Supplementary Materials: Application of a Universal Calibration Method for True Molar Mass Determination of Fluoro-Derivatized Technical Lignins by Size-Exclusion Chromatography

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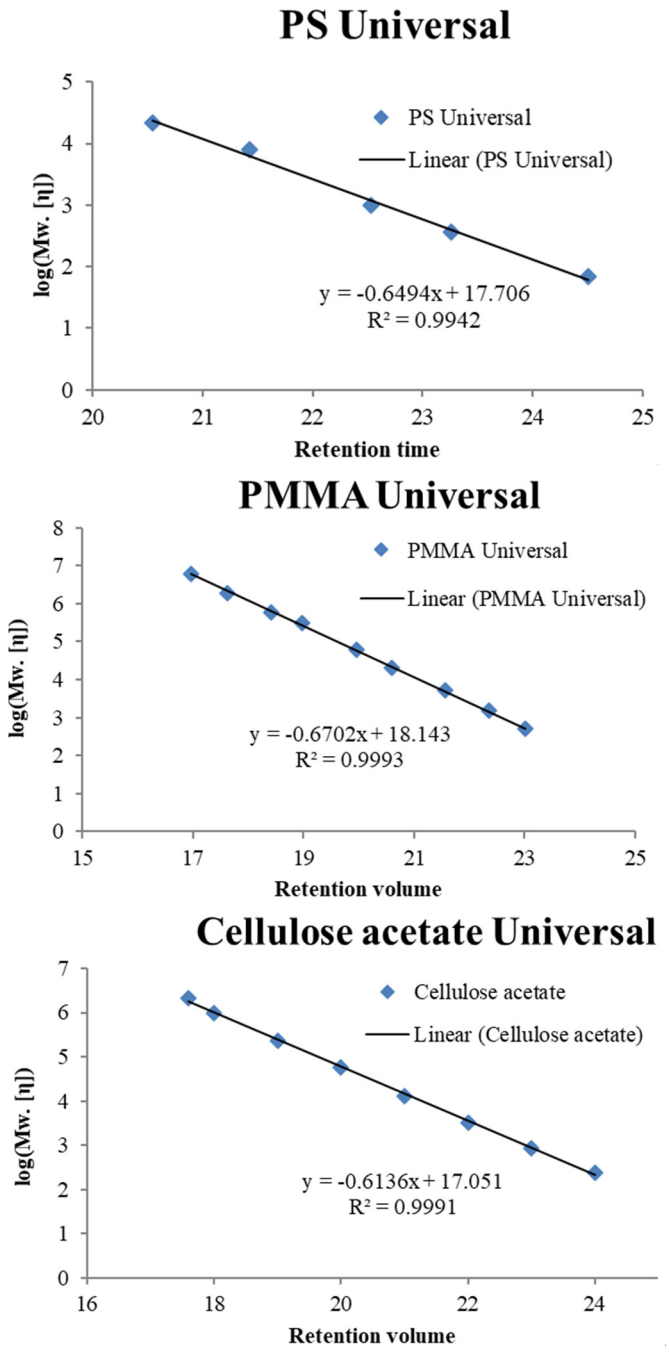

Figure S1. Individual universal calibration curves including data of each type of polymer.

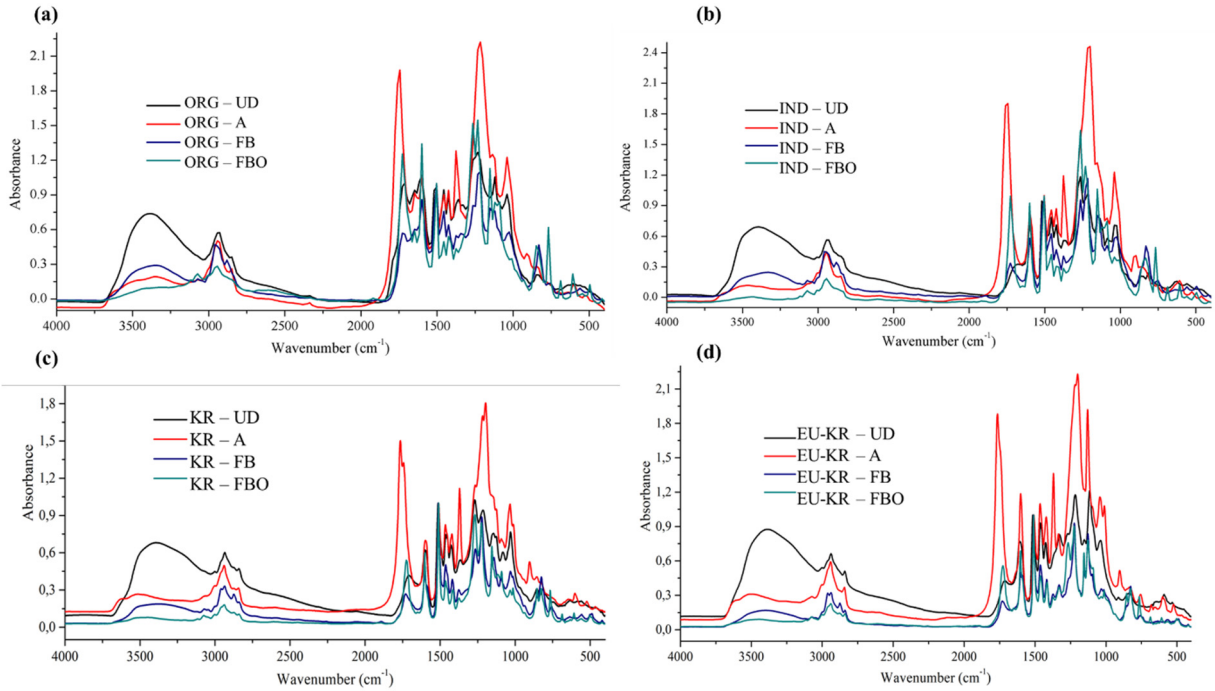

Figure S2. FTIR spectra of ethyl acetate washed (**a**) ORG, (**b**) IND, (**c**) KR, and (**d**) EU-KR (Eucalyptus kraft) lignins, UD—Underivatized, A—Acetylated, FB—Fluorobenzylated, FBO—Fluorobenzoylated.

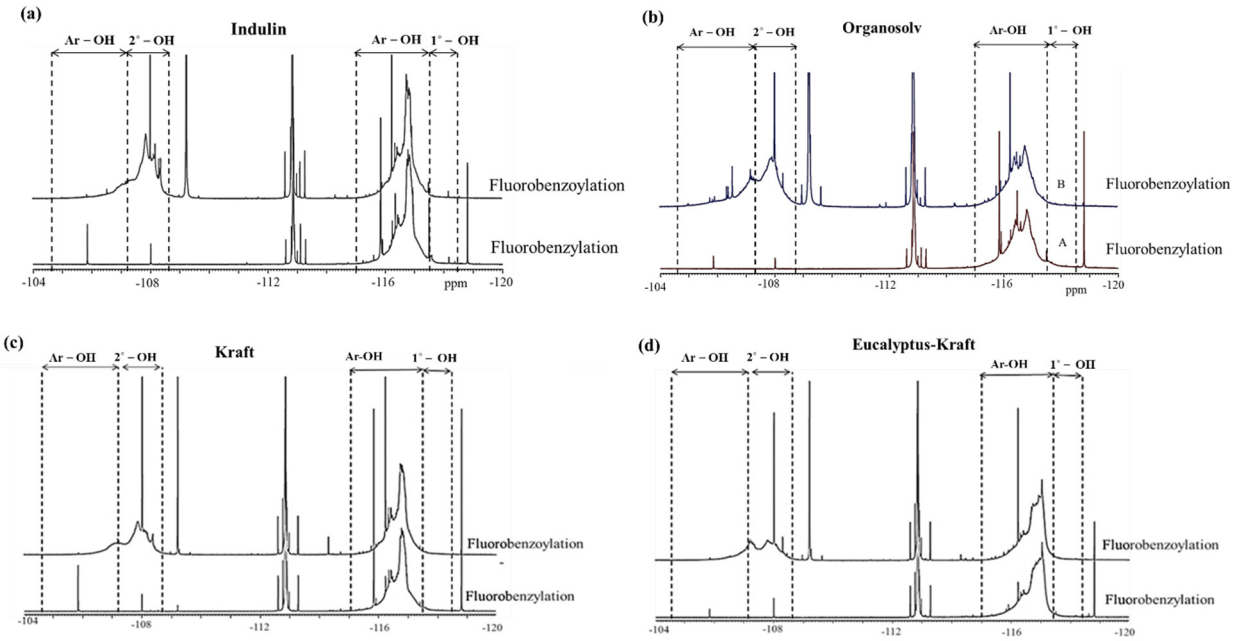

Figure S3. ^{19}F NMR spectra of fluorobenzylated and fluorobenzoylated, (**a**) ORG, (**b**) IND, (**c**) KR, and (**d**) EU-KR. Chemical shifts relative to $CFCl_3$. Internal standard: 2-fluoroacetophenone.

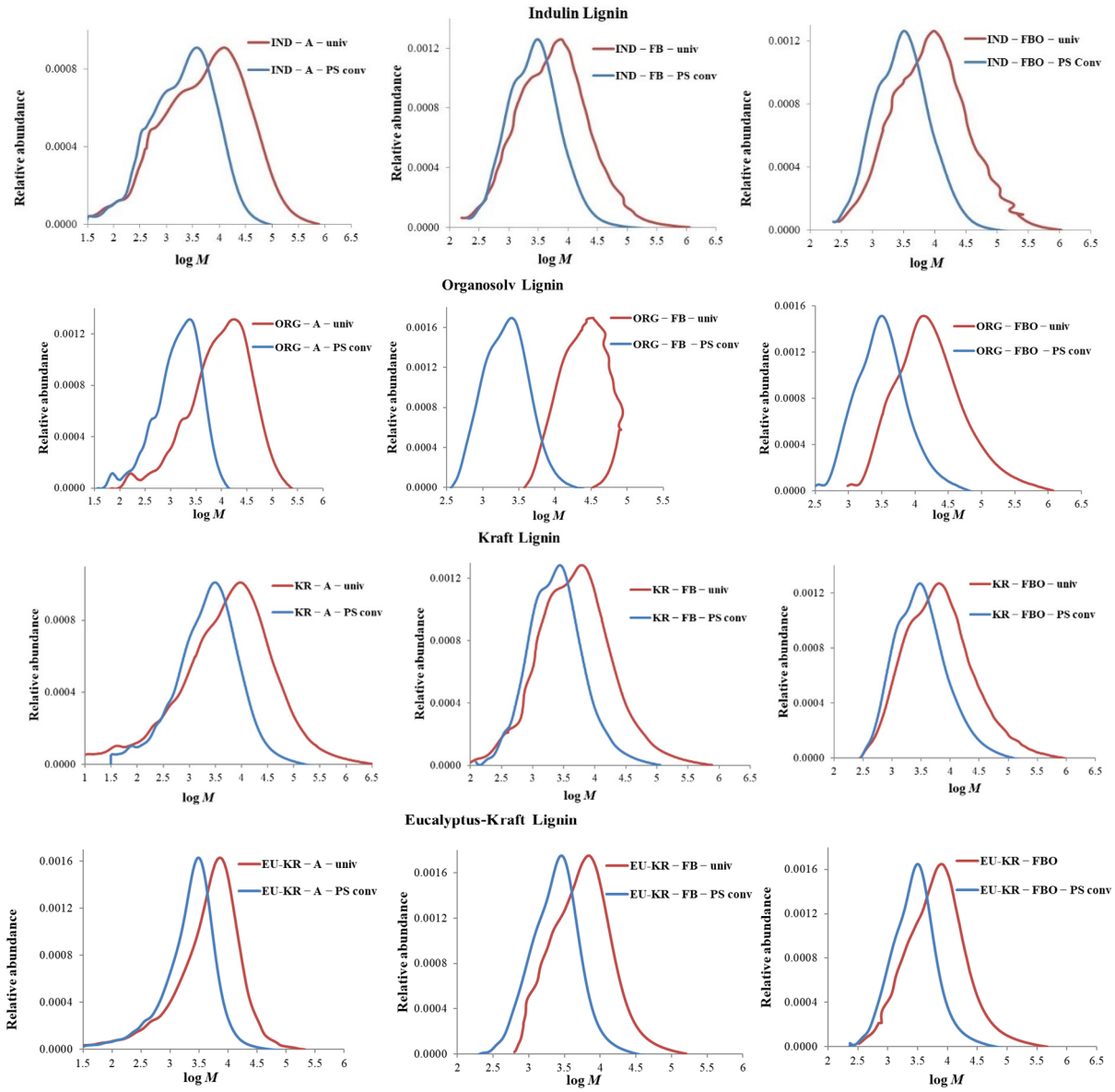

Figure S4. SEC—MMD curves (in THF): comparison of universal and standard calibration—calculations from global curve fit; A—acetylated, FB—fluorobenzylated and FBO—fluorobenzoylated lignin samples. Top to Bottom: IND, ORG, KR and EU-KR.

Equation S1 describes the calculation of the number of moles of hydroxyl groups. The following parameters are required: M_L = molar mass of lignin, M_{IS} = molar mass of internal standard, A_{Lar-OH} = Area of lignin aromatic hydroxyl group, $A_{LAli-OH}$ = Area of lignin aliphatic hydroxyl group, m_L = Mass of lignin, m_{IS} = mass of internal standard, M_F = molar mass of fluorobenzylated lignin, M_{HCl} = molar mass of HCl. The step-by-step explanation of this equation can be found in the reference [1].

$$n_{OH} = \frac{M_L}{M_{IS}} \times A_{LAr-OH} \times m_{IS}/m_L \times 1 \Big/ \left(1 - (M_F - M_{HCl}) \times \left(\frac{m_{IS}}{m_L}\right) \times A_{LAr-OH} \times \left(1 + \left(\frac{A_{LAli-OH}}{A_{LAr-OH}}\right)\right)\right) \quad \text{(S1)}$$

The mol/mol values of total hydroxyl groups in fluorobenzylated lignin samples are presented in Table S1. Among the investigated lignins, fluorobenzylation reaction predicted that IND lignin contains the highest total number of phenolic hydroxyls and little part of primary hydroxyls, totally about 0.697 mol/mol aromatic unit. It is followed by

EU-KR lignin (0.642 mol/mol) and by KR lignin (0.628 mol/mol). The least hydroxyl content was obtained for the case of ORG lignin (0.456 mol/mol). It can be pointed out that wheat straw or annual grass plants present lower hydroxyl groups content than hardwood and softwood lignins.

Table S1. Total OH groups (in mol/mol aromatic unit) in fluorobenzylated lignin samples, determined by ^{19}F NMR spectra.

Lignin (mg)	IS (mg)		FB Carbonylated–ArOH	FB Ar–OH	Total Ar–OH	1°–OH	Total OH
					x	y	
PB	4.3	A	0.328	0.561	0.889	0.053	
(15.5)		N			0.449	0.027	0.476
ORG	3.3	A	0.505	0.603	1.108	0.078	
(15.5)		N			0.426	0.030	0.456
IND	3.2	A	0.494	1.146	1.640	0.052	
(15.5)		N			0.675	0.021	0.697
KR	5.1	A	0.282	0.633	0.915	0.036	
(15)		N			0.604	0.024	0.628
EU-KR	5.2	A	0.251	0.699	0.95	0.024	
(15.4)		N			0.626	0.016	0.642

A—Area of the peak, N—number of moles, x and y represents number of moles of aromatic and aliphatic hydroxyl groups (mol/mol), IS—internal standard.

From the fluorobenzylation spectra, the number of moles of aromatic (x) and aliphatic hydroxyl groups (y) were calculated, and these values were used for the calculation of the monomer molar mass based on the equation below (Equation S2), with M_L = 200 g/mol, M_F = 144.5 g/mol (FBC) and M_{HCl} = 36.5 g/mol. The calculated "M" value represents the monomer molar mass of fluorobenzylated lignin which therefore will be used for determining the degree of polymerization of all lignin samples.

$$M = M_L + (x + y)M_F - (x + y)M_{HCl} \quad \text{(S2)}$$

From the analysis of fluorobenzoylated lignins ^{19}F NMR spectra and the determination of each peak area, the following Equation S3 was used to calculate the –OH content in derivatized lignins.

$$n_{OHx} = \frac{M_L}{M_{IS}} \times A_{Lix} \times (m_{IS}/m_{Li}) \times 1 \Big/ \left[(1 - (A_{Liu} \times m_{IS})/(M_{IS} \times m_{Li}) \times (M_{F1} - M_{HCl}) \times \left(1 + \left(\frac{A_{LiY}}{A_{Liu}}\right) + (M_{F2} - M_{FBA}) \times \frac{(A_{LiV} + A_{LiZ})}{A_{Liu}}\right)\right] \quad \text{(S3)}$$

The following parameters are used for the calculations: M_{FBC} = 144.5 g mol^{-1}, FBC = 4-fluorobenzyl chloride, M_{HCl} = 36.47 g mol^{-1}, HCl = hydrochloric acid, M_{FBAA} = 262.2 g mol^{-1}, FBAA = 4-fluorobenzoic acid anhydride, M_{FBA} = 140.1 g mol^{-1}, FBA = Fluorobenzoic acid, M_{IS} = 138.14 g mol^{-1}, IS = Internal standard (2-fluoroacetophenone), m_{IS} = mass of internal standard, m_{Li} = mass of lignin, A_{Liu} = Area corresponding to fluorobenzylated aromatic hydroxyl, A_{LiV} = Area corresponding to fluorobenzoylated aromatic hydroxyl, A_{Liy} = Area of primary hydroxyls, A_{LiZ} =Area of secondary hydroxyls.

The calculated total number of hydroxyl groups present in the lignin are reported in Table S2. From fluorobenzoylation, it is estimated that IND lignin possesses the highest total number of hydroxyl groups, followed by KR lignin. This trend differs from the results obtained by fluorobenzylation, where EU-KR lignin exhibited more hydroxyl groups than KR lignin. PB hydroxyl groups content is lower than for KR lignin, but higher than for EU-KR; ORG has the lowest content, typically about one-half of the IND lignin. Considering the extraction process employed for the investigated lignins, ORG lignin was extracted by the "lighter process", in comparison to the aqueous alkaline cooking processes that take place at much higher temperatures. This could play a significant role on the total hydroxyl groups quantity.

Table S2. Total OH groups in fluorobenzoylated lignin in (mol mol^{-1}), determined by ^{19}F NMR spectroscopic analysis.

Lignin (mg)	IS (mg)		FBO Ar–OH	FB Ar–OH	Total Ar–OH	2°–OH	1°–OH	TOTAL OH_{aliph}	Total OH
			v	u		z	y		
PB	4.4	A	0.196	0.945		0.446	0.027		
(16.7)		n	0.115	0.554	0.669	0.262	0.016	0.277	0.931
ORG	4.2	A	0.287	0.566		0.328	0.029		
(15.5)		n	0.155	0.305	0.460	0.177	0.016	0.193	0.637
IND	4.8	A	0.187	0.826		0.450	0.022		
(15.1)		n	0.141	0.623	0.764	0.339	0.017	0.356	1.119
KR	4.8	A	0.159	0.909		0.379	0.028		
(16.3)		n	0.105	0.601	0.706	0.251	0.019	0.269	0.957
EU-KR (16.2)	5.2	A	0.171	0.792		0.211	0.025		
		n	0.116	0.536	0.652	0.143	0.017	0.160	0.794

A—Area of the peak, n—number of moles, u and v describe the number of moles of aromatic hydroxyl groups fluorobenzylated (mol/mol) and fluorobenzoylated (mol/mol), y and z represent the number of moles of primary aliphatic hydroxyl groups fluorobenzylated (mol/mol) and fluorobenzoylated (mol/mol) respectively.

In order to calculate the monomer molar mass of fluorobenzoylated lignin samples, u, v, y and z values were extracted from the fluorobenzoylated lignin ^{19}F NMR spectra (in Table S2) and these values were further substituted in the Equation S4 to find out monomer molar mass of fluorobenzoylated lignin. The following components are considered for the monomer molar mass estimation: M_{F2} = molar mass of fluorobenzoic acid anhydride (FBAA), M_{FBA} = molar mass of fluorobenzoic acid produced as by-product during fluorobenzoylation, F_1= FBC molar mass of 4-fluorobenzyl chloride = 144.5 g mol^{-1}, M_L = 200 g mol^{-1} (molar mass of lignin monomer unit, assumed to 200 g/mol in all the calculations).

$$M = M_L + (u + y)(M_{F1} - M_{HCl}) + (v + z)(M_{F2} - M_{FBA}) \quad \text{(S4)}$$

These results can be compared to hydroxyl groups contents in each lignin samples (given in Table S1 and Table S2). Softwood KR and IND lignins exhibited higher total hydroxyl groups number than hardwood Kraft and annual plant lignins, in which most of the phenolic hydroxyl groups were derivatized during fluorobenzylation. IND lignin contains significant amount of aliphatic hydroxyl groups which are derivatized during fluorobenzoylation. It is interesting to note that the wheat straw PB lignin showed higher hydroxyl content than hardwood (Eucalyptus) Kraft lignin. ORG lignin (also from wheat straw) exhibited least total hydroxyl content; however its aliphatic hydroxyl content was higher than EUCA-Kraft lignin. Overall, these results revealed that Indulin lignin contains higher amount of total hydroxyl groups than all other lignins.

Table S3. Total number of OH groups (in mmol g^{-1}) in fluorobenzoylated lignin samples, determined by ^{19}F NMR spectra.

Lignin	Fluorobenzylated		Fluorobenzoylated		Total OH_{Phenol}	Total OH_{aliph}	Total $OH_{Calculated}$ mmol/g derivatized lignin
	Ar–OH	1°–OH	Ar–OH	2°–OH			
PB	1.802	0.051	0.374	0.851	2.176	0.902	3.078
ORG	1.110	0.057	0.563	0.643	1.673	0.700	2.373
IND	1.901	0.051	0.430	1.036	2.331	1.087	3.418
KR	1.938	0.060	0.339	0.808	2.277	0.868	3.145
EU-KR	1.840	0.058	0.397	0.490	2.237	0.548	2.785

Table S4. MMD of derivatized IND lignin from universal calibration with global curve fit and individual curve fit and comparison with standard calibration. (M values in g mol^{-1}).

	Universal calibration (Viscometric detector)				Standard calibration (RI detector)			
IND-Acetylated	M_n	M_w	M_w/M_n	M_{peak}	M_n	M_w	M_w/M_n	M_{peak}
Fit with Global curve[a]	1090	19920	18.3	12300	-	-	-	-
PS	950	17980	18.9	10990	820	4530	5.5	3770
PMMA	720	16860	23.4	9690	1040	5680	5.5	4750
Cellulose acetate	1880	25150	13.4	16970	1160	3940	3.4	3700
IND-Fluorobenzylated								
Fit with Global curve[a]	2440	15390	6.3	7600	-	-	-	-
PS	2150	13890	6.5	6780	1660	4490	2.7	3040
PMMA	1750	13020	7.4	5920	2110	5630	2.7	3830
Cellulose acetate	3790	19480	5.1	10670	1970	3980	2.0	3090
IND-Fluorobenzoylated								
Fit with Global curve[a]	3760	22270	5.9	9700	-	-	-	-
PS	3320	20100	6.1	8650	1910	5040	2.6	3210
PMMA	2730	18870	6.9	7570	2420	6310	2.6	4050
Cellulose acetate	5780	28080	4.9	13550	2210	4400	2.0	3230

a = fit with global curve (PS, PMMA, cellulose acetate).

Table S5. MMD of derivatized ORG lignin, from universal calibration with global curve fit and individual curve fit, and comparison with standard calibration. (M values in g mol^{-1}).

	Universal calibration (Viscometric detector)				Standard calibration (RI detector)			
ORG-Acetylated	M_n	M_w	M_w/M_n	M_{peak}	M_n	M_w	M_w/M_n	M_{peak}
Fit with Global curve[a]	2900	18950	6.5	17810	-	-	-	-
PS	2530	16930	6.7	15850	770	2100	2.7	2380
PMMA	1940	14920	7.7	13660	980	2650	2.7	3010
Cellulose acetate	4950	26270	5.3	25500	1070	2160	2.0	2520
ORG-Fluorobenzylated								
Fit with Global curve[a]	17981	27328	1.5	32630	-	-	-	-
PS	17480	29170	1.7	29050	1680	2690	1.6	2500
PMMA	14610	25550	1.7	25100	2130	3390	1.6	3150
Cellulose acetate	29610	45760	1.5	46540	1930	2700	1.4	2620
ORG-Fluorobenzoylated								
Fit with Global curve[a]	8600	34410	4.0	13540	-	-	-	-
PS	7620	31030	4.1	12080	2240	4800	2.1	3180
PMMA	6400	28970	4.5	10570	2850	6020	2.1	4015
Cellulose acetate	12800	43720	3.4	18940	2500	4270	1.7	3210

a = fit with global curve (PS, PMMA, cellulose acetate).

Table S6. MMD of derivatized KR lignin, from universal calibration with global curve fit and individual curve fit, and comparison with standard calibration. (M values in g mol^{-1}).

	Universal calibration (Viscometric detector)				Standard calibration (RI detector)			
KR-Acetylated	M_n	M_w	M_w/M_n	M_{peak}	M_n	M_w	M_w/M_n	M_{peak}
Fit with Global curve[a]	540	31060	57.5	9520	-	-	-	-
PS	470	28190	60.0	8490	750	5110	6.8	3100
PMMA	350	27270	77.9	7420	960	6380	6.6	3910
Cellulose acetate	990	37450	37.8	13340	1110	4290	3.9	3130
KR-Fluorobenzylated								
Fit with Global curve[a]	2190	13670	6.2	6250	-	-	-	-
PS	1930	12340	6.4	5570	1510	4170	2.8	2770
PMMA	1560	11550	7.4	4840	1920	5230	2.7	3490
Cellulose acetate	3430	17340	5.1	8840	1820	3710	2.0	2850
KR-Fluorobenzoylated								
Fit with Global curve[a]	3210	18190	5.7	6530	-	-	-	-
PS	2840	16450	5.8	5830	2020	5580	2.8	3040
PMMA	2350	15630	6.7	5090	2570	6980	2.7	3830
Cellulose acetate	4860	22540	4.6	9170	2310	4730	2.0	3090

a = fit with global curve (PS, PMMA, cellulose acetate).

Table S7. MMD of derivatized EU-KR lignin from universal calibration with global curve fit and individual curve fit, and comparison with standard calibration. (M values in g mol^{-1}).

	Universal calibration (Viscometric detector)				Standard calibration (RI detector)			
EU-KR-Acetylated	M_n	M_w	M_w/M_n	M_p	M_n	M_w	M_w/M_n	M_{peak}
Fit with Global curve[a]	1260	8460	6.7	7270	-	-	-	-
PS	1100	7580	6.9	6480	1010	3210	3.2	3070
PMMA	840	6820	8.1	5660	1300	4040	3.1	3870
Cellulose acetate	2150	11370	5.3	10200	1390	3080	2.2	3110
EU-KR-Fluorobenzylated								
Fit with Global curve[a]	3390	8580	2.5	7000	-	-	-	-
PS	3000	7680	2.6	6240	1780	3130	1.8	2870
PMMA	2500	6880	2.8	5430	2260	3940	1.7	3630
Cellulose acetate	5120	11610	2.3	9870	2040	3050	1.5	2940
EU-KR-Fluorobenzoylated								
Fit with Global curve[a]	3630	13210	3.6	7990	-	-	-	-
PS	3210	11890	3.7	7130	2040	4060	2.0	3200
PMMA	2670	10930	4.1	6240	2580	5100	2.0	4040
Cellulose acetate	5470	17200	3.1	11170	2300	3740	1.6	3230

a = fit with global curve (PS, PMMA, cellulose acetate).

Table S8. DP-average from standard calibration (solvent = THF; individual curve fit).

			Standard calibration in THF								
			Polystyrene			PMMA			Cellulose acetate		
		Molar mass of monomer	DP_n	DP_w	dispersity	DP_n	DP_w	dispersity	DP_n	DP_w	dispersity
PB	Acetylated	240	2	12	5	3	15	5	4	11	3
	FB	251*	6	13	2	7	16	2	7	12	2
	FBO	308*	7	18	3	9	22	2	8	15	2
IND	Acetylated	240	3	19	6	4	24	5	5	16	3
	FB	275*	6	16	3	8	20	3	7	14	2
	FBO	328*	6	15	3	7	19	3	7	13	2
ORG	Acetylated	240	3	9	2.7	4	11	2.7	4	9	2.0
	FB	249*	7	11	1.6	9	14	1.6	8	11	1.4
	FBO	275*	8	17	2.1	10	22	2.1	9	16	1.7
KR	Acetylated	240	3	21	6.8	4	27	6.7	5	18	3.9
	FB	268*	6	16	2.8	7	20	2.7	7	14	2.0
	FBO	310*	7	18	2.8	8	22	2.7	7	15	2.0
EU-KR	Acetylated	240	4	13	3.2	5	17	3.1	6	13	2.2
	FB	269*	7	12	1.8	8	15	1.7	8	11	1.5
	FBO	291*	7	14	2.0	9	18	2.0	8	13	1.6

* Monomer molar mass of FB and FBO lignins calculated from ^{19}F NMR; DP_w/DP_n = dispersity.

References

1. Esakkimuthu, E.S. Study of New Chemical Derivatization Techniques for Lignin Analysis by Size Exclusion Chromatography. Ph.D. Thesis, Université Grenoble Alpes, Grenoble, France, 2020.