

Application of a UHPLC-MS/MS method to investigate the metabolic pathways of Alzheimer's disease and dementia with Lewy bodies using postmortem cerebrospinal fluid and serum samples

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Materials and Methods

Chemicals and reagents

The analytical standards obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan) included 1-aminoacetone, glycine (Gly), β -alanine (β -Ala), cytosine, uracil, creatinine (CRE), valine (Val), threonine (Thr), taurine (Tau), thymine, 5-aminolevulinic acid (5-ALA), isoleucine (Ile), creatine (Cr), asparagine (Asn), aspartate (Asp), anthranilic acid (AA), tyramine, glutamine (Gln), histidine (His), methionine sulfoxide (MetSO), cysteate, arginine (Arg), citrulline (Cit), tyrosine (Tyr), N^G -monomethyl-L-arginine (NMMA), selenomethionine (Se-Met), tryptophan (Trp), N^1,N^8 -diacetylspermidine (Dac-SPD), cytidine, 1,3-diaminopropane (DAP), putrescine (PUT), acetylspermidine (Ac-SPD), cystathionine, carnosine (Car), cystine, N^1,N^{12} -diacetylspermine (Dac-SPM), spermidine (SPD), N^1 -acetylspermine (Ac-SPM), and spermine (SPM). Analytical standards purchased from SigmaAldrich (Buchs, Switzerland) included 1-amino-2-propanol, 4-aminobutanoic acid (GABA), 2-aminoethanesulfinic acid (hypotaurine), hydroxyproline (ProOH), methionine (Met), cysteinesulfinic acid, phenylalanine (Phe), 3-methoxy-tyramine (3-MeO-tyramine), acetylorithine (Ac-Orn), *N*-acetyl lysine (Ac-Lys), N^G,N^G -dimethyl-L-arginine (ADMA), kynurenine (Kyn), thymidine, uridine, histidinol, cystamine, 5-hydroxylysine (LysOH), 1-methylhistidine, (1-MeHis), 3-methylhistidine (3-MeHis), 3-hydroxykynurenine (3-KynOH), glutathione (GSH), and *S*-adenosyl-homocysteine (SAH). Alanine (Ala), serine (Ser), proline (Pro), leucine (Leu), glutamate (Glu), cadaverine (CAD), and lysine (Lys) were purchased from Kanto Chemicals (Tokyo, Japan). Ethanolamine (EA), *N*-methylglycine (Sarcosine, Sar), 2-aminobutyric acid (homoalanine), 5-aminovaleric acid (5-Ava),

homoserine (Hse), allothreonine (aThr), phenethylamine, *N*¹-acetylputrescine (Ac-PUT), agmatine (Agm), norleucine (Nle), 2-aminoacetophenone, phosphoethanolamine (EAP), *O*-acetylserine, *N*-methyl-anthranilatemethylanthranilate (MeAA), 3-hydroxyanthranilate (3-AAOH), tryptamine, 2-aminoadipate, 3-methoxyanthranilate (3-MeOAA), theanine, serotonin (5-HT), homophenylalanine (Hphe), methionine sulfone (MetSO₂), 5-methoxytryptamine (5-MeOT), indolelactate, 5-hydroxy-tryptophan (5-TrpOH), histamine (Hst), ornithine (Orn), 2,6-diaminopimelate, homocystine, and norspermidine (NorSPD) were obtained from Tokyo Kasei Co. (Tokyo, Japan). *N*-methylhistamine (MeHst) was obtained from Calbiochem (Darmstadt, Germany).

The ISs were labeled with deuterium, ¹³C, ¹⁵N, or a combination of stable isotopes. Arginine-¹³C₆ (Arg-¹³C₆) and 3-hydroxyanthranilate-*d*₃ (3-AAOH-*d*₃) were obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Glycine-¹³C₂¹⁵N₂, alanine-¹³C₃¹⁵N, GABA-*d*₆, serine-¹³C₃¹⁵N, proline-¹³C₅¹⁵N, valine-*d*₈, leucine-*d*₁₀, asparagine-¹³C₄¹⁵N₂, and tyrosine-¹³C₉¹⁵N were obtained from SigmaAldrich (Buchs, Switzerland). 1,6-Diaminohexane (DAH) was obtained from Kanto Chemicals Co. (Tokyo, Japan). *N*-methylglycine-*d*₃ (Sar-*d*₃), threonine-*d*₂, tryptophan-*d*₅, methionine-*d*₄, histidine-*d*₃, and ornithine-*d*₇ (Orn-*d*₇) were obtained from C/D/N Isotopes Co. (Quebec, Canada). Creatine-*d*₃, creatinine-*d*₃ (CRE-*d*₃), glutamine-*d*₅, glutamate-*d*₅, aspartate-¹³C, histamine-*d*₄, phenylalanine-*d*₆, guanosine-¹⁵N₅, lysine-¹³C₆¹⁵N₂, citrulline-*d*₄ (Cit-*d*₄), kynurenine-*d*₆ (Kyn-*d*₆), glutathione-¹³C₂¹⁵N (GSH-¹³C₂¹⁵N), cystathionine-*d*₄, spermidine-*d*₈ (SPD-*d*₈), and spermine-*d*₈ (SPM-*d*₈) were obtained from Cambridge Isotope Laboratories (Andover, USA).

FMOC derivatized UHPLC-MS/MS analysis

The LC-MS system consisted of an Acquity H-class UPLC coupled to a Xevo TQD triple quadrupole MS equipped with an ESI source (Waters, Milford, MA, USA). Chromatographic separation was achieved by performing gradient elution on a Waters reversed-phase Acquity BEH C₁₈ UPLC column (1.7 μm, 2.1 × 150 mm). The mobile phase, which consisted of 0.1% FA in water (solvent A) and 0.1% FA in acetonitrile (solvent B), was delivered at a flow rate of 0.4 mL/min. FMOC derivatives of the amine metabolites were detected *via* ESI-MS in positive or negative ion mode. The injection volume was 5 μL, and the total analytical run time was 10 min. The column was maintained at 50 °C in a column oven. The retention time and characteristic MS² transitions of the target compounds were used to confirm their identities in SRM mode.

The derivatization process are as follows: A stock solution containing 100 μM of each standard was prepared by diluting mixed standard solutions with 50:50 (v/v) ethanol/water. The working solutions and pretreated CSF and serum samples were derivatized using FMOC as previously described [21]. Freshly prepared 0.1 M NaHCO₃ in water (pH 9.0) and FMOC in acetonitrile (40 mM) were successively added to the reaction mixtures, and the mixtures were stored at room temperature for 10 min. The derivatization reaction was stopped by adding 1% FA in 50:50 (v/v) acetonitrile/water. The solvents were removed using a CVE-3110 centrifugal evaporator (EYELA, Tokyo, Japan), and the remaining residues were dissolved in the UHPLC mobile phase. A 5 μL aliquot of each solution was analyzed via UHPLC-MS/MS.

Table S1 UHPLC-MS/MS condition for 99 analytes and 33 ISs.

Analytes	Ionization	Retention time (min)	Precursor ions	Product ions	Cone Voltage (V)	Collision energy (eV)
EA	Positive	2.70	284	179	15	15
1-aminoacetone	Positive	3.56	296	179	20	20
Gly	Positive	2.84	298	179	15	20
1-amino-2-propanol	Positive	3.10	298	179	15	10
β -Ala	Positive	2.98	312	179	15	20
Ala	Positive	3.29	312	179	15	10
Sar	Positive	3.30	312	179	15	15
GABA	Positive	3.18	326	179	20	20
Homoalanine	Positive	3.75	326	179	20	20
Ser	Positive	2.32	328	179	20	20
Hypotaurine	Positive	2.12	332	179	20	20
Cytosine	Positive	1.96	334	179	30	30
Uracil	Positive	3.28	335	179	20	15
CRE	Positive	1.69	336	179	25	30
Pro	Positive	3.72	338	179	25	20
5-Ava	Positive	3.42	340	179	20	20
Val	Positive	4.34	340	179	25	25
Hse	Positive	2.26	342	179	20	20
aThr	Positive	2.51	342	179	20	20
Thr	Positive	2.71	342	179	20	20
Phenethylamine	Positive	6.26	344	179	15	20
Tau	Negative	1.73	346	124	35	25
Thymine	Positive	3.80	349	179	15	20
Ac-PUT	Positive	3.02	353	179	25	25
Agm	Positive	1.71	353	179	50	30
5-ALA	Positive	2.93	354	179	15	30
ProOH	Positive	2.18	354	179	20	25
Leu	Positive	4.98	354	179	25	20
Nle	Positive	5.04	354	179	15	15
Ile	Positive	4.92	354	179	20	15
Cr	Positive	1.41	354	179	40	35

Asn	Positive	1.98	355	179	25	30
Asp	Positive	2.37	356	179	20	20
2-aminoacetophenone	Positive	7.09	358	179	15	20
AA	Positive	5.91	360	179	15	20
Tyramine	Positive	4.48	360	179	20	15
EAP	Positive	1.08	364	179	20	20
Gln	Positive	1.98	369	179	25	25
O-acetyl-serine	Positive	3.25	370	179	15	25
Glu	Positive	2.42	370	179	20	20
Met	Positive	4.16	372	179	25	20
MeAA	Positive	4.65	374	179	25	25
3-AAOH	Positive	4.19	376	179	20	25
Cysteine sulfinate	Positive	1.68	376	179	20	25
His	Positive	7.74	379	179	50	25
Tryptamine	Positive	5.82	383	179	30	25
2-aminoadipate	Positive	2.53	384	179	20	20
MetSO	Positive	2.03	388	179	35	25
Phe	Positive	4.91	388	179	25	15
Cysteate	Negative	1.53	390	112	40	25
3-MeOAA	Positive	4.02	390	179	25	25
3-MeO-tyramine	Positive	4.65	390	179	20	20
Arg	Positive	1.29	397	179	45	35
Ac-Orn	Positive	2.51	397	179	25	30
Theanine	Positive	2.65	397	179	30	20
Cit	Positive	1.96	398	179	25	15
5-HT	Positive	4.03	399	179	30	30
Hphe	Positive	5.38	402	179	20	20
MetSO ₂	Positive	2.53	404	179	20	30
Tyr	Positive	3.23	404	179	25	20
NMMA	Positive	1.38	411	179	40	35
Ac-Lys	Positive	2.45	411	179	25	25
5-MeOT	Positive	5.44	413	179	30	30
Se-Met	Positive	4.38	420	179	20	25
ADMA	Positive	1.49	425	179	50	35
Trp	Positive	4.54	427	179	30	30
Indolelactate	Positive	5.20	428	179	20	30

Kyn	Positive	4.31	431	179	30	25
5-TrpOH	Positive	2.96	443	179	20	30
DAc-SPD	Positive	2.31	452	179	40	40
Thymidine	Positive	3.35	465	179	20	15
Cytidine	Positive	1.38	466	179	25	25
Uridine	Positive	1.89	467	179	20	40
DAP	Positive	7.04	519	179	30	35
PUT	Positive	7.09	533	179	25	25
CAD	Positive	7.31	547	179	30	25
Hst	Positive	7.10	556	179	40	40
MeHst	Positive	4.46	570	179	40	40
Orn	Positive	6.28	577	179	25	25
Histidinol	Positive	6.29	586	179	40	35
Lys	Positive	6.48	591	179	35	35
Cystamine	Positive	7.48	597	179	35	40
LysOH	Positive	5.57	608	179	30	30
1-MeHis	Positive	3.67	614	179	40	35
3-MeHis	Positive	4.03	614	179	40	40
Ac-SPD	Positive	6.42	633	179	45	40
2,6-diamino-pimelate	Positive	5.54	635	179	35	30
Cystathionine	Positive	5.71	667	179	35	30
3-KynOH	Positive	7.14	669	179	50	30
Car	Positive	5.88	672	179	50	35
Cystine	Positive	5.92	686	179	50	35
Homocystine	Positive	6.19	713	179	40	35
DAc-SPM	Positive	5.47	731	179	45	35
GSH	Positive	5.45	753	179	45	40
NorSPD	Positive	8.24	798	179	40	40
SPD	Positive	8.29	813	179	45	40
SAH	Positive	4.35	830	179	40	40
Ac-SPM	Positive	7.93	912	179	50	35
SPM	Positive	8.92	1092	179	60	50
Gly- ¹³ C ₂ ¹⁵ N ₂	Positive	2.84	301	179	15	20
Sar- <i>d</i> ₃	Positive	3.28	315	179	15	15
Ala- ¹³ C ₃ ¹⁵ N	Positive	3.29	316	179	15	15
GABA- <i>d</i> ₆	Positive	3.18	332	179	20	15

Ser- ¹³ C ₃ ¹⁵ N	Positive	2.32	332	179	20	20
CRE- <i>d</i> ₃	Positive	1.65	339	179	25	20
Thr- <i>d</i> ₂	Positive	2.71	344	179	20	20
Pro- ¹³ C ₅ ¹⁵ N	Positive	3.72	344	179	25	25
Val- <i>d</i> ₈	Positive	4.34	348	179	25	20
Cr- <i>d</i> ₃	Positive	1.35	357	179	40	15
Asp- ¹³ C	Positive	2.37	357	179	20	20
Asn- ¹³ C ₄ ¹⁵ N ₂	Positive	1.98	361	179	25	25
Leu- <i>d</i> ₁₀	Positive	4.98	364	179	25	10
Gln- <i>d</i> ₅	Positive	1.98	374	179	25	25
Glu- <i>d</i> ₅	Positive	2.42	375	179	25	25
Met- <i>d</i> ₄	Positive	4.16	376	179	25	20
3-AAOH- <i>d</i> ₃	Positive	4.19	379	179	20	20
His- <i>d</i> ₃	Positive	7.74	382	179	50	15
Phe- <i>d</i> ₆	Positive	4.91	396	179	25	20
Cit- <i>d</i> ₄	Positive	1.96	402	179	25	20
Arg- ¹³ C ₆	Positive	1.29	403	179	45	35
Tyr- ¹³ C ₉ ¹⁵ N	Positive	3.23	414	179	25	20
Trp- <i>d</i> ₅	Positive	4.54	432	179	30	30
Kyn- <i>d</i> ₆	Positive	4.22	437	179	30	25
Guanosine- ¹⁵ N ₅	Positive	1.64	511	179	25	25
Hst- <i>d</i> ₄	Positive	7.10	560	179	40	40
DAH	Positive	7.52	562	179	35	30
Orn- <i>d</i> ₇	Positive	6.28	584	179	25	15
Lys- ¹³ C ₆ , ¹⁵ N ₂	Positive	6.48	599	179	35	35
Cystathionine- <i>d</i> ₄	Positive	5.71	671	179	35	40
GSH- ¹³ C ₂ , ¹⁵ N	Positive	5.45	755	179	45	35
SPD- <i>d</i> ₈	Positive	8.29	821	179	45	40
SPM- <i>d</i> ₈	Positive	8.92	1100	179	50	25

Table S2 Method validation of LOQs, LODs, linearity, and ISs

Analytes	LOD (nM)	LOQ (nM)	Ranges	r^2	Internal standard
EA	10.0	25.0	50-100000	0.999	Gly- ¹³ C ₂ ¹⁵ N ₂
1-aminoacetone	10.0	25.0	50-100000	0.995	Gly- ¹³ C ₂ ¹⁵ N ₂
Gly	1.0	10.0	100-100000	1.000	Gly- ¹³ C ₂ ¹⁵ N ₂
1-amino-2-propanol	1.0	10.0	50-25000	0.999	Gly- ¹³ C ₂ ¹⁵ N ₂
β-Ala	0.1	1.0	100-100000	1.000	Ala- ¹³ C ₃ ¹⁵ N
Ala	1.0	3.0	100-100000	1.000	Ala- ¹³ C ₃ ¹⁵ N
Sar	10.0	25.0	100-50000	0.999	Sar- <i>d</i> ₃
GABA	10.0	25.0	50-100000	0.999	GABA- <i>d</i> ₆
Homoalanine	100.0	250.0	50-37500	1.000	Ala- ¹³ C ₃ ¹⁵ N
Ser	0.1	1.0	500-100000	1.000	Ser- ¹³ C ₃ ¹⁵ N
Hypotaurine	500.0	1000.0	1000-25000	0.997	Ser- ¹³ C ₃ ¹⁵ N
Cytosine	500.0	1000.0	1000-100000	0.998	Ser- ¹³ C ₃ ¹⁵ N
Uracil	10.0	25.0	100-25000	0.999	His- <i>d</i> ₃
CRE	1.0	10.0	250-100000	1.000	CRE- <i>d</i> ₃
Pro	1.0	10.0	100-100000	1.000	Pro- ¹³ C ₅ ¹⁵ N
5-Ava	1.0	5.0	10-7500	0.999	Val- <i>d</i> ₈
Val	1.0	10.0	250-100000	0.999	Val- <i>d</i> ₈
Hse	1.0	10.0	50-7500	0.990	Ser- ¹³ C ₃ ¹⁵ N
aThr	1.0	10.0	10-25000	0.999	Thr- <i>d</i> ₂
Thr	1.0	10.0	500-100000	1.000	Thr- <i>d</i> ₂
Phenethylamine	1.0	10.0	1-1000	0.999	Phe- <i>d</i> ₆
Tau	1.0	10.0	100-100000	0.999	Ser- ¹³ C ₃ ¹⁵ N
Thymine	1.0	5.0	10-5000	0.999	His- <i>d</i> ₃
Ac-PUT	1.0	10.0	10-5000	0.999	Orn- <i>d</i> ₇
Agm	0.1	1.0	1-100	0.996	Arg- ¹³ C ₆
5-ALA	10.0	50.0	50-37500	0.999	Gly- ¹³ C ₂ ¹⁵ N ₂
ProOH	25.0	50.0	250-50000	0.999	Pro- ¹³ C ₅ ¹⁵ N
Leu	1.0	10.0	100-100000	1.000	Leu- <i>d</i> ₁₀
Nle	1.0	5.0	10-100000	1.000	Leu- <i>d</i> ₁₀
Ile	1.0	10.0	1000-100000	0.999	Leu- <i>d</i> ₁₀
Cr	100.0	250.0	500-100000	1.000	Cr- <i>d</i> ₃
Asn	25.0	50.0	2500-100000	0.999	Asn- ¹³ C ₄ ¹⁵ N ₂

Asp	1.0	3.0	10-25000	1.000	Asp- ¹³ C
2-aminoacetophenone	25.0	50.0	50-100000	0.998	3-AAOH- <i>d</i> ₃
AA	1.0	5.0	5-1000	1.000	Trp- <i>d</i> ₅
Tyramine	5.0	10.0	10-25000	0.999	Tyr- ¹³ C ₉ ¹⁵ N
EAP	10.0	25.0	100-10000	0.999	Ser- ¹³ C ₃ ¹⁵ N
Gln	0.5	1.0	50-100000	0.999	Gln- <i>d</i> ₅
O-acetyl-serine	50.0	100.0	500-100000	1.000	Ser- ¹³ C ₃ ¹⁵ N
Glu	1.0	3.0	500-75000	0.999	Glu- <i>d</i> ₅
Met	1.0	10.0	100-100000	1.000	Met- <i>d</i> ₄
MeAA	1.0	5.0	500-75000	0.999	3-AAOH- <i>d</i> ₃
3-AAOH	250.0	500.0	500-100000	0.999	3-AAOH- <i>d</i> ₃
Cysteine sulfinat	1.0	10.0	100-50000	0.999	Ser- ¹³ C ₃ ¹⁵ N
His	1.0	10.0	50-100000	0.999	His- <i>d</i> ₃
Tryptamine	0.1	1.0	1-1000	0.999	Trp- <i>d</i> ₅
2-aminoadipate	10.0	50.0	250-100000	0.999	Glu- <i>d</i> ₅
MetSO	25.0	50.0	250-25000	0.999	Met- <i>d</i> ₄
Phe	5.0	10.0	1000-50000	0.999	Phe- <i>d</i> ₆
Cysteate	25.0	500.0	2500-75000	0.997	Ser- ¹³ C ₃ ¹⁵ N
3-MeOAA	0.1	1.0	25-75000	1.000	3-AAOH- <i>d</i> ₃
3-MeO-tyramine	5.0	10.0	10-10000	0.999	Tyr- ¹³ C ₉ ¹⁵ N
Arg	1.0	3.0	500-100000	1.000	Arg- ¹³ C ₆
Ac-Orn	1.0	5.0	5-1000	0.999	Orn- <i>d</i> ₇
Theanine	1.0	5.0	5-1000	0.995	Glu- <i>d</i> ₅
Cit	1.0	3.0	25-100000	1.000	Cit- <i>d</i> ₄
5-HT	0.5	1.0	50-100000	0.999	Trp- <i>d</i> ₅
Hphe	1.0	5.0	50-2500	0.999	Phe- <i>d</i> ₆
MetSO ₂	5.0	10.0	10-10000	0.999	Met- <i>d</i> ₄
Tyr	250.0	500.0	500-100000	1.000	Tyr- ¹³ C ₉ ¹⁵ N
NMMA	0.5	1.0	500-25000	0.999	Arg- ¹³ C ₆
Ac-Lys	1.0	5.0	1-100	0.999	Lys- ¹³ C ₆ , ¹⁵ N ₂
5-MeOT	0.1	0.1	5-1000	0.999	Trp- <i>d</i> ₅
Se-Met	25.0	50.0	25-1000	0.999	Met- <i>d</i> ₄
ADMA	3.0	5.0	100-25000	0.992	Arg- ¹³ C ₆
Trp	10.0	25.0	500-25000	0.999	Trp- <i>d</i> ₅
Indolelactate	50.0	100.0	100-100000	0.999	Trp- <i>d</i> ₅
Kyn	1.0	5.0	50-100000	0.999	Kyn- <i>d</i> ₆

5-TrpOH	1.0	5.0	10-37500	0.999	Trp- <i>d</i> ₅
DAc-SPD	0.5	1.0	1-10000	0.999	SPD- <i>d</i> ₈
Thymidine	50.0	100.0	1000-100000	0.999	Guanosine- ¹⁵ N ₅
Cytidine	250.0	500.0	1000-25000	0.999	His- <i>d</i> ₃
Uridine	250.0	500.0	500-75000	1.000	His- <i>d</i> ₃
DAP	0.5	1.0	25-5000	0.999	DAH
PUT	0.5	1.0	10-5000	0.998	DAH
CAD	0.5	1.0	1-100000	0.999	Orn- <i>d</i> ₇
Hst	0.5	1.0	1-5000	0.999	Hst- <i>d</i> ₄
MeHst	0.5	1.0	1-37500	0.999	His- <i>d</i> ₃
Orn	5.0	10.0	500-50000	0.999	Orn- <i>d</i> ₇
Histidinol	5.0	10.0	25-7500	0.999	His- <i>d</i> ₃
Lys	1.0	3.0	50-10000	0.999	DAH
Cystamine	5.0	10.0	25-75000	0.997	Ser- ¹³ C ₃ ¹⁵ N
LysOH	5.0	10.0	2500-100000	0.999	DAH
1-MeHis	1.0	5.0	10-7500	0.999	His- <i>d</i> ₃
3-MeHis	100.0	250.0	500-75000	0.998	His- <i>d</i> ₃
Ac-SPD	5.0	10.0	10-10000	1.000	SPD- <i>d</i> ₈
2,6-diamino-pimelate	10.0	25.0	50-100000	1.000	DAH
Cystathionine	25.0	50.0	50-10000	0.999	Cystathionine- <i>d</i> ₄
3-KynOH	0.5	1.0	1-1000	0.998	Kyn- <i>d</i> ₆
Car	0.5	1.0	10-10000	0.997	His- <i>d</i> ₃
Cystine	100.0	250.0	100-7500	0.999	Ser- ¹³ C ₃ ¹⁵ N
Homocystine	50.0	100.0	250-7500	0.999	Ser- ¹³ C ₃ ¹⁵ N
DAc-SPM	500.0	1000.0	250-100000	0.999	SPM- <i>d</i> ₈
GSH	100.0	250.0	25-100000	0.999	GSH- ¹³ C ₂ , ¹⁵ N
NorSPD	1.0	5.0	100-5000	0.999	SPD- <i>d</i> ₈
SPD	3.0	5.0	500-100000	1.000	SPD- <i>d</i> ₈
SAH	50.0	100.0	100-10000	0.999	Ser- ¹³ C ₃ ¹⁵ N
Ac-SPM	10.0	25.0	1-10000	0.998	SPM- <i>d</i> ₈
SPM	10.0	25.0	10-5000	0.999	SPM- <i>d</i> ₈

Table S3 Recovery and repeatability of several analytes spiked in 10-fold diluted serum samples (pooled) at low and high concentrations (nM).

Analytes	Spike conc. (nM)	10-fold diluted serum recovery (CV %)
EA	5000	97.3 (4.7)
	10000	103.6 (6.9)
Gly	5000	97.3 (4.7)
	10000	103.6 (6.9)
β -Ala	1000	111.9 (2.7)
	5000	116.0 (3.5)
Ala	5000	94.1 (2.7)
	10000	96.6 (7.7)
Sar	5000	75.2 (3.3)
	10000	99.8 (2.5)
GABA	5000	104.0 (1.6)
	10000	107.4 (2.3)
Homoalanine	1000	107.9 (3.2)
	5000	110.6 (3.0)
Ser	5000	96.3 (5.3)
	10000	107.6 (2.7)
Hypotaurine	5000	71.5 (3.0)
	10000	79.4 (7.2)
Uracil	1000	87.1 (7.0)
	5000	96.5 (6.2)
CRE	5000	104.5 (5.5)
	10000	111.6 (7.5)
Pro	5000	102.6 (2.2)
	10000	103.5 (3.6)
5-Ava	1000	76.6 (4.6)
	5000	78.0 (5.4)
Val	5000	104.7 (2.3)
	10000	104.0 (3.2)
Hse	1000	93.9 (4.8)
	5000	103.0 (6.5)
aThr	1000	97.6 (6.7)
	5000	111.7 (3.1)
Thr	1000	100.2 (2.9)
	5000	102.7 (4.6)
Phenethylamine	1000	100.5 (7.2)
	5000	75.6 (3.4)
Tau	5000	110.0 (2.8)
	10000	111.2 (3.5)
Thymine	1000	87.8 (5.1)
	5000	109.3 (4.4)

Table S3 (continued)

Analytes	Spike conc. (nM)	10-fold diluted serum recovery (CV %)
Agm	1000	105.8 (3.7)
	5000	113.1 (3.4)
Nle	1000	105.2 (5.1)
	5000	106.5 (2.1)
Ile	5000	90.0 (2.8)
	10000	88.6 (3.4)
Leu	5000	98.1 (2.3)
	10000	98.8 (1.6)
Asn	1000	101.0 (4.2)
	5000	104.6 (1.7)
Asp	1000	104.5 (3.9)
	5000	99.6 (1.7)
Tyramine	1000	117.3 (3.6)
	5000	97.7 (2.9)
Gln	5000	99.3 (3.9)
	10000	98.6 (3.4)
Glu	5000	88.3 (4.4)
	10000	94.9 (3.7)
Met	5000	112.3 (4.8)
	10000	105.7 (5.7)
MeAA	5000	111.1 (4.6)
	10000	93.6 (3.2)
Cysteine sulfinate	1000	100.7 (11.5)
	5000	97.4 (2.8)
His	5000	102.8 (2.3)
	10000	103.8 (1.8)
Tryptamine	5000	109.4 (4.1)
	10000	111.5 (2.6)
Phe	1000	101.3 (2.3)
	5000	88.7 (2.0)
3-MeOAA	1000	99.5 (8.4)
	5000	83.5 (3.9)
Arg	5000	104.3 (2.6)
	10000	103.6 (2.7)
Cit	1000	101.3 (1.6)
	10000	101.4 (1.9)
5-HT	1000	103.1 (2.4)
	5000	103.5 (2.6)
Hphe	1000	83.6 (2.0)
	5000	74.2 (4.0)
MetSO ₂	1000	108.2 (8.0)
	5000	113.7 (3.0)

Table S3 (continued)

Analytes	Spike conc. (nM)	10-fold diluted serum recovery (CV %)
Tyr	5000	83.1 (2.7)
	10000	75.7 (3.4)
5-MeOT	5000	115.7 (3.3)
	10000	116.4 (3.0)
ADMA	5000	109.5 (3.7)
	10000	117.8 (5.2)
Trp	1000	110.9 (3.0)
	5000	101.6 (1.8)
TrpOH	5000	77.2 (3.5)
	10000	105.3 (3.3)
Cytidine	1000	113.7 (7.9)
	5000	92.1 (7.6)
Hst	1000	82.9 (3.0)
	5000	67.7 (1.6)
MeHst	1000	111.1 (8.2)
	5000	111.1 (9.1)
Orn	5000	96.3 (2.1)
	10000	95.9 (3.7)
KynOH	1000	113.5 (4.9)
	5000	68.3 (6.1)
Car	1000	114.5 (4.9)
	5000	68.4 (6.1)
DAc-SPM	5000	89.5 (6.5)
	10000	113.5 (9.2)
GSH	5000	73.9 (10.0)
	10000	99.6 (7.8)
SPD	5000	102.4 (2.8)
	10000	99.9 (4.4)
Ac-SPM	5000	113.9 (3.6)
	10000	114.6 (10.1)
SPM	1000	98.8 (5.4)
	5000	86.7 (7.0)

Table S4 The CSF/serum concentration levels (μM) of ninety-nine targeted compounds in DLB and AD subjects (mean \pm SD) using LC-ESI-MS/MS.

Analytes	DLB		AD	
	CSF	Serum	CSF	Serum
EA	23.87 \pm 5.58 ^a	81.97 \pm 34.25 ^a	44.84 \pm 40.91 ^a	215.69 \pm 102.71 ^a
1-aminoacetone	0.361 \pm 0.269	0.138 \pm 0.075	0.553 \pm 0.205	0.231 \pm 0.214
Gly	18.41 \pm 6.36 ^a	477.61 \pm 167.12 ^a	58.19 \pm 77.30 ^a	704.64 \pm 240.80 ^a
1-amino-2-propanol	0.243 \pm 0.083	0.932 \pm 0.679	0.376 \pm 0.254	2.32 \pm 1.99
β -Ala	1.24 \pm 0.601	14.89 \pm 6.12 ^a	3.17 \pm 3.84	43.61 \pm 6.63 ^a
Ala	132.79 \pm 32.45 ^a	818.59 \pm 302.95 ^a	188.50 \pm 121.67 ^a	1295.06 \pm 245.25 ^a
Sar	< LOD	< LOD	< LOD	< LOD
GABA	5.61 \pm 4.53	0.514 \pm 0.063	4.09 \pm 3.50	1.69 \pm 1.05
Homoalanine	3.39 \pm 1.61 ^a	24.56 \pm 12.79 ^a	4.37 \pm 2.19 ^a	47.55 \pm 29.76 ^a
Ser	197.16 \pm 55.91 ^a	632.41 \pm 231.74 ^a	259.13 \pm 144.10 ^a	806.23 \pm 293.55 ^a
Hypotaurine	6.09 \pm 1.38	41.91 \pm 32.56 ^a	6.61 \pm 2.44	161.02 \pm 118.55 ^a
Cytosine	< LOD	< LOD	< LOD	< LOD
Uracil	1.25 \pm 0.602	8.51 \pm 11.13	3.23 \pm 0.927	9.54 \pm 5.75
CRE	121.07 \pm 46.40	64.82 \pm 37.42	63.46 \pm 41.15	81.58 \pm 75.20
Pro	8.53 \pm 2.11	424.54 \pm 136.22 ^a	16.20 \pm 16.65	430.92 \pm 133.34 ^a
5-Ava	0.031 \pm 0.0088	1.44 \pm 1.00	0.029 \pm 0.024	0.558 \pm 0.279
Val	30.83 \pm 7.95 ^a	340.60 \pm 89.08 ^a	35.92 \pm 19.19 ^a	347.55 \pm 74.31 ^a
Hse	12.52 \pm 1.65 ^a	24.65 \pm 6.96 ^a	11.48 \pm 5.64 ^a	35.31 \pm 8.21 ^a
aThr	1.55 \pm 0.960	1.79 \pm 0.751	1.78 \pm 0.785	1.84 \pm 0.456
Thr	97.96 \pm 31.42 ^a	437.00 \pm 126.43 ^a	106.19 \pm 40.13 ^a	485.17 \pm 106.27 ^a
Phenethylamine	< LOD	< LOD	< LOD	< LOD
Tau	19.83 \pm 15.79 ^a	216.07 \pm 258.51 ^a	40.29 \pm 43.30 ^a	243.18 \pm 160.38 ^a
Thymine	0.012 \pm 0.0059	0.525 \pm 0.574	0.037 \pm 0.019	0.682 \pm 0.749
Ac-PUT	0.280 \pm 0.201	3.66 \pm 2.94	0.175 \pm 0.037	3.91 \pm 2.34
Agm	< LOD	< LOD	< LOD	< LOD
5-ALA	0.058 \pm 0.0056	0.697 \pm 0.180	0.075 \pm 0.021	1.02 \pm 0.292
ProOH	2.19 \pm 1.38	61.22 \pm 41.21	1.75 \pm 1.17	46.11 \pm 15.25
Leu	71.80 \pm 17.02 ^a	834.14 \pm 166.48 ^a	79.88 \pm 56.18 ^a	862.06 \pm 193.76 ^a
Nle	< LOD	< LOD	< LOD	< LOD
Ile	12.22 \pm 3.51	183.60 \pm 35.93 ^a	14.48 \pm 12.19	181.11 \pm 55.71 ^a
Cr	94.45 \pm 32.94 ^a	96.34 \pm 69.64 ^a	117.26 \pm 40.10 ^a	113.96 \pm 79.98 ^a
Asn	63.61 \pm 19.74 ^a	624.46 \pm 228.04 ^a	92.48 \pm 74.70 ^a	1217.42 \pm 559.41 ^a
Asp	2.89 \pm 2.70 ^a	35.40 \pm 17.62 ^a	18.33 \pm 32.10 ^a	42.85 \pm 17.64 ^a
2-aminoacetophenone	< LOD	< LOD	< LOD	< LOD
AA	0.037 \pm 0.026	0.090 \pm 0.048	0.026 \pm 0.017	0.078 \pm 0.032

Analytes	DLB		AD	
	CSF	Serum	CSF	Serum
Tyramine	< LOD	< LOD	< LOD	< LOD
EAP	< LOD	< LOD	< LOD	< LOD
Gln	1471.75 ± 385.12 ^a	782.95 ± 258.78 ^a	1187.25 ± 346.08 ^a	1051.73 ± 295.57 ^a
O-acetyl-serine	< LOD	< LOD	< LOD	< LOD
Glu	1.88 ± 1.39	67.08 ± 28.77	6.44 ± 7.35	110.32 ± 20.05
Met	9.89 ± 1.86	72.47 ± 36.07 ^a	11.20 ± 9.00	83.91 ± 34.26 ^a
MeAA	< LOD	< LOD	< LOD	< LOD
3-AAOH	< LOD	< LOD	< LOD	< LOD
Cysteine sulfinate	2.33 ± 2.25	< LOD	2.80 ± 1.01	0.166 ± 0.084
His	15.64 ± 2.94 ^a	115.22 ± 9.95 ^a	16.99 ± 8.92 ^a	139.13 ± 37.87 ^a
Tryptamine	< LOD	< LOD	< LOD	< LOD
2-aminoadipate	1.14 ± 0.757	0.742 ± 0.158	0.681 ± 0.231	0.653 ± 0.054
MetSO	9.34 ± 2.57 ^a	22.05 ± 16.04 ^a	7.36 ± 4.75 ^a	21.35 ± 12.42 ^a
Phe	64.16 ± 12.01 ^a	301.20 ± 73.78 ^a	50.82 ± 21.58 ^a	286.10 ± 24.47 ^a
Cysteate	< LOD	< LOD	< LOD	< LOD
3-MeOAA	< LOD	< LOD	< LOD	< LOD
3-MeO-tyramine	0.078 ± 0.023	0.040 ± 0.033	0.166 ± 0.061	0.079 ± 0.053
Arg	36.29 ± 7.65 ^a	277.78 ± 63.03 ^a	32.63 ± 15.98 ^a	154.84 ± 81.55 ^a
Ac-Orn	< LOD	1.34 ± 1.25	< LOD	0.559 ± 0.228
Theanine	< LOD	< LOD	< LOD	< LOD
Cit	3.10 ± 0.783	26.75 ± 11.63	3.74 ± 2.51	23.14 ± 5.01
5-HT	< LOD	0.130 ± 0.083	< LOD	0.073 ± 0.020
Hphe	< LOD	< LOD	< LOD	< LOD
MetSO ₂	0.140 ± 0.113	0.291 ± 0.166	0.190 ± 0.240	1.45 ± 2.00
Tyr	13.01 ± 1.71 ^a	45.95 ± 11.84 ^a	11.00 ± 2.19 ^a	49.58 ± 9.73 ^a
NMMA	1.03 ± 0.413	5.08 ± 1.80	1.55 ± 0.501	6.87 ± 1.86
Ac-Lys	0.0045 ± 0.0019	0.036 ± 0.023	0.0036 ± 0.0018	0.022 ± 0.0079
5-MeOT	< LOD	< LOD	< LOD	< LOD
Se-Met	< LOD	< LOD	< LOD	< LOD
ADMA	7.05 ± 8.26 ^a	38.77 ± 25.85 ^a	9.30 ± 6.40 ^a	49.67 ± 15.73 ^a
Trp	6.27 ± 1.30 ^a	31.84 ± 6.35 ^a	4.22 ± 1.54 ^a	29.61 ± 5.16 ^a
Indolelactate	0.155 ± 0.029	6.89 ± 6.88	0.351 ± 0.272	3.72 ± 1.46
Kyn	0.647 ± 0.556	3.83 ± 2.33	0.776 ± 0.511	4.93 ± 0.857
5-TrpOH	< LOD	< LOD	< LOD	< LOD
DAc-SPD	0.159 ± 0.073	0.159 ± 0.173	0.180 ± 0.058	0.152 ± 0.035
Thymidine	< LOD	< LOD	< LOD	< LOD
Cytidine	< LOD	4.78 ± 5.15	< LOD	13.00 ± 13.06
Uridine	3.46 ± 1.86	175.48 ± 205.65	2.51 ± 0.955	30.48 ± 17.53
DAP	0.864 ± 0.375	1.42 ± 0.798	1.71 ± 0.465	3.14 ± 2.08
PUT	0.835 ± 0.496	4.03 ± 3.43 ^a	1.03 ± 0.933	9.12 ± 8.21 ^a

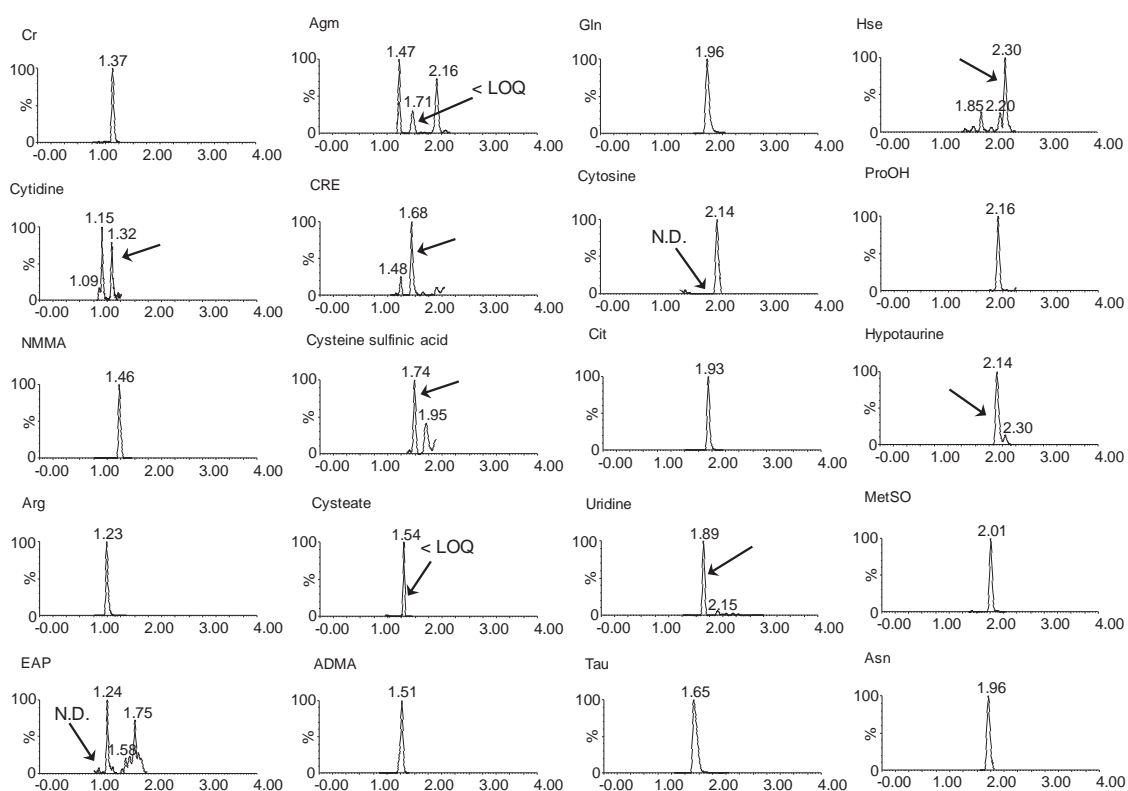
Analytes	DLB		AD	
	CSF	Serum	CSF	Serum
CAD	0.039 ± 0.017	4.33 ± 2.40	0.029 ± 0.030	3.31 ± 3.64
Hst	0.0038 ± 0.0020	0.0097 ± 0.0059	0.0044 ± 0.0033	0.024 ± 0.012
MeHst	0.137 ± 0.114 ^a	2.73 ± 3.10 ^a	0.334 ± 0.409 ^a	1.42 ± 0.461 ^a
Orn	17.16 ± 7.52 ^a	206.81 ± 88.56 ^a	14.69 ± 8.59 ^a	286.41 ± 118.11 ^a
Histidinol	< LOD	< LOD	< LOD	< LOD
Lys	57.37 ± 18.71 ^a	340.05 ± 70.68 ^a	47.83 ± 22.91 ^a	344.82 ± 50.63 ^a
Cystamine	< LOD	0.753 ± 0.515	0.622 ± 0.599	0.900 ± 0.452
LysOH	2.81 ± 0.106	3.98 ± 0.874	2.77 ± 0.048	4.96 ± 0.609
1-MeHis	5.46 ± 5.21 ^a	29.50 ± 20.69 ^a	6.12 ± 3.43 ^a	50.42 ± 17.04 ^a
3-MeHis	< LOD	< LOD	< LOD	< LOD
Ac-SPD	0.272 ± 0.033	2.12 ± 1.17	0.275 ± 0.144	3.09 ± 1.35
2,6-diamino-pimelate	< LOD	< LOD	< LOD	< LOD
Cystathionine	2.61 ± 1.37	3.39 ± 2.49	5.43 ± 5.64	21.95 ± 32.62
3-KynOH	0.0048 ± 0.0036	0.0078 ± 0.0073	0.0060 ± 0.0019	0.043 ± 0.033
Car	0.019 ± 0.0067	< LOD	0.044 ± 0.055	< LOD
Cystine	40.82 ± 22.83 ^a	17.14 ± 16.87 ^a	59.53 ± 66.24 ^a	14.24 ± 7.62 ^a
Homocystine	3.48 ± 1.53	< LOD	5.45 ± 2.88	< LOD
DAc-SPM	1.84 ± 1.06	46.57 ± 55.39	0.358 ± 0.054	42.30 ± 18.22
GSH	9.31 ± 3.98 ^a	< LOD	7.11 ± 3.51 ^a	< LOD
NorSPD	< LOD	< LOD	< LOD	< LOD
SPD	4.25 ± 2.35	4.66 ± 3.67	9.96 ± 9.03	12.56 ± 9.00
SAH	0.430 ± 0.161	2.14 ± 2.77	0.857 ± 0.766	1.19 ± 0.887
Ac-SPM	0.015 ± 0.017	1.30 ± 0.903	0.051 ± 0.058	1.37 ± 0.493
SPM	0.365 ± 0.201	0.636 ± 0.681	1.05 ± 1.40	2.00 ± 1.59

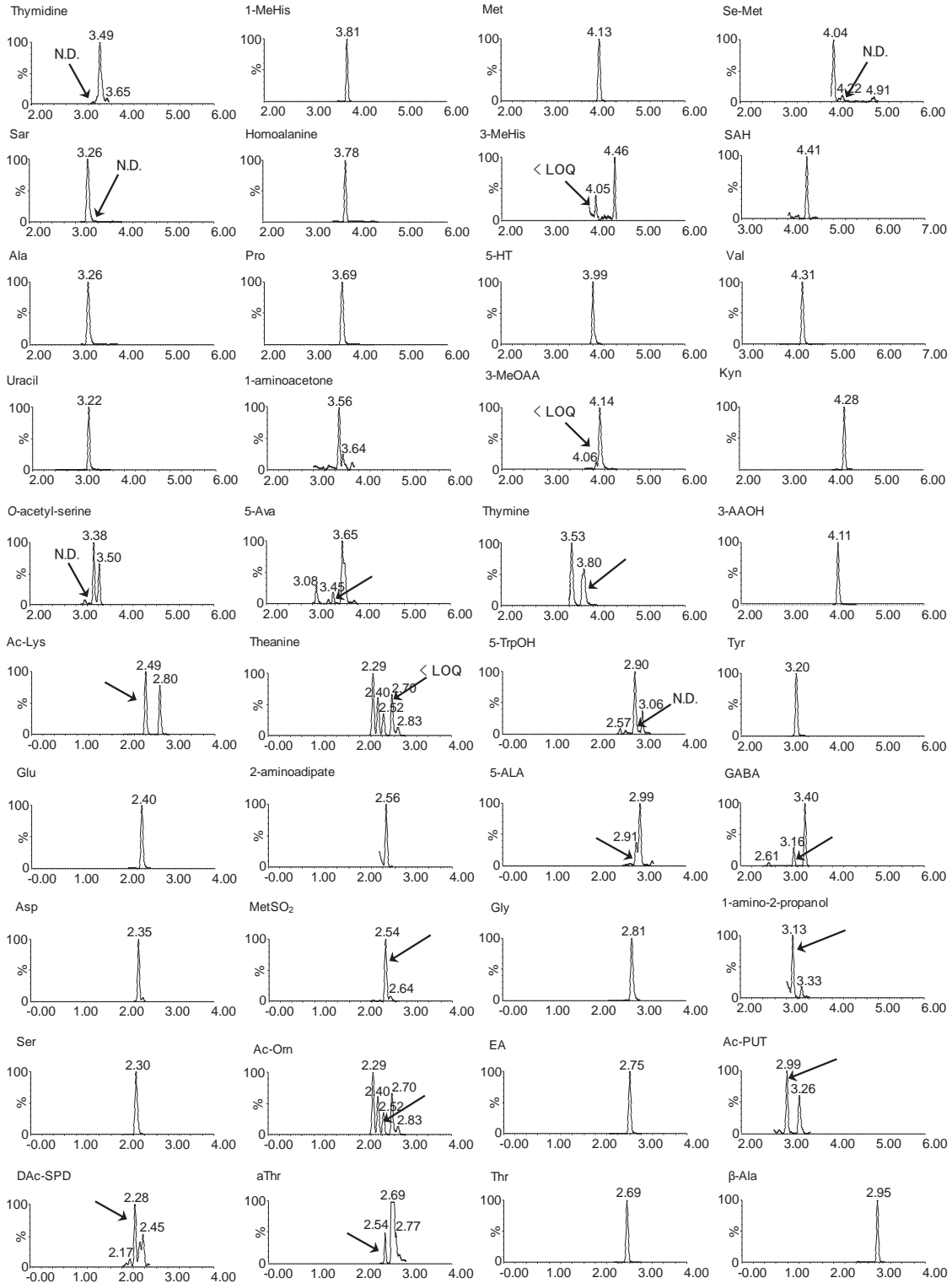
* : P < 0.05, ** : P < 0.01, ***: P < 0.005, ****: P < 0.001 (AD vs DLB)

^a 10-fold dilution

Fig. S1. Fmoc-derivatized UHPLC-ESI-MS/MS chromatograms of all analytes from serum in SRM .

Fmoc-derivatized amines were analyzed using an ACQUITY ultra-performance liquid chromatography coupled to a Xevo TQD triple quadrupole mass spectrometer equipped with an ESI source. The RP analysis was performed using an ACQUITY UPLC BEH C18 column at 50°C. The injection volume of 5 μ L was used, and the total run time of analysis was 10 min. The mobile phase consisted of solvent A: 0.1% FA in water, and solvent B: 0.1% FA in acetonitrile, was delivered at a flow rate of 0.4 mL/min. The gradient elution was set as follows: solvent A/B = 60/40 (0 min), 30/70 (5 min), 2/98 (7 min), 2/98 (9 min). Detailed MS/MS conditions were shown in Table 1. These chromatographic monitoring is time-cut for the detected retention time of each analytes.





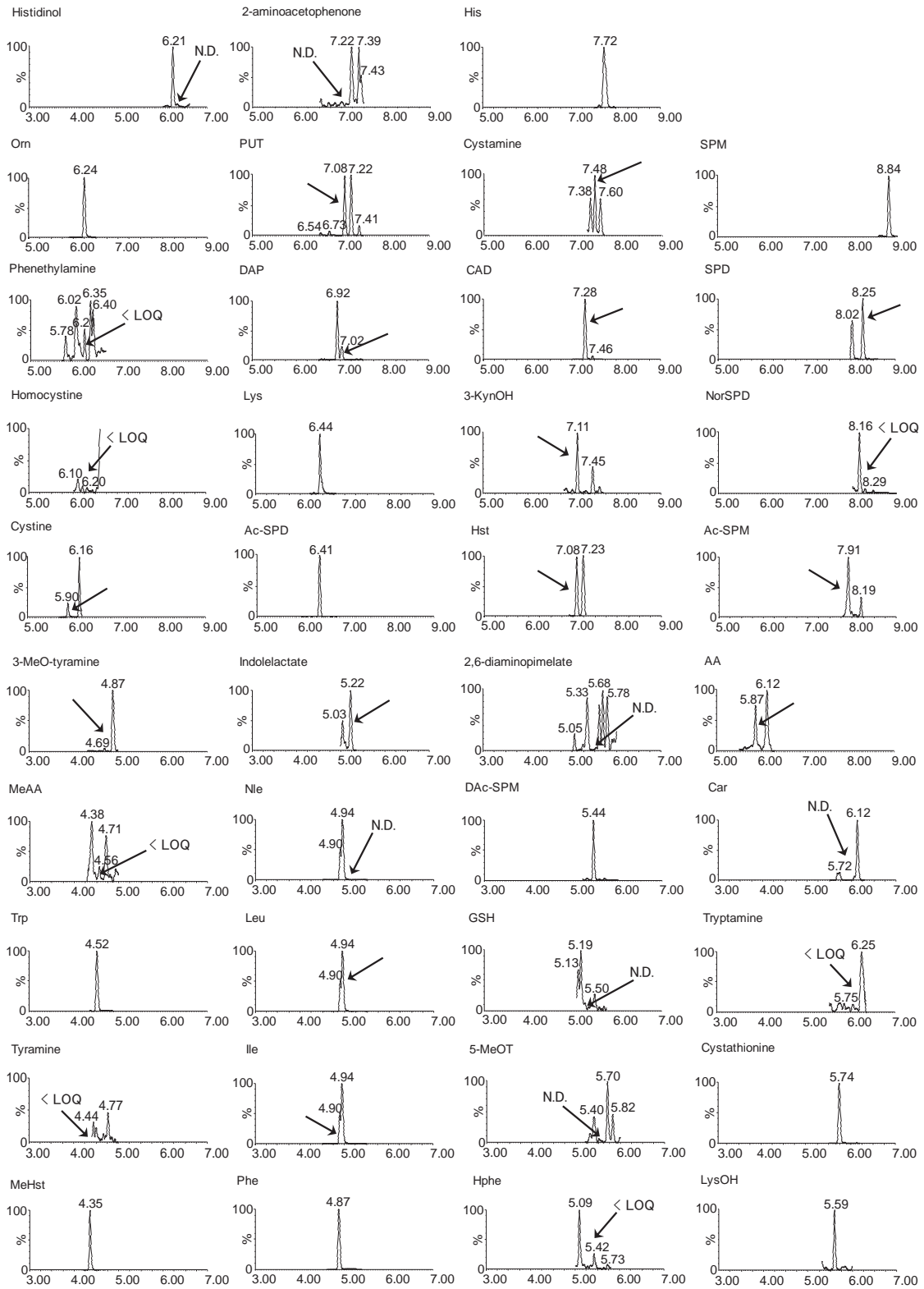


Fig. S2. Trends of concentration levels expressed by metabolic pathway analysis in CSF.

Gradation color shows the increased (red) or decreased (blue) concentration levels calculated by dividing the median (AD / DLB).

