Supplemental information

Quantitative profiling of endocannabinoids and related N-acylethanolamines in human CSF using nano LC-MS/MS

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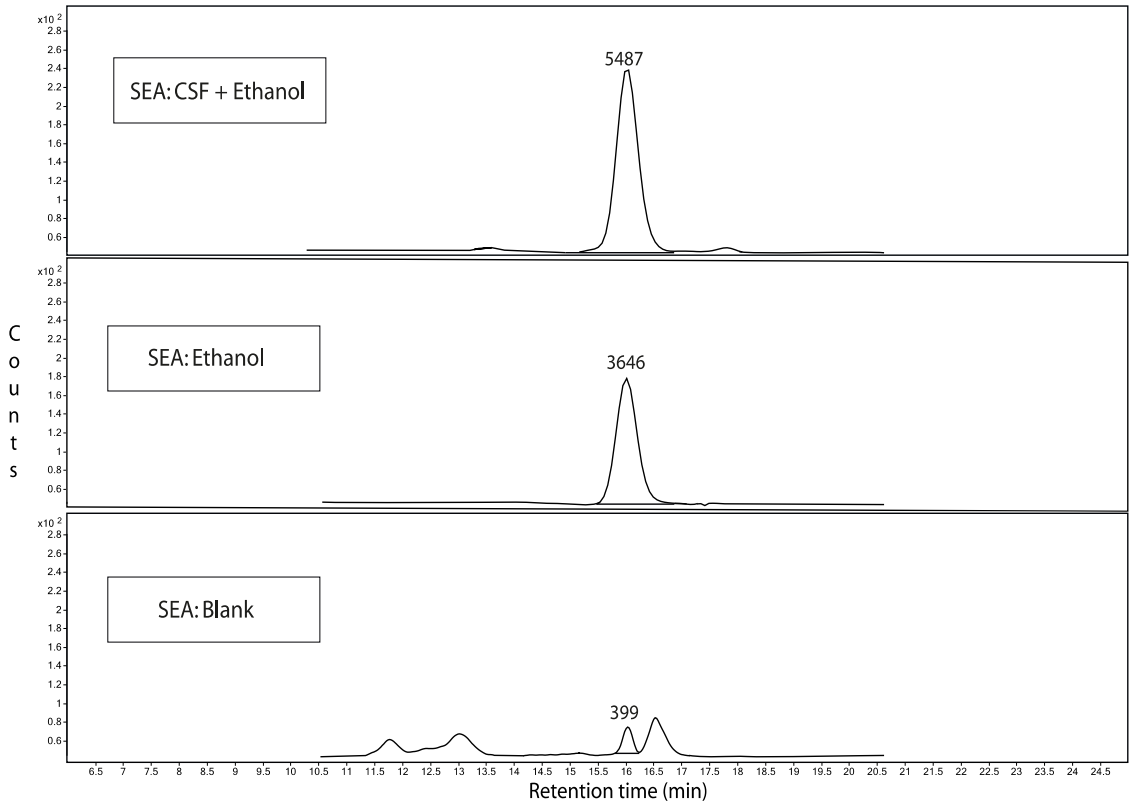
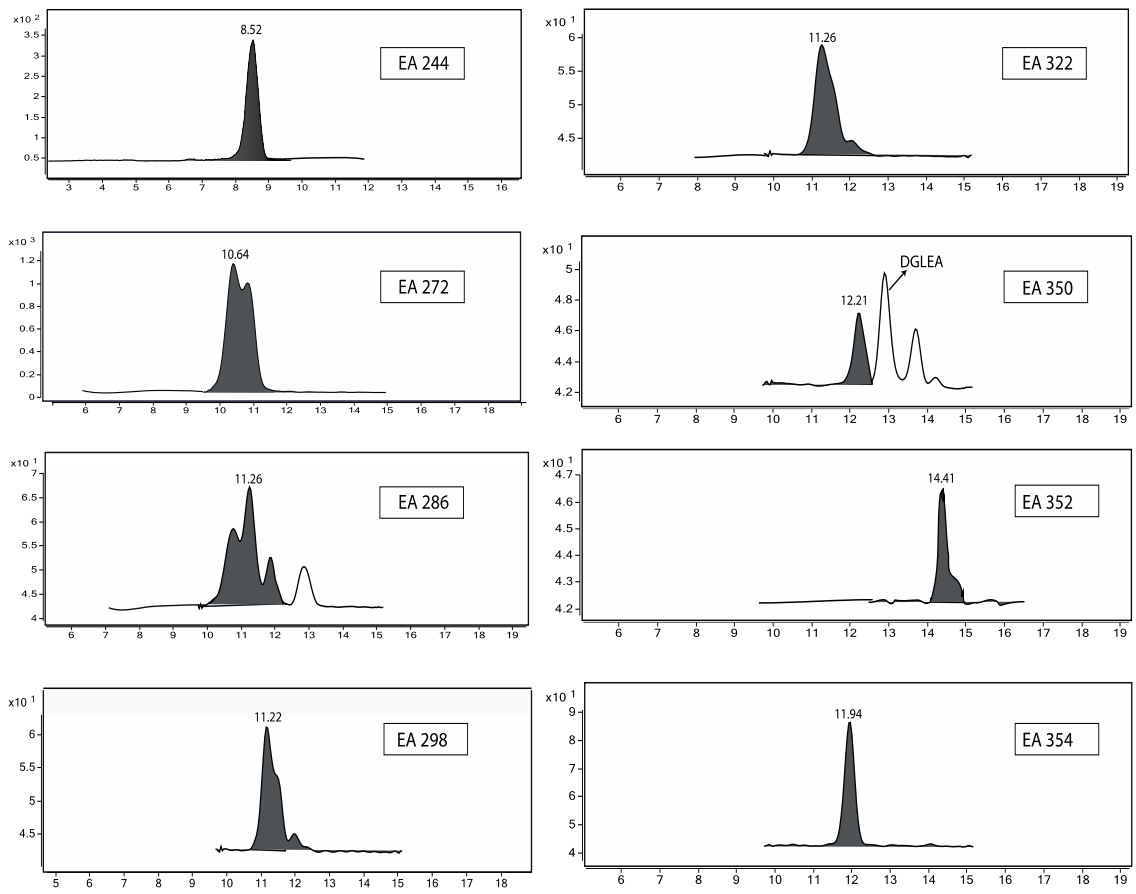
Running title: Quantitative profiling of endocannabinoids in human CSF



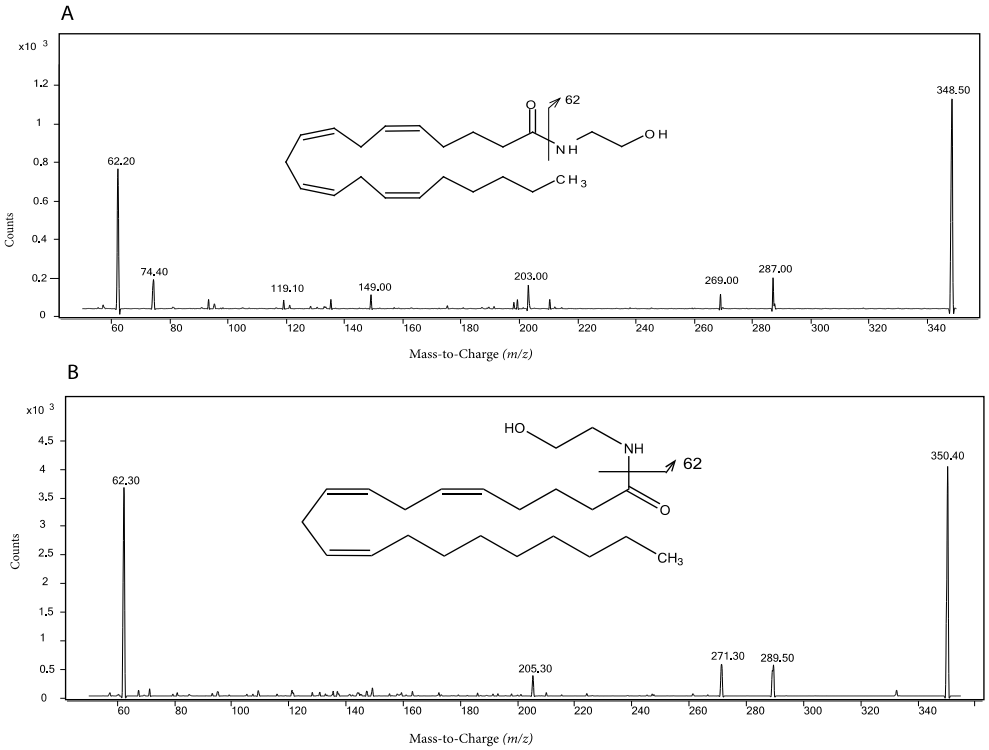
Supplemental Figure S1: Contaminant of SEA from ethanol and blank(MeOH/H2O)

Top window: chromatogram of SEA in sample with ethanol. Middle window: chromatogram of background SEA in pure ethanol. Bottom window: Chromatogram of background SEA in blank. X-axis is retention time and Y-axis is response signal.



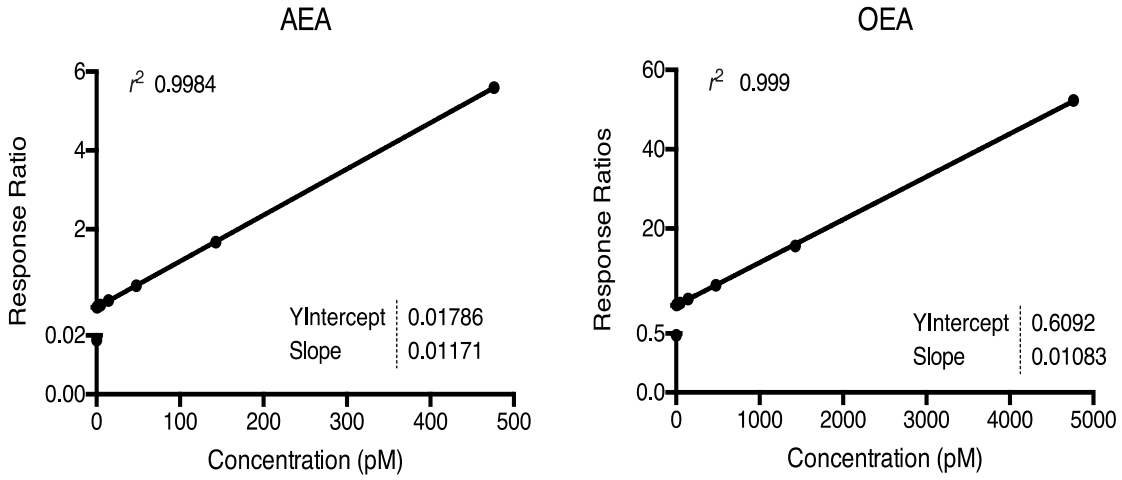
Supplemental Figure S2: Chromatograms of putatively annotated NAEs in CSF.

Putatively annotated NAEs are labeled as EA (from ethanolamine) followed by molecular mass (m/z) value. X-axis is retention time and Y-axis is response signal.



Supplemental Figure S3: MS/MS fragmentation mass spectra of anandamide and EA350

Fragmentation pattern of (A) anadamide (384.2>62.3) and putatively annotated NAE, (B) EA350 (350.4>62.3) in positive mode at a collision energy of 10V. X-axis is m/z and Y-axis is response signal.



Supplemental Figure S4: Calibration curves for AEA and OEA compounds

The displayed calibration curves are constructed in matrix (standard-addition method) in their respective dynamic ranges and the y-intercept of the curve represents the endogenous concentrations.

Supplemental Table S1: MRM (Multiple reaction monitoring) parameters of the target list

Compound Name	C	C = C	MW g/mole	Compound name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell time (msec)	CE
Identified								
PEA	16	0	299.5	*N*-palmitoylethanolamide	300.3	62.1	20	10
LEA	18	2	323.5	*N*-linoleoylethanolamide	324.3	62.1	50	10
OEA	18	1	325.5	*N*-oleoylethanolamide	326.3	62.1	50	10
SEA	18	0	327.6	*N*-stearoylethanolamide	328.3	62.1	300	10
AEA	20	4	347.5	*N*-arachidonoylethanolamide	348.3	62.1	200	10
DGLEA	20	3	349.6	*N*-dihomo-γ-linolenoylethanolamide	350.3	62.1	400	10
DHEA	22	6	371.6	*N*-docosahexaenoylethanolamide	372.3	62.1	100	10
DEA	22	4	375.6	*N*-docosatetraenoylethanolamide	376.3	62.1	400	10
1&2-AG[a]	20	4	378.6	*N*-arachidonoylglycerol	379.3	287.1	100	5
Internal Standards								
PEA - d4	16	0	303.5	*N*-palmitoylethanolamide - d4	304.3	62.1	20	10
LEA - d4	18	2	327.5	*N*-linoleoylethanolamide - d4	328.3	66.1	20	10
OEA - d4	18	1	329.6	*N*-oleoylethanolamide - d4	330.3	66.1	20	10
SEA - d3	18	0	330.6	*N*-stearoylethanolamide - d3	331.3	62.1	100	10
AEA - d8	20	4	355.6	*N*-arachidonoylethanolamide - d8	356.3	62.1	20	10
DHEA - d4	22	6	375.6	*N*-docosahexaenoylethanolamide- d4	376.3	66.1	20	10
2-AG - d8	20	4	386.6	*N*-arachidonoylglycerol - d8	387.3	294.1	100	10

Compound Name	C	C=C	MW g/mole	Compound name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell time (msec)	CE
Putatively annotated								
EA244	12	0	243.5	*N*-laurylethanolamide	244.3	62.1	800	10
EA272	14	0	271.5	*N*-myristylethanolamide	272.3	62.1	400	10
EA286	15	0	285.5	*N*-pentadecanoylethanolamide	286.3	62.1	800	10
EA298	16	1	297.5	*N*-palmitoleoylethanolamide	298.3	62.1	50	10
EA322	18	3	321.5	*N*-α-linolenylethanolamide	322.3	62.1	100	10
EA350	20	3	349.5	*N*-eicosatrienoylethanolamide	350.3	62.1	150	10
EA352	20	2	351.5	*N*-eicosadienoylethanolamide	352.3	62.1	150	10
EA354	20	1	353.5	*N*-eicosenoylethanolamide	354.3	62.1	50	10
Not detected in CSF								
O-AEA	20	4	347.5	*O*-arachidonoylethanolamine	348.3	62.1	200	10
NADA	20	4	439.6	*N*-arachidonoyldopamine	440.4	137	50	25
2-AGE	20	4	364.6	2-arachidonyl glyceryl ether	365.2	273.1	50	13

The target list includes identified compounds, deuterated ISTDs, putatively annotated compounds and compounds which are included in the list but are not detected in the sample. All compounds are analyzed in positive mode. The fragmentor voltage is at 100V for all transitions. "C" and "C=C" = number of carbon atoms and number of double bonds in the fatty acid chain of the molecule, respectively; MW= molecular weight (g/mole); m/z = mass-to-charge ratio; CE= Collision energy (volts); [a] Parameters are same for 1-AG and 2-AG.

Supplemental Table S2: Results from linear regression analysis (n=45 healthy controls)

(A)

Compound	**Gender**			**Age**			**R^2**
	Coefficient	*95% C.I.*	*P-value*	*Coefficient*	*95% C.I.*	*P-value*	
DHEA	**1.1795**	**[0.9351; 1.4878]**	**0.1588**	**1.0097**	**[1.0020; 1.0175]**	***0.015**	14.4%
DEA	0.9857	[0.7902; 1.2296]	0.8960	1.0004	[0.9931; 1.0077]	0.919	0.1%
DGLEA	1.1698	[0.9230; 1.4826]	0.1889	1.0018	[0.9939; 1.0097]	0.653	4.1%
AEA	0.9270	[0.7483; 1.1484]	0.4790	1.0040	[0.9969; 1.0111]	0.267	5.4%
1&2-AG	1.0694	[0.8331; 1.3726]	0.5906	1.0044	[0.9961; 1.0127]	0.295	2.8%
LEA	1.0738	[0.7618; 1.5137]	0.6776	1.0009	[0.9896; 1.0123]	0.876	0.4%
PEA	0.9989	[0.7705; 1.2950]	0.9932	1.0000	[0.9915; 1.0087]	0.991	0.0%
OEA	1.0917	[0.8712; 1.3680]	0.4368	1.0016	[0.9942; 1.0091]	0.663	1.6%
SEA	1.0338	[0.8182; 1.3063]	0.7755	0.9981	[0.9904; 1.0058]	0.615	1.1%
EA354	0.9867	[0.9509; 1.0238]	0.4672	0.9994	[0.9982; 1.0006]	0.322	2.8%
EA350	0.9667	[0.7446; 1.2552]	0.7951	1.0052	[0.9966; 1.0139]	0.232	4.2%
EA352	1.0897	[0.8656; 1.3716]	0.4557	1.0039	[0.9963; 1.0116]	0.309	3.0%
EA298	1.1462	[0.8317; 1.5796]	0.3956	1.0116	[1.0009; 1.0224]	*0.034	10.4%
EA286	1.1764	[0.8131; 1.7021]	0.3798	1.0066	[0.9944; 1.0190]	0.284	3.6%
EA244	0.9283	[0.5816; 1.4816]	0.7496	0.9982	[0.9829; 1.0138]	0.817	0.3%
EA272	0.9624	[0.6535; 1.4172]	0.8425	0.9969	[0.9842; 1.0097]	0.623	0.6%
EA322	1.0782	[0.6364; 1.8266]	0.7747	0.9961	[0.9788; 1.0136]	0.651	0.9%

(B)

		Age			**R^2**
		Coefficient	*95% C.I.*	*P-value*	
Males	DHEA	1.0141	[1.0036; 1.0246]	0.0106	27.3%
Females	DHEA	1.0023	[0.9902; 1.0146]	0.6969	7.7%

(A) For each compound, its relative concentration was set as dependent variable with gender and age as predictors. Regression coefficients were exponentiated because the dependent variable was log-transformed. **(B)** Regression analysis split by gender for DHEA.