

## Mitigating trade barriers by standardized determination of lignin molar mass

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### Abstract

In order to prepare a draft for an official ISO method for size-exclusion chromatography (SEC) of kraft lignin samples, four samples were analysed by the three participating laboratories using a harmonized analytical setup. Harmonization of aqueous SEC was successful, but organic SEC proved more complex and further investigations are needed. Furthermore, even though the aqueous SEC analysis was repeatable, a strategy for data evaluation should be developed in order to achieve a method applicable for quality assessments.

### Introduction

Harmonized and standardized characterization methods are essential to avoid trade barriers by facilitating communication between lignin manufacturers and purchasers. No official standardized test methods for technical lignin are currently available. As a consequence, the results from analysis of lignin properties largely depend on which method or laboratory is used.

Among the most important lignin properties are average molar mass, molar mass distribution and molecular size and shape. Size-exclusion chromatography (SEC) is commonly used for lignin molar mass determination, but there is a wide variation in eluents, columns and processing methods used by different laboratories. Attempts to harmonize the SEC methods have indeed been made, for example within the framework of the EuroLignin network [1]. A number of draft analytical protocols are available for ILI members on [<http://www.ili-lignin.com/projects/norms.php>]. However, still, much remains to be done.

In order to take the next step towards an official standard for the determination of lignin molar mass, a joint project has been carried out by RISE (former Innventia), FPInnovations and VTT, aiming at writing a draft for an official ISO method. The draft will include two simple and robust methods that can be used by different labs as reference methods. The two methods are based on, respectively, organic and aqueous (SEC). The work was focused on kraft lignins of different origin. Four samples were analysed by the three laboratories using the current in-house methods as well as a harmonized analytical setup.

## Experimental

### Materials

All reagents used were of analytical grade. The lignin samples were obtained from kraft black liquor using the LignoForce<sup>TM</sup> [2] and LignoBoost<sup>TM</sup> [3] processes (Table 1).

Table 1. Lignin samples.

Sample	Process	Black liquor origin
LF-SW	LignoForce	Softwood
LF-EUC	LignoForce	Eucalyptus
LB-SW/HW	LignoBoost	Softwood/hardwood mixture
LB-EUC	LignoBoost	Eucalyptus

### SEC conditions

SEC was performed using current in-house methods as well as tentative harmonized analytical set-ups, according to Table 2. Samples were dissolved in the mobile phase at a concentration of 0.5 – 1 mg/mL and filtered prior to analysis using syringe filters with a 0.45- $\mu$ m pore size.

Table 2. SEC parameters

Setup	Mobile phase	Derivatization	Columns
Aq1	0.1M NaOH	none	PSS MCX 1000Å + 1000Å
Aq2	0.1M NaOH	none	PSS MCX 1000Å + 100000Å
Aq3	NaH <sub>2</sub> PO <sub>4</sub> /NaOH <sup>a</sup>	none	PSS MCX 1000Å + 1000Å
Aq4	NaH <sub>2</sub> PO <sub>4</sub> /NaOH <sup>a</sup>	none	PSS MCX 1000Å + 100000Å
THF1	THF	acetobromination <sup>b</sup>	Agilent PLgel 5um Mixed-C, 2 columns
THF2	THF	acetobromination <sup>b</sup>	Waters HR4 + HR4E + HR1
THF3	THF	acetobromination <sup>b</sup>	Waters HR4 + HR4E + HR2
THF4	THF	acetylation <sup>c</sup>	Agilent PLgel 5um Mixed-C, 2 columns
THF5	THF	acetylation <sup>c</sup>	Waters HR4 + HR2 + HR1
THF6	THF	acetylation <sup>c</sup>	Waters HR4 + HR4E + HR1
THF7	THF	acetylation <sup>c</sup>	Waters HR4 + HR4E + HR2

<sup>a</sup>) 6.9 g/L NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O + 3.2 g/L NaOH. Adjusted to pH 12 with 1 M NaOH.

<sup>b</sup>) according to [4]

<sup>c</sup>) according to [5]

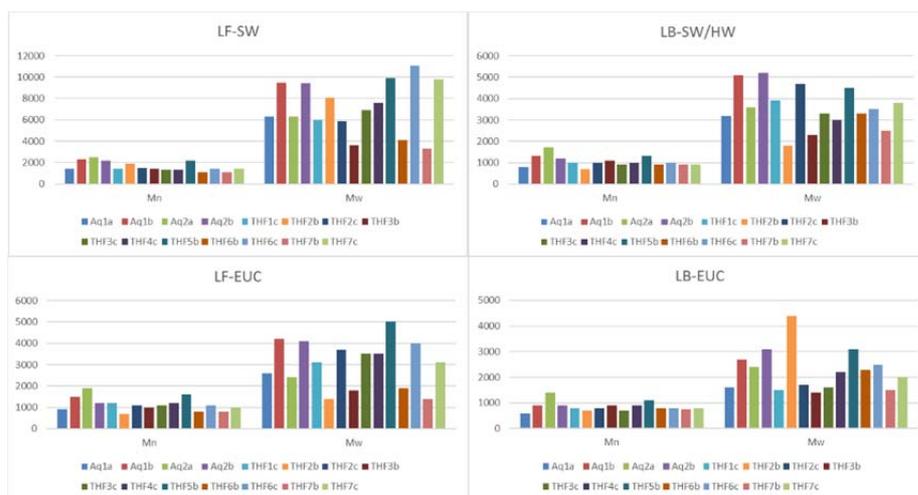
### Calibration and calculation

The relative average molar masses were calculated from the chromatograms using commercial calibration standards. Aqueous SEC was calibrated using various polystyrene sulfonate standards within the molar mass range from 891 to 400000 g/mol. Organic SEC was calibrated using various polystyrene standards within the molar mass range from 162 to 900000 g/mol. The selection of calibrants was not harmonized at this stage.

In the case of aqueous SEC, both linear and 3<sup>rd</sup> degree polynomial curve fits were evaluated, whereas only a 3<sup>rd</sup> degree polynomial curve fit was used for organic SEC.

### Results & discussion

The results from analysis using the different SEC parameters and in different labs are shown in Figure 1. As expected, the results differed largely between the different methods. For example, the weight-average molar mass ( $M_w$ ) for the LignoForce<sup>TM</sup> softwood lignin (LF-SW) ranged from 3300 to 11100 g/mol, employing the different methods. In order to understand the differences and eventually suggest a harmonized setup, the chromatographic behaviour of the aqueous and organic SEC systems was studied in more detail.

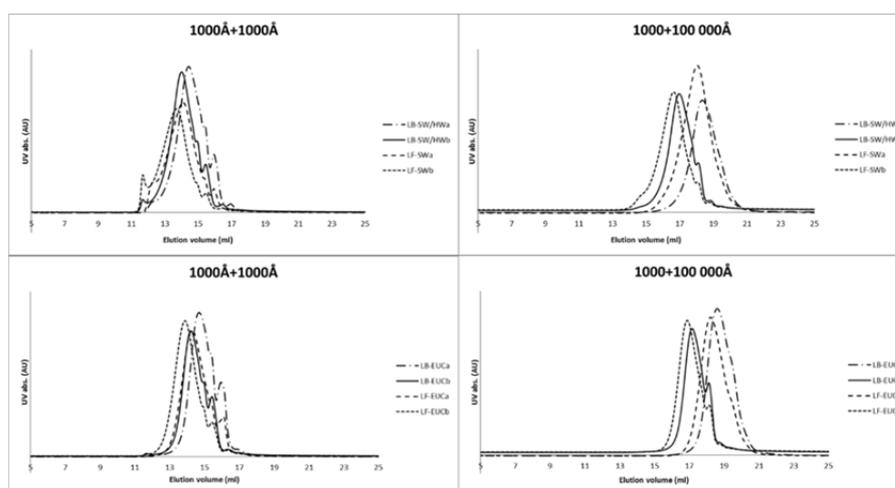


**Figure 1.** Average molar masses obtained in the three labs ( $VTT=a$ ,  $RISE=b$  and  $FPInnovations=c$ ) using different setups, as explained in Table 2.

### Aqueous SEC

Initial trials showed that the two mobile phases (0.1M NaOH and  $\text{NaH}_2\text{PO}_4/\text{NaOH}$ ) studied behaved in a similar fashion. The remaining study was, therefore, carried out only using 0.1M NaOH as the mobile phase.

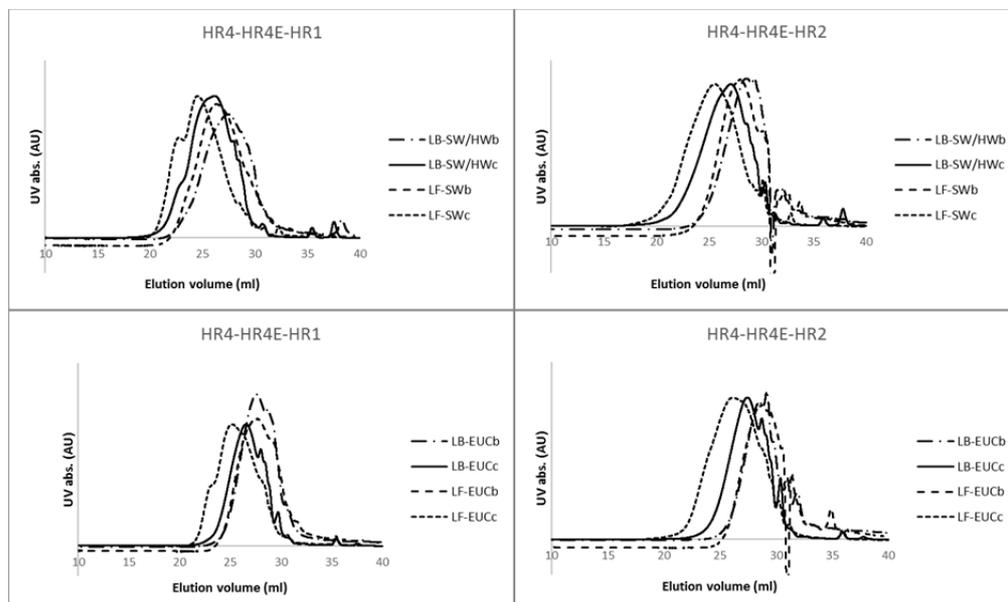
In Figure 2, it can be seen that both column combinations gave similar chromatograms in both labs. However, for the two lignins with higher relative molar mass, LF-SW and LB-SW/HW, the chromatograms from setup Aq 1 show an exclusion peak in the high molar mass end, indicating that the separation efficiency of this setup is insufficient for samples in this molar mass range. Setup Aq 2 was, therefore, chosen as the recommended harmonized method.



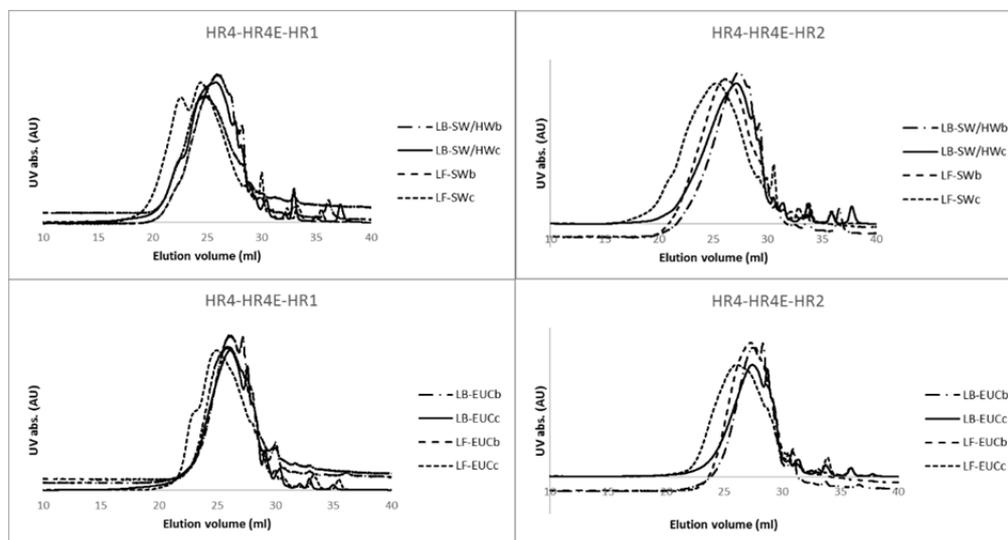
**Figure 2.** SEC chromatograms obtained in two labs ( $VTT=a$ ,  $RISE=b$ ) using 0.1M NaOH and the two different column setups, MCX 1000Å+1000Å and MCX 1000Å+100000Å (setups Aq 1 and Aq 2, Table 2).

### Organic SEC

Comparison of the different organic SEC systems proved somewhat more complicated. The chromatograms obtained for acetobrominated samples (Figure 3) and acetylated samples (Figure 4) show different chromatographic behavior in the two labs.



**Figure 3.** SEC chromatograms obtained from acetobrominated samples in two labs (RISE=b, FPInnovations=c) using THF and two different column setups; Waters HR4+HR4E+HR1 and HR4+HR4E+HR2 (setup THF2 and THF3, Table 2).



**Figure 4.** SEC chromatograms obtained from acetylated samples in two different labs (RISE=b, FPInnovations=c) using THF and two different column setups; Waters HR4+HR4E+HR1 and HR4+HR4E+HR2 (setup THF6 and THF7, Table 2).

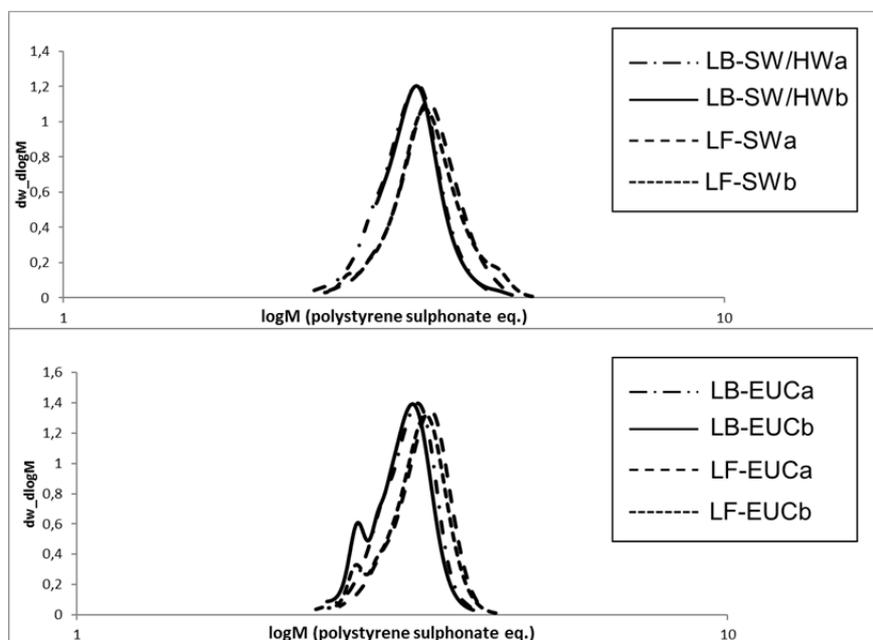
The chromatograms obtained from analysis of acetylated lignins (Figure 4) show a tendency of “front tailing”. This phenomenon has been described previously [1], and has been explained to be an effect of the, sometimes, insufficient solubility and/or aggregation of acetylated lignin in THF. We, therefore, concluded that, for kraft lignins, acetobromination seems preferable compared to acetylation.

In both Figures 3 and 4, it can be seen that the chromatograms obtained in the two labs are not similar. The reason for this remains to be investigated.

It is known that, at certain conditions, the styrene divinylbenzene column material in the Styragel columns may be degraded, or contaminated with adsorbed lignin [1, 4, 6], which affects the chromatographic behavior. Whether this, or something else, is the reason for the differences found here will be the subject of the remaining part of the project. For the time being, it is not possible to decide which column setup is to be recommended for molar mass determination of kraft lignins.

### Integration and calculation

For the aqueous SEC system, calibration using polystyrene sulfonate standards was used to convert the chromatograms to the corresponding relative molar mass distributions (Figure 5). As can be seen, the relative molar mass distributions obtained in the two labs are almost similar. This means the calibration further compensated for the (relatively small) differences in chromatographic performance between the two labs observed in Figure 2.



**Figure 5.** Relative molecular mass distributions calculated using linear calibration by polystyrene sulfonate standards from SEC employing setup Aq2 (Table 2). ( $VTT=a$ ,  $RISE=b$ .)

The resulting average molar masses are given in Table 3. As can be seen, the harmonized method gave results that were much more similar compared to the initial analyses (Figure 1). In fact, for  $M_p$  and  $M_n$ , the difference in results between labs is of the same magnitude as the difference between replicates in one lab. The variation in results is the most pronounced for  $M_w$ . One major reason for the variations is that the analytical results are obtained on a logarithmic scale, whereas the calculated relative average molar mass values are given in linear values. This means, very small analytical differences give large differences in calculated average molar mass.

In order to achieve a method applicable for quality assessments, i.e. mitigating trade barriers, an improved strategy for data evaluation must be developed in addition to the harmonized SEC parameters.

Table 3. Results using the harmonized aqueous SEC system

Sample	M <sub>n</sub>		M <sub>n</sub>		M <sub>w</sub>		M <sub>w</sub> /M <sub>n</sub>	
	VTT	RISE	VTT	RISE	VTT	RISE	VTT	RISE
LF-SW	3600	3300	2500	2600	6300	7600	2.5	2.9
LF-EUC	3100	2800	1900	1700	2400	3100	1.3	1.8
LB-SW/HW	2700	2600	1700	1700	3600	3800	2.1	2.2
LB-EUC	2200	1900	1400	1200	2400	2000	1.7	1.6

## Conclusions

- The results from size-exclusion chromatography largely depend on the instrumental parameters and must be interpreted with caution.
- Harmonization of aqueous SEC has been successful. In short, the method comprises SEC using 0.1M NaOH as the mobile phase, a mobile phase flow rate of 0.5 mL/min, Waters columns MCX 1000Å and 100000Å in series, and calibration/calculation using polystyrene sulfonate standards using a linear fit.
- Even though the (aqueous) SEC analysis can be repeated, very small analytical differences give large differences in calculated average molar mass. In order to achieve a method applicable for quality assessments, a strategy for data evaluation must be developed.
- In the next step a draft ISO Technical Specification will be prepared.
- Organic SEC has proven more complex and further investigations are needed.
- Acetobromination prior to organic SEC seems preferable compared to acetylation.

## References

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