Supplemental Material

Enhanced lipid isomer separation in human plasma using reversed-phase UPLC with ion-mobility / high-resolution MS detection

Carola W.N. Damen,[1,2*], Giorgis Isaac[3], James Langridge[4], Thomas Hankemeier,[1,2] Rob J. Vreeken[1,2]

[1] Netherlands Metabolomics Centre, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands
[2] Division of Analytical Biosciences, Leiden Academic Centre for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands
[3] Waters Corporation, 34 Maple Street, Milford, MA 01757, USA
[4] Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX, UK

* Corresponding author:
Carola W.N. Damen
Netherlands Metabolomics Centre
Division Analytical Biosciences / LACDR
P.O. Box 9502,
2300 RA Leiden
The Netherlands
Tel. +31 (0)71-527 4321
Fax. +31 (0)71-527 4277
e-mail: c.w.n.damen@lacdr.leidenuniv.nl

Legend to Supplemental Tables:

Table 1: Results of robustness test on CSH column with 20 min gradient. For each run 5 injections of a human plasma extract were performed.

Table 2: Results of robustness test on CSH column with 10 min gradient. For each run 5 injections of a human plasma extract were performed.

Table 1: Results of robustness test on CSH column with 20 min gradient. For each run 5 injections of a human plasma extract were performed.

Compound	LPC (20:4)		PC (36:2)		Cer (d18:1/16:0)		SM (C20)		CE (20:4)		MG (20:3)		DG (36:3)		TG (52:1)	
m/z	544.334		786.601		520.509		759.637		690.618		403.282		641.512		878.817	
ion	$[M+H]^+$		$[M+H]^+$		$[M-H_2O+H]^+$		$[M+H]^+$		$[M+NH_4]^+$		$[M+Na]^+$		$[M+Na]^+$		$[M+NH_4]^+$	
	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)
Intra-run 1	1.00	0.000	8.19	0.067	5.64	0.385	9.85	0.144	15.49	0.000	0.65	0.00	10.82	0.124	16.11	0.056
Intra-run 2	1.00	0.000	8.30	0.108	5.70	0.351	10.00	0.205	15.52	0.058	0.65	0.00	10.99	0.119	16.12	0.068
Intra-run 3	0.99	0.000	8.24	0.221	5.66	0.237	9.89	0.143	15.49	0.000	0.65	0.00	10.90	0.112	16.11	0.000
Intra-run 4	1.00	0.000	8.27	0.132	5.69	0.157	9.95	0.174	15.50	0.000	0.65	0.00	10.97	0.122	16.11	0.000
Intra-run 5	1.00	0.000	8.24	0.054	5.68	0.193	9.91	0.111	15.49	0.035	0.65	0.00	10.93	0.000	16.11	0.000
Intra-run 6	1.00	0.000	8.32	0.000	5.71	0.096	10.02	0.109	15.50	0.058	0.65	0.00	11.05	0.181	16.11	0.000
Inter-run	**1.00**	**0.380**	**8.26**	**0.564**	**5.68**	**0.498**	**9.94**	**0.622**	**15.50**	**0.069**	**0.65**	**0.00**	**10.94**	**0.673**	**16.11**	**0.045**

Table 1 (continued)

Compound	FA arachidonic acid (20:4)		LPE (20:1)		PE (40:3)		PI(34:1)		PG (34:1)		ST d18:1/20:0		HexCer (d18:1/16:0)	
m/z	303.233		506.325		796.586		835.53		747.518		834.5770		698.5576	
ion	$[M-H]^-$		$[M-H]^-$		[M-H]-		[M-H]-		[M-H]-		[M-H]-		[M-H]-	
	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)
Intra-run 1	1.89	0.000	1.27	0.000	8.93	0.218	5.91	0.000	5.58	0.196	6.19	0.000	7.70	0.071
Intra-run 2	1.89	0.000	1.27	0.000	8.94	0.158	5.92	0.076	5.58	0.098	6.20	0.088	7.71	0.071
Intra-run 3	1.89	0.290	1.27	0.000	8.82	0.000	5.85	0.187	5.53	0.198	6.12	0.146	7.61	0.186
Intra-run 4	1.88	0.000	1.27	0.000	8.86	0.050	5.86	0.143	5.55	0.197	6.16	0.267	7.65	0.215
Intra-run 5	1.89	0.000	1.27	0.000	8.87	0.000	5.87	0.076	5.54	0.302	6.17	0.000	7.65	0.175
Intra-run 6	1.89	0.000	1.27	0.000	8.96	0.200	5.92	0.076	5.62	0.195	6.22	0.227	7.73	0.216
Inter-run	**1.89**	**0.228**	**1.27**	**0.000**	**8.90**	**0.582**	**5.89**	**0.507**	**5.57**	**0.580**	**6.18**	**0.523**	**7.68**	**0.579**

Table 2: Results of robustness test on CSH column with 10 min gradient. For each run 5 injections of a human plasma extract were performed.

Compound	LPC (20:4)		PC (36:2)		Cer (d18:1/16:0)		SM (C20)		CE (20:4)		MG (20:3)		DG (36:3)		TG (52:1)	
m/z	544.334		786.601		520.509		759.637		690.618		403.282		641.512		878.817	
ion	$[M+H]^+$		$[M+H]^+$		$[M-H_2O+H]^+$		$[M+H]^+$		$[M+NH_4]^+$		$[M+Na]^+$		$[M+Na]^+$		$[M+NH_4]^+$	
	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)
Intra-run 1	0.99	0.000	6.75	0.000	4.93	0.000	7.10	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.94	0.000
Intra-run 2	0.99	0.451	6.75	0.000	4.93	0.181	7.10	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.94	0.000
Intra-run 3	0.99	0.000	6.79	0.000	4.89	0.204	7.08	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.92	0.000
Intra-run 4	0.99	0.000	6.79	0.000	4.89	0.091	7.08	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.92	0.000
Intra-run 5	0.99	0.000	6.79	0.000	4.89	0.145	7.08	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.92	0.000
Intra-run 6	1.00	0.000	6.80	0.081	4.93	0.000	7.10	0.000	8.72	0.000	0.65	0.00	7.20	0.000	8.93	0.123
Inter-run	**0.99**	**0.410**	**6.78**	**0.318**	**4.91**	**0.439**	**7.09**	**0.143**	**8.72**	**0.000**	**0.65**	**0.00**	**7.20**	**0.000**	**8.93**	**0.113**

Table 2 (continued)

Compound	FA arachidonic acid (20:4)		LPE (20:1)		PE (40:3)		PI(34:1)		PG (34:1)		ST d18:1/20:0		HexCer (d18:1/16:0)	
m/z	303.233		506.325		796.586		835.53		747.518		834.5770		698.5576	
ion	$[M-H]^-$		$[M-H]^-$		[M-H]-		[M-H]-		[M-H]-		[M-H]-		[M-H]-	
	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)	Rt (min)	RSD (%)
Intra-run 1	1.81	0.000	1.25	0.000	6.92	0.000	5.06	0.000	4.68	0.096	5.30	0.103	6.58	0.204
Intra-run 2	1.81	0.000	1.27	0.000	6.92	0.000	5.06	0.000	4.68	0.096	5.31	0.084	6.57	0.136
Intra-run 3	1.80	0.000	1.25	0.000	6.90	0.000	5.01	0.000	4.62	0.362	5.25	0.209	6.51	0.168
Intra-run 4	1.80	0.000	1.25	0.000	6.90	0.130	5.01	0.000	4.64	0.236	5.27	0.208	6.53	0.168
Intra-run 5	1.80	0.248	1.25	0.000	6.92	0.000	5.03	0.178	4.63	0.361	5.28	0.085	6.53	0.306
Intra-run 6	1.81	0.000	1.27	0.000	6.92	0.000	5.06	0.000	4.69	0.191	5.33	0.308	6.59	0.166
Inter-run	**1.81**	**0.281**	**1.26**	**0.763**	**6.91**	**0.135**	**5.04**	**0.468**	**4.66**	**0.612**	**5.29**	**0.540**	**6.55**	**0.515**

Legend to Supplemental Figures.

Figure 1: Mobilogram of the mobility separation of PC 18:1(9Z)/18:1(9Z) (red line) and PC 18:1(6Z)/18:1(6Z) (green line). The CCS values for these lipids are 302.8 and 305.6 A^2, respectively.

Figure 2: XIC for a Porcine brain extract (Avanti Lipids) for PC36:2 ([M+H]+ m/z 786.6007 with 0.05 Da extraction window). Clearly 3 peaks are visible. Peaks with Rt 7.74 and 8.49 min are PC 18:1/18:1 and show reasonable resemblance in terms of Rt and CCS compared to the standards PC 18:1(9Z)/18:1(9Z) was PC 18:1(6Z)/18:1(6Z), respectively. The peak with Rt 8.03 min is either PC 18:0/18:2 or PC 18:2/18:0.

Figure 3: Spectrum of a human plasma sample at retention time 7.66 min (+/- 0.08 min) of low (A) and elevated (B) energy spectra without mobility data used and the same low (C) and elevated energy (D) spectra using the mobility data making structural elucidation possible.

Figure 1: Mobilogram of the mobility separation of PC 18:1(9Z)/18:1(9Z) (red line) and PC 18:1(6Z)/18:1(6Z) (green line). The CCS values for these lipids are 302.8 and 305.6 A^2, respectively.

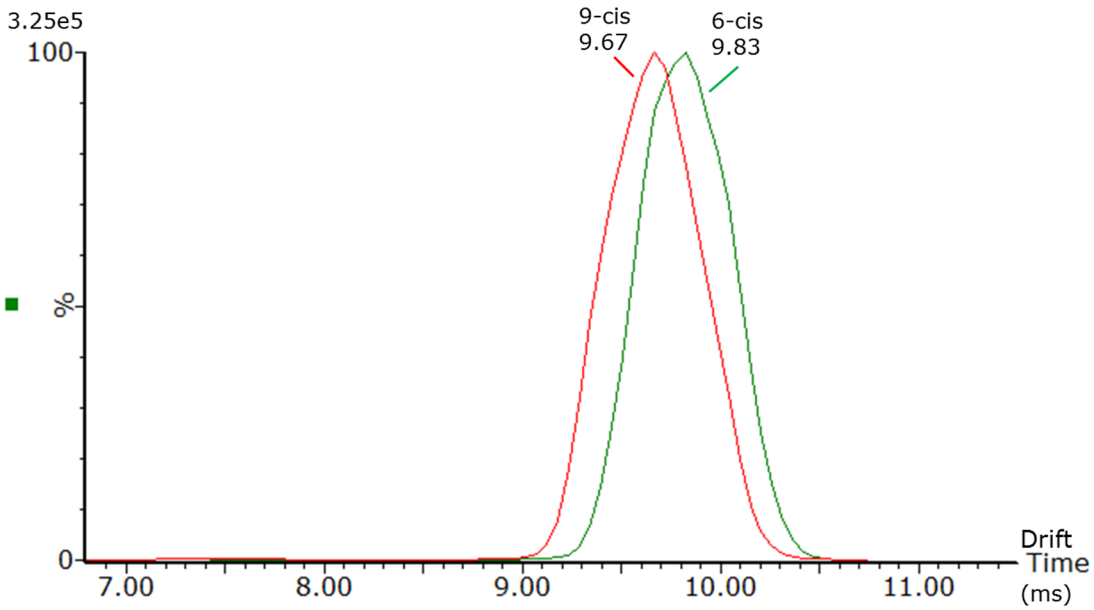

Figure 2: XIC for a Porcine brain extract (Avanti Lipids) for PC36:2 ([M+H]+ m/z 786.6007 with 0.05 Da extraction window). Clearly 3 peaks are visible. Peaks with Rt 7.74 and 8.49 min are PC 18:1/18:1 and show reasonable resemblance in terms of Rt and CCS compared to the standards PC 18:1(9Z)/18:1(9Z) was PC 18:1(6Z)/18:1(6Z), respectively. The peak with Rt 8.03 min is either PC 18:0/18:2 or PC 18:2/18:0.

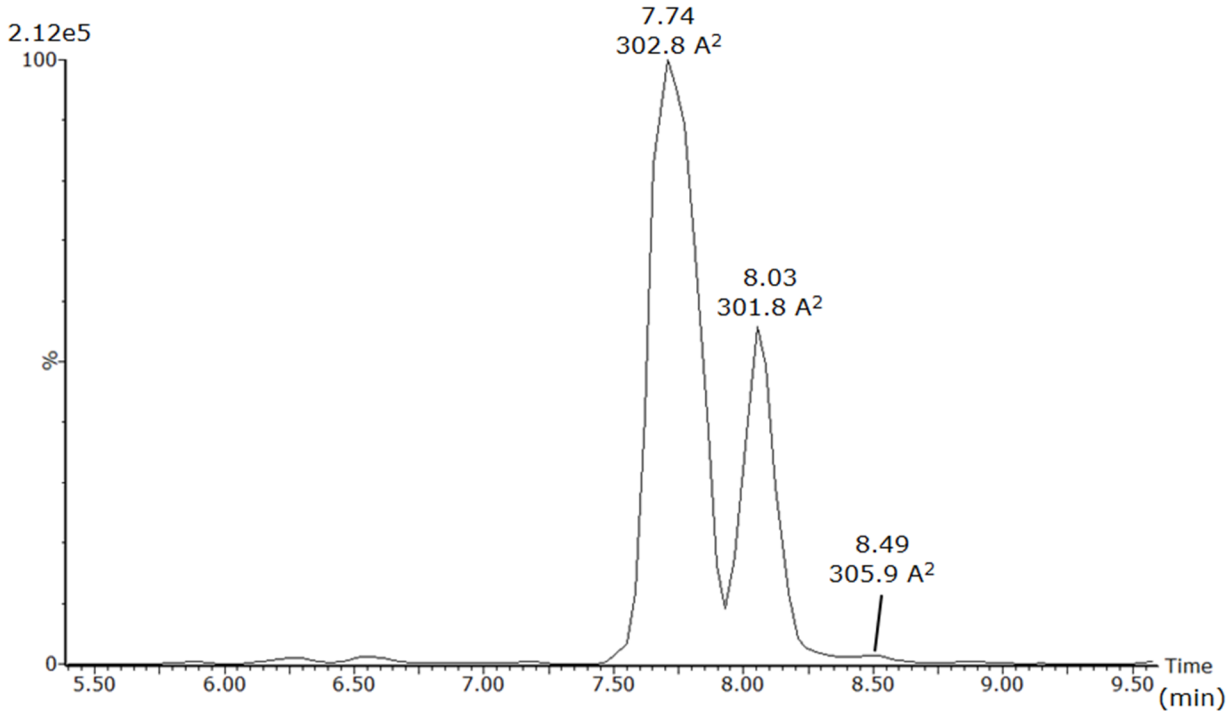

Figure 3: Spectrum of a human plasma sample at retention time 7.66 min (+/- 0.08 min) of low (A) and elevated (B) energy spectra without mobility data used and the same low (C) and elevated energy (D) spectra using the mobility data making structural elucidation possible.