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Determination of Oversulfated Chondroitin Sulfate and Dermatan Sulfate in Heparin Sodium Using Anion-Exchange Chromatography with UV Detection

INTRODUCTION

Heparin, a complex sulfated glycosaminoglycan, is a well-known anticoagulant used to stop or prevent blood from clotting in certain types of surgeries and dialysis treatments.¹⁻³ In January 2008, the FDA was notified that patients using the heparin-active pharmaceutical ingredient experienced higher incidences of severe allergic reactions, including difficulty in breathing, nausea, vomiting, hypotension, and even death. An FDA investigation revealed that the heparin was adulterated with a highly sulfated version of chondroitin sulfate (a galactosamine glycosaminoglycan), subsequently referred to as oversulfated chondroitin sulfate (OSCS).^{4,5}

The assay for heparin sodium in the U.S. Pharmacopeia (USP) monograph measured heparin anti-clotting activity.⁶ This assay could not detect OSCS, which is believed to be synthetic. Therefore, the USP published a revision of the monograph (Stage 1 revision, USP 32) to address the immediate health issue by identifying OSCS in heparin by capillary electrophoresis (CE).⁷ Based on the public comments received from the Stage 1 revision, the USP replaced the CE method with an anion-exchange chromatography method (Identification B) in the Stage 2 revision. The chromatography method improves the resolution of heparin from impurities, such as OSCS and dermatan

sulfate.⁸ In addition, the USP added a high-performance anion-exchange with pulsed amperometric detection method (HPAE-PAD) to identify the percent of organic impurities (discussed in Application Note 233).⁹ The Stage 2 monograph is scheduled to be implemented in August 2009.

This Application Note (AN) describes the separation of dermatan sulfate and OSCS from heparin using anion-exchange chromatography (AE) with UV detection according to the USP Stage 2 revision. Dermatan sulfate, OSCS, and heparin sodium are separated on a 2 mm IonPac® AS11 anion-exchange column (USP L61 type column) using a gradient separation, with sodium phosphate monobasic and sodium perchlorate mobile phase solutions at 0.22 mL/min and detected by UV absorbance at 202 nm. This method takes advantage of the hydrophilic character and low capacity of the IonPac AS11 column stationary phase to separate 1% (v/v) concentrations of dermatan sulfate and OSCS from a large, single heparin peak. The column diameter and low flow rate minimizes mobile phase consumption and therefore, waste. The method ruggedness and precision are discussed in this AN. This method can detect <1% (v/v) dermatan sulfate and OSCS in adulterated heparin samples.

EQUIPMENT

Dionex ICS-3000 Chromatography system consisting of:

SP Single Pump or DP Dual Pump module with a GM-4 (P/N 049135, 2 mm) gradient mixer column

TC Thermal Compartment with a 6-port injection valve

VWD Variable Wavelength Detector 3400 with PEEK™ semi-micro flow cell, 2.5 µL, 7 mm (P/N 6074-0300)

AS Autosampler with Sample Tray Temperature Controlling option, 100 µL sample syringe (P/N 055064), and 1.5 mL sample tray

Chromeleon® 6.8 Chromatography Data System Bottles, 1 L or 2 L (two each), glass coated, GL45 (Dionex P/N 045900, 045901) for mobile phase solutions

Vial Kit, 1.5 mL glass with caps and septa (P/N 055427) or 0.3 mL polypropylene sample vials with caps and slit septa (P/N 055428)

Nalgene® Media-Plus with 90 mm, 0.45 µm nylon filter (Nalge Nunc International P/N 164-0020) or equivalent nylon filter

Vacuum pump

PEEK Tubing:

Black (0.25 mm or 0.01 in i.d., P/N 052306 for 5 ft) tubing: liquid line connections from the pump to the injection valve

Red (0.127 mm or 0.005 in i.d., P/N 052310 for 5 ft) tubing: liquid line connections from the injection valve to columns and cell.

10 µL PEEK sample loop (P/N 042949)

pH Meter with pH electrode

Magnetic stirrer

REAGENTS AND STANDARDS

Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity

Use ACS reagent grade chemicals for all reagents and standards, unless otherwise specified.

Heparin Sodium Identification Standard (9.3 mg; USP P/N 1304038)

Heparin Sodium System Suitability Standard (>90% heparin, <10% oversulfated chondroitin sulfate; 50 mg; USP P/N 1304049)

Chondroitin sulfate B (dermatan sulfate, sodium salt), sodium salt from porcine intestinal mucosa, >90% lyophilized powder (Sigma-Aldrich P/N C3788)

Sodium perchlorate, monohydrate ($\text{NaClO}_4 \cdot \text{H}_2\text{O}$, VWR International P/N EM-SX0693)

Sodium phosphate, monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, VWR International P/N JT3818)

Sodium phosphate, monobasic dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, VWR International P/N JT3819)

Phosphoric acid, 85–87% (H_3PO_4 , VWR International P/N JT0260)

pH 7 (yellow) buffer solution (VWR International P/N BDH5046) to calibrate the pH meter

pH 4 (red) buffer solution (VWR International P/N BDH5018) to calibrate the pH meter

SAMPLES

Heparin, sodium salt, grade 1A, porcine intestinal mucosa (Sigma-Aldrich P/N H3393)

Heparin, sodium salt (Sigma-Aldrich P/N H4784)

Chondroitin sulfate A, sodium salt, bovine trachea (Sigma-Aldrich P/N 9819)

CONDITIONS

Column: IonPac AG11 Guard, 2 × 50 mm
(P/N 044079)
IonPac AS11 Analytical, 2 × 250 mm
(P/N 044077)

Flow Rate: 0.22 mL/min

Mobile Phase: A: 2.6 mM Sodium phosphate
monobasic, pH = 3.0
B: 1.0 M Sodium perchlorate in 2.6 mM
sodium phosphate monobasic, pH = 3.0

Gradient: See Table 1

Column Temp.: 30 °C*

Tray Temp.: 10 °C

Inj. Volume: 10 µL

Detection: UV absorbance at 202 nm

Typical System
backpressure: 1100–1300 psi
Run time: 75 min

*Since the completion of this work, the USP has published a revision that raises the temperature to 40 °C.¹²

Table 1: Gradient Conditions

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Elution
0	80	20	Start linear gradient
60	10	90	End linear gradient. Start the linear gradient to the initial conditions
61	80	20	Equilibrate at the initial conditions
75	80	20	End run

PREPARATION OF SOLUTIONS AND REAGENTS

Caution: Sodium perchlorate is a strong oxidizer and is incompatible with reducing agents and flammable solvents. Review the Material Safety Data Sheets (MSDS) and consult local safety personnel for proper handling, safety precautions, and waste disposal.

General Tips

When preparing mobile phase solutions, it is essential to use high quality, Type 1 water, 18.2 MΩ-cm resistivity, deionized water. Prepare 1 L of degassed, deionized water weekly for the AS flush solution using vacuum filtration. Standard solutions and Mobile Phase A were prepared with deionized water (unless otherwise specified) according to the proposed USP Monograph described below.

Use glass containers whenever possible for storing and preparing mobile phase solutions to prevent UV-detectable leachable compounds from plastic containers that can result in increased detector noise and contaminant peaks.

3 M Phosphoric Acid for pH Adjustment

Add 1 mL of 85% phosphoric acid to 4 mL of deionized water. Mix thoroughly. This solution will be used to adjust the pH of both mobile phase solutions. Dispense 10–100 µL aliquots of 3 M phosphoric acid using a micropipettor with a filtered pipette tip to prevent the acid from damaging the micropipettor.

Mobile Phase Solutions

Mobile Phase A: 2.6 mM Sodium Phosphate Monobasic Dihydrate, pH = 3.0

Prepare 2 L of Mobile Phase A by weighing 0.80 g of sodium phosphate monobasic dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, FW 155.99) into a 2 L volumetric flask, dissolving it in ~1000 mL of deionized water, and then diluting to the mark with deionized water. Add a stir bar, stir with a magnetic stirrer while adjusting the pH to 3.0 using incremental 50 µL additions of 3 M H_3PO_4 to pH 3.2, and then smaller increments (5–20 µL) to pH 3.0. Typically, 300–350 µL of 3 M H_3PO_4 is used to adjust a 2 L solution to pH 3.0. Set aside 1 L as the diluent for Mobile Phase B to dissolve the sodium perchlorate reagent. The remaining 1 L will be filtered and degassed after Mobile Phase B is prepared.

Mobile Phase B: 1.0 M Sodium Perchlorate Monohydrate in 2.6 mM NaH₂PO₄ Dihydrate, pH = 3.0

Prepare 1 L of Mobile Phase B by weighing 140 g of sodium perchlorate monohydrate (NaClO₄ • H₂O, FW 140.46) into a 1 L volumetric flask, and dissolving the reagent in ~ 500 mL of pH-adjusted Mobile Phase A. The dissolution of sodium perchlorate is endothermic so allow at least 10 min to equilibrate to room temperature before diluting to 1 L with pH-adjusted Mobile Phase A. Add a stir bar and stir with a magnetic stirrer while adjusting the pH to 3.0 with 3 M H₃PO₄. Typically, 5–40 µL is used to adjust a 1 L solution to pH 3.0.

Filtering and Degassing Mobile Phase Solutions

Filter and degas each mobile phase separately using vacuum filtration with applied vacuum for 10 min. Transfer each mobile phase to a separate 1 L glass bottle, immediately cap the bottle, connect it to the corresponding mobile phase line, and place the mobile phase solutions under ~ 4–5 psi of nitrogen or other inert gas. Prime the pump with the new solutions and equilibrate the column for 1 h at the starting conditions prior to use.

Standard Stock Solutions

Stock solutions were prepared at higher concentrations than proposed by the USP to prepare the 1% (v/v) System Suitability standard.

Dermatan Sulfate

To prepare a 60 mg/mL dermatan sulfate stock solution, dissolve 30 mg of chondroitin sulfate B (dermatan sulfate) in 500 µL of deionized water.

Heparin System Suitability RS

This stock solution was prepared with USP System Suitability RS, the only reference standard containing OSCS available at the time of our experiments. To prepare a 60 mg/mL solution of USP System Suitability stock standard, dissolve 30 mg of the USP System Suitability RS in 500 µL of deionized water. The final solution was expected to contain approximately 54 mg/mL USP heparin and 6 mg/mL USP OSCS based on the composition of >90% heparin and <10% OSCS listed in the MSDS. This stock solution was used to prepare the 1% (v/v) System Suitability standard and to add OSCS to other standards and sample solutions.

Heparin Standard Solution

To prepare a 31 mg/mL heparin standard solution, dissolve the contents of the USP Heparin Sodium Identification RS (9.3 mg) ampoule with 300 µL of deionized water. Gently mix until fully dissolved. Transfer the standard solution into a 300 µL autosampler vial.

Standard Solutions

Dermatan Sulfate Standard Solution

A 20 mg/mL dermatan sulfate standard was prepared by adding 100 µL of the 60 mg/mL dermatan sulfate stock solution and 200 µL of deionized water into a 300 µL autosampler vial. Mix the solution thoroughly.

1% (v/v) System Suitability Solution

To prepare 300 µL of 1% (v/v) of dermatan sulfate and OSCS in 20 mg/mL heparin sodium standard solution, pipette 3 µL of the 20 mg/mL dermatan sulfate, 10 µL of the 60 mg/mL System Suitability stock solution, 176 µL of the 31 mg/mL heparin standard solution, and 111 µL deionized water into a 300 µL autosampler vial. Mix thoroughly. This solution can be prepared more easily when the OSCS RS is available by diluting 3 µL each of 20 mg/mL dermatan sulfate and OSCS in 294 µL of 20 mg/mL heparin standard solution. This 300 µL volume is sufficient for six full loop injections of 10 µL (2x the sample loop volume plus 25 µL used for small loops) or 15 partial loop injections of 10 µL from 25 µL loop with a 5 µL cut volume (2x cut volume plus the injection volume).

SAMPLE PREPARATION

Heparin Samples

To prepare 20 mg/mL heparin samples, first prepare a 60 mg/mL heparin solution by dissolving 30 mg of the (Sigma-Aldrich grade 1A or Sigma) heparin sample in 500 µL of deionized water, and then gently mixing until fully dissolved. Add 100 µL of 60 mg/mL heparin to 200 µL of deionized water and then transfer the 20 mg/mL heparin sample into a 300 µL autosampler vial.

To prepare 20 mg/mL heparin with 0.2 mg/mL dermatan sulfate and OSCS samples similar to the 1% (v/v) System Suitability standard solution, pipette 3 µL of 20 mg/mL dermatan sulfate, 10 µL of 60 mg/mL System Suitability stock solution, 273 µL of 20 mg/mL heparin sample, and 14 µL deionized water. Transfer the 1% (v/v) System Suitability solution to a 300 µL sample vial. These heparin samples contain 1.8 mg/mL of USP heparin with the remainder of heparin from the Sigma heparin sample.

SYSTEM PREPARATION AND SETUP

Plumbing the Chromatography System

To plumb the chromatography system, use black PEEK tubing (See description under Equipment section.) between the pump and injection valve, and red PEEK tubing after the injection valve (in the TC module) and before the flow cell. Install ~ 15 cm (~ 6 in) of black PEEK tubing from the pump to the GM-4 gradient mixer, and ~ 61 cm (~ 24 in) of black PEEK tubing from the gradient mixer to port P (or 2) in System 1 injection valve. Install the sample loop into the injection valve ports labeled L (1 and 4), the AS Autosampler transfer line into port S (or 5), and the autosampler waste line into port W (or 6). To install the IonPac AS11 column set, connect ~ 28 cm (~ 11 in) of red PEEK tubing into port C (or 3) followed by guard and the analytical column according to the product manual.¹⁰ Connect ~ 43 cm (~ 17 in) of red PEEK tubing from the end of the analytical column to the flow cell inlet. Install a 100 μ L sample syringe on the AS Autosampler for a 10 μ L injection. Enter the sample syringe and the sample loop sizes into the AS autosampler module under module setup menu and plumbing configuration.

Assembling the UV Semi-Micro PEEK Cell

Install the semi-micro PEEK flow cell according to the product manual.¹¹ To reduce noise, install the backpressure line from the flow cell kit on the flow cell outlet and before the waste line. After the mobile phase is flowing through the cell, turn on the cell and allow 60 min to warm up the lamp. To prevent salt build-up in the cell when the cell is inactive overnight or over the weekend, manually flush the cell with five 1 mL aliquots of deionized water using a 1 mL disposable syringe, as a precaution to prevent damage to the tubing.

The PEEK semi-micro flow cell is preferred over a stainless steel cell or standard cell because the PEEK material is inert to the mobile phase solutions and the smaller flow path of this cell is designed for the low flow rates used in this application.

Post-Acquisition, Background Subtraction

The gradient of sodium perchlorate causes a rise in the baseline of the chromatogram. If desired, one can subtract the baseline from a water injection by using the Chromeleon post-acquisition function, that is the *Arithmetic Combination*. The new chromatogram is

shown as a separate channel without overwriting the original data. Right click on the open chromatogram to select *Arithmetic Combination*. Channels A and B are the chromatograms of the sample and the water injection to be subtracted, respectively. Select *UV_Vis_1* for both chromatograms. For the most accurate subtraction, select the water sample injection closest to the sample injection. The subtracted chromatogram will be shown in a channel, typically labeled *Sub_UV_Vis_1_water_US_Vis_1* and will be automatically saved with the data file.

RESULTS AND DISCUSSION

Separation

Figure 1 demonstrates that 20 mg/mL of USP heparin is eluted from the 2 \times 250 mm IonPac AS11 anion exchange column within 35 min using 2.6 mM NaH_2PO_4 (pH 3) and 0.2 to 0.9 M NaClO_4 (pH 3) from 0 to 60 min at 0.22 mL/min. The baseline increases as the gradient increases. Heparin is the large broad peak eluting from 20 to 35 min with a maxima at 25.6 min. The width of this peak is attributed to the distribution of chain lengths of heparin in the heparin sodium sample and the variation in the number of sulfate groups per molecule.

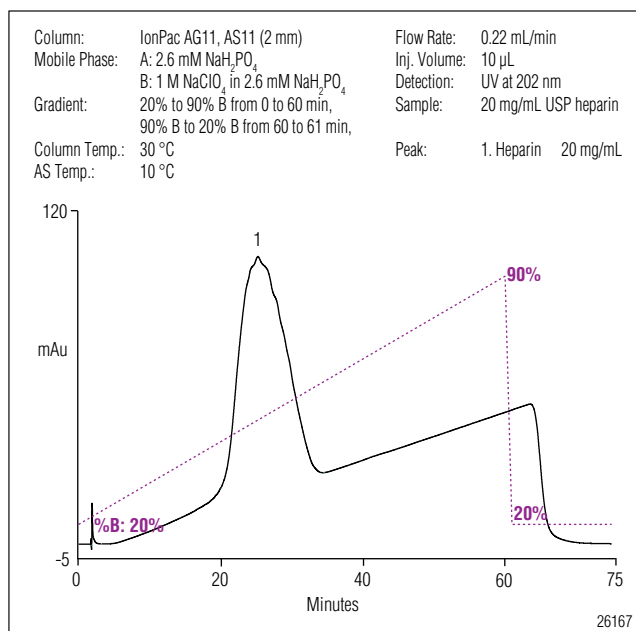


Figure 1. 20 mg/mL USP heparin identification standard.

Modifying the Mobile Phase Solutions

Initially, the analytes eluted 7–10 min earlier than expected when using mobile phase solutions prepared according to the proposed Stage 2 revision of the Identification B monograph. Two liters of Mobile Phase A were prepared with 0.80 g of the anhydrous salt of NaH_2PO_4 and two liters of Mobile Phase B were prepared in the same way with the same amount and type of sodium phosphate plus 280 g of the anhydrous salt of NaClO_4 (pH 3). To increase the retention of the analytes, we reduced the concentrations of the mobile phase solutions in collaboration with the USP. This was accomplished by adding the same weight of the monohydrated forms of each reagent as listed above without adjusting for the differences in formula weight. We also evaluated the use of the dihydrate salt of NaH_2PO_4 , which effectively reduced the molarity from 3.3 to 2.6 mM NaH_2PO_4 and from 1.1 to 1.0 M NaClO_4 , resulting in increased retention times and resolution of the other analytes from heparin. To further improve the consistency of the mobile phase preparation, 1 L of pH-adjusted Mobile Phase A from a 2 L preparation was used as the diluent to prepare 1 L of Mobile Phase B.

Sample Analysis

Figure 2 shows the separation of 0.2 mg/mL dermatan sulfate, with and without 20 mg/mL grade 1A heparin. In these chromatograms, we applied the background subtraction of a water injection to clearly demonstrate that dermatan sulfate has a smaller peak response when 20 mg/mL heparin is present. This lower response when heparin is present indicates that the heparin may be slightly overloading the column. The resolution of dermatan sulfate (1.1 ± 0.1 USP) in our experiments marginally meets the USP specification of NLT (not less than) 1.0. (In the later heparin monograph, the column temperature conditions were changed to 40 °C to improve resolution of dermatan sulfate and OSCS from heparin.)¹² These results show that the 20 mg/mL heparin peak starts eluting from the column within 2 min after the dermatan peak is eluted and further implies that dermatan sulfate may be unresolved from heparin at >20 mg/mL concentrations.

Figure 3B shows the separation of 0.2 mg/mL dermatan sulfate and OSCS from 20 mg/mL heparin in the 1% (v/v) System Suitability Standard. The resolution (USP) of heparin from the critical contaminant OSCS is 1.8 ± 0.1 , well within the USP specification of NLT 1.5.

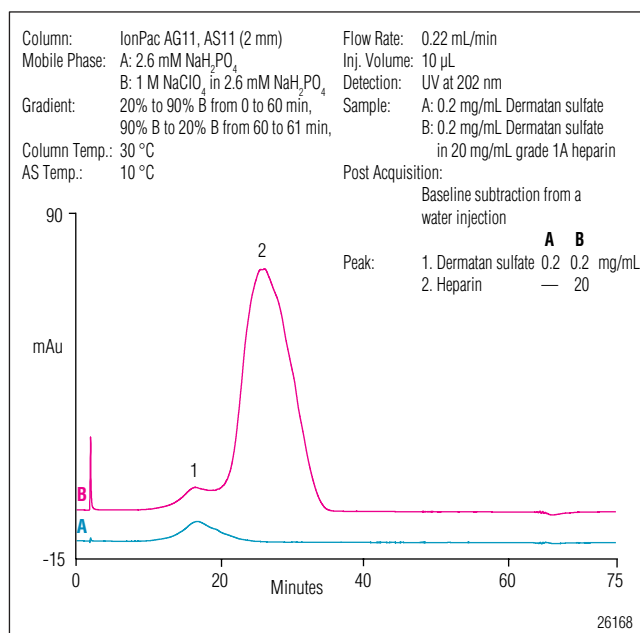


Figure 2. Comparison of 0.2 mg/mL dermatan sulfate A) without and B) with 20 mg/mL heparin.

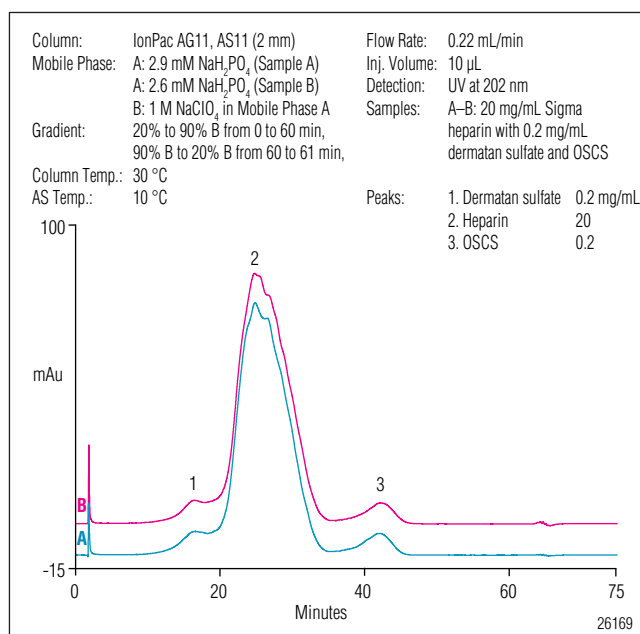


Figure 3. Comparison of 1% System Suitability sample separated with Mobile Phase A prepared with A) monohydrate and B) dihydrate salts of sodium phosphate monobasic.

However, the resolution of dermatan sulfate from heparin (1.1 ± 0.1) is marginally within the USP specification of NLT 1.0. The retention times of dermatan sulfate, heparin, and OSCS were 16.8 ± 0.6 , 25.1 ± 0.3 , and 41.7 ± 0.2 min ($n = 6$), respectively. These retention times are 3–8 min earlier than reported by the USP (20, 30, 50 min). To determine the elution of other glycosaminoglycans, we tested commercially available chondroitin sulfate A at 0.2 mg/mL in 20 mg/mL heparin (not shown). We found that chondroitin sulfate A elutes at the same time as dermatan sulfate.

Precision and Reproducibility

To evaluate the method precision, we determined the heparin peak area and retention time RSDs of dermatan sulfate, OSCS, and heparin with triplicate injections of 1% (v/v) System Suitability samples containing 0.2 mg/mL dermatan sulfate, 0.2 mg/mL OSCS, and 20 mg/mL heparin over three days with mobile phase solutions prepared daily. During this experiment, we used the monohydrate salt of NaH_2PO_4 . During this period of time, we evaluated the effect of the salt hydration form on resolution and retention times (Figure 3). The mobile phase solutions in these 3-day experiments were prepared with 0.8 g of NaH_2PO_4 per 2 L without adjusting for formula weight (2.9 mM NaH_2PO_4).

The retention times of the analytes were slightly lower with this mobile phase than with the mobile phase prepared with the dihydrate salt. The intra-day and between-day heparin peak area RSDs averaged 0.67 ± 0.12 and 0.60 respectively, well within the USP specification of NMT (not more than) 2. The intra-day retention time RSDs of dermatan sulfate, heparin, and OSCS were <1.2 for all three peaks, whereas the between-day RSDs were 3.6, 1.1, and 0.5 respectively. Although the monohydrate salt of NaH_2PO_4 was used in this experiment, we recommend the dihydrate salt of NaH_2PO_4 for mobile phase preparation (used for all other work in this AN except the column study, including the method ruggedness studies that follow). We did not repeat these experiments with using the dihydrated form of the salt to prepare the mobile phase.

Method Ruggedness

Effect of Mobile Phase pH

Mobile phase solutions prepared with the dihydrate salt of NaH_2PO_4 and monohydrate salt of NaClO_4 were adjusted to pH 2.9 and 3.1 to compare to similar solutions adjusted to pH 3.0. These differences in mobile phase pH had very little effect on retention times and a small effect on the resolution (USP) of dermatan sulfate (0.93 ± 0.06) and OSCS (1.68 ± 0.08) from heparin.

Column Reproducibility

Two IonPac AS11 columns were compared from the same lot using mobile phase solutions prepared from the monohydrate salts and performing triplicate injections of 0.2 mg/mL dermatan sulfate in 20 mg/mL grade 1A heparin. We found negligible differences in the retention times of dermatan sulfate (<0.7 min) and heparin (<1.2 min) and no change in resolution of dermatan sulfate from heparin (1.1 ± 0.1 USP).

Method Stability

The IonPac AS11 column life was evaluated for this method by performing 310 injections of heparin over 27 consecutive days. Injections of the 1% System Suitability standards were interspersed on average every 15 injections, to monitor the change in the resolution of dermatan sulfate and OSCS from heparin. This experiment showed that the IonPac AS11 column was not affected by the 310 heparin injections (Figure 4). The resolution of dermatan sulfate from heparin at the start of the experiment was 1.02 ± 0.07 ($n = 3$), which was similar to the resolution at the end of the experiment (1.09 ± 0.06 , $n = 3$). The resolution averaged 1.04 ± 0.07 ($n = 25$) for the experiment, marginally exceeding the USP specification (NLT 1). The resolution of heparin from OSCS was also similar at the beginning and end of the experiment with resolution values of 1.74 ± 0.07 ($n = 3$) and 1.84 ± 0.06 ($n = 3$), respectively. The average resolution was 1.76 ± 0.12 ($n = 25$). The retention time of heparin averaged 25.43 ± 0.52 min (2.0% RSD; $n = 310$). The changes in mobile phase solutions had minimal effect on the retention times. Communication with a customer since the completion of this work indicates that a column temperature of 40 °C increases both resolution factors.

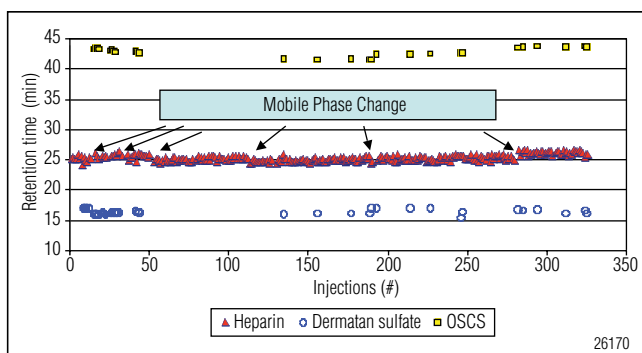


Figure 4. Retention time stability of 20 mg/mL heparin, 0.2 mg/mL dermatan sulfate, and 0.2 mg/mL OSCS over 27 days.

CONCLUSION

In this AN, 1% (v/v) dermatan sulfate and OSCS were separated from heparin using the USP Identification B method, Stage 2 revision of the Heparin Sodium monograph. This application takes advantage of the unique properties of the IonPac AS11 column to resolve dermatan sulfate and the critical OSCS contaminant from heparin, thereby allowing OSCS-contaminated heparin to be detected and quarantined from worldwide supplies. Additionally, the 2 mm column format provides the advantage of operating at low flow rates, which reduces the mobile phase consumption and waste.

PRECAUTIONS

Consistent mobile phase preparation is critical to consistent retention times and separation. Do not prepare >20 mg/mL concentrations of heparin in working solutions or standards, as dermatan sulfate may not be resolved.

SUPPLIERS

Sigma-Aldrich, Inc., P.O. Box 951524, Dallas, TX
75395-1524, 1-800-325-3010
www.sigmaaldrich.com

U.S. Pharmacopeia, 12601 Twinbrook Parkway,
Rockville, MD, USA 20852-1790
1-800-227-8772 www.usp.org

VWR International, Inc., Goshen Corporate Park West,
1310 Goshen Parkway, West Chester, PA 19380
1-800-932-5000 www.vwrsp.com

REFERENCES

- Goldstein, J. Making Heparin is a Dirty Job. *The WSJ*. February 21, 2008.
- Tyrrell, D.; Horne, A. P.; Holme, K. R.; Preuss, J. M. H.; Page, C. P. Heparin in Inflammation: Potential Therapeutic Applications Beyond Anticoagulation. *Adv. Pharmacol.* **1999**, *46*, 151–162.
- Rutty, C. J., Ph.D. Miracle Blood Lubricant: Connaught and the Story of Heparin *Contact* (Employee News of Connaught Laboratories Ltd.) **1996**, *9* (4), 1928–1937.
- FDA Press Release, Baxter's Multiple-Dose Vial Heparin Linked to Severe Allergic Reactions. Federal Drug Administration. February 11, **2008**.
- Guerrini, M.; Beccati, D.; Shriver, Z.; Naggi, A.; Viswanathan, K.; Bisio, A.; Capila, I.; Lansing, J. C.; Guglieri, S.; Fraser, B.; Al-Hakim, A.; Gunay, N. S.; Zhang, Z.; Robinson, L.; Buhse, L.; Nasr, M.; Woodcock, J.; Langer, R.; Venkataraman, G.; Linhardt, R. J.; Casu, B.; Torri, G.; Sasisekharan, R. Oversulfated Chondroitin Sulfate is a Contaminant in Heparin Associated with Adverse Clinical Events. *Nat. Biotechnol.* **2008**, *26*, 669–675.
- Heparin Sodium monograph in The United States Pharmacopeia 31, Supplement 2, NF 26; American Pharmaceutical Association, Washington, DC, 2008.
- Heparin Sodium monograph in The United States Pharmacopeia 32, Supplement 2, NF 25; American Pharmaceutical Association, Washington, DC, 2009.
- Heparin Sodium, *Pharmacoepial Forum* **2009**, *35* (2), 1–10.
- Dionex Corporation. *Determination of Organic Impurities in Heparin by HPAE-PAD using CarboPac PA20 Column*, Application Note 233; LPN 2286. Sunnyvale, CA, 2009.
- Dionex Corporation. *Product Manual for IonPac AG11 Guard and AS11 Analytical Column*; Document No. 034791. Sunnyvale, CA, 2006.
- Dionex Corporation. *Product Manual for ICS Series Variable Wavelength Detector*; Document No. 065141. Sunnyvale, CA, 2006.
- Heparin Sodium, *Pharmacoepial Forum* **2009**, *35* (5), 1–4.

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