Characterization of liquid-liquid extraction fractions from lignocellulosic biomass by high performance liquid chromatography hyphenated to tandem high-resolution mass spectrometry (HPLC/MSⁿ)

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Supplementary material

Table S1: Experimental log K obtained by GC-FID for the system MTBE/acetate buffer solution at pH = 2.8 and theoretical values log P related to chemical structures.

chemical family	analyte	MTBE		acetate buffer solution (pH 2.8)		K	log K	log P (chemicalize.com)
		t _R	area	t _R	area			
aliphatic acids	acetic acid	4.8	1.3	4.7	2.7	0,5	-0.3	-0.2
	propanoic acid	5.8	5.9	5.7	1.9	3,1	0.5	0.5
	butanoic acid	6.9	9.5	6.8	0.7	13,6	1.1	0.9
	levulinic acid	9.9	0.8	9.9	2.7	0,3	-0.6	-0.1
aromatic acids	ferulic acid	13.2	9.7	13.2	0.2	44,1	1.6	1.7
	sinapic acid	15.3	7.3	15.3	0.6	12,2	1.1	1.5
phenols	phenol	9.4	28.5	9.4	0.3	86,5	1.9	1.7
	4-ethylphenol	11.5	20.5	11.5	0.01	1709	3.2	2.6
	trimethylphenol	12.1	21.4	12.1	0.01	2138	3.3	3.2
	p-cresol	11.0	21.4	11.0	0.02	1068	3.0	2.2
	catechol (benzenediol)	11.7	13.3	11.7	0.1	101	2.0	1.4
	2-methoxyphenol	10.8	16.3	10.8	0.4	36,9	1.6	1.5
	methoxybenzene	8.9	18.6	8.9	0.04	442	2.6	1.8
	isoeugenol	14.4	20.2	14.4	0.02	1008	3.0	2.6
aldehydes	2-methyl-2-butanal	6.6	13.5	6.6	1.2	11,5	1.1	1.2
	acetaldehyde	3.7	1.6	3.7	3.1	0,5	-0.3	-0.4
ketones	hydroxyacetone	5.3	11.6	5.3	101	0,1	-0.9	-0.7
	2-hexanone	7.1	15.4	7.2	0.4	39,9	1.6	1.7
	2-pentanone	5.9	21.4	5.9	2.2	9,9	1.0	1.3
	3-methylcyclopent-2-	9.0	128	9.0	114	1,1	0.1	1.3
	2,5-hexanedione	8.6	5.4	8.6	8.4	0,6	-0.2	0.3
esters	ethyl acetate	5.1	10.3	5.1	1.4	7,6	0.9	0.3
	propyl acetate	6.2	13.7	6.2	0.5	29,8	1.5	0.8
	butyl acetate	7.5	12.9	7.5	0.1	180	2.3	1.3
	methyl butanoate	6.3	10.9	6.3	0.3	37,6	1.6	1.1
furans	furan	4.3	5.5	4.3	0.04	147	2.2	1.1
	furfural	7.6	8.4	7.6	1.9	4,5	0.7	0.8
	hydroxymethylfurfural	11.9	5.8	11.9	13.5	0,4	-0.4	-0.1
	furfurylic alcohol	7.9	9.4	7.9	2.4	4,0	0.6	0.3
	3-ethyl dihydro 2 (3H)	10.2	10.3	10.2	3.4	3,0	0.5	0.4
	2-methyl	5.8	10.6	5.8	1.8	5,9	0.8	1.0
alcohols	methanol	3.7	1.4	3.7	5.6	0,3	-0.6	-0.5
	ethanol	3.9	3.5	3.9	6.2	0,6	-0.2	-0.2
	propan-2-ol	4.2	6.3	4.2	5.2	1,2	0.1	0.3
	hexan-1-ol	8.2	18.3	8.2	0.2	84,6	1.9	1.7

Figure S1: Partition coefficients of 35 model molecules representative of biomass chemical families in MTBE/water solvent system at pH 2.8.

Figure S2: Mass distribution in the four fractions and the lost masses. Histograms correspond to the average mass percentage of each chemical family obtained after 3 repetitions on model molecules.

Figure S3: Venn diagrams representing the number of detected compounds with each ionization modes and those detected by several modes. Only the formulae of the compounds were used and compared between ionization modes to build this diagram. Thus isomers with different retention times were not taking into account and were considered as only one compound. (traced on http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.html)

Figure S4: DBE of AQ1 components as a function of their molecular mass (blue points: compounds eluted with less than 12% of MeOH, black points: eluted with more than 12% of MeOH)

Figure S5: Main losses for MS² to MS⁷ fragmentation stages in negative-ion mode ESI according to HPLC retention time (AQ1)

Figure S6: MS² spectrum obtained in negative-ion ESI mode with annotation of compounds a, b and c present in fraction AQ1

Figure S7: ORG2 van Krevelen diagram (1), DBE as a function of molecular mass (2) and neutral losses in positive-ion mode ESI for MS² until MS3 according to retention time (3)

Figure S8: ORG3 van Krevelen diagram (1), DBE as a function of molecular mass (2) and losses in negative-ion mode ESI for MS² (red dots) until MS6 (black outline circles) according to retention time (3)

Table S2: Fragments obtained in negative-ion mode ESI for the most intense ions in AQ3

<i>m/z</i>	<i>t_R</i> (min)	Ion formula (<i>m/z</i>)	DBE	MS ² loss	MS ³ loss	MS ⁴ loss	MS ⁵ loss	MS ⁶ loss	Putative identification
197.0456	12.1	C ₉ H ₉ O ₅	5	-·CH ₃ (100%) -CO ₂ (50%)	-·CH ₃ (100%)				Syringic acid (DBE 5)
163.0400	13.2	C ₉ H ₇ O ₃	6	-CO ₂ (100%)					Coumaric acid (DBE 6)
193.0502	14.2	C ₁₀ H ₉ O ₄	6	-·CH ₃ (100%) -CO ₂ (75%) -(·CH ₃ +CO ₂) (5%)	-CO ₂ (100%)				Ferulic acid (DBE 6)
187.0974	17.0	C ₉ H ₁₅ O ₄	2	-H ₂ O (100%) -(H ₂ O+CO ₂) (30%)					Aliphatic acid (DBE 2)
327.0873	13.0	C ₁₈ H ₁₅ O ₆	11	-CO ₂ (100%) -(2×CO ₂) (30%)	-CO ₂ (100%)				Di-aromatic compounds (DBE between 8 and 12)
389.1242	13.2	C ₂₀ H ₂₁ O ₈	10	-H ₂ O (100%) -(CO ₂ +H ₂ O) (10%) -C ₇ H ₈ O ₂ (5%) -CO ₂ (5%) -(CO ₂ +H ₂ O+CH ₂ O) (5%)	-CO ₂ (100%) -(CO ₂ +CH ₂ O) (5%)	-CH ₂ O (100%) -·CH ₃ (40%)	-·CH ₃ (100%)	-·CH ₃ (100%)	
357.0978	13.5	C ₁₉ H ₁₇ O ₇	11	-CO ₂ (100%) -2×CO ₂ (15%) -(2×CO ₂ +·CH ₃) (10%)	-(CO ₂ +·CH ₃) (100%) -CO ₂ (30%)				
387.1083	13.8	C ₂₀ H ₁₉ O ₈	11	-CO ₂ (100%) -(2×CO ₂ +·CH ₃) (5%) -(2×CO ₂) (5%)	-·CH ₃ -CO ₂ (100%) -CO ₂ (25%)	-·CH ₃ (100%)			
371.1133	14.4	C ₂₀ H ₁₉ O ₇	11	-H ₂ O (100%) -CO ₂ (40%) -CH ₂ O (25%) -(H ₂ O+CO ₂) (5%)	-CO ₂ (100%) -CH ₂ O (30%) -(CO ₂ +·CH ₃) (20%)	-·CH ₃ (100%)	-·CH ₃ (100%) -CO (20%)	-·CH ₃ (100%) -CO (10%)	

401.1240	17.7	$C_{21}H_{21}O_8$	11	-H ₂ O (100%) -CH ₂ O (30%) -CO ₂ (20%) -C ₁₀ H ₁₀ O ₅ (5%) -C ₉ H ₁₀ O ₃ (5%)	-·CH ₃ (100%)	-·CH ₃ (100%)		
353.1030	17.8	$C_{20}H_{17}O_6$	12	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO ₂ (100%)	-CO (100%)	
537.1395	15.8	$C_{28}H_{25}O_{11}$	16	-CO ₂ (100%) -2×CO ₂ (30%) -C ₉ H ₈ O ₆ (5%)	-CO ₂ (100%)	-·CH ₃ (100%)	-·CH ₃ (100%)	-·CH ₃ (100%)
477.1192	18.5	$C_{26}H_{21}O_9$	16	-CO ₂ (100%) -C ₂ H ₄ O ₃ (10%) -C ₈ H ₈ O ₄ (10%)	-·CH ₃ (100%) -CH ₂ O (10%)	-·CH ₃ (100%)		Tri-aromatic compounds (DBE between 12 and 18)
523.1248	18.8	$C_{27}H_{23}O_{11}$	16	-2×CO ₂ (100%) -CO ₂ (95%)	-·CH ₃ (100%) -C ₈ H ₈ O ₂ (60%)	-·CH ₃ (100%)		
731.1973	21.5	$C_{38}H_{35}O_{15}$	21	-CO ₂ (100%) -H ₂ O-CO ₂ (45%)				Tetra- aromatic compounds (DBE between 18 and 24)
699.2073	22.2	$C_{38}H_{35}O_{13}$	21	-CO ₂ (100%)				
741.2182	22.4	$C_{40}H_{37}O_{14}$	22	-CO ₂ (100%) -2×CO ₂ (20%) -C ₂₀ H ₂₀ O ₇ (15%)	-CO ₂ (100%) -C ₁₉ H ₂₀ O ₅ (15%)			

Table S3: Fragments obtained in negative-ion mode ESI for the most intense ions in ORG3

<i>m/z</i>	tr (min)	Ion formula (<i>m/z</i>)	DBE	MS ² loss	MS ³ loss	MS ⁴ loss	MS ⁵ loss	MS ⁶ loss	Putative identification
121.0293	10.1	C ₇ H ₅ O ₂	5	-CO (100%)					Hydroxybenzaldehyde (DBE 5)
151.0400	11.8	C ₈ H ₇ O ₃	5	-·CH ₃ (100%)	-CO ₂ (100%)				Vanillin (DBE 5)
181.0505	12.8	C ₉ H ₉ O ₄	5	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO (100%)			Syringaldehyde (DBE 5)
195.0661	13.8	C ₁₀ H ₁₁ O ₄	5	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO (100%)	-CO (100%)		Acetosyringone (DBE 5)
177.0556	14.5	C ₁₀ H ₉ O ₃	6	-·CH ₃ (100%) - (·CH ₃ +CO) (60%)	-CO (100%)				Coniferaldehyde (DBE 6)
207.0661	14.8	C ₁₁ H ₁₁ O ₄	6	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO (100%)	-CO (100%)		Sinapaldehyde (DBE 5)
273.0765	12.6	C ₁₅ H ₁₃ O ₅	9	-(CO+H ₂ O) (100%) -H ₂ O (60%) -(·CH ₃ +H ₂ O) (30%) -CO (25%) -C ₂ H ₄ O ₃ (20%) -C ₂ H ₃ O ₂ (20%)	-·CH ₃ (100%) -CO (20%)				Di-aromatic compounds (DBE between 8 and 12)
303.0871	13.2	C ₁₆ H ₁₅ O ₆	9	-(CO+H ₂ O) (100%) -H ₂ O (95%) -(·CH ₃ +H ₂ O) (60%) -·CH ₃ (50%) -CO (30%) -C ₂ H ₄ O ₃ (10%) -C ₂ H ₃ O ₂ (5%)	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO (20%)		
333.0975	13.4	C ₁₇ H ₁₇ O ₇	9	-(CO+H ₂ O) (100%) -H ₂ O (95%) -·CH ₃ (65%)	-·CH ₃ (100%)	-·CH ₃ (100%)	-·CH ₃ (100%)	-CO (20%)	

				-CO (20%) -(·CH ₃ +H ₂ O) (20%) -C ₂ H ₃ O ₂ (10%)				
273.0765	17.3	C ₁₅ H ₁₃ O ₅	9	-·CH ₃ (100%)	-CH ₃ (100%)	-CO (100%)	-CO (100%)	
271.061	17.5	C ₁₅ H ₁₁ O ₅	10	-(CO+CO ₂) (100%) -CO (40%)	-H ₂ O (100%) -CO ₂ (50%)			
301.0716	17.7	C ₁₆ H ₁₃ O ₆	10	-(CO+CO ₂ +CH ₃) (100%) -·CH ₃ (80%) -(CO+C ₂ H ₂ O) (45%) -(CO+CO ₂) (30%) -CO (20%)	-CHO (100%) -C ₂ H ₂ O (30%) -HO (20%)			
299.0923	20.8	C ₁₇ H ₁₅ O ₅	10	-·CH ₃ (100%)	-CH ₃ (100%)	-CO (100%) -H ₂ O (15%)	-CO (100%) -CO ₂ (25%)	
329.0664	21.1	C ₁₇ H ₁₃ O ₇	11	-·CH ₃ (100%)	-CH ₃ (100%)	-CO (100%) -(CO+CO ₂) (25%)	-CO ₂ (100%) -CO (70%)	
509.1454	18.6	C ₂₇ H ₂₅ O ₁₀	15	-H ₂ O (100%) -C ₇ H ₈ O ₂ (40%) -(C ₇ H ₈ O ₂ +CH ₂ O) (20%)	-CH ₃ (100%)	-CH ₃ (100%)		
507.1299	21.9	C ₂₇ H ₂₃ O ₁₀	16	-·CH ₃ (100%) -C ₇ H ₈ O ₂ (85%) -(2×·CH ₃ +CO) (45%) -(·CH ₃ +C ₇ H ₈ O ₂) (40%) -C ₁₀ H ₁₀ O ₃ (20%)	-(CO+·CH ₃) (100%) -CH ₃ (80%)			Tri-aromatic compounds (DBE between 12 and 18)
657.1983	21.4	C ₃₆ H ₃₃ O ₁₂	20	-C ₁₈ H ₁₆ O ₆ (100%) -C ₇ H ₈ O ₂ (35%)	-CH ₃ (100%)	-CH ₃ (100%)	-CH ₃ (100%) -(·CH ₃ +CO) (30%)	Tetra-aromatic compounds (DBE between 18 and 24)
683.2144	22.0	C ₃₈ H ₃₅ O ₁₂	21	-C ₁₈ H ₁₆ O ₆ (100%) -C ₂₀ H ₁₈ O ₆ (90%)	-CH ₃ (100%)	-CH ₃ (100%)	-CH ₃ (100%)	