Supplementary data to:

Skonberg et al, Pitfalls in the sample preparation and analysis of *N*-acylethanolamines

Table text:

Table S1A. Calculated concentrations (pM) of PEA in examined chloroforms, hexane and ethyl acetate. Concentrations are calculated relative to injected standards of 1 µM PEA in MeCN, taking into account the volume of solvent evaporated (15, 30 or 60 mL) and the reconstitution volume (100 µL MeOH). (< LOQ = below quantification limit: PEA = 34 pM).

Table S1B. Calculated concentrations (pM) of SEA in examined chloroforms, hexane and ethyl acetate. Concentrations are calculated relative to injected standards of 1 µM PEA in MeCN, taking into account the volume of solvent evaporated (15, 30 or 60 mL) and the reconstitution volume (100 µL MeOH). (< LOQ = below quantification limit: SEA = 39 pM).

Figure legends:

Figure S1A. MS/MS-chromatogram of *m/*z 300.25 in positive electrospray ionisation mode of PEA standard (1 µM in MeCN) with corresponding MS/MS-spectrum (*m/z* 30-325).

Figure S1B. MS/MS-chromatogram of *m/*z 300.25 in positive electrospray ionisation mode of representative sample from evaporated chloroform (Fluka-brand, stabilised with amylene) with corresponding MS/MS-spectrum (*m/z* 30-325).

Figure S2A. MS/MS-chromatogram of *m/*z 328.25 in positive electrospray ionisation mode of SEA standard (1 µM in MeCN) with corresponding MS/MS-spectrum (*m/z* 30-325).

Figure S2B. MS/MS-chromatogram of *m/*z 328.25 in positive electrospray ionisation mode of representative sample from evaporated chloroform (Fluka-brand, stabilised with amylene) with corresponding MS/MS-spectrum (*m/z* 30-350).

Figure S3A. LC-MS chromatograms (SIM on both *m/z* 326.25 and 396.25) from synthesis of 9,10-dichloro-SEA and from OEA in Merck LiChroSolv chloroform. From top to bottom: OEA in CH_2Cl_2 after standing 120 min at ambient temperature, showing no formation of 9,10-dichloro-SEA; start of synthesis before addition of chlorine gas to the OEA solution; 15 min sample from the synthesis of 9,10-dichloro-SEA by chlorination of OEA, showing almost complete disappearance of OEA and subsequent formation of the product; sample of OEA in Merck LiChroSolv chloroform, showing disappearance of OEA and formation of a product with similar mass and retention time to the product from the chlorination synthesis, i.e. 9,10-dichloro-SEA.

Figure S3B. MS/MS-spectra of *m/z* 396.25. Top: Product from chlorination of OEA in CH_2Cl_2; Bottom: Product from reaction of OEA in Merck LiChrosolv chloroform.

Figure S4. ^{1}H-NMR of OEA

Figure S5A. 2D-^{1}H-NMR spectrum (COSY) of 9,10-dichloro-SEA (reaction product from OEA in Merck LiChrosolv)

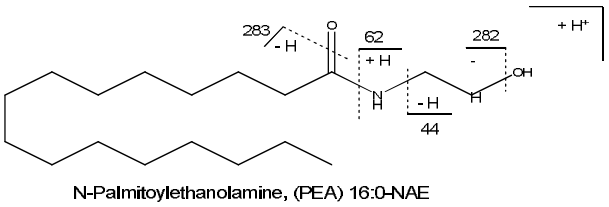
Figure S5B. 2D-^{1}H-NMR spectrum (COSY) of 9,10-dichloro-SEA (reaction product from OEA in Merck LiChrosolv); expansion of 0-4.5 ppm range with coupling between K+N and L+M protons shown.

Solvent, conc. pM PEA	15 mL		30 mL		60 mL		Avg.	SD	CV%
Sigma ACS w. amylene, batch A	355,8	398,3	379,1	340,8	356,1	499,9	388,3	58,3	15,0
Sigma ACS w. amylene, batch B	< LOQ								
Sigma ACS w. EtOH, batch A	429,0	408,0	372,0	399,0	341,0	343,0	382,0	36,0	9,4
Sigma ACS w. EtOH, batch B	< LOQ								
Sigma ACS w. EtOH, batch C	< LOQ								
Sigma CHROMASOLV w. amylene	< LOQ								
Sigma CHROMASOLV w. EtOH	< LOQ								
Fluka w. amylene, batch A	11715,2	11858,0	10042,5	9976,6	8302,3	7672,9	9927,9	1712,5	17,2
Fluka w. amylene, batch B	445,4	472,9	356,3	280,6	385,3	399,3	390,0	68,1	17,5
Fluka w. amylene, batch C	383,8	413,2	365,9	343,8	305,5	296,0	351,4	45,4	12,9
Fluka w. EtOH, batch A	342,5	322,3	308,2	280,4	285,7	285,8	304,2	24,7	8,1
Fluka w. EtOH, batch B	< LOQ								
Fluka w. EtOH, batch C	2254,4	2197,7	1977,3	1993,6	1708,3	1712,5	1974,0	231,5	11,7
Merck EMSURE w. EtOH, batch A	84,0	76,8	66,1	72,6	125,4	113,1	89,7	24,0	26,7
Merck EMSURE w. EtOH, batch B	< LOQ								
Merck LiChroSolv w. amylene	< LOQ								
Alpha-Aesar w. amylene	< LOQ								
Alpha-Aesar w. EtOH	< LOQ								
Merck n-Hexane p.a.	< LOQ								
Merck ethyl acetate p.a.	< LOQ								

Table S1A

Solvent, conc. pM PEA	15 mL		30 mL		60 mL		Avg.	SD	CV%
Sigma ACS w. amylene, batch A	269,4	341,1	331,0	305,7	289,2	293,7	305,0	26,92	8,83
Sigma ACS w. amylene, batch B	< LOQ								
Sigma ACS w. EtOH, batch A	408,1	414,7	397,7	538,9	457,1	379,8	432,7	58,01	13,41
Sigma ACS w. EtOH, batch B	< LOQ								
Sigma ACS w. EtOH, batch C	< LOQ								
Sigma CHROMASOLV w. amylene	< LOQ								
Sigma CHROMASOLV w. EtOH	< LOQ								
Fluka w. amylene, batch A	11145,6	11723,4	8916,2	9689,8	6728,2	6656,8	9143,3	2146,25	23,47
Fluka w. amylene, batch B	619,4	630,9	424,2	317,3	411,5	398,3	466,9	128,15	27,45
Fluka w. amylene, batch C	730,6	704,0	551,8	569,8	462,4	454,3	578,8	117,11	20,23
Fluka w. EtOH, batch A	308,7	281,0	389,0	318,9	379,1	327,4	334,0	41,91	12,55
Fluka w. EtOH, batch B	< LOQ								
Fluka w. EtOH, batch C	3485,0	3302,7	2829,8	2686,2	2352,9	2012,8	2778,2	557,22	20,06
Merck EMSURE w. EtOH, batch A	106,3	110,3	111,6	124,3	124,5	81,5	109,8	15,76	14,36
Merck EMSURE w. EtOH, batch B	< LOQ								
Merck LiChroSolv w. amylene	< LOQ								
Alpha-Aesar w. amylene	< LOQ								
Alpha-Aesar w. EtOH	< LOQ								
Merck n-Hexane p.a.	< LOQ								
Merck ethyl acetate p.a.	< LOQ								

Table S1B



N-Palmitoylethanolamine, (PEA) 16:0-NAE

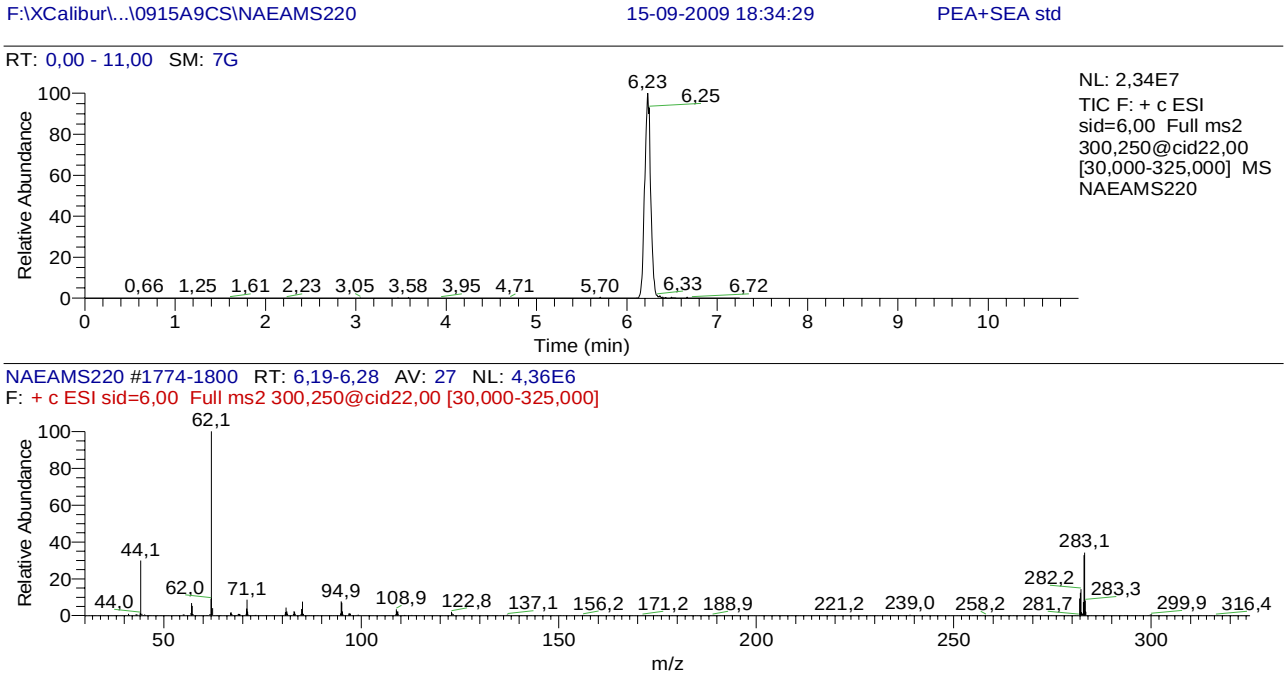


Figure S1A

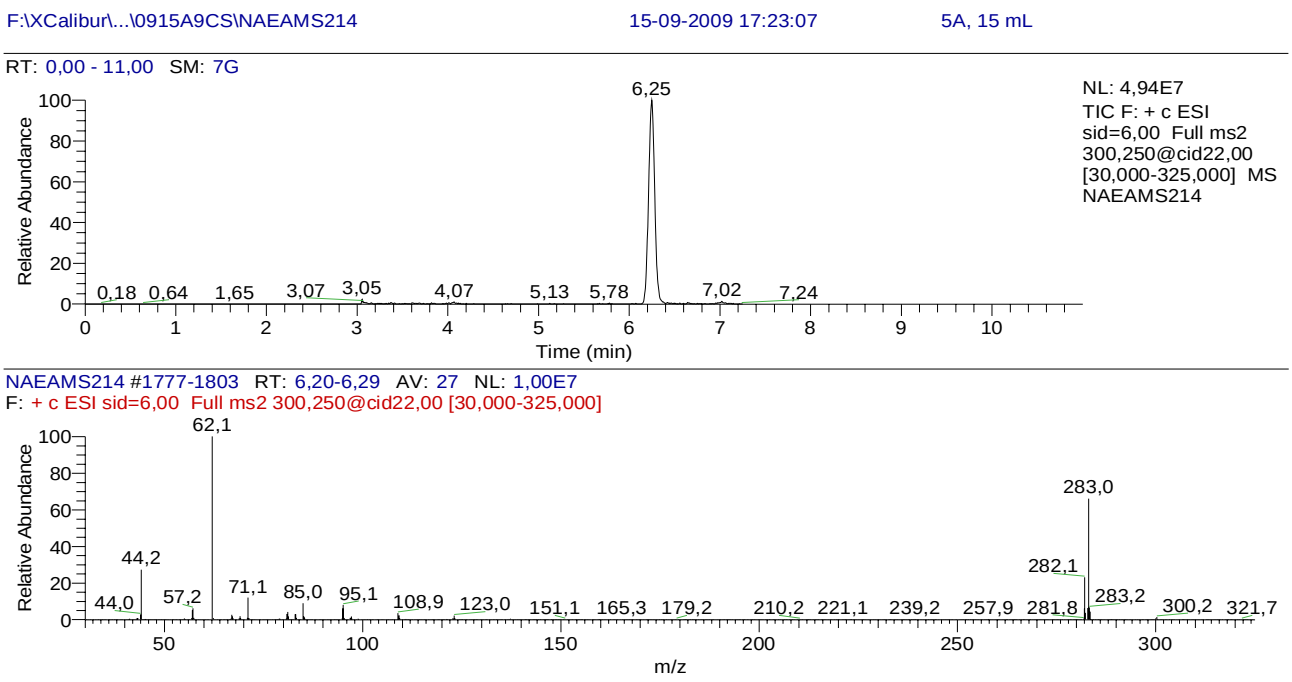
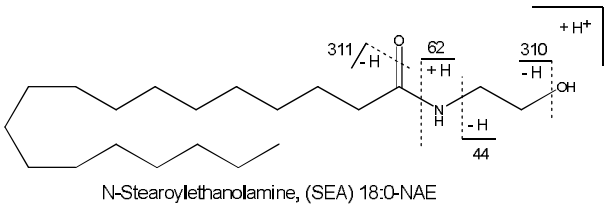


Figure S1B



N-Stearoylethanolamine, (SEA) 18:0-NAE

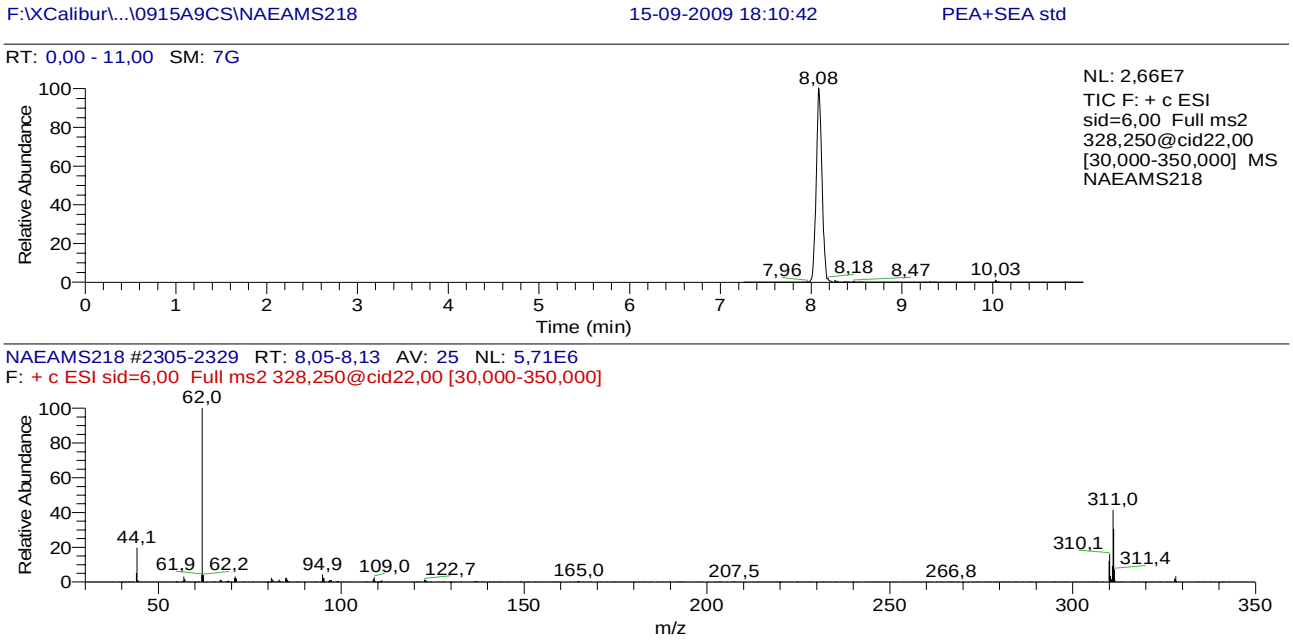


Figure S2A

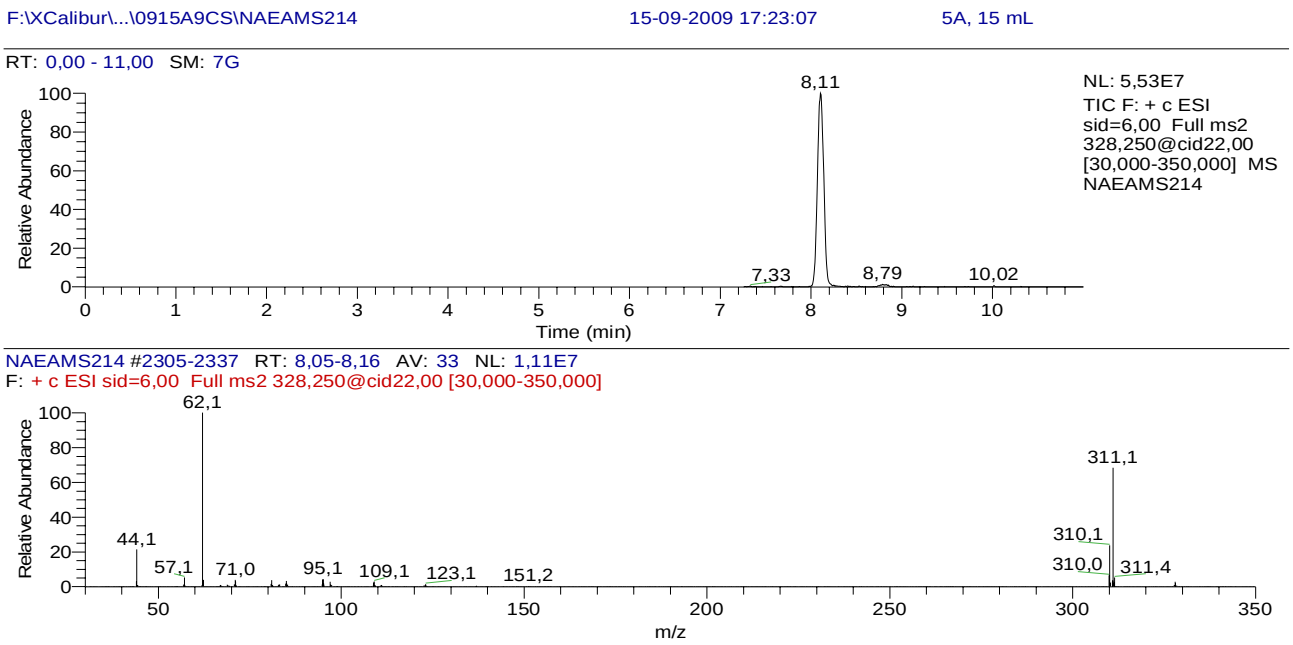


Figure S2B

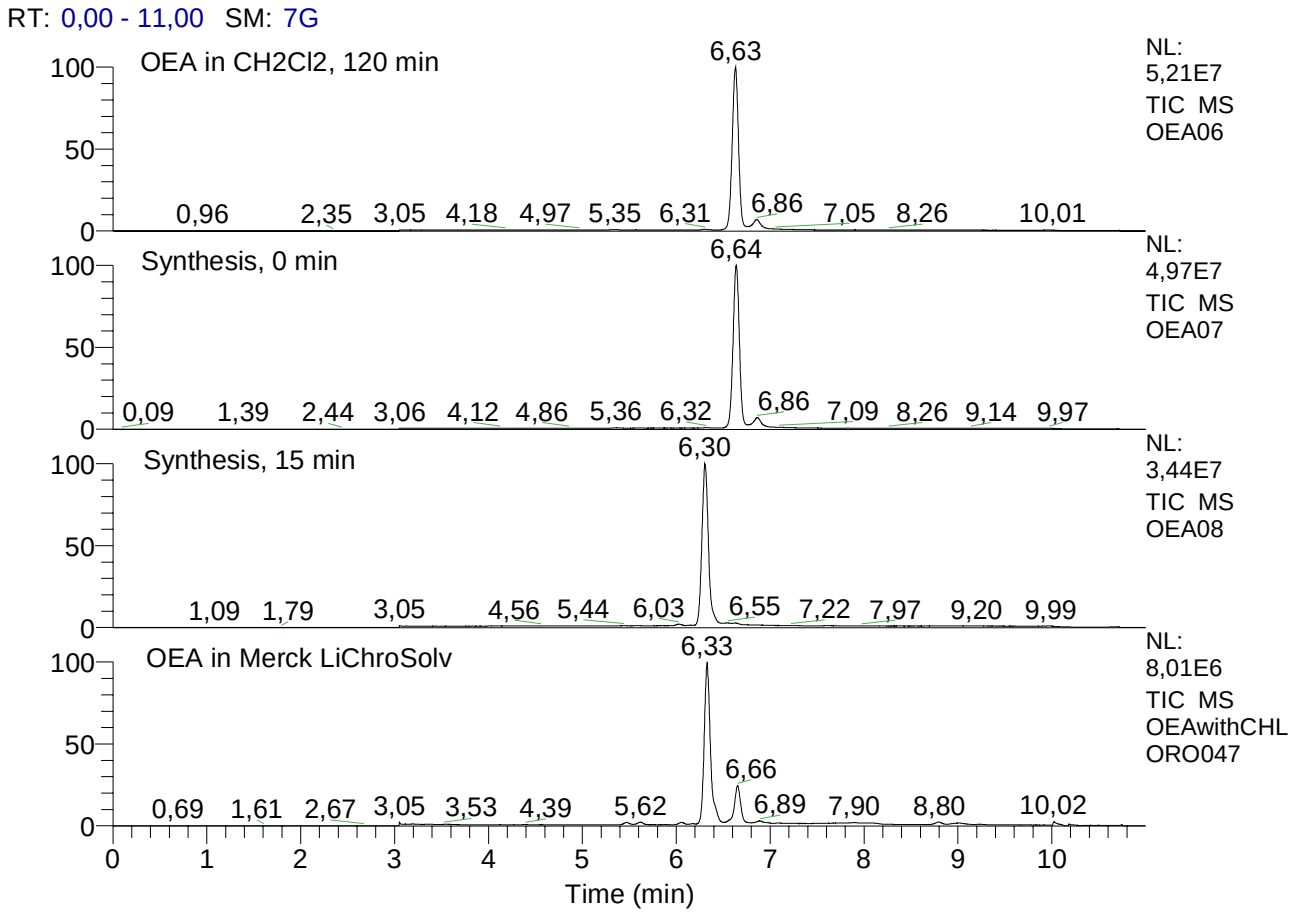


Fig. S3A

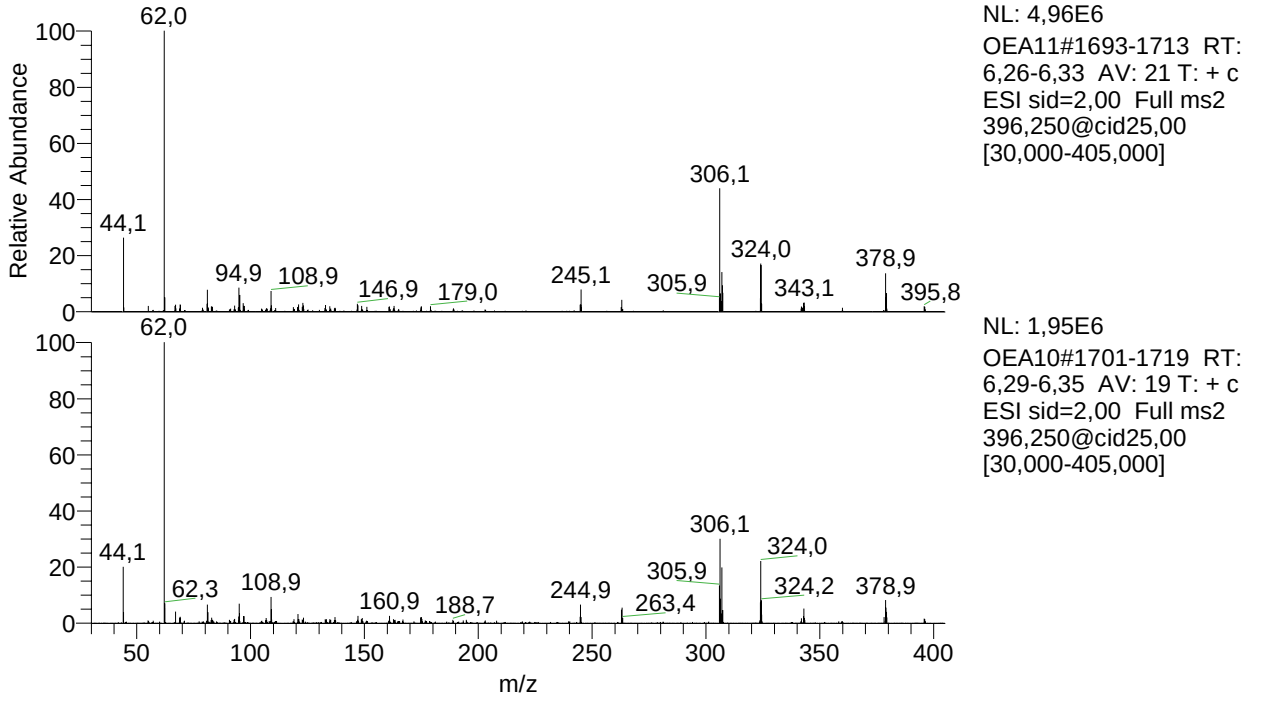


Fig. S3B

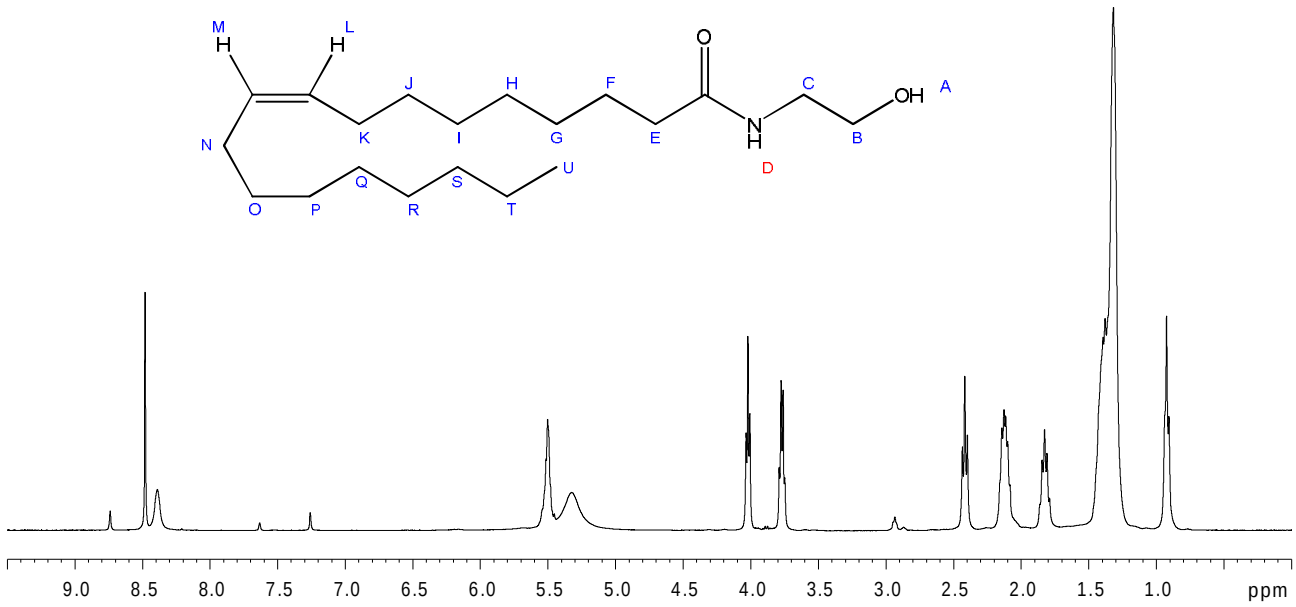

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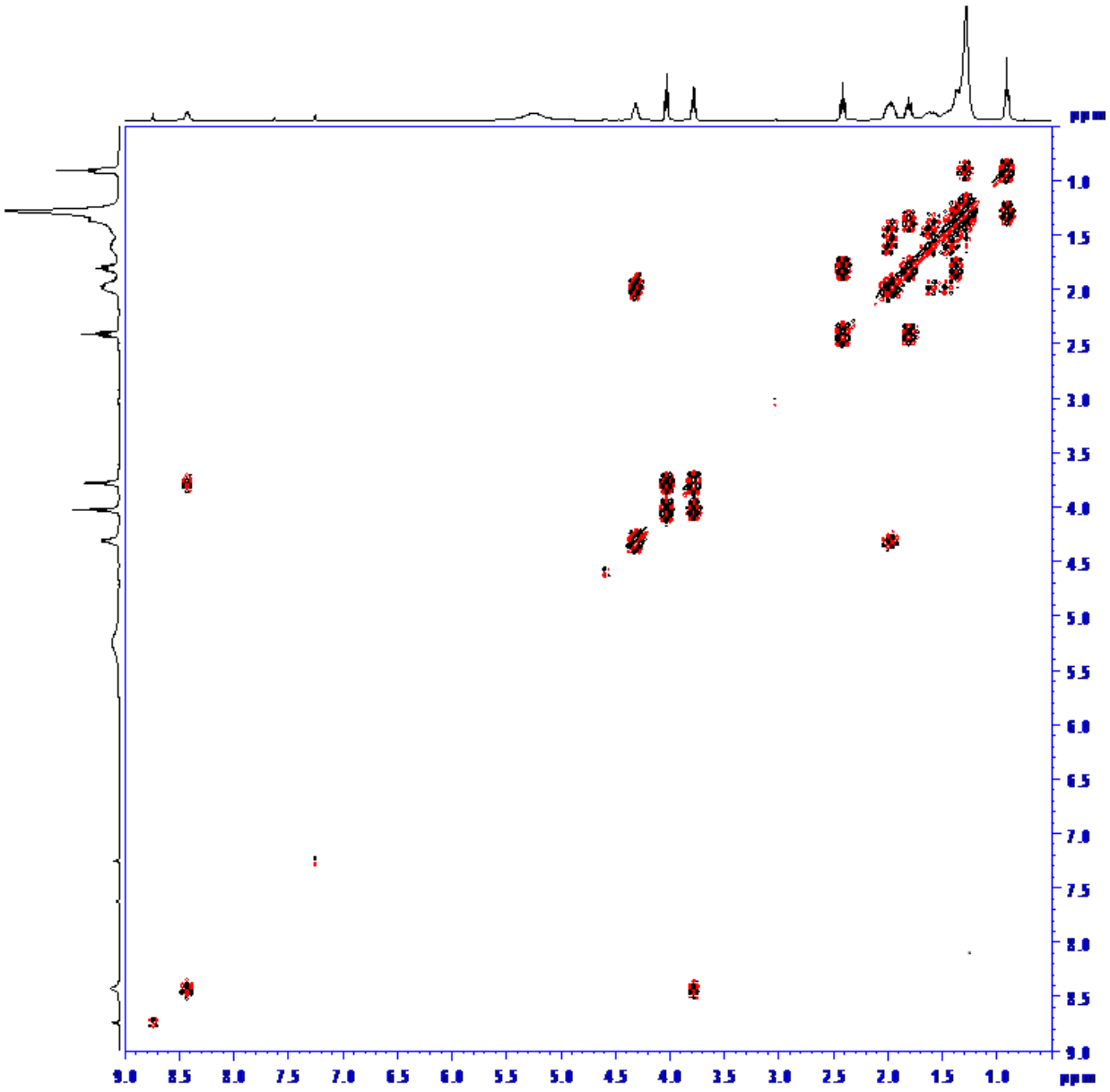
Fig. S4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ppm

Fig. S5A

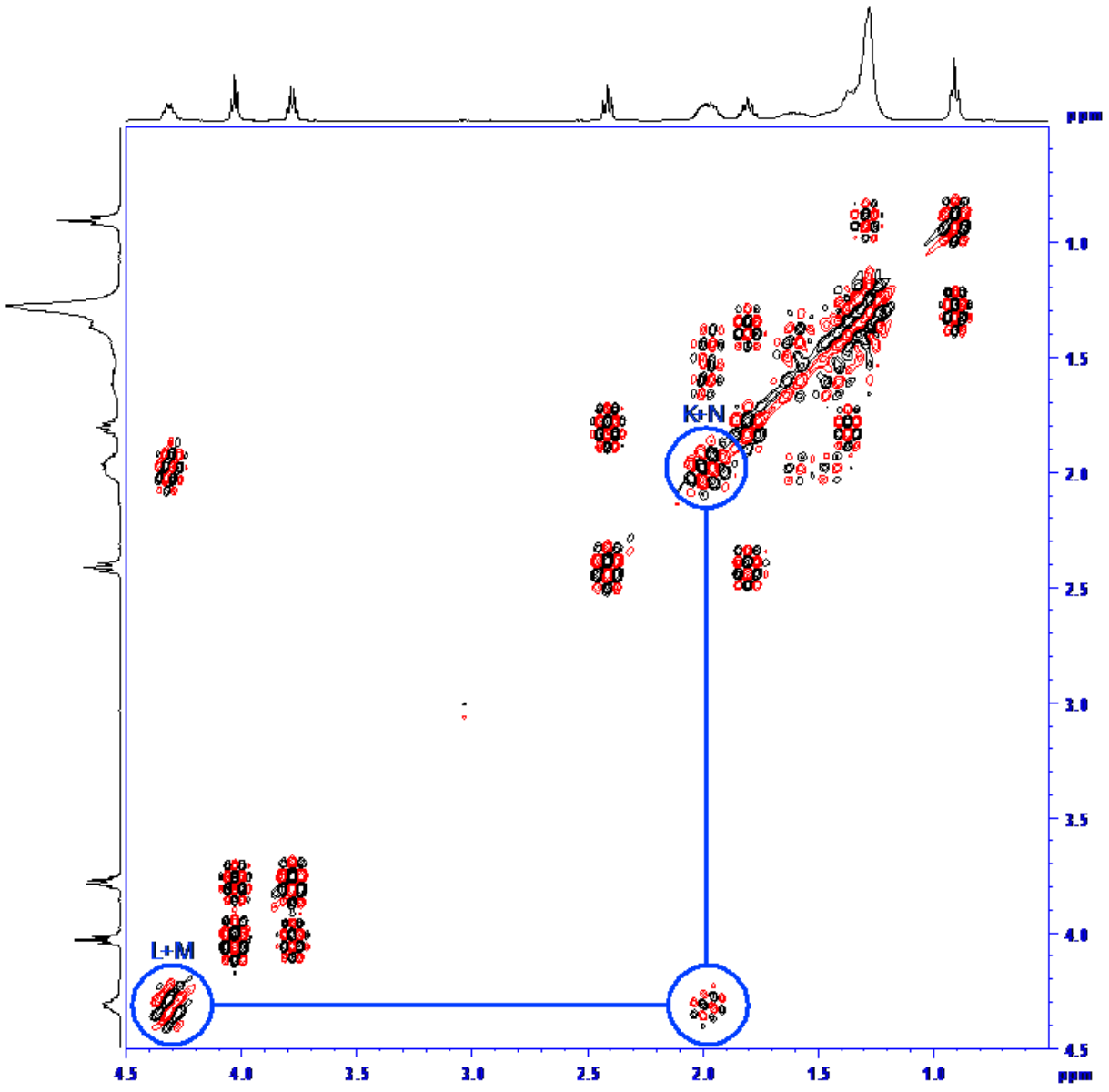

Fig. S5B