# Guidance for Industry

## Immunogenicity-Related Considerations for the Approval of Low Molecular Weight Heparin for NDAs and ANDAs

***DRAFT GUIDANCE***

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

**April 2014**
**CMC**

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**U.S. Department of Health and Human Services**
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## TABLE OF CONTENTS

**I. INTRODUCTION ..... 1**

**II. BACKGROUND ..... 2**

**III. SUBMISSION RECOMMENDATIONS ..... 3**

**A. Characterization of Active Ingredient Sameness (ANDAs only) ..... 3**

**B. Impurities and Immunogenicity Risk (NDAs/ANDAs) ..... 6**

*1. Studies Assessing the Interaction of LMWH with PF4 ..... 6*

*2. Characterization of Impurities ..... 7*

*3. Use of In Vitro and In Vivo Immunological Models ..... 8*

*4. Selection and Specification of Product Lots Used for Studies to Assess the Risk of Immunogenicity ..... 8*

# Guidance for Industry[1]
# Immunogenicity-Related Considerations for the Approval of Low Molecular Weight Heparin for NDAs and ANDAs

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

## I. INTRODUCTION

This draft guidance discusses immunogenicity-related approval considerations for low molecular weight heparin (LMWH) products. 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)[2] 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 approval of the LMWH[3] or after approval of the LMWH. 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.[4]

FDA's guidance documents, including this guidance, 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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[1] This guidance has been prepared by the Office of Pharmaceutical Science in the Center for Drug Evaluation and Research (CDER) at FDA.

[2] 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 generally 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.

[3] 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.

[4] See 21 CFR 314.420.

37 **II. BACKGROUND**
38
39 LMWH products are anticoagulants used for prevention and treatment of thrombosis (blood clots).
40 These products are produced by depolymerization of the anticoagulant heparin; complex, naturally
41 occurring polysaccharides found in certain animal species and whose backbone consists of repeating
42 disaccharide building blocks.[5] Treatment with heparin or LMWH products is associated with a
43 potentially fatal adverse event, heparin-induced thrombocytopenia (HIT). This occurs when the
44 patient produces antibodies to heparin or LMWH in complex with the chemokine platelet factor
45 (PF4), leading to irreversible aggregation and depletion of blood platelets (thrombocytopenia). In the
46 clinical trials supporting the approval of the LMWH Lovenox, HIT occurred in 1.3 percent of patients
47 receiving Lovenox, 1.2 percent of patients receiving heparin sodium, and 0.7 percent of patients
48 receiving placebo.[6] However, in subsequent reports, the risk of HIT in patients treated with Lovenox
49 and heparin sodium was estimated to be 0.2 percent[7] and 2 – 3 percent, respectively.[8] Although the
50 rate of HIT is relatively low for LMWHs, there are potentially serious consequences associated with
51 HIT. Because LMWHs can be administered in out-patient settings, it is particularly important that
52 applicants document how the risk of immunogenicity is assessed and managed. The review of
53 LMWH applications includes a review of the immunogenicity-related information.
54
55 In general, clinical trials are used to assess the risk of immunogenicity for products approved under
56 original NDAs. Clinical trials are also used in some cases to address the risk of immunogenicity
57 following postapproval source-material or manufacturing changes for NDAs. This guidance discusses
58 an alternative approach that can be used, once the risk of immunogenicity has been evaluated through
59 clinical trials in the first instance, to assess the effect of certain changes (including postapproval
60 changes) on the product's immunogenicity risk. Because it is important that duplicate versions of
61 LMWHs (i.e., ANDAs) are as safe and effective as their brand name counterparts, including
62 immunogenicity, this guidance also provides recommendations on meeting the requirement for active
63 ingredient sameness for ANDAs for LMWHs as well as addressing impurities and their effect on
64 forming complexes with PF4 and eliciting an immune response.
65
66 For a product that is the subject of an ANDA or sANDA, the relevant reference product is the
67 reference listed drug (RLD). For a product that is the subject of an NDA or sNDA, the relevant
68 reference product in the case of postapproval changes is the approved LMWH product; or in the case
69 of an original NDA for which manufacturing changes are made after completion of clinical trials, the
70 product used in clinical trials.
71
72 The risk of immunogenicity for the LMWH products subject to this draft guidance[9] can be adequately
73 characterized in comparison to their relevant reference products by addressing three principal critical
74 elements: (1) the sameness of the active pharmaceutical ingredient (API) (ANDAs only); (2) the
75 impurities in the product that may impact on the association of the LMWH product with the

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[5] 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.

[6] See WARNINGS AND PRECAUTIONS section of Lovenox Product labeling, NDA-20-164, revised June 2013.

[7] 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.

[8] Arepally GM, and Ortel TL, 2010, Heparin-Induced Thrombocytopenia, Annual Review of Medicine, 61:77-90.

[9] See the Introduction.

76 chemokine PF4, as well as the size and charge of the complexes formed with PF4 (NDAs/ANDAs);
77 and (3) the impurities in the product that could modify the detection, uptake, processing or
78 presentation of the product (or the complexes it forms with PF4) to the immune system
79 (NDAs/ANDAs). The methods used can vary provided the methods used are sensitive to changes in
80 the LMWH product. Because bioanalytical characterization may be insufficient to confirm these three
81 critical elements as they relate to immunogenicity considerations, we also recommend using in vitro
82 and/or in vivo studies of the immune system to detect differences in the LMWH product as compared
83 to its relevant reference product. Differences in any of the principal elements described above
84 between the LMWH product and its relevant reference product could suggest increased risk for
85 immune responses and the need to perform other studies (e.g., clinical studies). Furthermore, as
86 science and technology evolve, there may be different methods available for evaluating the
87 immunogenicity of LMWH products. Similarly, the Agency's approval considerations may evolve
88 based on greater scientific knowledge, such as a better understanding of potential causes of increased
89 risk of immunogenicity of these products.
90
91 ## III. SUBMISSION RECOMMENDATIONS
92
93 ### A. Characterization of Active Ingredient Sameness (ANDAs only)
94
95 A demonstration of the sameness of an active ingredient is critical to addressing the risk of
96 immunogenicity in the context of ANDAs.
97
98 The characterization of sameness of the active ingredient or API[10] contained in the LMWH product
99 and relevant reference product can be established by demonstrating equivalence with respect to the
100 following: (1) physicochemical properties; (2) heparin source material and mode of
101 depolymerization; (3) disaccharide building blocks, fragment mapping and sequence of
102 oligosaccharide species; (4) biological and biochemical assay; and (5) in vivo pharmacodynamic
103 profile.[11,12] The comparative approach we describe below has been shown to be sufficient to
104 characterize the heterogeneity of the LMWH API. These criteria are highly sensitive to minor
105 changes in manufacturing conditions and able to identify differences in a number of attributes[13]
106 among LMWH products found to meet relevant compendial standards (e.g., anti-Xa activity, anti-IIa
107 activity, and anti-Xa/anti-IIa).
108
109 **Criterion 1: Equivalence of Physicochemical Properties**
110

[10] For purposes of this guidance "active ingredient" and API are used interchangeably.
[11] 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.
[12] 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.
[13] These differences included, for example, the levels of modified disaccharide building blocks and sequences of some short oligosaccharides.

111 You should characterize the relative abundance of oligosaccharides of different molecular weights in
112 the LMWH API and the molecular weight distribution of the relevant reference product API to
113 demonstrate equivalence. Such a comparison can be achieved by size exclusion chromatography
114 (SEC), in conjunction with the method commonly referred to as the *chain mapping* method[14,15] that
115 provides complementary information on a fingerprint profile of oligosaccharide molecular weights at
116 a higher resolution.
117
118 In addition to molecular weight, you should demonstrate equivalence for key features of the LMWH
119 oligosaccharides by analyzing their overall chemical composition. These analyses should include
120 nuclear magnetic resonance (NMR) analysis of characteristic structures, such as the epimerization
121 state of the uronic acid structure (i.e., iduronic versus glucuronic acid) and the modified structures at
122 the non-reducing end of the oligosaccharide chains, ultraviolet (UV) specific absorbance that
123 demonstrates the presence of unique functional groups such as the $\Delta^{4,5}$-uronate structure of
124 enoxaparin, and certain USP tests (e.g., $^{13}C$ NMR spectra, sodium content, and the ratio of sulfate to
125 carboxylate).
126
127 **Criterion 2: Equivalence of Heparin Source Material and Mode of Depolymerization**
128
129 The distribution of sequences of LMWH API is a function of both the sequences found naturally in
130 the parent heparin and the site(s) where the cleavage reaction occurs in the polysaccharide chain.
131 Chemical structures introduced at the terminal ends of the cleaved oligosaccharide chains are a result
132 of the cleavage reaction by which the heparin polysaccharide chains are depolymerized into the
133 LMWH API oligosaccharide chains. Because the diversity of disaccharide building block sequences
134 within heparin results from its biosynthetic pathway, the use of equivalent heparin source material
135 and equivalent methods of depolymerization is expected to ensure that LMWH API will be at least
136 similar with respect to both the distribution of natural sequences of dissacharide units in the
137 oligosaccharide chains and the diversity of the modified disaccharide building blocks at the terminal
138 ends of the oligosaccharide chains. Therefore, as the applicant, you should use equivalent heparin
139 source material (i.e., heparin derived from porcine intestinal mucosa and that meets USP monograph
140 standards for heparin sodium). For equivalent mode of depolymerization, you should utilize the same
141 depolymerization chemistry (e.g., alkaline β-elimination of the benzyl ester derivative of heparin for
142 enoxaparin) to cleave the heparin polysaccharides, as used to manufacture the relevant reference
143 product.
144
145 **Criterion 3: Equivalence in Disaccharide Building Blocks, Fragment Mapping, and**
146 **Sequence of Oligosaccharide Species**
147
148 You should demonstrate equivalence in the identity and quantitative levels of the disaccharide
149 building blocks,[16] including their modifications, between the LMWH API and its relevant reference
150 product API. This can be achieved by exhaustive digestion of the LMWH API with purified

[14] 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.

[15] 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.

[16] 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).

151 digesting enzymes (e.g., heparinases I, II, and III) and/or chemical reagents (e.g., nitrous acid) to
152 yield the disaccharide building blocks comprising the LMWH API. These disaccharide building
153 blocks can then be separated and quantified by a variety of analytical approaches, such as capillary
154 electrophoresis (CE), reversed-phase high performance liquid chromatography (RP-HPLC) and
155 strong anion exchange high performance liquid chromatography (SAX-HPLC).[17,18,19] The identification
156 of these disaccharide building blocks can be achieved by using a combination of several techniques,
157 including (but not limited to) comparison to structurally assigned disaccharide building blocks in the
158 literature, mass spectroscopy (MS), NMR spectroscopy, and/or chemical approaches such as analysis
159 with modifying reagents (e.g., sodium borohydride and nitrous acid) or modifying enzymes (e.g., 2-
160 O-sulphatase, 6-O-sulphatase, and $\Delta^{4,5}$-glycuronidase).[20,21, 22,23]
161
162 You should demonstrate equivalence in the fragment map of digested oligosaccharides (representing
163 the signature of recurring oligosaccharide sequences within the LMWH) between the LMWH API
164 and its relevant reference product API. This can be achieved by partial digestion using enzymatic
165 reagents that cleave in a structurally specific fashion (e.g., heparinase I), followed by a qualitative
166 and quantitative analysis using sensitive analytical methods (e.g., RP-HPLC or SAX-HPLC).[24,25]
167
168 You should analyze sequences of a subset of oligosaccharides in the LMWH API and demonstrate
169 their equivalence to those present in the relevant reference product API. The direct sequencing of
170 oligosaccharides from the LMWH API can be done by isolating particular oligosaccharide species
171 from the mixture through size and/or charge separation, and then analyzing their sequence using
172 high-resolution analytical techniques (e.g., approaches based on property-encoded nomenclature
173 (PEN) in conjunction with matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-
174 MS), iterative chemical and enzymatic digestion of fluorescent tagged oligosaccharides in
175 conjunction with analysis by polyacrylamide gel electrophoresis, and/or enzymatic digestion in
176 conjunction with NMR spectroscopy).[26,27,28,29] In this part of the evaluation, it is not necessary to

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[17] 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.
[18] 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.
[19] 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.
[20] Ibid.
[21] 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.
[22] Stringer, S.E., Balbant, S.K., Pye, D.A., Gallagher, J.T., 2003, Heparin Sequencing, Glycobiology, 13(2):97-103.
[23] Myette, J.R., Shriver, Z., Kisiltepe, T., McLean, M.W., Venkataraman, G., Sasisekharan, R., 2002, Molecular Cloning of the Heparin/Heparin Sulfate Δ 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.
[24] 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.
[25] 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.
[26] 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.
[27] Sasisekharan, R., Venkataraman, G., Shriver, Z. & Raman, R., 1999, Sequencing Complex Polysaccharides, Science, 286:537-542.

177 sequence all oligosaccharides in the LMWH API; the focus should be on sequencing short
178 oligosaccharides that provide a sensitive measure of changes in process conditions.[30]
179
180 **Criterion 4: Equivalence in Biological and Biochemical Assays**
181
182 You should demonstrate that the LMWH API is equivalent to its relevant reference product API with
183 respect to in vitro biological assays for relevant markers of anticoagulant activity, such as the
184 activated partial thromboplastin time (aPPT) and the Heptest prolongation time. To meet the criterion
185 of equivalence with respect to biochemical assays, you should demonstrate that the LMWH API is
186 equivalent to its relevant reference product API in terms of factor Xa inhibition (anti-Xa) and factor
187 IIa inhibition (anti-IIa).
188
189 **Criterion 5: Equivalence of In Vivo Pharmacodynamic Profile**
190
191 You should demonstrate equivalence in the in vivo pharmacodynamic profiles based upon
192 measurements of in vivo plasma anti-Xa and anti-IIa activities. For this purpose, you should conduct
193 a fasting, single-dose, two-way crossover in vivo study in normal subjects as described in FDA's
194 individual drug product bioequivalence guidance for enoxaparin and dalteparin.[31,32]
195
196 **B. Impurities and Immunogenicity Risk (NDAs/sNDAs and ANDAs/sANDAs)**
197
198 *1. Studies Assessing the Interaction of LMWH with PF4*
199
200 Heparin-induced thrombocytopenia (HIT) is mediated by antibodies to the LMWH-PF4 complex.
201 The primary immunogenicity risk factor associated with LMWHs is thought to involve the interaction
202 of the active ingredient with PF4 and the presence of impurities may affect the interaction of the
203 LMWH with PF4. Therefore, the association of the LMWH with PF4, as well as the size and charge
204 of the LMWH-PF4 complexes formed under specified conditions, should be assessed and compared
205 to that of the relevant reference product.
206
207 The characteristics of the complexes formed by LMWH with PF4 could be affected by the ratio and
208 concentration of the two components and; therefore, the association of the proposed LMWH and PF4
209 should be characterized at different ratios and concentrations. The ratios and concentrations selected
210 should encompass those previously described in the literature as being immunogenic, including those

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[28] 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.
[29] 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.
[30] 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.
[31] 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.
[32] 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.

211 that lead to the formation of ultralarge complexes.[33] The methods used to assess association of the
212 LMWH with PF4 (e.g., surface plasmon resonance) should be sensitive to differences in the LMWH
213 and the development and validation studies that support the suitability of the method(s) selected
214 should be submitted to the application.
215
216 Because size and charge of the various complexes formed between PF4 and the LMWH are expected
217 to change depending on the ratio and concentration of the two components, for each set of
218 concentrations, the size and charge and relative concentrations of small, intermediate and ultralarge
219 complexes[34,35] formed should be characterized using suitable bioanalytical methods. Several
220 methods may be needed to accurately characterize both small and large complexes. Suitable methods
221 include SEC-UV and SEC-multi-angle light scattering analysis, photon correlation spectroscopy,
222 analytical ultracentrifugation, field flow fractionation, and atomic force microscopy. The chosen
223 method(s) should be shown to be suitable for identifying differences in the size and charge of the
224 LMWH-PF4 complexes, and the development and validation studies that support the suitability of the
225 method(s) selected should be submitted to the application. The results obtained should be confirmed
226 using an orthogonal method, whenever possible.
227
228 *2. Characterization of Impurities*
229
230 Impurities in a LMWH can foster product immunogenicity by catalyzing changes in the product,
231 acting as innate immune agonists, or changing the interaction of the LMWH with PF4. Impurities
232 may be either process or product related. They can be of known structure, partially characterized, or
233 unidentified.[36] You should characterize impurities (e.g., residual proteins, nucleic acids, and lipids)
234 present in the LMWH that could potentially modify the detection, uptake, processing, or presentation
235 of the LMWH, or the complexes it forms with PF4, to the immune system. Studies should
236 demonstrate that the LMWH is free of such impurities or contains similar levels and quality of such
237 substances as its relevant reference product.
238
239 FDA recommends three complementary approaches using a variety of suitable bioanalytical methods
240 to address impurities: (1) testing the LMWH, as well as the unfractionated heparin source material
241 and other raw materials for the presence of impurities (e.g., proteins, lipids, and nucleic acids);[37] (2)
242 assessing the capacity of the manufacturing process to remove potential impurities; and (3)
243 characterizing the amount and nature of product impurities in the LMWH relative to those in its
244 relevant reference product. The chosen method(s) should be shown to be suitable for identifying
245 impurities and the development and validation studies supporting the suitability of the method(s)
246 selected should be submitted to the application.
247

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[33] 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.

[34] 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.

[35] 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.

[36] International Conference on Harmonisation guidances for industry *Q3A (R2) Impurities in New Drug Substances and Q3B (R2) Impurities in New Drug Products*.

[37] However, these impurities are usually controlled during the preparation of unfractionated heparin sodium.

248 In addition, information should be provided on extractables and leachables from the container closure
249 system over the shelf life of the LMWH product.
250
251 *3. Use of In Vitro and In Vivo Immunological Models*
252
253 The immune system can be an effective tool to detect small changes in product impurities and active
254 ingredients that are missed by current analytical methods. Assessment of multiple parameters of
255 immune activation and characterization of the immune response elicited by the LMWH and its
256 relevant reference product using in vitro and/or in vivo models may complement other bioanalytical
257 techniques designed to assess the potential of the LMWH to generate greater immune responses as
258 compared to its relevant reference product. The chosen method(s) should be shown to be suitable for
259 detecting changes in product impurities and the LMWH and the development and validation studies
260 that support the suitability of the method(s) selected should be submitted to the application.
261
262 *4. Selection and Specification of Product Lots Used for Studies to Assess the Risk*
263 *of Immunogenicity*
264
265 For each lot of the LMWH used in the experiments in comparison to the relevant reference product,
266 the documentation should include the identification name, date, and site of manufacture;
267 manufacturing process (if more than one exists); container closure system; and results from the
268 release and stability testing. The rationale for the selection of lots should be provided. We also
269 recommend that you provide to FDA freshly manufactured, mid-expiry-cycle, and close-to-expiry
270 product lots for the LMWH, and similar-stage lots of the relevant reference product.
271
272