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# Immunogenicity- Related Considerations for Low Molecular Weight Heparin Guidance for Industry

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)**

**February 2016  
Pharmaceutical Quality/CMC**

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## Guidance for Industry

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**U.S. Department of Health and Human Services**  
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*Contains Nonbinding Recommendations*

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# **Immunogenicity-Related Considerations for Low Molecular Weight Heparin Guidance for Industry<sup>1</sup>**

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

## **I. INTRODUCTION**

This guidance discusses immunogenicity-related considerations for low molecular weight heparin (LMWH) products and finalizes the draft guidance titled *Immunogenicity-Related Considerations for the Approval of Low Molecular Weight Heparin for NDAs and ANDAs* that was published on April 9, 2014 (79 FR 19621). Section II includes background information. Section IIIA includes recommendations on meeting the requirement for active ingredient sameness for abbreviated new drug applications (ANDAs) for LMWHs, which helps to address immunogenicity-related considerations in the context of ANDAs. Section IIIB includes recommendations on addressing impurities and their potential effect on immunogenicity for ANDAs. Section IIIB also includes recommendations on impurities for new drug applications (NDAs) and supplemental NDAs (sNDAs) or supplemental ANDAs (sANDAs)<sup>2</sup> in instances where the source material (Heparin Sodium, USP) or another component is changed, or when there are alterations in the manufacturing process for the LMWH either before or after approval of the LMWH drug application.<sup>3,4</sup> Drug Master File (DMF) holders should also be mindful of the recommendations in this guidance and ensure DMFs are current. DMF holders must notify authorized applicants of changes.<sup>5</sup>

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

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<sup>1</sup> This guidance has been prepared by the Office of Pharmaceutical Quality in the Center for Drug Evaluation and Research (CDER) at FDA.

<sup>2</sup> If you make changes to an approved NDA or ANDA, you can submit those changes in one of three ways, depending on whether the change is a minor change, a moderate change, or a major change (section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70 and 21 CFR 314.97). For LMWH products, generally any changes to the source material (Heparin Sodium USP) or other components are considered major changes and require a prior approval supplement (PAS). This type of change must be approved by FDA before you can distribute the modified drug product.

<sup>3</sup> This would occur, for example, when you have conducted clinical studies of a LMWH product for an NDA with a particular source of heparin, and, before approval of the NDA by FDA, you change the source of the heparin.

<sup>4</sup> See the guidances for industry *Immunogenicity Assessment for Therapeutic Protein Products* and the *International Conference on Harmonization, Q9 Quality Risk Management*.

<sup>5</sup> See 21 CFR 314.420.

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### **II. BACKGROUND**

LMWH products are anticoagulants used for the prevention and treatment of thrombosis (blood clots). These products are produced by depolymerization of the anticoagulant heparin, a complex, naturally occurring polysaccharide found in certain animal species and whose backbone consists of repeating disaccharide building blocks.<sup>6</sup> Treatment with heparin or LMWH products can be associated with a potentially fatal adverse event, heparin-induced thrombocytopenia (HIT). This occurs when the patient produces antibodies to heparin or LMWH in complex with the chemokine platelet factor (PF4), leading to irreversible aggregation and depletion of blood platelets (thrombocytopenia). The risk of HIT in patients treated with Lovenox (enoxaparin) and heparin sodium has been estimated to be 0.2 percent<sup>7</sup> and 2 – 3 percent, respectively.<sup>8</sup> Although the rate of HIT is relatively low for LMWHs, there are potentially serious consequences associated with HIT. Because LMWHs can be administered in out-patient settings, it is particularly important that applicants document how the risk of immunogenicity is assessed and managed. The review of LMWH applications includes a review of the immunogenicity-related information.

In general, clinical trials are used to assess the risk of immunogenicity for products approved under original NDAs. Clinical trials are also used in some cases to address the risk of immunogenicity following postapproval source-material or manufacturing changes for NDAs.<sup>9</sup> This guidance discusses an alternative approach that can be used, once the risk of immunogenicity has been evaluated through clinical trials in the first instance, to assess the effect of certain changes (including postapproval changes) on the product's immunogenicity risk. Because it is important that generic versions of LMWHs (i.e., products approved in ANDAs) are as safe and effective as their brand name counterparts, including immunogenicity, this guidance also provides recommendations on meeting the requirement for active ingredient sameness for ANDAs for LMWHs as well as addressing impurities and their effect on forming complexes with PF4 and eliciting an immune response.

For a product that is the subject of an ANDA or sANDA, the relevant reference product is the reference listed drug (RLD). For a product that is the subject of an NDA or sNDA, the relevant reference product in the case of postapproval changes is the approved LMWH product, or in the case of an original NDA for which manufacturing changes are made after completion of clinical trials, the product used in clinical trials.

The risk of immunogenicity for the LMWH products subject to this guidance<sup>10</sup> can be adequately characterized in comparison to their relevant reference products by addressing three principal critical elements: (1) the sameness of the active pharmaceutical ingredient (API) (ANDAs only); (2) the impurities in the product that may impact on the association of the LMWH product with the chemokine PF4, as well as the size and charge of the complexes formed with PF4 (NDAs/ANDAs); and (3) the impurities in the product that could modify the detection, uptake, processing or presentation of the product (or the complexes it forms with PF4) to the immune system

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<sup>6</sup> Depolymerization refers to the breaking (or cleavage) of polysaccharide chains into smaller oligosaccharide fragments by chemical or enzymatic means. Because LMWH chains are shorter than the parent heparin chains, in this draft guidance we generally use the term *oligosaccharides* in connection with LMWHs and the term *polysaccharides* in connection with heparin.

<sup>7</sup> Martel N, Lee J, Wells PS, 2005, Risk for Heparin-induced Thrombocytopenia with Unfractionated and Low-Molecular-Weight Heparin Thromboprophylaxis: A Meta-Analysis, *Blood*, 106:2710-2715.

<sup>8</sup> Arepally GM, and Ortel TL, 2010, Heparin-Induced Thrombocytopenia, *Annual Review of Medicine*, 61:77-90.

<sup>9</sup> See footnote 4.

<sup>10</sup> See the Introduction.

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(NDAs/ANDAs). The methods used can vary provided the methods used are sensitive to changes in the LMWH product. Because bioanalytical characterization may be insufficient to confirm these three critical elements as they relate to immunogenicity considerations, we also recommend using in vitro and/or in vivo studies of the immune system to detect differences in the LMWH product as compared to its relevant reference product. Differences in any of the principal elements described above between the LMWH product and its relevant reference product could suggest increased risk for immune responses and the need to perform other studies (e.g., clinical studies). Furthermore, as science and technology evolve, there may be different methods available for evaluating the immunogenicity of LMWH products. Similarly, the Agency's approval considerations may evolve based on greater scientific knowledge, such as a better understanding of potential causes of increased risk of immunogenicity of these products.

### **III. SUBMISSION RECOMMENDATIONS**

#### **A. Characterization of Active Ingredient Sameness (ANDAs only)**

A demonstration of the sameness of an active ingredient is critical to addressing the risk of immunogenicity in the context of ANDAs.

The characterization of sameness of the active ingredient or API<sup>11</sup> contained in the LMWH product and relevant reference product can be established by demonstrating equivalence with respect to the following: (1) physicochemical properties; (2) heparin source material and mode of depolymerization; (3) disaccharide building blocks, fragment mapping and sequence of oligosaccharide species; (4) biological and biochemical assay; and (5) in vivo pharmacodynamic profile.<sup>12,13</sup> The comparative approach we describe below has been shown to be sufficient to characterize the heterogeneity of the LMWH API. These criteria are highly sensitive to minor changes in manufacturing conditions and are able to identify differences in a number of attributes<sup>14</sup> among LMWH products found to meet relevant compendial standards (e.g., anti-Xa activity, anti-IIa activity, and anti-Xa/anti-IIa).

#### **Criterion 1: Equivalence of Physicochemical Properties**

You should characterize the relative abundance of oligosaccharides of different molecular weights in the LMWH API and the molecular weight distribution of the relevant reference product API to demonstrate equivalence. Such a comparison can be achieved by size exclusion chromatography (SEC), in conjunction with the method commonly referred to as the *chain mapping* method,<sup>15,16</sup> that

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<sup>11</sup> For purposes of this guidance “active ingredient” and API are used interchangeably.

<sup>12</sup> These five criteria and the basis by which they ensure sameness of the active ingredient were previously addressed in FDA’s response to the citizen petition pertaining to the approval of a generic version of Lovenox (enoxaparin sodium) injection. See pages 11 – 23 of the letter dated July 23, 2010, to Peter Safir, Covington & Burling, from Douglas Throckmorton, Deputy Director, CDER, available at <http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM220083.pdf>.

<sup>13</sup> Lee, S., Raw, A., Yu, L., Lionberger, R., Ya, Naiqi, Verthelyi, D., Rosenberg, A., Kozlowski, S., Webber, K., Woodcock, J., 2013, Scientific Considerations in the Review and Approval of Generic Enoxaparin in the United States, *Nature Biotechnology*, 31:220-226.

<sup>14</sup> These differences included, for example, the levels of modified disaccharide building blocks and sequences of some short oligosaccharides.

<sup>15</sup> Mourier, P.A.J., Viskov, C. 2004, Chromatographic Analysis and Sequencing Approach of Heparin Oligosaccharides Using Cetyltrimethylammonium Dynamically Coated Stationary Phases, *Analytical Biochemistry*, 332:299-313.

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provides complementary information on a fingerprint profile of oligosaccharide molecular weights at a higher resolution.

In addition to molecular weight, you should demonstrate equivalence for key features of the LMWH oligosaccharides by analyzing their overall chemical composition. These analyses should include nuclear magnetic resonance (NMR) analysis of characteristic structures, such as the epimerization state of the uronic acid structure (i.e., iduronic versus glucuronic acid) and the modified structures at the non-reducing end of the oligosaccharide chains, ultraviolet (UV) specific absorbance that demonstrates the presence of unique functional groups such as the  $\Delta^{4,5}$ -uronate structure of enoxaparin, and certain USP tests (e.g.,  $^{13}\text{C}$  NMR spectra, sodium content, and the ratio of sulfate to carboxylate).<sup>17</sup>

#### **Criterion 2: Equivalence of Heparin Source Material and Mode of Depolymerization**

The distribution of sequences of LMWH API is a function of both the sequences found naturally in the parent heparin and the site(s) where the cleavage reaction occurs in the polysaccharide chain. Chemical structures introduced at the terminal ends of the cleaved oligosaccharide chains are a result of the cleavage reaction by which the heparin polysaccharide chains are depolymerized into the LMWH API oligosaccharide chains. Because the diversity of disaccharide building block sequences within heparin results from its biosynthetic pathway, the use of equivalent heparin source material and equivalent methods of depolymerization is expected to ensure that LMWH API will be at least similar with respect to both the distribution of natural sequences of disaccharide units in the oligosaccharide chains and the diversity of the modified disaccharide building blocks at the terminal ends of the oligosaccharide chains. Therefore, you should use equivalent heparin source material (i.e., heparin derived from porcine intestinal mucosa and that meets USP monograph standards for heparin sodium). For equivalent mode of depolymerization, you should utilize the same depolymerization chemistry (e.g., alkaline  $\beta$ -elimination of the benzyl ester derivative of heparin for enoxaparin) to cleave the heparin polysaccharides, as used to manufacture the relevant reference product.

#### **Criterion 3: Equivalence in Disaccharide Building Blocks, Fragment Mapping, and Sequence of Oligosaccharide Species**

You should demonstrate equivalence in the identity and quantitative levels of the disaccharide building blocks,<sup>18</sup> including their modifications, between the LMWH API and its relevant reference product API. This can be achieved by exhaustive digestion of the LMWH API with purified digesting enzymes (e.g., heparinases I, II, and III) and/or chemical reagents (e.g., nitrous acid) to yield the disaccharide building blocks comprising the LMWH API. These disaccharide building blocks can then be separated and quantified by a variety of analytical approaches, such as capillary electrophoresis (CE), reversed-phase high performance liquid chromatography (RP-HPLC) and

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<sup>16</sup> Thanawiroon, C., Rice, K.G., Toida, T., Linhardt, R.J. 2004, Liquid Chromatography/Mass Spectrometry Sequencing Approach for Highly Sulfated Heparin Derived Oligosaccharides, *The Journal of Biological Chemistry*, 279:2608-2615.

<sup>17</sup> Keire DA, Buhse LF and al-Hakim, A, 2013, Characterization of currently marketed heparin products: Composition analysis by 2D-NMR, *Analytical Methods*, 5, 2984-2994, Ye H., Toby T.K., Sommers C.D., Ghasriani H., Trehy M.L., Ye W., Kolinski R.E., Buhse L.F., al-Hakim A. and Keire D.A., 2013, Characterization of currently marketed heparin products: key tests for LMWH quality assurance. *J. Pharm. Biomed. Anal.*, 85C:99-107.

<sup>18</sup> Other small oligosaccharide units that should be considered in this type of compositional analysis include trisaccharide units (which derive from LMWH oligosaccharides having an odd number of saccharide units) and tetrasaccharide units (which occur due to the inherent resistance of some tetrasaccharide units to further cleavage).

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strong anion exchange high performance liquid chromatography (SAX-HPLC).<sup>19,20,21</sup> The identification of these disaccharide building blocks can be achieved by using a combination of several techniques, including (but not limited to) comparison to structurally assigned disaccharide building blocks in the literature, mass spectroscopy (MS), NMR spectroscopy, and/or chemical approaches such as analysis with modifying reagents (e.g., sodium borohydride and nitrous acid) or modifying enzymes (e.g., 2-O-sulphatase, 6-O-sulphatase, and  $\Delta^{4,5}$ -glycuronidase).<sup>22,23, 24,25</sup>

You should demonstrate equivalence in the fragment map of digested oligosaccharides (representing the signature of recurring oligosaccharide sequences within the LMWH) between the LMWH API and its relevant reference product API. This can be achieved by partial digestion using enzymatic reagents that cleave in a structurally specific fashion (e.g., heparinase I), followed by a qualitative and quantitative analysis using sensitive analytical methods (e.g., RP-HPLC or SAX-HPLC).<sup>26,27</sup>

You should analyze sequences of a subset of oligosaccharides in the LMWH API and demonstrate their equivalence to those present in the relevant reference product API. The direct sequencing of oligosaccharides from the LMWH API can be done by isolating particular oligosaccharide species from the mixture through size and/or charge separation, and then analyzing their sequence using high-resolution analytical techniques (e.g., approaches based on property-encoded nomenclature (PEN) in conjunction with matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), iterative chemical and enzymatic digestion of fluorescent tagged oligosaccharides in conjunction with analysis by polyacrylamide gel electrophoresis, and/or enzymatic digestion in conjunction with NMR spectroscopy).<sup>28,29,30,31</sup> In this part of the evaluation, it is not necessary to

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<sup>19</sup> Sundarem, M., Qi, Y., Shriver, Z., Liu, D., Zhao, G., Venkataraman, G., Langer, R., Sasisekharan, R., 2003, Rational Design of Low-Molecular Weight Heparins with Improved In Vivo Activity, *Proc Natl Acad Sci USA*, 100:651-656.

<sup>20</sup> Toyoda, H., Yamamoto, H., Ogino, N., Toida, T., Imanari, T., 1999, Rapid and Sensitive Analysis in Heparin and Heparin Sulfate of Reversed-Phase Ion-Pair Chromatography on a 2 mm Porous Silica Gel Column, *J Chromatography, A* 830:197-201.

<sup>21</sup> Mourier P., Viskov C., June 2, 2005, Method for Determining Specific Groups Constituting Heparins or Low Molecular Weight Heparins, US Patent Application Publication US2005/0119477 A1.

<sup>22</sup> Ibid.

<sup>23</sup> Saad, O.M., Leary, J.A., 2003, Compositional Analysis and Quantification of Heparin and Heparin Sulfate by Electrospray Ionization Ion Trap Mass Spectrometry, *Analytical Chemistry*, 75:2985-2995.

<sup>24</sup> Stringer, S.E., Balbant, S.K., Pye, D.A., Gallagher, J.T., 2003, Heparin Sequencing, *Glycobiology*, 13(2):97-103.

<sup>25</sup> Myette, J.R., Shriver, Z., Ksiltepe, T., McLean, M.W., Venkataraman, G., Sasisekharan, R., 2002, Molecular Cloning of the Heparin/Heparin Sulfate  $\Delta$  4,5 Unsaturated Glycuronidase From Flavobacterium Heparinum, Its Recombinant Expression in Escherichia Coli, and Biochemical Determination of Its Unique Substrate Specificity, *Biochemistry*, 41:7424-7434.

<sup>26</sup> Linhardt, R.J., Rice, K.O., Kim, Y.S., Lohse, D.L., Wang, H.M., Loganathan, D., 1988, Mapping and Quantification of the Major Oligosaccharide Components of Heparin, *Biochemical Journal*, 254:781-787.

<sup>27</sup> Chuang, W.L., McAllister, H., Rabenstein, D.L., 2001, Chromatographic Methods for Product-Profile Analysis and Isolation of Oligosaccharides Produced by Heparinase-Catalyzed Depolymerization of Heparin, *Journal of Chromatography, A* 932:65-74.

<sup>28</sup> Sasisekharan, R. et al., 2000, Sequencing of 3-O Sulfate Containing Heparin Decasaccharides with a Partial Antithrombin III Binding Site, *P Natl Acad Sci USA*, 97:10359-10364.

<sup>29</sup> Sasisekharan, R., Venkataraman, G., Shriver, Z. & Raman, R., 1999, Sequencing Complex Polysaccharides, *Science*, 286:537-542.

<sup>30</sup> Turnbull, J.E., Hopwood, J.J. & Gallagher, J.T., 1999, A Strategy for Rapid Sequencing of Heparan Sulfate and Heparin Saccharides, *P Natl Acad Sci USA*, 96:2698-2703.

<sup>31</sup> Sugahara, K. et al., 1999, Structural Studies of Octasaccharides Derived from the Low-sulfated Repeating Disaccharide Region and Octasaccharide Serines Derived from the Protein Linkage Region of Porcine Intestinal Heparin, *Biochemistry*, 38:838-847.



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sequence all oligosaccharides in the LMWH API; the focus should be on sequencing short oligosaccharides that provide a sensitive measure of changes in process conditions.<sup>32</sup>

#### **Criterion 4: Equivalence in Biological and Biochemical Assays**

You should demonstrate that the LMWH API is equivalent to its relevant reference product API with respect to *in vitro* biological assays for relevant markers of anticoagulant activity, such as the activated partial thromboplastin time (aPTT) and the Heptest prolongation time. To meet the criterion of equivalence with respect to biochemical assays, you should demonstrate that the LMWH API is equivalent to its relevant reference product API in terms of factor Xa inhibition (anti-Xa) and factor IIa inhibition (anti-IIa).

#### **Criterion 5: Equivalence of In Vivo Pharmacodynamic Profile**

You should demonstrate equivalence in the *in vivo* pharmacodynamic profiles based upon measurements of *in vivo* plasma anti-Xa and anti-IIa activities. For this purpose, you should conduct a fasting, single-dose, two-way crossover *in vivo* study in normal subjects as described in FDA's individual drug product bioequivalence guidance for enoxaparin and dalteparin.<sup>33,34</sup>

#### **B. Impurities and Immunogenicity Risk (NDAs/sNDAs and ANDAs/sANDAs)**

##### *1. Studies Assessing the Interaction of LMWH with Platelet Factor 4*

Heparin-induced thrombocytopenia (HIT) is mediated by antibodies to the LMWH-PF4 complex. The primary immunogenicity risk factor associated with LMWHs is thought to involve the interaction of the active ingredient with PF4 and the presence of impurities may affect the interaction of the LMWH with PF4. Therefore, the association of the LMWH with PF4, as well as the size and charge of the LMWH-PF4 complexes formed under specified conditions, should be assessed and compared to that of the relevant reference product.

The characteristics of the complexes formed by LMWH with PF4 could be affected by the quality of the PF4 itself (e.g., its ability to form tetramers) and the ratio and concentration of the two components. Therefore, the association of the proposed LMWH and PF4 should be characterized at different ratios and concentrations. The ratios and concentrations selected should encompass those previously described in the literature as being immunogenic, including those that lead to the formation of ultra large complexes.<sup>35</sup> The methods used to assess association of the LMWH with PF4

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<sup>32</sup> Ozug, J.; Wudyka S.; Gunay N.S. et al. 2012, Structural elucidation of the tetrasaccharide pool in enoxaparin sodium, *Anal Bioanal Chem* 403:2733-2744.

<sup>33</sup> We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

<sup>34</sup> The general study design for the *in vivo* pharmacodynamic study described in the individual drug product bioequivalence guidances for enoxaparin and dalteparin are applicable to other similar LMWH products. See <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075207.htm>; Cochran S., Li P., Ferro V., 2009, A surface plasmon resonance-based solution affinity assay for heparan sulfate-binding proteins, *Glycoconj J.* 26:577–587; and Brandt S., Krauel K., Gottschalk K. E.; Renné T., Helm C.A., Greinacher A., Block S., 2014, Characterisation of the conformational changes in platelet factor 4 induced by polyanions: towards *in vitro* prediction of antigenicity, *Thromb Haemost*, 112: 53–64.

<sup>35</sup> Rauova, L, Poncz, M, McKenzie, SE et al., 2005, Ultra Large Complexes of PF4 and Heparin are Central to the Pathogenesis of Heparin-induced Thrombocytopenia. *Blood*, 05:131-8, and Suvarna S, Espinasse, B, Qi R, Lubica R,

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(e.g., electron microscopy, surface plasmon resonance or circular dichroism) should be sensitive to differences in the LMWH and the development and validation studies that support the suitability of the method(s) selected should be submitted to the application.

Because size and charge of the various complexes formed between PF4 and the LMWH are expected to change depending on the ratio and concentration of the two components, for each set of concentrations, the size and charge and relative concentrations of small, intermediate and ultra large complexes<sup>36,37</sup> formed should be characterized using suitable bioanalytical methods. Several methods may be needed to accurately characterize both small and large complexes. Examples of suitable methods include SEC-UV and SEC-multi-angle light scattering analysis, photon correlation spectroscopy, analytical ultracentrifugation, field flow fractionation, circular dichroism, and atomic force microscopy. The chosen method(s) should be shown to be suitable for identifying differences in the size and charge of the LMWH-PF4 complexes, and the development and validation studies that support the suitability of the method(s) selected should be submitted to the application. The results obtained should be confirmed using an orthogonal method, whenever possible.

#### *2. Characterization of Impurities*

Impurities in a LMWH can foster product immunogenicity by catalyzing changes in the product, acting as innate immune agonists, or changing the interaction of the LMWH with PF4. Impurities may be either process- or product-related. They can be of known structure, partially characterized, or unidentified.<sup>38</sup> You should characterize impurities (e.g., residual proteins, nucleic acids, and lipids) present in the LMWH that could potentially modify the detection, uptake, processing, or presentation of the LMWH, or the complexes it forms with PF4, to the immune system. Studies should demonstrate that the LMWH is free of such impurities or contains similar levels and quality of such substances as its relevant reference product.

FDA recommends three complementary approaches using a variety of suitable bioanalytical methods to address impurities: (1) testing the LMWH, as well as the unfractionated heparin source material and other raw materials for the presence of impurities (e.g., proteins, lipids, and nucleic acids);<sup>39</sup> (2) assessing the capacity of the manufacturing process to remove potential impurities; and (3) characterizing the amount and nature of product impurities in the LMWH relative to those in its relevant reference product. The chosen method(s) should be shown to be suitable for identifying impurities and the development and validation studies supporting the suitability of the method(s) selected should be submitted to the application.

In addition, information should be provided on extractables and leachables from the container closure system over the shelf life of the LMWH product.

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Poncz M, Cines D B, Wiesner M R, and Arepally G M, 2007, Determinants of PF4/heparin immunogenicity, *Blood*, 110: 4253.

<sup>36</sup> Greinacher, A, Alban, S, Omer-Adam, M A et. al., 2008, Heparin-Induced Thrombocytopenia: A Stoichiometry-Based Model to Explain the Differing Immunogenicities of Unfractionated Heparin, Low-Molecular-Weight Heparin, and Fondaparinux in Different Clinical Settings, *Thrombosis Research*, TR-03320.

<sup>37</sup> Suvarna, S, QI R, and Arepally, G M, 2009, Optimization of a Murine Immunization Model for Study of PF4/Heparin Antibodies, *Journal of Thrombosis and Haemostasis*, 7:857–864.

<sup>38</sup> International Conference on Harmonisation guidances for industry *Q3A (R2) Impurities in New Drug Substances and Q3B (R2) Impurities in New Drug Products*.

<sup>39</sup> While these impurities are usually controlled during the preparation of unfractionated heparin sodium, the data should be submitted to FDA for review.

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### ***3. Use of In Vitro and In Vivo Immunological Models***

The immune system can be an effective tool to detect small changes in product impurities and active ingredients that are missed by current analytical methods. Assessment of multiple parameters of immune activation and characterization of the immune response elicited by the LMWH and its relevant reference product using in vitro and/or in vivo models may complement other bioanalytical techniques designed to assess the potential of the LMWH to generate greater immune responses as compared to its relevant reference product. The chosen method(s) should be shown to be suitable for detecting changes in product impurities and the LMWH and the development and validation studies that support the suitability of the method(s) selected should be submitted to the application.

### ***4. Selection and Specification of Product Lots Used for Studies to Assess the Risk of Immunogenicity***

For each lot of the LMWH used in the experiments in comparison to the relevant reference product, the documentation should include the identification name, date, and site of manufacture; manufacturing process (if more than one exists); container closure system; and results from the release and stability testing. The rationale for the selection of lots should be provided. We also recommend that you provide to FDA data on multiple freshly manufactured, mid-expiry-cycle, and close-to-expiry product lots for the LMWH, and similar-stage lots of the relevant reference product.