

SUPPORTING INFORMATION TO:

Single-step rapid diagnosis of dopamine and serotonin metabolism disorders

Aurélien Lo,¹ Pierre Guibal,¹ Diane Doummar,² Diana Rodriguez,² Jean-Yves Hautem,² Rémy Couderc,² Thierry Billette De Villemeur,² Emmanuel Roze,^{3,4} Pierre Chaminade,¹ and Fathi Moussa^{*,1,2}

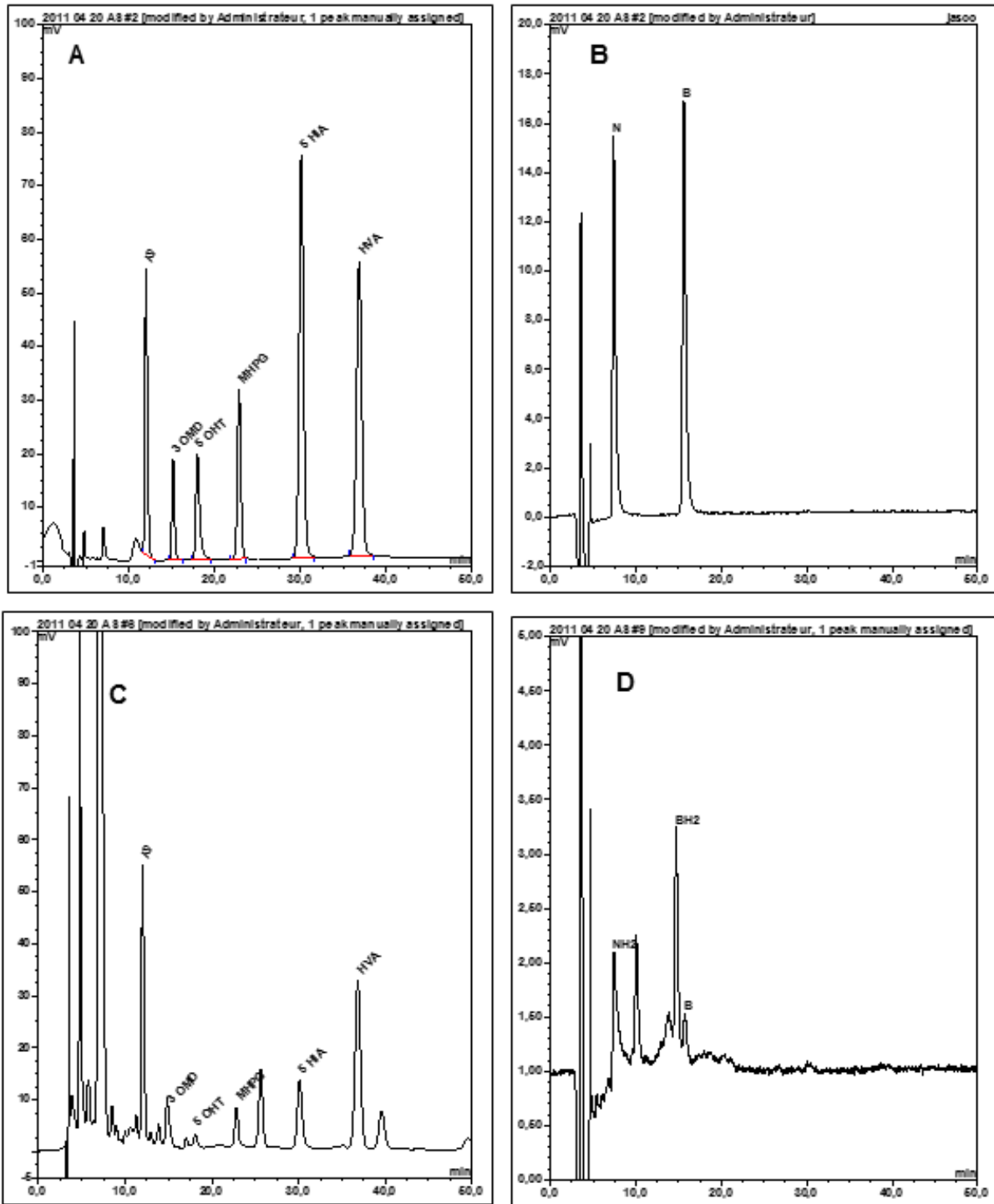


Figure S1: HPLC profiles of a standard mixture with (A) electrochemical detection followed by (B) sequential fluorescence detection, and a CSF sample of a patient from the control group with (C) electrochemical detection and (D) sequential fluorescence detection. Chromatographic conditions: column 4.6 x 150 mm, 3 μ m Atlantis T3 (Waters, France); mobile phase: pH 5.2, 0.05 M citrate buffer/methanol (97/3, v/v). Flow rate: 0.5 mL/min at 30°C

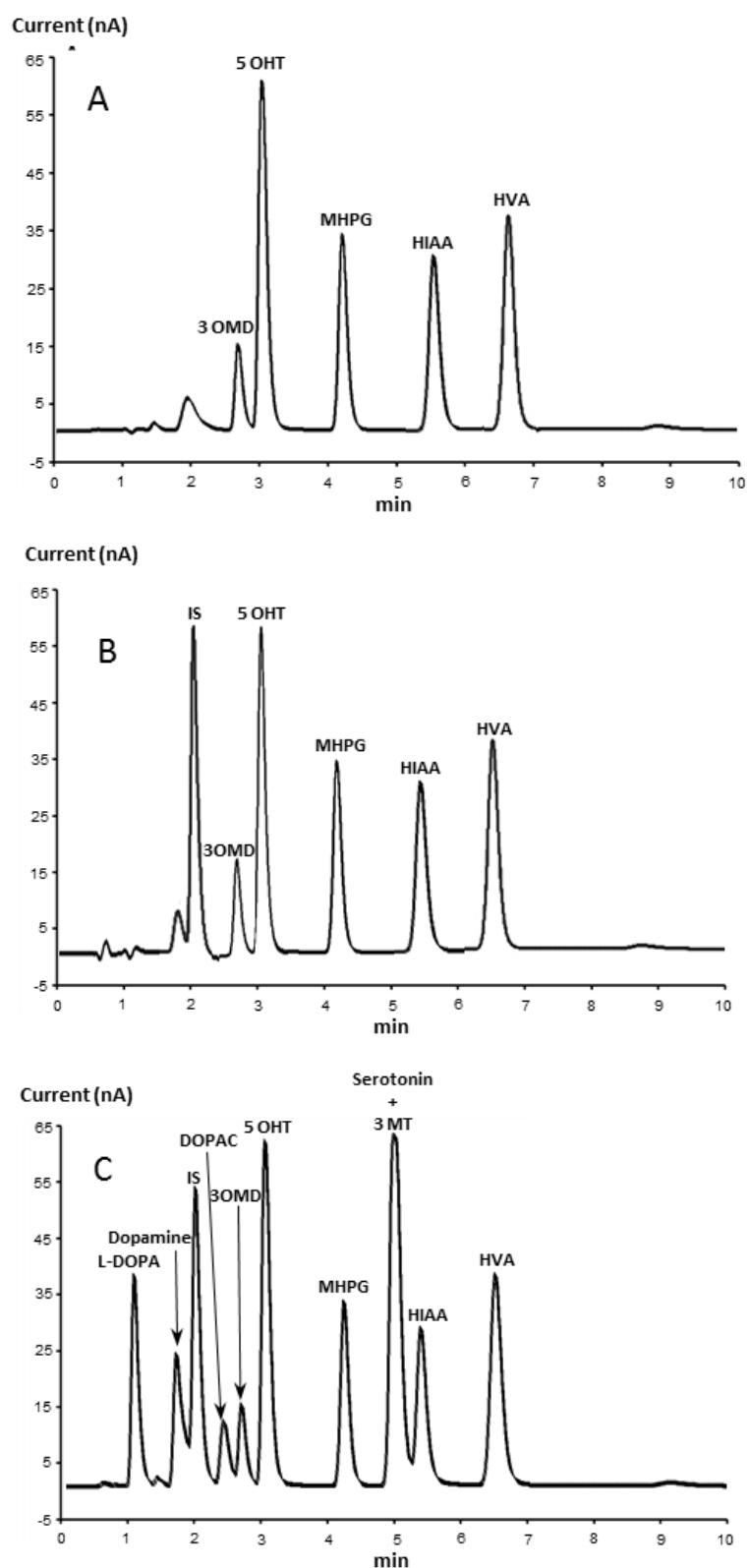


Figure S2. Chromatographic profiles of several metabolites (500 nM) obtained with the proposed method: (A) Standard mixture containing the targeted metabolites 3 OMD, 5 OHT (or 5-HTp), MHPG, HIAA, and HVA; (B) Addition of the IS to mixture A, and (C) Addition of L-DOPA, dopamine, DOPAC, serotonin, and 3 methoxy-tyramine (MT) to mixture B.

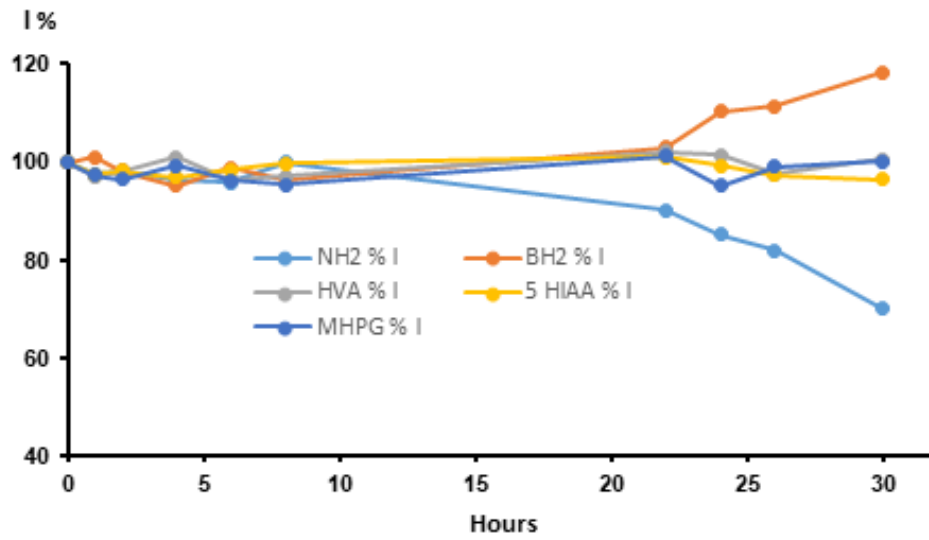


Figure S3: Stability of the analytes in the auto-sampler (10 °C, in the dark). I % = relative intensity vs initial intensity (Time 0; 100 %)

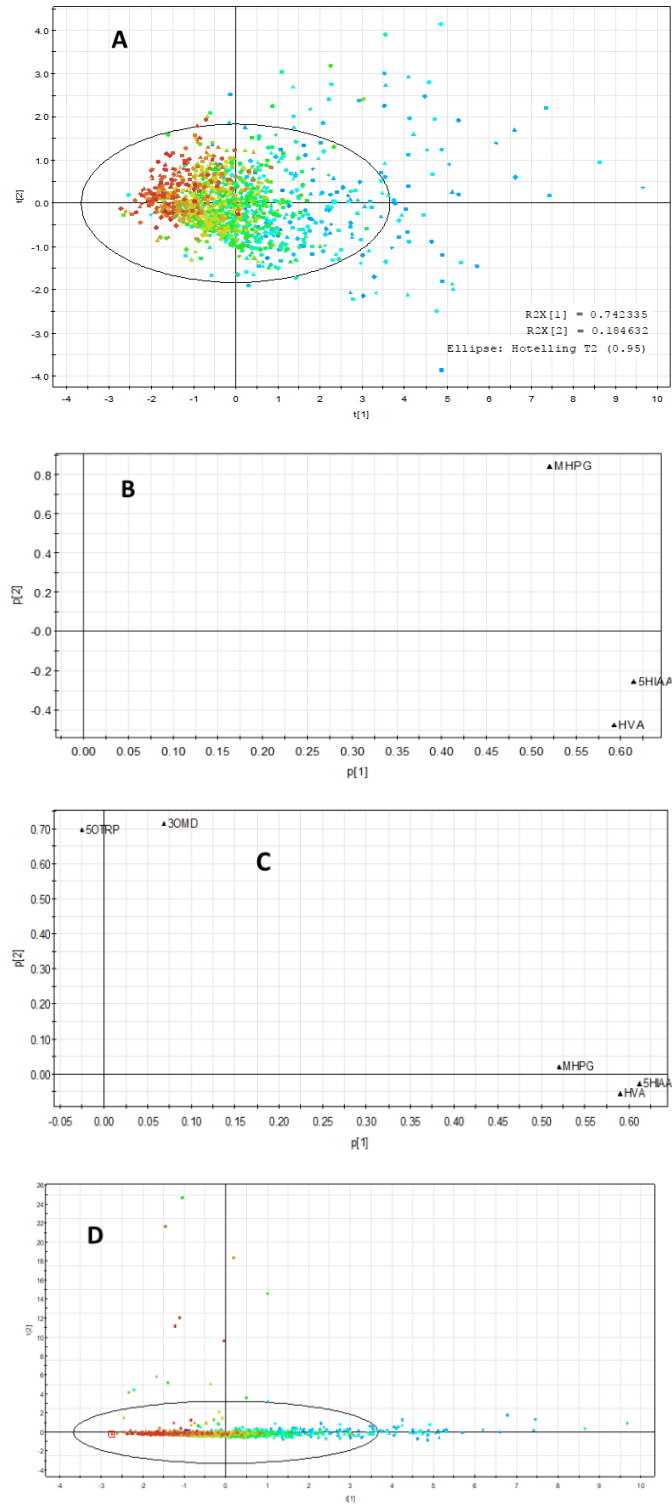


Figure S4. PCA results: (A) Score plot and (B) loading plot obtained after considering HVA, 5 HIAA, and MHPG, only, (C) loading plot after adding 3-OMD and 5-HTrp, and (D) score plot evolution after adding 3-OMD, 5-HTrp, and BH2.

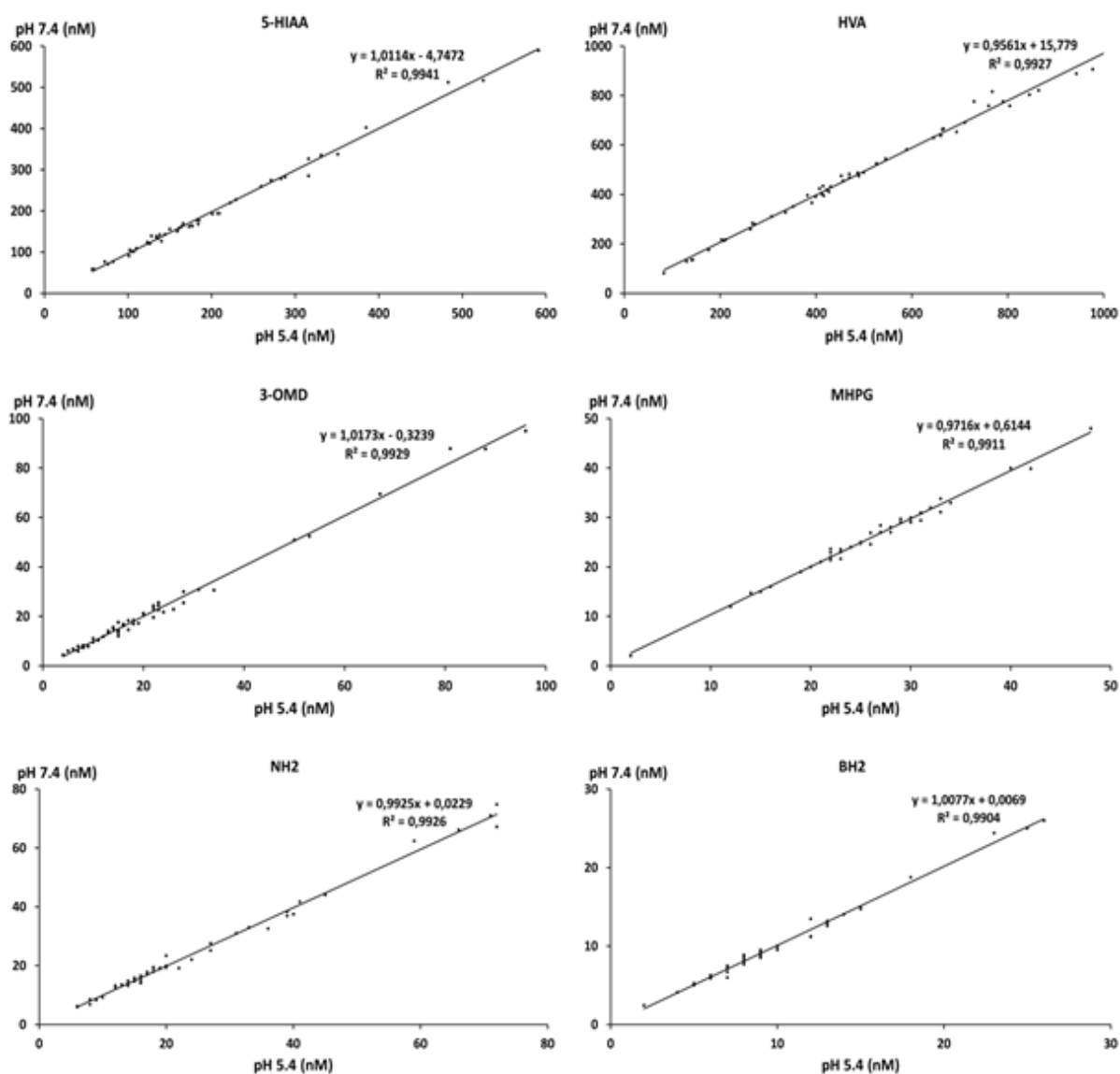


Figure S5: Correlation between the results obtained with the proposed methods at pH 5.2 and at pH 7.4.

Table S1: Linearity of the proposed method at pH 5.2

Analyte	LOQ nM	Slope	Intercept	R ²	Sy/x ^a	Sa ^b	Sb ^c
3 OMD	5	976	- 2822	0.9997	2939	2.9	672
5 OHT	5	1079	- 8492	0.9993	4892	4.8	1119
MHPG	5	2012	- 873	0.9998	4535	4.4	1037
5 HIAA	10	1131	- 24621	0.9970	22153	10.8	5121
HVA	15	970	- 3464	0.9999	3325	1.1	761
N	5	1958	- 1801	0.9968	3969	21.1	1089
NH2	5	1386	- 1550	0.9918	3859	22.3	1168
BH2	5	1339	- 1622	0.9947	3436	20.0	1029
B	5	2471	- 1059	0.9936	7048	37.5	1933

LOQ: limit of quantification; ^a Standard error of the estimate; ^b Standard error of the slope; ^c Standard error of the intercept. 5 OHT (or 5-HTp).

Table S2: Analytical recovery and accuracy of the proposed method at pH 5.2. (5-OHT = 5HT_{1p})

Analytes	Standard solutions (n = 6)			Authentic CSF (n = 6)				p-values
	Concentration (nM)	Within-run (CV%)	Between-run (CV %)	Added concentration (nM)	Measured concentration (nM)	Recovery (%)	Within-run CV (%)	
3-OMD	0	-	-	0	30	-	8.9	
	5	6.8	6.5	5	-	-	-	
	25	5.0	7.5	25	56	101.8	7.5	0.0515
	62.5	3.0	4.0	62.5	91	98.4	8.7	0.0536
	125	1.5	2.0	125	-	-	-	
	250	0.9	2.0	250	266	95.0	4.8	0.0576
5-OHT	0	-	-	0	24	-	7.2	
	5	7.2	9.3	5	-	-	-	
	25	4.0	8.6	25	48	98.6	9.5	0.2571
	62.5	2.7	7.1	62.5	88	101.7	8.6	0.9098
	125	2.9	4.5	125	-	-	-	
	250	1.5	2.8	250	263	96.0	7.7	0.0508
MHPG	0	-	-	0	21	-	9.4	
	5	2.7	6.2	5	-	-	-	
	25	2.6	7.4	25	48	104.3	6.9	0.6734
	62.5	1.3	3.5	62.5	81	97.0	5.1	0.8737
	125	0.9	2.7	125	-	-	-	
	250	0.9	2.6	250	266	98.2	1.4	0.0527
5 HIAA	0	-	-	0	113	-	8.5	
	10	6.1	9.7	10	-	-	-	
	50	5.5	8.9	50	158	96.9	8.6	0.8418
	125	5.0	5.6	125	240	100.8	6.8	0.0860
	250	4.2	8.8	250	-	-	-	
	500	4.2	6.6	500	596	97.2	2.8	0.3197
HVA	0	-	-	0	370	-	8.1	
	15	2.3	5.7	15	-	-	-	
	75	1.1	5.7	75	437	98.2	6.6	0.1700
	187.5	0.7	3.7	187.5	560	100.3	5.1	0.3810
	375	0.6	3.0	375	-	-	-	
	750	0.4	2.6	750	1105	98.7	2.8	0.0613
NH ₂	0	-	-	0	11	-	2.8	
	5	4.9	7.5	5	15	93.8	7.4	0.4965
	12.5	7.6	7.1	12.5	22	93.6	3.8	0.0628
	25	6.8	8.7	25	-	-	-	
	50	5.4	6.4	50	56	91.8	5.7	0.0556
	100	3.4	7.4	100	-	-	-	
BH ₂	0	-	-	0	22	-	4.5	
	5	5.6	7.9	5	26	96.3	6.4	0.6612
	12.5	6.3	5.7	12.5	33	95.7	6.3	0.5649
	25	5.9	8.5	25	-	-	-	
	50	6.7	6.3	50	69	95.8	8.7	0.2674
	100	4.7	6.2	100	-	-	-	
B	0	-	-	0	10	-	9.0	
	5	8.9	9.7	5	14	93.3	4.2	0.6776
	12.5	5.4	7.0	12.5	23	102.2	3.4	0.2423
	25	8.8	6.3	25	-	-	-	
	50	7.4	6.5	50	59	98.3	4.3	0.4988
	100	4.9	7.2	100	-	-	-	

Table S3: p value as a result of Shapiro Wilk normality test on population and log transformed population.

p - value	HVA		5 HIAA		HVA / 5 HIAA		MHPG		3 OMD		NEOP		BH2	
	nom	log	nom	log	nom	log	norm	log	nom	log	nom	log	norm	log
0 – 30 days	0.5296	0.0884	0.2902	0.0139	0.3094	0.6882	0.4354	0.7996	0.3916	0.3094	0.3876	0.5830	0.0639	0.3504
1 – 6 months	0.2688	0.1908	0.0012	0.9786	0.0001	0.5465	< 0.0001	0.0053	< 0.0001	0.0306	< 0.0001	0.0779	< 0.0001	0.1327
0.5 – 3 years	0.0012	0.4707	< 0.0001	0.0012	< 0.0001	0.3737	< 0.0001	0.6965	< 0.0001	0.0758	< 0.0001	0.0044	< 0.0001	0.0087
3 – 7 years	< 0.0001	0.8886	< 0.0001	0.5082	0.0003	0.3644	< 0.0001	0.0170	< 0.0001	0.0008	< 0.0001	0.8843	< 0.0001	0.0805
7- 11 years	0.0018	0.0931	0.0001	0.3896	0.0011	0.0699	< 0.0001	0.1525	< 0.0001	0.0188	0.0001	0.5168	< 0.0001	0.8617
11- 16 years	0.1294	0.3268	0.1084	0.4421	0.0001	0.0777	0.0001	0.5520	< 0.0001	0.0144	0.0002	0.7117	< 0.0001	0.4109
Over 16 years	< 0.0001	0.9252	< 0.0001	0.0074	0.0001	0.6569	0.001	0.1986	< 0.0001	0.4773	0.0006	0.9284	< 0.0001	0.1130

Table S4. Linearity of the method with a mobile phase pH set at 7.4. (5 OHT = 5-HTrp)

Analyte	LOQ nM	Slope	Intercept	R ²	S _{y/x}	S _a	S _b
3-OMD	6,1	1971	-2973	0,9998	3499	967	7,1
5-OHT	6,6	1682	705	0,9998	3451	954	6,9
5-HIAA	9,4	1651	-1816	0,9989	5969	1650	6,5
HVA	10,0	1803	6852	0,9999	6242	1725	6,5
MHPG	3,5	4421	-1013	0,9999	7466	2063	17,5
N	2,4	10842	-60626	0,9991	2660	735	14,4
NH2	3,2	6055	-1070	0,9995	3086	2070	83,4
B	1,6	11291	-67127	0,9994	2930	810	15,7
BH2	3,2	6584	-2313	0,9997	1923	533	12,8
BH4	4,2	4817	7871	0,998	3481	1626	97,2

Table S5: Analytical recovery and accuracy of the method with mobile phase pH set at 7.4.

Analytes	Standard solution (n = 6)			Authentic CSF (n = 6)			
	Concentration (nM)	Within-run (CV%)	Between-run (CV %)	Added concentration (nM)	Measured concentration (nM)	Recovery (%)	Within-run CV (%)
3-OMD	0	-	-	0	15,1	-	5,5
	17,4	0,8	2,6	16,5	-	-	-
	33,1	0,4	3,0	33	48,5	101,1	4,4
	65,4	0,7	2,8	66	83,7	103,9	1,9
	131,0	2,5	2,6	132	142,7	96,6	4,6
	264,6	0,4	2,0	264	280,6	100,6	3,3
5-OHT	0	-	-	0	14,6	-	0,0
	17,4	3,4	8,1	16,6	-	-	-
	33,3	1,0	9,2	33,3	48,2	101,2	10,4
	66,0	0,5	7,6	66,5	87,5	109,6	4,9
	132,2	2,5	9,0	133	146,9	99,5	5,3
	266,5	0,7	12,3	266	279,3	99,5	7,6
5-HIAA	0	-	-	0	100,6	-	6,9
	31,6	3,2	4,2	31	-	-	-
	61,8	0,9	4,4	61,9	166,0	105,5	5,0
	123,3	0,6	4,7	123,9	231,4	105,5	3,8
	247,6	2,1	3,6	247,8	347,8	99,8	5,3
	495,7	0,7	5,1	495,5	594,3	99,6	3,4
HVA	0	-	-	0	251,7	-	4,7
	32,4	0,3	2,9	31,9	-	-	-
	63,9	0,2	2,8	63,8	318,0	103,9	1,4
	128,2	0,6	3,9	127,6	391,7	109,7	1,5
	253,4	2,4	3,1	255,3	504,6	99,1	3,4
	511,2	0,4	6,4	510,5	760,0	99,6	2,0
MHPG	0	-	-	0	12,4	-	7,5
	15,2	0,4	3,2	14,3	-	-	-
	28,8	0,3	3,2	28,6	42,0	103,2	8,1
	56,8	0,7	4,0	57,3	69,6	99,9	4,8
	113,2	2,5	2,4	114,5	126,3	99,5	6,4
	229,7	0,4	3,6	229	241,6	100,1	3,3
N	0	-	-	0	n.d.	-	n.d.
	6,9	4,7	2,3	6,2	-	-	-
	12,5	2,1	7,2	12,3	13,3	107,6	8,3
	24,4	2,4	5,0	24,7	25,5	103,1	6,8
	48,2	1,9	7,0	49,4	50,5	102,3	3,8
	99,3	0,8	1,0	98,8	97,9	99,1	1,2
NH2	0	-	-	0	n.d.	-	n.d.
	7,2	4,9	9,9	6,2	-	-	-
	12,7	3,2	5,9	12,3	12,5	101,1	8,9
	24,5	2,1	6,3	24,7	26,4	106,9	4,3
	47,0	3,2	5,1	49,4	49,5	100,3	3,6
	99,8	0,4	5,8	98,7	98,2	99,5	3,9
B	0	-	-	0	n.d.	-	n.d.
	6,5	6,2	6,1	6,3	-	-	-
	12,2	3,7	4,8	12,5	11,6	92,8	5,7
	25,3	2,8	5,0	25	25,8	103,2	7,0
	49,6	1,8	6,1	50	49,9	99,8	2,4
	100,1	0,6	0,6	100	100,0	100,0	3,0
BH2	0	-	-	0	n.d.	-	n.d.
	6,1	5,0	6,6	6,2	-	-	-
	12,3	4,5	3,1	12,3	15,4	124,6	2,8
	24,6	2,8	5,7	24,7	28,0	113,6	7,3
	49,7	2,7	9,0	49,3	47,2	95,8	11,3
	98,4	2,4	9,1	98,6	98,4	99,8	5,9
BH4	0	-	-	0	n.d.	-	n.d.
	12,9	5,7	9,9	12,4	13,6	109,8	6,9
	19,6	9,0	8,9	18,6	-	-	-
	23,7	2,7	7,7	24,8	26,4	106,7	9,2
	35,2	3,3	3,3	37,1	-	-	-
	51,0	2,2	4,2	49,5	56,9	114,9	7,9
	-	-	-	99	94,7	95,7	2,5