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September 26, 2005

SEP 26 2005

BY HAND DELIVERY

Dockets Management Branch (HFA-305)
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

CITIZEN PETITION SUPPLEMENT No. 2 **(03P-0064)**

The undersigned, on behalf of Aventis Pharmaceuticals, Inc., a subsidiary of sanofi-aventis, successor in interest to Aventis Pharmaceuticals, SA ("sanofi-aventis") submits this Supplement No. 2 (the "Supplement") to its Citizen Petition filed February 19, 2003 (03P-0064/CP1) (the "Citizen Petition") and Supplement No. 1 filed February 13, 2004 (03P-0064/Supp1) ("Supplement No. 1").¹ The Citizen Petition requests that the Commissioner of Food and Drugs withhold approval of any abbreviated new drug application ("ANDA") for a generic version of Lovenox[®] (enoxaparin sodium injection) ("enoxaparin") until the conditions set forth in the Citizen Petition are satisfied. This Supplement is submitted under sections 505(b) and 505(j) of the Federal Food, Drug, and Cosmetic Act ("FDCA" or the "Act") (21 U.S.C. §§ 355 (b) and (j)) and 21 C.F.R. § 10.30.

This Supplement serves two primary purposes. Part I provides important new information about the distinctive biological properties of enoxaparin. These newly discovered features are a product of sanofi-aventis' manufacturing process for enoxaparin, and may have clinical significance. This further underscores the importance of withholding approval of any ANDA for a generic enoxaparin product made with a manufacturing process that is not equivalent to sanofi-aventis' process.

¹ See FDA docket numbers 2003P-0064/CP1 (February 19, 2003) (hereinafter "Citizen Petition") and 2003P-0064/Supp1 (February 13, 2004) (hereinafter "Supplement No. 1").

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Absent that, the proposed generic product must be supported by proof of equivalent safety and effectiveness demonstrated through clinical trials. Part II of this Supplement responds to recent comments filed by Amphastar Pharmaceuticals, Inc. (“Amphastar”).

I. Newly Discovered Biological Properties of Enoxaparin

In its Citizen Petition, sanofi-aventis demonstrated that its manufacturing process for enoxaparin creates a highly complex collection of macromolecules with a chemical structure that is unique among currently approved low molecular weight heparins (“LMWHs”). This structure is marked by distinct polysaccharide sequences and structural modifications (or “fingerprints”) that are highly sensitive to sanofi-aventis’ process. The recognition of these fingerprints has only become possible due to recent advances in the field of analytical technology.²

The Citizen Petition and Supplement No. 1 describe several structural fingerprints of enoxaparin that may have clinical significance. Most important among these are the presence of the 1,6-anhydro ring structure at the reducing end of all oligosaccharides bearing 6-O-sulfo groups on the glucosamine moiety, and antithrombin III (“ATIII”) binding sequences each of which are highly dependent on sanofi-aventis’ manufacturing process for enoxaparin. The Citizen Petition also pointed out that about 30% of enoxaparin has not yet been fully characterized directly, leading to the possibility that additional structural fingerprints with pharmacological activity will be discovered in the future.³

Through continued testing, sanofi-aventis has discovered additional biological properties of enoxaparin that may have clinical significance. Other new data confirms the existence and significance of biological properties that have been previously identified. These studies, discussed in the remainder of this Part I, below, add further strength to the argument that any product claiming to be generic enoxaparin that does not employ a manufacturing process that is equivalent to sanofi-aventis’ process may not share enoxaparin’s safety and effectiveness profile.

A. Enoxaparin’s Effect on Tissue Factor Pathway Inhibitor

In a comment dated October 13, 2004, sanofi-aventis suggested that in addition to anti-Xa/IIa activity, endogenous release of the Kunitz-type inhibitor, Tissue Factor Pathway Inhibitor (“TFPI”), may contribute to Enoxaparin’s anti-thrombotic properties.⁴ Tissue factor (“TF”), a membrane-bound glycoprotein that initiates blood

² See Citizen Petition, at 3.

³ See *id.*

⁴ See FDA docket number 2003P-0064/RC1 (Oct. 13, 2004) (citing J Fareed & D Hoppensteadt, et al. Heterogeneity in low molecular weight heparins. Impact on the therapeutic profile. *Current Pharm. Design* 2004; 10:983-999, 986; GT Gerotziafas, A (continued...)

coagulation by allosteric activation of factor VII, is regulated primarily by TFPI.⁵ So, TFPI represents an important regulatory mechanism of blood coagulation.⁶

A recent study by Professor Shaker Mousa, Ph.D., has focused on the effect of enoxaparin on TFPI endothelial cell release inhibition induced by the bacterial endotoxin lipopolysaccharide (“LPS”). This study demonstrated that enoxaparin reverses the LPS inhibition of TFPI release, thus enhancing the TFPI release from the endothelium. The extent of enoxaparin’s reversal of TFPI inhibition is a function of the length of the polysaccharide chains within the enoxaparin mixture as well as the concentration of the 1,6-anhydro ring structure. As was previously discussed in the Citizen Petition and Supplement No. 1, both the 1,6-anhydro ring structure concentration and the mixture of polysaccharide chain lengths within enoxaparin is dependant upon sanofi-aventis’ manufacturing process.⁷ As a result, enoxaparin’s overall effect on TFPI inhibition reversal is process dependent.

Dr. Mousa’s study, attached hereto as Appendix A, compared the extent of TFPI inhibition reversal exhibited by enoxaparin fractions versus fractions from an LMWH that was similar to enoxaparin in molecular weight, anti-Xa activity, and anti-Xa/anti-IIa ratio, but contained the 1,6-anhydro ring structure in only minimal (< 7%) amounts (the “< 7% 1,6-anhydro LMWH”).⁸ In Table 1, reprinted below from Dr. Mousa’s study report, compounds 1, 2, 3, and 4 are, respectively, hexasaccharide, octasaccharide, decasaccharide, and dodecasaccharide fractions from a < 7% 1,6-anhydro LMWH (“Group A”). Compounds 5, 6, 7, and 8 are, respectively, the same fractions taken from enoxaparin (i.e. containing 15-25% 1,6-anhydro concentration) (“Group B”). Compound 9 is enoxaparin, compound 10 is a < 7% 1,6-anhydro LMWH, and compound 11 is a 40-50% 1,6-anhydro LMWH. Compounds 12, 13, and 14 are pure octasaccharides displaying different affinities to ATIII, and compound 15 is a heptasaccharide fraction of enoxaparin.

Zafiroopoulos, et al. Inhibition of factor VIIa generation and prothrombin activation by treatment with enoxaparin in patients with unstable angina. *British J. of Haematol.* 2003; 120:611-617).

⁵ S Mousa and B Kaiser. Tissue factor pathway inhibitor in thrombosis and beyond: role of heparin. *Drugs of the Future* 2004; 29(7):751-766; Q Tobu, et al. Comparative tissue factor pathway inhibitor release, potential of heparins. *Clin. Appl. Thrombosis Haemostasis* 2005; 11(1):37-47.

⁶ *See id.*

⁷ *See* Citizen Petition, at 10-19.

⁸ As discussed in the Citizen Petition, enoxaparin contains the 1,6-anhydro ring structure at the reducing ends of between 15% and 25% of its polysaccharide chains. *See* Citizen Petition, at 13.

Table 1: LPS-mediated impairment of Endothelial TFPI-release (reprinted from [Appendix A](#))

Heparanoid (1.0 ug/ml)	LPS (0.1ug/ml) Mean TFPI (ng / 10 ⁵ cells) ± SD
Control	4.03 ± 0.4
LPS	0.54 ± 0.1
+ 1	1.22 ± 0.2
2	2.02 ± 0.3
3	2.33 ± 0.2
4	4.12 ± 0.4
5	3.26 ± 0.2
6	2.92 ± 0.3
7	3.26 ± 0.3
8	4.92 ± 0.3
9	7.51 ± 0.6
10	7.16 ± 0.7
11	8.99 ± 0.6
12	4.28 ± 0.4
13	3.94 ± 0.3
14	3.77 ± 0.4
15	6.49 ± 0.5
Enoxaparin	7.94 ± 0.6 ⁹

Data represent mean ± SD, n = 3.

These results make clear that both polysaccharide chain length and 1,6-anhydro ring structure concentration affect enoxaparin's reversal of TFPI inhibition. The effect of chain length on TFPI inhibition reversal is demonstrated by comparing inhibition levels for individual chains within a given group (A or B). Regardless of 1,6-anhydro ring structure concentration, TFPI inhibition reversal increases in both Groups A and B as chain length increases from hexasaccharide through dodecasaccharide. Similarly, the effect of the 1,6-anhydro ring structure on TFPI inhibition reversal can be seen by comparing individual chains in Group A (< 7% 1,6-anhydro) to the corresponding chains in Group B (enoxaparin chains with 15-25% 1,6-anhydro). For any given chain length (e.g., octasaccharide compounds 2 and 6), reversal of TFPI inhibition is stronger in the version of the fraction containing the higher concentration of the 1,6-anhydro ring structure. Further, as is evident from comparing compounds 9, 10, and 11 these effects are strong enough to be evident in entire LMWH mixtures.

These results make clear, therefore, that reversal of TFPI inhibition constitutes yet another process-dependent biological property of enoxaparin that may have clinical significance. Professor Mousa's data show that enoxaparin's reversal of

⁹ The entry for "Enoxaparin" at the bottom of Table 1 represents an un-blinded sample of enoxaparin from the same lot as component 9 (enoxaparin). All other components, including component 9 (enoxaparin) were blinded.

TFPI inhibition is dependent upon sanofi-aventis' manufacturing process. Enhancement of TFPI release may have significant clinical relevance. TFPI release plays a major role in neutralization of the TF/FVIIa complex initiating coronary thrombosis after artery injury or plaque rupture. TFPI release not only elevates blood TFPI concentrations but also induces high TFPI concentrations at sites of tissue damage and ongoing thrombosis.¹⁰ Thus, a product claiming to be enoxaparin that did not employ a manufacturing process equivalent to sanofi-aventis' process might exhibit a different anti-thrombotic profile than does enoxaparin.

B. Process-Dependent Effect on Inhibition of Factor VIIa

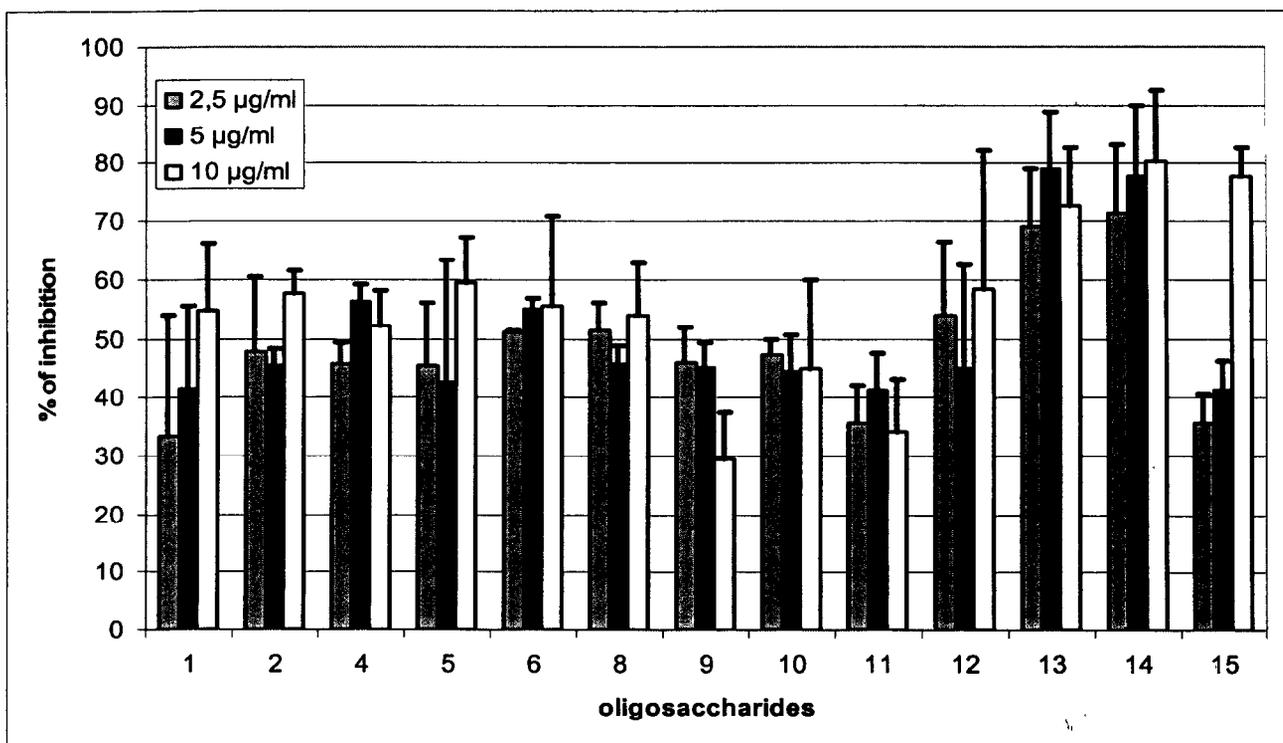
A recent study conducted by Drs. Michel Samama and Ismael Elalamy of Hotel-Dieu Hospital in Paris, France, has revealed that enoxaparin has a process-dependent inhibitory effect on Factor VIIa generation. Factor VIIa is linked to arterial thrombogenesis. .

In this study, attached hereto as Appendix B, Drs. Samama and Elalamy compared the Factor VIIa inhibitory effect of selected polysaccharide fractions bearing varying concentrations of 1,6-anhydro ring structure as well as pure octasaccharides displaying different affinity toward ATIII. In Figure 1 from the study report, reprinted below, compounds 1, 2, and 4 are hexa, octa, and dodecasaccharide fractions from a < 7% 1,6-anhydro LMWH (which contain only small amounts of ATIII binding site content). Compounds 5, 6, and 8 are corresponding fractions of the same lengths, from a 40-50% 1,6-anhydro LMWH, again with only small amounts of ATIII binding site content.¹¹ Compound 9 is enoxaparin, compound 10 is a < 7% 1,6-anhydro LMWH, and compound 11 is a 40-50% 1,6-anhydro LMWH. Compounds 12, 13, and 14 are pure octasaccharides bearing ATIII binding sites displaying different affinities to ATIII.

¹⁰ See Mousa, *supra* note 5.

¹¹ Compounds 3 and 7 were not requested by Drs. Samama and Elalamy and therefore were not provided by sanofi-aventis.

Figure 1. Percentage of FVIIa inhibition in the presence of different oligosaccharides at high concentrations (2.5, 5 and 10 $\mu\text{g/ml}$). Mean \pm SD ($n=3$).



Although Figure 1 does not suggest a relationship between 1,6-anhydro ring structure concentration and inhibition of Factor VIIa generation, it does demonstrate that the concentration of ATIII binding sites within enoxaparin influences Factor VIIa generation inhibition. For example, in Figure 1, compounds 12, 13, and 14 are pure octasaccharides that bear an ATIII binding site. They show significantly greater inhibition of Factor VIIa generation at all concentrations than do compounds 1, 2, 4, 5, 6, and 8, which contain only smaller amounts of ATIII binding oligosaccharides (fractions of defined size of oligosaccharide mixture that contain ATIII binding compounds as well as non-ATIII binding oligosaccharides).

Enoxaparin's inhibition of Factor VIIa generation thus represents another biological property of enoxaparin with possible clinical significance. As discussed in the Citizen Petition and Supplement No. 1, the proportion and structure of ATIII binding sites in enoxaparin's polysaccharides is a function of sanofi-aventis' manufacturing process.¹² Factor VIIa, in turn, plays a crucial role in arterial thrombogenesis. The plasma concentration of Factor VIIa is higher in thrombotic

¹² See Supplement No. 1, at Part II.

states. This blood coagulation process is initiated when cryptic tissue factor is exposed to circulated blood. Tissue factor binds to Factor VIIa, and the complex initiates thrombus formation.¹³ Enoxaparin disrupts the formation of the TF/FVIIa complex by inhibiting generation of Factor VIIa, thus reducing arterial thrombogenesis.

This adds further support to the arguments sanofi-aventis made in its Citizen Petition. A product claiming to be generic enoxaparin that did not employ an equivalent manufacturing process might contain within its fractions a different distribution and structure of ATIII binding sites, which could lead, in turn, to a different effect on inhibition of Factor VIIa. Such a product might exhibit a different effect on arterial thrombosis, which could have clinical consequences for patients switched to the generic product.

C. Process Dependent ATIII Binding Sites Are Ubiquitous in Enoxaparin's Oligosaccharides

In Supplement No. 1, sanofi-aventis identified the presence of process-dependant variations in the structure of the ATIII binding sites in enoxaparin's oligosaccharides. These variations are caused by differences in the position at which the ATIII domain of the unfractionated chain may be cleaved during sanofi-aventis' depolymerization step, and may change oligosaccharide affinity for ATIII. Thus, two ATIII binding oligosaccharides of the same chain length may demonstrate different binding potency, which is not necessarily correlated in the same way to the *in vitro* anti-Xa activity. These sequence variations may cause differences in the half-lives of the anti-Xa activity, leading to different overall anti-coagulation profiles.¹⁴

Although sanofi-aventis speculated that these process dependant variations would exist in all of enoxaparin's oligosaccharide fractions, Supplement No. 1 established the variations only in enoxaparin's octasaccharide fractions. Based on a recent study by Dr. Christian Boudier of Laboratoire d'Enzymologie in Illkirch, France, however, the affinity measurement of a collection of pure ATIII binding deca-saccharides now demonstrates that these process-dependent structural variations also exist in this higher fraction. This demonstration reinforces the fact that in general, the nature and the proportion of enoxaparin's ATIII binding oligosaccharides is process dependent. With current analytical techniques, sanofi-aventis can explore these structural variations of the ATIII binding oligosaccharides in all of enoxaparin's fractions from hexasaccharides to dodecasaccharides.

Dr. Boudier's study, attached hereto as Appendix C, measured the change of fluorescence of ATIII upon its complexation with different oligosaccharides

¹³ See Mousa, *supra* note 5.

¹⁴ See Supplement No. 1, at Part II.

and more particularly with deca-saccharides isolated from enoxaparin. Fluorescence differences of the complex establish different degrees of affinity, which, in turn demonstrates the importance of the process-related structural variations. As a reference compound, the ATIII binding affinity of the synthetic pentasaccharide Arixtra™ was initially established and the tested oligosaccharides were then compared on the basis of their affinity constants to the pentasaccharide. Using this technique, Dr. Boudier discovered that among the enoxaparin oligosaccharides, 8 have higher affinity (as established by Kd values) than the pentasaccharide Arixtra™ including one oligosaccharide with a Kd more than 20 times lower. In addition, several have lower affinity than the pentasaccharide, including one oligosaccharide with a Kd about 2 times higher than Arixtra™.

Sanofi-aventis' manufacturing process for enoxaparin creates variations in the structure of the ATIII binding sequences. As a result of Dr. Boudier's study, it is now clear that the structural changes affect affinity towards the ATIII protein within enoxaparin's larger oligosaccharide fractions, not just in the octasaccharides. This has potentially significant ramifications for patients. As discussed in Supplement No. 1, oligosaccharide fractions with a lower affinity for ATIII (due to sequence variations) may have a different duration of action *in vivo* as a result of a different half-life of anti-Xa activity. This could result in anti-coagulant effects that differ significantly more than a superficial assessment of anti-Xa activity might suggest.¹⁵ As a result, a product claiming to be enoxaparin that does not employ a manufacturing process equivalent to sanofi-aventis' process may have an anti-Xa/anti-IIa ratio that is similar to enoxaparin, but may nevertheless exhibit a different profile of bleeding safety and antithrombotic effectiveness.

D. Enoxaparin Has a Specific Effect on FGF2 Pro-angiogenic Activity

In both the Citizen Petition and Supplement No. 1, sanofi-aventis discussed enoxaparin's influence on fibroblast growth factor FGF-1.¹⁶ The proteins of the fibroblast growth factor ("FGF") family are extremely efficient mitogens that can influence angiogenesis through stimulation of endothelial cell proliferation. Neoangiogenesis, in turn, can alleviate and ameliorate diseases characterized by microvascular insufficiency such as ischemic heart disease.¹⁷

Recent studies conducted by Professor B.U. von Specht of the Center of Clinical Research, Freiburg University Hospital, Germany, have shown that enoxaparin has a distinct effect on another growth factor involved in the angiogenesis process,

¹⁵ See *id.*

¹⁶ See Citizen Petition, at 17, 18; Supplement No. 1, at 9, 10.

¹⁷ See Citizen Petition, at 17 (citing Isner JM. Angiogenesis for revascularization of ischemic tissues. *European Heart J.* 1997; 18:1).

FGF2, which is specifically related to the presence of the 1,6-anhydro ring structure. FGF2 is a heparin binding protein that promotes angiogenesis but is inhibited by heparin.

In his study, attached hereto as Appendix D, Professor von Specht compared the FGF2 inhibiting effects of three groups of compounds: oligosaccharides with the 1,6-anhydro ring structure, oligosaccharides without the 1,6-anhydro ring structure, and a third group consisting of heparin, enoxaparin, and an ultra low molecular weight heparin. Professor von Specht found that oligosaccharides bearing the 1,6-anhydro ring structure inhibited FGF2-induced BHK proliferation less than did oligosaccharides that did not contain the 1,6-anhydro ring structure. In addition, the study demonstrated that heparin inhibited FGF2-induced BHK proliferation more than did enoxaparin, which in turn had a greater inhibitory effect than did the ultra low molecular weight heparin.

In light of this study it now appears that FGF2 is sensitive not only to variations in molecular weight and saccharide chain length, but also to the presence or absence of the 1,6-anhydro ring structure. The 1,6-anhydro ring structure reduces the inhibitory action on FGF2-induced cell proliferation, thus stimulating greater angiogenesis. Thus, a generic product claiming to be enoxaparin that did not contain the 1,6-anhydro ring structure or contained it in a different concentration than is found in enoxaparin might have a different effect on important conditions such as ischemic heart disease and myocardial infarction.

E. Summary of Part I

The data presented in Part I establish at least two new process-dependent biological properties of enoxaparin that may have clinical significance. First, enoxaparin enhances TFPI release, which neutralizes the Tissue Factor/Factor VIIa thrombogenic complex. The extent of enoxaparin's reversal of TFPI inhibition is process dependant (Pr Mousa study). Second, Factor VIIa generation is inhibited by several of enoxaparin's oligosaccharides depending on their ATIII binding site expression, which is again dependent on sanofi-aventis' manufacturing process (Pr Samama).

The data above also adds strength to previous arguments sanofi-aventis has made in other submissions to this docket. Dr. Boudier's study demonstrates that the process-dependent ATIII binding sites originally discussed in Supplement No. 1 are present in enoxaparin's larger oligosaccharide fractions, not just in the octasaccharides. As previously submitted, Professor von Specht presented FGF-1 data showing the pro-angiogenic effect of the 1,6- anhydro ring moieties, which is desirable. The growth factor FGF-2 also has a desirable pro-angiogenic effect. However, Dr. Boudier's study demonstrates that Enoxaparin inhibits the pro-angiogenic effect of FGF2. Furthermore, it is shown that the levels of 1,6-anhydro ring moieties can alter the level of inhibition of FGF2. All of these recent studies add further strength to the central argument that

enoxaparin is characterized by structural fingerprints that are dependant upon sanofi-aventis' manufacturing process and may have clinical significance.

II. Amphastar's July 18, 2005 Comment Fails to Establish Equivalence

On July 18, 2005, Amphastar submitted a third comment to this Citizen Petition docket (the "July 2005 Amphastar Comment").¹⁸ Amphastar's latest comment provides chromatograms of commercially acquired Lovenox and its proposed generic product, and claims that they are identical. Amphastar claims that these chromatograms prove that its proposed generic product is the same as enoxaparin.

Amphastar has attempted to establish "sameness" in this manner before. In its first comment to this docket on June 4, 2004, Amphastar provided direct analysis HPLC-SAX chromatographic comparisons of its product to enoxaparin. Sanofi-aventis responded in its October 2004 and March 2005 comments by pointing out that (a) Amphastar's chromatograms of its product were not identical to enoxaparin and were in any case too poorly resolved for meaningful comparison, and (b) even if Amphastar's chromatograms of its product were identical to chromatograms of enoxaparin, this would not establish "sameness" as required by the FDCA.¹⁹

Amphastar's latest attempt to demonstrate sameness through chromatographic comparison appears to employ CTA-Sax/UV chromatography technology. Nevertheless, Amphastar again fails to establish sameness for the same reasons that hampered its previous attempt. First, even a cursory glance at Amphastar's chromatograms of its product clearly reveals that they are not identical to Amphastar's chromatograms of enoxaparin. Second, as sanofi-aventis has pointed out on several occasions, even were Amphastar's chromatograms identical, they would not establish that Amphastar's proposed product is "the same as" enoxaparin.

A. Amphastar's Chromatograms Are Not Identical

In its first comment to this docket on June 4, 2004 Amphastar attempted to establish sameness between enoxaparin and its proposed generic product by comparing poorly resolved chromatograms using HPLC-SAX technology. After sanofi-aventis' October 13, 2004 comment pointed out the flaws in Amphastar's analytical data, Amphastar has now returned with chromatograms using CTA-Sax/UV technology similar to the technology sanofi-aventis uses for its chromatograms.²⁰

¹⁸ See FDA docket number 2003P-0064/C7 (July 18, 2005).

¹⁹ See FDA docket numbers 2003P-0064/RC1 (October 13, 2004) and 2003P-0064/RC2 (March 17, 2005).

²⁰ It is actually unclear what method Amphastar used to develop its chromatograms. The July 18, 2005 Amphastar Comment labels its chromatograms only as LC/UV.

Despite Amphastar's claim that "[b]oth of these studies demonstrate that the two products have the same chromatogram profiles," even a cursory examination of Amphastar's most recent chromatograms reveals significant differences. For example, Figure 1 of the July 18, 2005 Amphastar Comment (reprinted below as Figure 2) compares Amphastar's proposed generic product (upper chromatogram) with an Amphastar chromatogram of (presumably) commercially acquired Lovenox using CTA-Sax/UV (or similar method).

Figure 2: Fig. 1, Reprinted from Amphastar's July 18, 2005 Comment (original in colour)

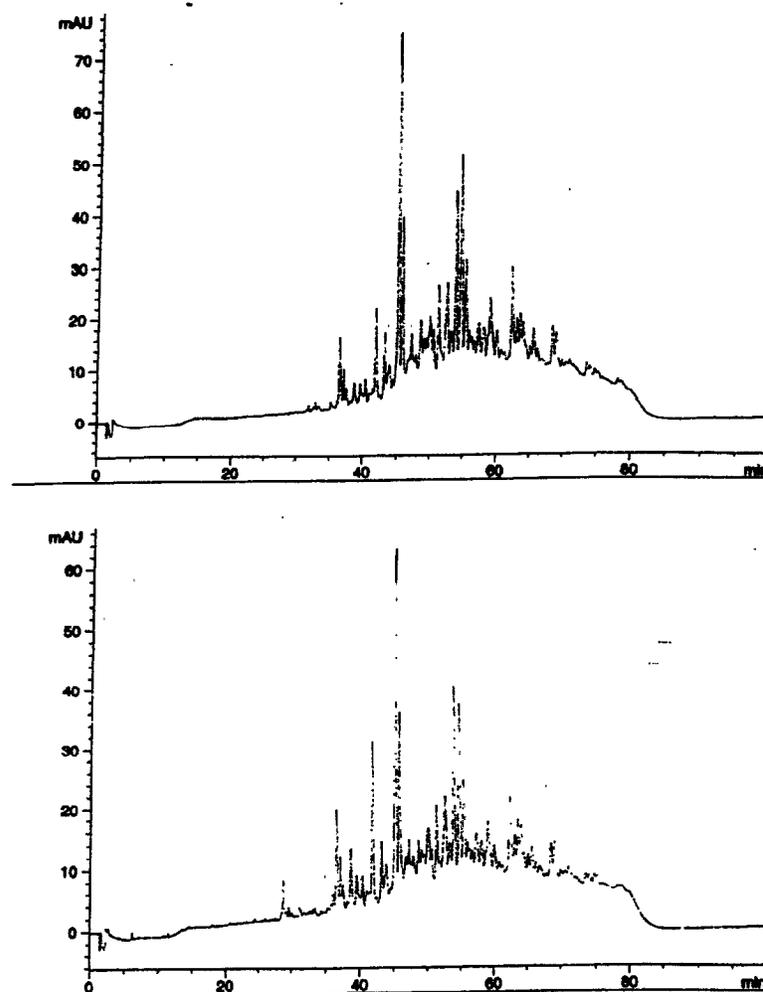


Fig.1 A comparison of chromatogram of entire distribution of oligosaccharides between Amphastar's Enoxaparin (above blue) and Aventis' Lovenox (below red).

Several differences are apparent in this comparison:

- Amphastar's Lovenox chromatogram contains a peak at 29 minutes not present in Amphastar's chromatogram of its own product;
- The ratio of the three peaks at 39, 40, and 41 minutes, respectively, are different. In Amphastar's Lovenox chromatogram, the first peak at 39 minutes is roughly twice the height of the peaks at 40 and 41. In Amphastar's chromatogram of its own product, the three peaks are of equal height;
- The peak at 42 minutes is significantly higher in Amphastar's Lovenox chromatogram (30 mAU) than it is in Amphastar's chromatogram of its own product (20 mAU);
- The ratios of peaks at 54 and 55 minutes are reversed between the two products. In Amphastar's Lovenox chromatogram, the peak at 54 minutes is higher than the peak at 55 minutes. In Amphastar's chromatogram of its own product, the peak at 55 minutes is higher than the peak at 54 minutes.

Figure 2 of the July 18, 2005 Comment (reprinted below as Figure 3) presents a similar chromatographic comparison after heparinase-digestion, with Amphastar's product on top, and Amphastar's Lovenox chromatogram at bottom:

Figure 3: Fig. 2, Reprinted from Amphastar's July 18, 2005 Comment (original in colour)

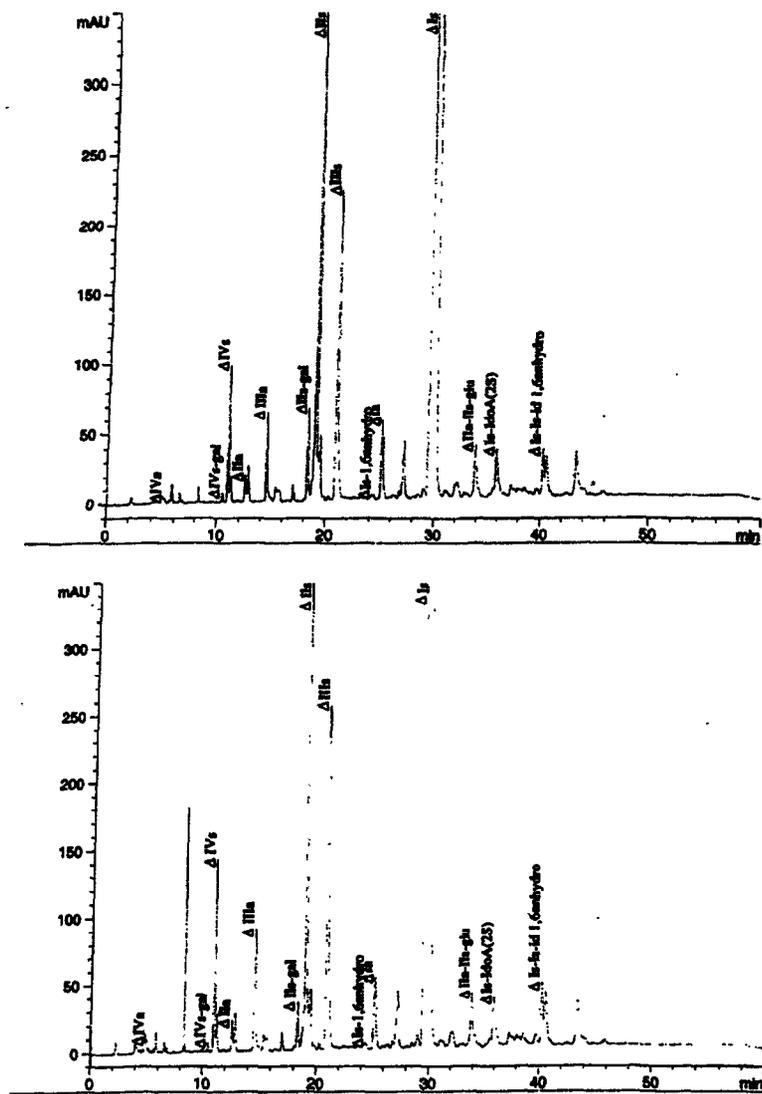


Fig. 2 A comparison of chromatogram of Heparinase-hydrolyzed Enoxaparin between Amphastar's Enoxaparin (above blue) and Aventis' Lovenox (below red).

Once again, multiple differences are apparent in the comparison:

- The Lovenox sample shows a clear peak at 8.5 minutes that is observed only as a very minor peak in Amphastar's sample of its own product;
- The ratio between the peaks in the two chromatograms is different in several respects.

It is also interesting to note the differences between the "Lovenox" chromatograms provided by Amphastar in the July 18, 2005 Amphastar Comment and the Lovenox chromatogram samples provided by sanofi-aventis in its March 17, 2005 comment.²¹ Figures 2 and 4 in sanofi-aventis' March 17, 2005 comment present the same chromatographic analyses as the "Lovenox" samples in Figures 1 and 2, respectively, of the July 18, 2005 Amphastar Comment. Yet Amphastar's "Lovenox" chromatograms look significantly different from sanofi-aventis' chromatograms of its own product. These differences may arise partly from a non-optimal/uncontrolled use of the heparinase enzymes by Amphastar. The "Lovenox" sample that Amphastar analyzed appears to have a different level of depolymerization than sanofi-aventis' sample, and therefore is not fully representative of the digestible part of the polysaccharides chains.²²

For example, Amphastar's 1,6-anhydro oligosaccharides are found only in the tetrasaccharide form or longer. This shows an incomplete digestion because the Δ 1,6-anhydro is present only in trace amounts on that oligosaccharide. As mentioned in sanofi-aventis' March 17 comment, the disaccharide building block methodology only enables one to quantify the disaccharides from which the heparinoid is made, but does not demonstrate how to reassemble the building blocks. Therefore, the specific process-dependent oligosaccharides (for example the ATIII binding sequences) are not revealed by this kind of analytical methodology. However, even at this basic level of analysis, Amphastar fails to show both the qualitative and quantitative comparability of the building block chromatograms. Again, these results are not comparable on either a qualitative or quantitative basis. This demonstrates the lack of analytical methodology and Amphastar's inability to handle rigorous analysis of such complex products.

²¹ See FDA docket number 2003P-0064/RC2 (March 17, 2005).

²² In its November 23rd comment, Amphastar criticized one of sanofi-aventis' chromatograms by arguing that it was truncated and therefore misleading when used as a comparison to Amphastar's complete chromatogram. It is interesting to note that now in its most recent comment, Amphastar has provided its own comparison of truncated chromatograms.

Amphastar has failed to account for any of these differences between its “Lovenox” chromatograms and its chromatograms of its own product. As a result, Amphastar has not demonstrated that Amphastar’s proposed generic product and enoxaparin have the “same chromatogram profile.”

B. Even Identical Chromatograms Would Not Establish Sameness

As sanofi-aventis has repeatedly stated, even if Amphastar’s chromatograms of enoxaparin and its proposed generic product were identical, this would not establish “sameness.”

First, current analytical techniques simply are not sufficiently sophisticated to resolve complex molecules such as enoxaparin in sufficient detail to ensure identity. In this chromatogram analysis, one has to remember that resolution diminishes as molecular weight increases. Moreover, the UV signal response is molar and it decreases as the molecular weight increases. Very simply, the number of different compounds dramatically increases with the molecular weight and the resolution of such chromatographic system finds its limitation at approximately 3000 Da.

As a result, Amphastar’s chromatograms do not achieve baseline resolution between approx 30 and 65 min. Indeed, at this time, chromatographic technology is not sufficiently sophisticated to achieve baseline resolution for a complex product such as enoxaparin. It is therefore impossible to generate chromatograms that could identify each chemical structure in enoxaparin’s composition and serve as a useful comparator for sameness. Despite this analytical limitation, it is clear from the discussion in Part II(A), above, that even just in the analyzable part, Amphastar’s proposed generic product contains a different oligosaccharide mixture than does enoxaparin. Therefore, it is obvious that the overall mixture is not the same. As sanofi-aventis has repeatedly pointed out, these differences in the oligosaccharide mixtures are significant, and may have real therapeutic consequences for patients.

Second, even identical chromatograms with baseline resolution may not be sufficient to ensure sameness in complex products such as enoxaparin. Although such chromatograms might demonstrate that the two products contained the same mixture of polysaccharide chains (i.e. the same number and location of hexasaccharides, octasaccharides, dodecasaccharides, etc.) this might not ensure that the structure of each such chain would be the same.

III. Conclusion

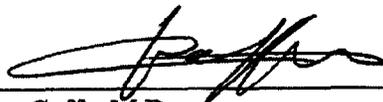
The scientific data sanofi-aventis has presented in the Citizen Petition and Supplements make clear that enoxaparin is characterized by various process-dependent structural fingerprints and biological properties that may make significant contributions to enoxaparin’s overall therapeutic effect. These fingerprints and

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biological properties (both discovered and as yet undiscovered) are distinct products of the manufacturing process utilized by sanofi-aventis. Because of the complexity of the enoxaparin macromolecule, a generic manufacturer cannot demonstrate that its product is equivalent through simple chemical testing such as chromatographic comparison. It is therefore critical that FDA deny any ANDA for a generic enoxaparin product that is not manufactured through a process that is equivalent to sanofi-aventis' process for enoxaparin. Barring that, FDA must require the applicant to establish an equivalent safety and effectiveness profile through clinical testing.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this Supplement includes all information and views on which the Supplement relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.



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Respectfully submitted,



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Attachments