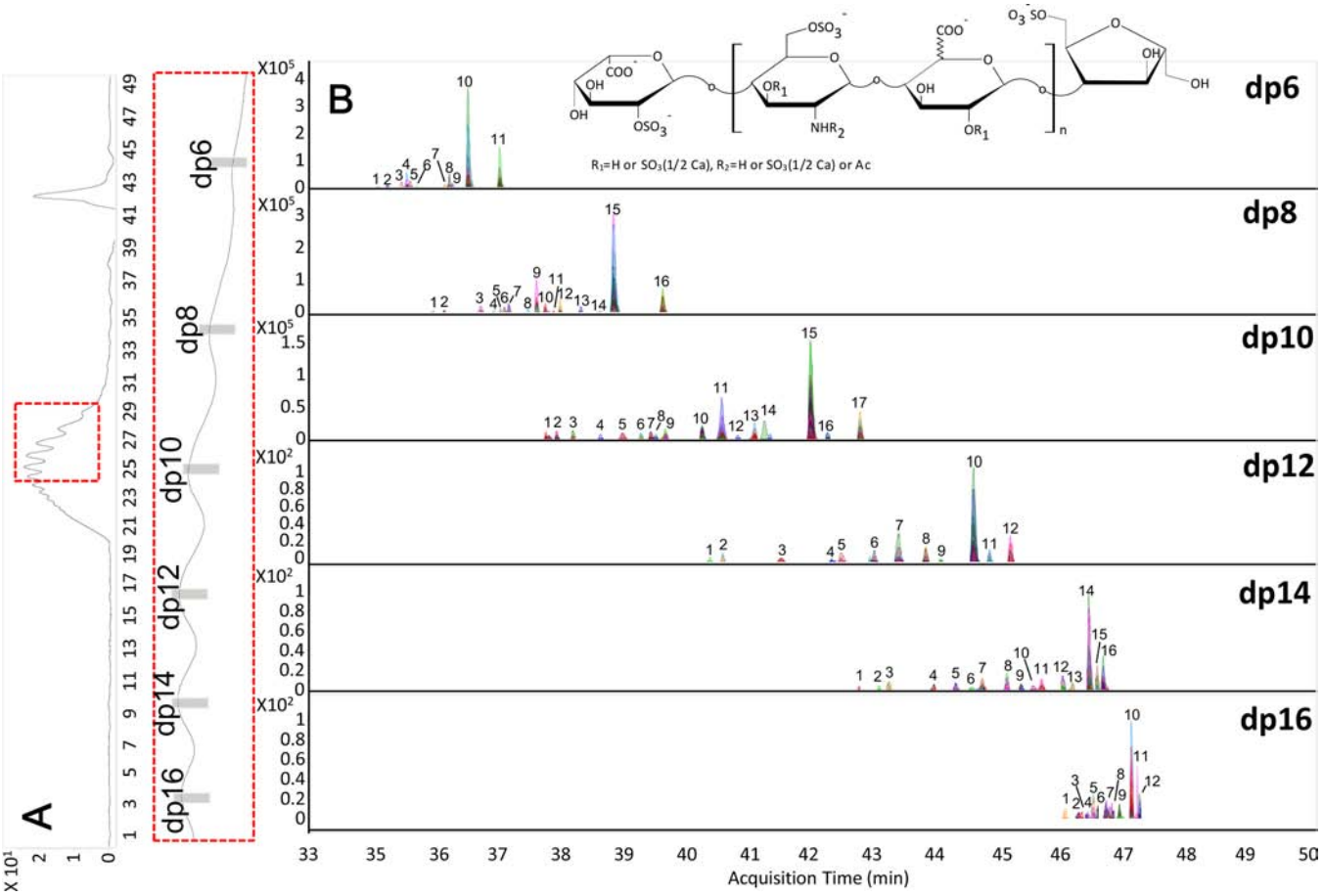

Figure 2. Profiling analysis of nadroparin using MHC-2D-LC-Q/TOF-MS. (A) The first dimensional chromatogram of nadroparin and the cutting position of each dp from 6 to 16 is shown. (B) The second dimensional chromatograms (ECC) for dp6 to dp16 are shown. In these fractions, the 11 to 17 peaks observed are numbered based on their retention times.

providing much improved resolution of heparin oligosaccharides (Supporting Information, Figure S2).

Stability of the First Dimensional Chromatography. The stability of the ^{1}D chromatography was very important in developing this automated 2D LC method. ^{1}D stability guarantees each peak in the ^{1}D chromatography can be identified, selected, and accurately and precisely transferred to ^{2}D for further separation. The result of stability of the ^{1}D chromatography is described in the Supporting Information, Figure S3.

Selection of the Loop Size. The size of the loop linking ^{1}D to ^{2}D decides the volume of each targeted peak in the ^{1}D chromatography transferred to the ^{2}D chromatography. Three loops with different sizes (6-, 20-, and 60-μL) were used to analyze the octasaccharide in nadroparin to determine the proper volume to transfer. The 6-μL loop was selected in the optimized method, as the chromatograms obtained had higher resolution and showed no peak splitting (Detailed information is provided in the Supporting Information, Figure S4). The loop size is typically selected based on the ^{2}D column capacity and size. The column used in this work has small particle size (1.7 μm) and a high capacity. A larger ^{2}D column is better fit with a bigger loop, but analytical efficiency decreases and its separation, flow rate, and the corresponding MS sensitivity are worse than those obtained with the current column.

Investigation of the Cutting Position. LMWH oligosaccharides of each dp afforded a ^{1}D chromatogram that showed relatively broad and overlapped peaks as they contain multiple components (Figure S3). Only 6 μL of eluent was selected and transferred from ^{1}D to ^{2}D chromatography. Thus, different cutting positions of a broad peak in ^{1}D chromatogram can result in different components being transferred. The cutting position of each dp in nadroparin was investigated using an octasaccharide mixture as a representative component. Five positions were cut and transferred to the ^{2}D chromatography (Supporting Information, Figure S6A). These five ^{2}D chromatograms were compared with the chromatogram generated with the nadroparin octasaccharide mixture prepared offline (Figure S6B–G). We conclude that the only 6 μL of eluent at the top/middle of a broad peak most accurately reflected its composition, consistent with the expected diffusional behavior of glycans on a SEC column (^{1}D). Since the cut volume and the selection of the top/middle position can alter the distribution of components, caution must be taken in quantification as early or late eluting components might be underrepresented. The details are described in Supporting Information and in Figure S6.

Profiling Analysis of Nadroparin. In Figure 2, nadroparin was analyzed using this developed MHC-2D LC-Q/TOF MS method. Oligosaccharides of dp6 to dp24 in nadroparin were observed in the ^{1}D chromatogram, of which dp6 ~ dp16 were transferred to the ^{2}D chromatography for further separation and MS analysis. The volume transferred from the ^{1}D to ^{2}D was 6 μL, and the cutting positions were all selected at the top

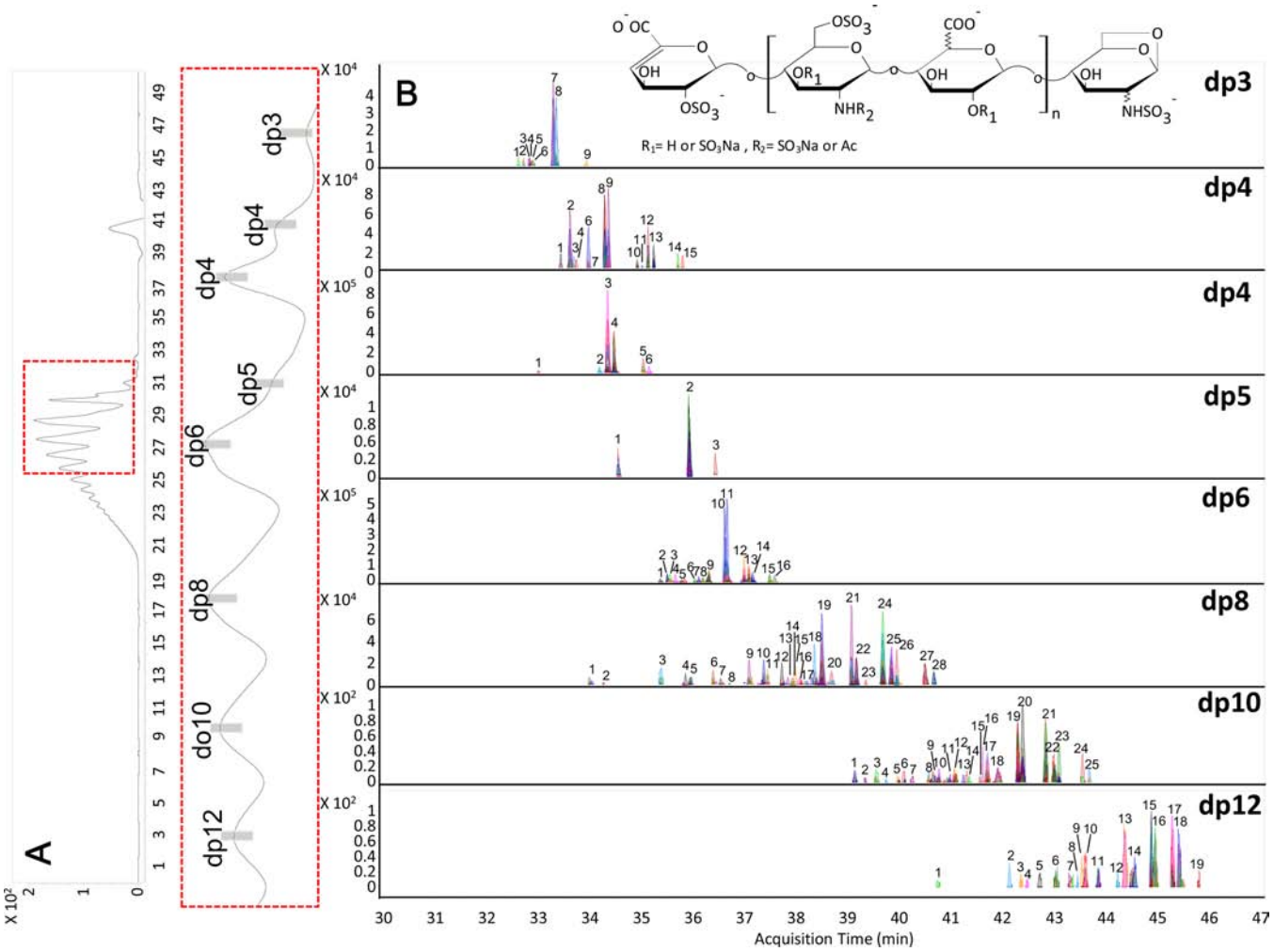

Figure 3. Profiling analysis of enoxaparin using MHC-2D-LC-Q/TOF-MS. (A) The first dimensional chromatogram of enoxaparin and the cutting position of each dp from 3 to 12 is shown. (B) The second dimensional chromatograms (ECC) for dp3 to dp12 are shown. In these fractions, the 9 to 28 peaks observed are numbered based on their retention times.

of each peak in the ^{1}D chromatogram (Figure 2A). In the ^{2}D chromatogram of nadroparin, 11, 16, 17, 12, 16, and 12 oligosaccharides were observed in dp6, dp8, dp10, dp12, dp14, and dp16, respectively. The structural assignments for the oligosaccharides are listed in Table S2 using standard nomenclature. Each oligosaccharide in nadroparin can be described using five numbers in square brackets corresponding to the number of uronic acid residues (IdoA or GlcA), glucosamine residues (GlcN), 2,5-anhydro-mannitol residues, acetyl groups (Ac), and sulfo groups (S).[28] The components in each dp of nadroparin were unambiguously observed using this analytical method. Their relative contents, calculated from the total volume of each peak in ECC, after combining the mass signals of different charge states, different PTA adducts, and fragments corresponded to in-source sulfo group loss.

Two major components were observed in dp6. They have same composition [3;2;1;0;8] and account for over 80% of dp 6 (Supporting Information, Table S2), one at ~67% and one at ~16%. Their different retention times suggest different sequences, such as different sulfo group positions and/or different distribution patterns of IdoA and GlcA. Minor components eluted earlier as they contained fewer sulfo groups, including peaks **4** to **6** having compositions of [3;2;1;0;7] (Figure 2B and Supporting Information Table S2). In addition, some oligosaccharides with rare compositions in nadroparin were also observed, such as hexasaccharides with intact glucosamine at the reducing end, peaks **1** and **2** both having a composition of [3;3;0;3;5], and peaks **7**–**9** having compositions of [3;3;0;3;6], [3;3;0;3;6] and [3;3;0;1;6], respectively. One of the octasaccharides with the lowest degree of sulfation was also observed in the ^{2}D chromatogram of dp6 (peak **3**) with a composition of [4;3;1;1;6].

The two major octasaccharides with 11 sulfo groups eluting late, both had a composition of [4;3;1;0;11]. The other 13 minor oligosaccharides with lower sulfation were eluted earlier, including two octasaccharides with 8 sulfo groups, five octasaccharides with 9 sulfo groups (with or without acetyl group), six octasaccharides with 10 sulfo groups. Some of these were isomers having the same composition (Figure 2B and Table S2). An octasaccharide with an intact reducing end residue, observed in peak **13**, had a composition of [4;4;0;1;9].

The detailed assignments of oligosaccharides having other dp values within nadroparin are provided in Figure 2B and Supporting Information Table S2. In all, 84 oligosaccharide peaks with high quality MS signals were observed from dp6 to dp16 in nadroparin. Many of these are believed to consist of a single structure.

Profiling Analysis of Enoxaparin. Enoxaparin was next analyzed using this MHC-2D LC-Q/TOF MS (Figure 3). On the basis of the profile in the ^{1}D chromatogram (Figure 3A), enoxaparin contained a greater number of smaller oligosaccharides than did nadroparin. Furthermore, oligosaccharides with an odd number of sugar residues were observed in significantly greater amounts in enoxaparin. Oligosaccharides up to dp24 were observed in the ^{1}D chromatogram of enoxaparin. Oligosaccharides between dp3 and dp12 were transferred to the ^{2}D chromatography for further separation and MS analysis. The volume transferred from the ^{1}D to ^{2}D was 6 μL, and the cutting positions were all selected from the top of each peak in the ^{1}D chromatogram (Figure 3A). In enoxaparin, oligosaccharides bigger than dp12 showed lower content and MS intensity, so their MS signals lacked sufficient intensity for MS analysis. The assignment of structure was based on the known structural properties of enoxaparin. Each oligosaccharide in enoxaparin can be described using six numbers in square brackets corresponding to the number of unsaturated uronic acid residues (ΔUA, 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid), saturated uronic acid residues (IdoA or GlcA), glucosamine residues (GlcN), acetyl groups (Ac), sulfo groups (S) and anhydro sugar residues, respectively (Supporting Information Table S3).

Nine peaks were observed in the ^{2}D chromatogram of dp3. Most of these were trisaccharides, and their backbones are all composed of an unsaturated, a glucosamine, and a saturated uronic acid. Two major components with the composition of [1;1;1;0;4;0] represented more than 87% of the trisaccharides. The sequences of these two trisaccharides are different based on sulfo group location and/or the form of uronic acid present. The other components (less than 13%) include four trisaccharides with the same composition of [1;1;1;1;4;0] and three tetrasaccharides present at ∼5%. The compositions of these tetrasaccharides are [1;1;2;0;4;0] or [0;2;2;0;4;1] for peaks **1** and **5** and [1;1;2;0;4;1] for peak **6**. The GlcN residue, located at the middle of the sugar chain in the odd oligosaccharides, cannot be dehydrated to form 1,6-anhydro-structure, and dehydration only occurs at the nonreducing end during β-elimination (used to prepare enoxaparin) to form ΔUA. Thus, the assignment of these trisaccharides is unambiguous. In the case of oligosaccharides, with an even number of sugar residues, the composition and unambiguous assignment based on mass is not possible as dehydration could occur at either the nonreducing end, during β-elimination to form ΔUA or at the reducing end to form 1,6-anhydro-glucosamine, and these cannot be distinguished based on one-dimensional MS analysis. Thus, two possible compositions for each such even-numbered oligosaccharide are provided in the Supporting Information Table S3, except in cases where dehydration occurs at both reducing and nonreducing ends or at neither the reducing nor nonreducing ends.

Two peaks corresponding to tetrasaccharides of enoxaparin were observed at ∼29.8 and ∼30.1 min in the ^{1}D chromatogram. The different retention times imply their different sizes or shapes, which could result from different sequences of their backbones. Both of these were cut and analyzed with the ^{2}D chromatography and the ^{1}D peak at 29.8 min contained 6 tetrasaccharides while the ^{1}D peak at 30.1 min contained 15 tetrasaccharides. The oligosaccharides with contents more than 10% from the dp4 peak (30.1 min, ^{1}D) were peak **2** with the composition of [1;1;2;0;5;0] or [0;2;2;0;5;1], peak **8** and **9** both with the composition of [1;1;2;0;5;1], peak **12** with the composition of [1;1;2;0;6;0] or [0;2;2;0;6;1]. The oligosaccharides with the contents more than 10% from another dp4 peak (29.8 min, ^{1}D) are peak **3** and **4** with the composition of [1;1;2;0;6;0] or [0;2;2;0;6;1]. In all, 24 different tetrasaccharides were observed in enoxaparin.

Three peaks, containing four pentasaccharides, were observed with the compositions of [1;2;2;0;6;0] for peak **1**, [1;2;2;0;7;0] for peak **2**, and [0;3;2;0;7;0] + [1;2;2;1;8;0] for peak **3**. Their contents corresponded to 10.5, 85.4, and 4.1%, respectively.

Four major hexasaccharides with contents more than 10% were observed with the compositions of [1;2;3;0;8;1] for peaks **11** and **12** and [1;2;3;0;9;0] or [0;3;3;0;9;1] for peaks **13** and **14**. In all, a total of 17 hexasaccharides were observed.

In the ^{2}D chromatogram of dp8, 28 peaks were observed. The first three were assigned as heptasaccharides with the same composition of [1;3;3;0;10;1], accounting for 2.6%. Four major octasaccharides were observed with contents more than 10%, respectively. Their compositions are [1;3;4;0;11;0] or [0;4;4;0;11;1] for peak **19**, [1;3;4;0;11;1] for peak **21**, and [1;3;4;0;12;0] or [0;4;4;0;12;1] for peaks **24** and **25**.

In the ^{2}D chromatograms of dp10 and dp12, 25 and 19 peaks corresponding to deca and docecasaccharides were observed including four and five major oligosaccharides with contents higher than 10%, respectively (Figure 3B and Supporting Information Table S3). No oligosaccharides with an odd number of residues were observed in these peaks. The lower contents of odd-numbered oligosaccharides at higher dp values and/or the poor sensitivity of MS for larger oligosaccharides could be the reason for our failure to detect these.

The detailed assignments of all oligosaccharides in enoxaparin are labeled in Figure 3 B and listed in Supporting Information Table S3. Totally, 122 oligosaccharide peaks were observed from dp3 to dp12 in enoxaparin with good quality of MS signal. It is believed that many of these contain a single structure.

■ DISCUSSION AND CONCLUSIONS

Profiling analysis is important for developing a deeper structural insight into LMWHs. None of the previous oligosaccharide mapping methods provides as complete a picture, due to their relatively low resolution and the inherent complexity of LMWHs. In this work, a method with loop-based MHC-2D LC-Q/TOF MS was developed. This represents an efficient, automated, and robust method to profile LMWHs. SEC and IPRP are two dimensions that can most effectively separate LMWHs based on size, charge, and polarity. LMWHs separated by size alone afford complex mixtures of many components with different charges (number of sulfo groups) and different sequences (isomers). Thus, size separation alone, using ^{1}D chromatography (SEC), can result in the mis-assignment of many oligosaccharides. ^{2}D chromatography (IPRP) has higher resolution than SEC and separates oligosaccharides primarily by charge and polarity. However, if IPRP is used alone some components are severely overlapped, such as oligosaccharides with high dp and low sulfation and those with low dp and high sulfation (Figures 2B and 3B). The 2D LC method developed combines these two orthogonal modes of separation. The components with different size and the components with the same size but different sequences in LMWHs were all resolved and shown in a single analysis and structural information on each component provided using Q/TOF MS.

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In all, 84 oligosaccharides ranging from dp 6 to dp 14 were observed in nadroparin using this MHC-2D LC-Q/TOF MS method. The compositions of these oligosaccharides were all unambiguously assigned. Furthermore, there was a major structure (>50%) present within each dp grouping in nadroparin. This major structure is always the one with the highest number of sulfo group compared to other components of the same dp. Its sulfo group number follows the relationship: $s = n \times 3 - 1$, in which "n" equals to the number of disaccharides. In addition, no acetyl group was observed in the oligosaccharides with the highest sulfation, rather, a single acetyl group is commonly observed in oligosaccharides having relatively lower sulfation. Most oligosaccharides in nadroparin have an even number of sugar residues and contain a saturated uronic acid (IdoA or GlcA) at their nonreducing ends and 2,5-anhydro-mannitol at their reducing ends. Exceptions include minor (less than 6%) components, observed in dp6, in which several had intact glucosamine residues at the reducing end and contained three acetyl groups.

In total, 122 oligosaccharide peaks were observed from dp3 to dp12 of enoxaparin using this MHC-2D LC-Q/TOF MS method. The components in each dp of enoxaparin are more complicated than those observed in nadroparin. First of all, oligosaccharides having an odd- number of sugar residues, including dp3, dp5, and dp7, are present in enoxaparin but not in nadroparin. In the odd-numbered oligosaccharides, the numbers of uronic acid residues were always one more than the number of glucosamine residues. Larger oligosaccharides with an odd-number of sugar residues were not observed. The reason for this could be their lower contents at higher dp values and/or the poor sensitivity of MS detection for larger oligosaccharides. Since the positions of dehydration in enoxaparin can be at either the nonreducing end, during β-elimination affording a ΔUA or at the reducing end affording a 1,6-anhydro-glucosamine, it was not possible to unambiguously make MS assignments for many of the components of enoxaparin. The site of dehydration in the vast majority of enoxaparin oligosaccharides is the ΔUA at the nonreducing end of the chain. The oligosaccharides in enoxaparin include those having the highest sulfo group number (three sulfo groups per disaccharide), which were not observed in nadroparin. The nitrous acid releases the sulfo group from the N-sulfated glucosamine when heparin is converted to nadroparin, so the highest sulfo group number in nadroparin oligosaccharides is always $n \times 3 - 1$ (n = number of disaccharide). In contrast, the reaction to produce enoxaparin does not impact the sulfo groups in heparin. The number of sulfo groups in odd-number oligosaccharides was relatively low. Several major components present at each dp of enoxaparin included some oligosaccharides with the highest sulfo group number and some of oligosaccharides with the sulfo group number of $n \times 3 - 1$ (n = number of disaccharides). This result was different from that observed for nadroparin.

Thus, a fuller picture of the LMWH, such as nadroparin and enoxaparin, was obtained using MHC-2D LC−Q/TOF MS. Compared to previously reported oligosaccharide mapping using one-dimensional chromatography, this method provided much higher resolution and provides structural information on a greater number of the oligosaccharide components of these LMWHs.[22,24] It may be possible to analyze even larger oligosaccharides by MHC-2D with mass detection using an Orbitrap mass spectrometer. In addition, MHC-2D LC-Q/Tof MS provides analysis with high efficiency, simple optimization, robustness, and low contamination. MS/MS detection using MHC-2D might be applied to provide sequence information on the oligosaccharide components of LMWHs. The current method might be useful in the quality control of LMWHs and could also be a powerful tool for heparin-related glycomics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02218.

Preparation of nadroparin dp8, description of 2-D chromatographic conditions, investigation of pore size in ^{2}D columns, stability, cutting volume, and cutting position of ^{1}D chromatography, and tables of mass spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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