

# EP/USP GPC/SEC Molar Mass Determination for Low Molecular Weight Heparin (LMWH)

## Application Note Pharmaceutical Analysis

### Authors

Daniela Held and Friedhelm Gores

contact: [DHeld@pss-polymer.com](mailto:DHeld@pss-polymer.com) and [FGores@pss-polymer.com](mailto:FGores@pss-polymer.com)

### Introduction

Natural heparin is a polysaccharide and consists of molecular chains of varying lengths. Low molecular weight heparin (LMWH) consists of short chains and is used as anticoagulant in diseases that feature thrombosis and for prophylaxis against it. Because LMWH has more predictable anticoagulant effect and pharmacokinetics as well as less side-effects, it is recommended over unfractionated heparin. Various methods of heparin depolymerization are used to manufacture LMWH.

LMWHs are defined as heparin salts having an average molecular weight of less than 8000 Da and for which at least 60% of all chains have a molecular weight less than 8000 Da. GPC/SEC is the method of choice to measure the molecular weight (molar mass) and the molecular weight fractions above/below a molecular weight limit. Both, the Pharmaeuropa and the US Pharmacopeia, require GPC/SEC to characterize e.g. Enoxaparin Sodium or other low molar mass heparins with respect to molecular weight. As recently again demonstrated, these methods allow to measure true molar masses with inexpensive standard lab equipment and lowest experimental effort.



### System Requirements

	USP <sup>1</sup>	EP <sup>2</sup>
SECcurity GPC1260 isocratic pump	<ul style="list-style-type: none"> <li>flow rate [mL/min]: 0.6</li> <li>mobile phase: 1M Ammonium acetate</li> </ul>	<ul style="list-style-type: none"> <li>flow rate [mL/min]: 0.5</li> <li>mobile phase: Na<sub>2</sub>PO<sub>4</sub> 28.4 g/L</li> </ul>
PSS SECcurity GPC1260 Autosampler		
Columns	column packing L59 <ul style="list-style-type: none"> <li>PSS PROTEEMA precolumn 8*50mm</li> <li>PSS PROTEEMA 5µm 100Å (8*300mm)</li> <li>PSS PROTEEMA 5µm 100Å (8*300mm)</li> </ul>	silica based, 5µm <ul style="list-style-type: none"> <li>PSS PROTEEMA 5µm 100Å (7.5*300mm)</li> </ul>
Detectors	Refractive index <ul style="list-style-type: none"> <li>SECcurity 1260 RI</li> </ul>	Refractive index <ul style="list-style-type: none"> <li>SECcurity 1260 RI</li> <li>additional for calibration UV:</li> <li>SECcurity 1260 UV @ 234 nm</li> </ul>
Calibration	<ul style="list-style-type: none"> <li>Low Molecular Weight Heparin Molecular Weight Calibrant</li> <li>Verification: USP System Suitability</li> </ul>	<ul style="list-style-type: none"> <li>Heparin Low-Molecular-Mass for calibration CRS batch 2</li> <li>M<sub>n</sub> = 3800</li> </ul>
Software	GPC software <ul style="list-style-type: none"> <li>PSS WinGPC UniChrom UniChrom with heparin module</li> <li>optional: Compliance Pack</li> </ul>	<ul style="list-style-type: none"> <li>PSS WinGPC UniChrom with heparin module</li> <li>optional: Compliance Pack</li> </ul>

## Procedure, Results & Discussion

Although the calibration procedures described in the EP and USP are totally different, a dedicated GPC/SEC software can be used to analyze the heparin data in accordance with both standards precisely. The following figures show PSS WinGPC UniChrom raw data of the EP standard (Fig.1a), the EP elugram data overlaid with the corresponding automatically determined calibration curves (Fig.1b) as well as the raw data of the US calibration standard (Fig. 2a) and the procedure to construct the USP calibration curve (Fig 2b).

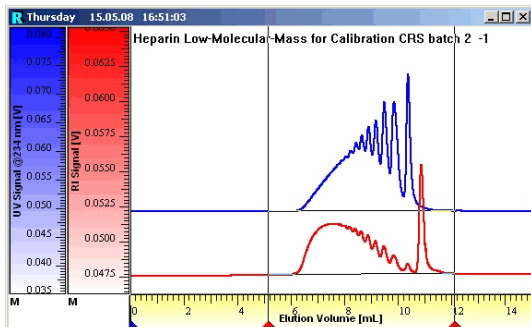


Fig. 1a: Raw data EP standard CRS 2, UV and RI signal

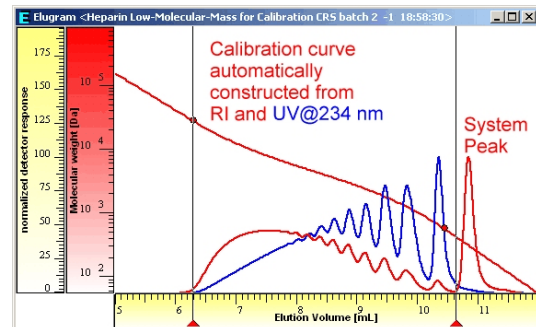


Fig.1b: Elugram CRS2 overlaid with the calibration curve constructed from RI and UV signal

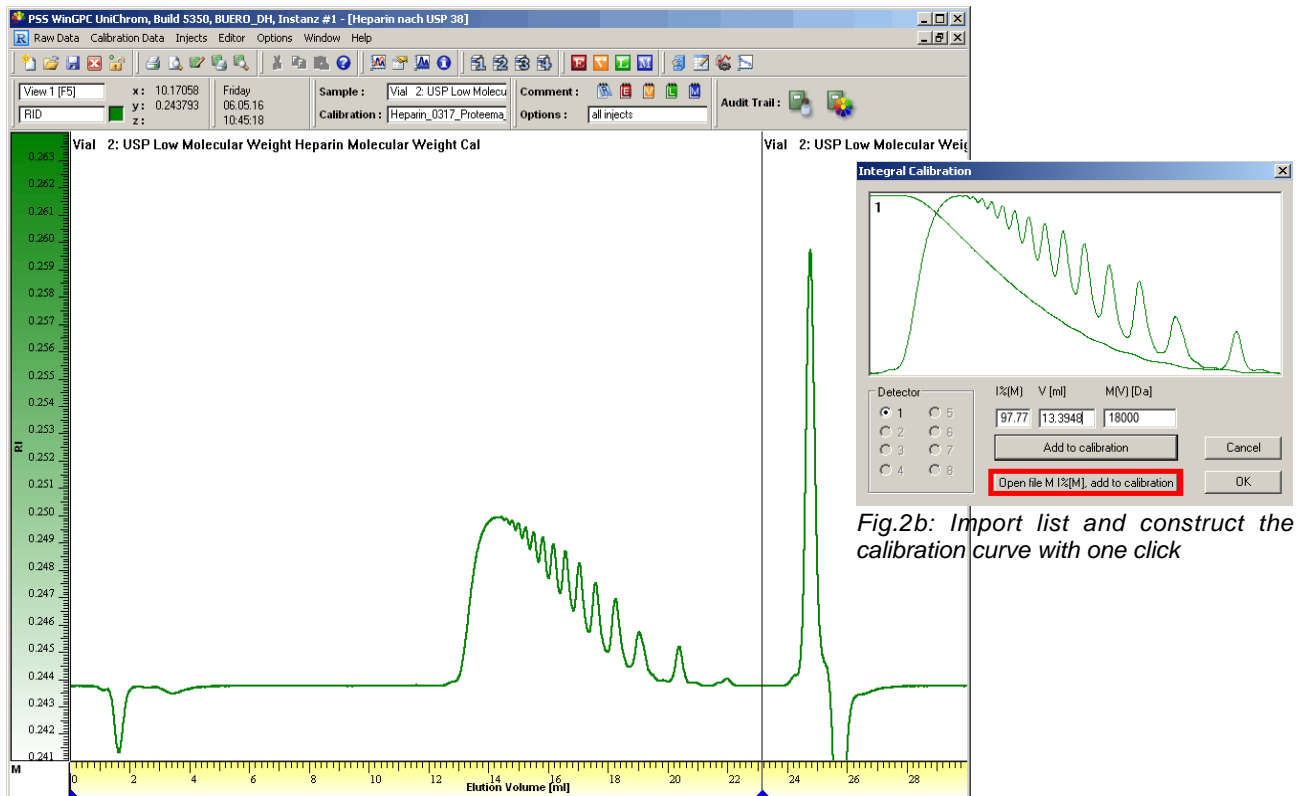


Fig. 2a: Raw data Standard USP, RI signal

Fig.2b: Import list and construct the calibration curve with one click

Further Information:

Video Application Note:

YouTube

<https://www.youtube.com/watch?v=e1KD2iV7YZ0>



Once the calibration curve is constructed, the analysis of the data is comparable. All required molar mass information based on true heparin values are obtained. Figure 3 shows the final results for the analysis of unknown low molecular weight heparin. PSS WinGPC UniChrom GPC/SEC software allows to set additional markers such as 2000 and 8000 Da, to display the measured percent below 2000 Da, above 8000 Da and in between these values.

Of utmost importance is the y-axis of the result window. WinGPC UniChrom shows a true molar mass distribution with a  $w(\log M)$  axis. Only this guarantees correct fractions above/below the user selectable molecular weight borders.<sup>3</sup>

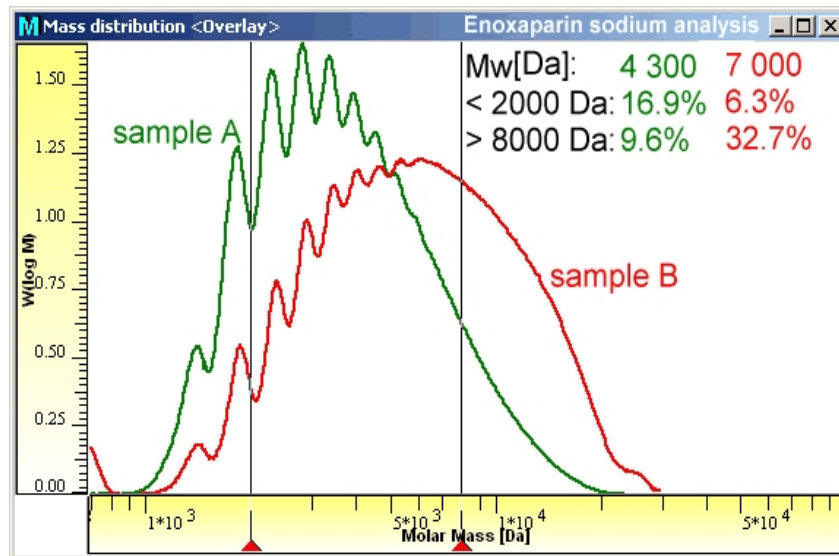


Fig.3: Result comparison Enoxaparin sodium, sample A and sample B

## Literature

<sup>1</sup> USP 209 Low Molecular Weight Heparin Molecular Weight Determination

<sup>2</sup> EP Low-Molecular-Mass (LMM) heparins Monograph 0828

<sup>3</sup> D. Held, P. Kilz: "Qualification of GPC/SEC/SEC Data and results" chapter 13 in H.-J. Kuss/ S. Kromidas (eds), Quantification in LC and GC - A Practical Guide to Good Chromatographic Data, Wiley-VCH, Weinheim (2009)