Supplementary Material

Short-term exposure to environmental levels of nicotine and cotinine impairs visual motor response in zebrafish larvae through a similar mode of action: exploring the potential role of zebrafish α7 nAChR

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Quality assurance

All quality parameters are summarized in Supplementary Table ST2. First, no signal of any analyte was observed in the blank sample concluding that no cross-contamination was observed. Excellent correlation coefficients ($r^2 > 0.99$) were obtained for all target metabolites over the range of concentrations studied. Calibration and quantification were performed using isotopically labelled internal standards (ISM), to correct for all possible losses throughout the extraction process and chromatographic analysis. Besides, IDLs ranged from 1.1 (GABA and His) to 10.5 (DA) pg head^{-1}, and MDLs varied from 3.6 (GABA) to 58.5 (NE) pg head^{-1}. Intra-day precision values ranged from 0.9% to 5.9%, while inter-day precision values ranged from 1.9% to 19.5%. Regarding the assessment of method recovery, all target metabolites showed recoveries between 60 and 123%, except for ACh that reported 140% recovery. Finally, Glu presented signal suppression (ME<70%), while DA and NE suggested a signal enhancement (ME<130%).

Supplementary Tables

Supplementary Table ST1. MS parameters for all target compounds, including the tested alkaloids and the main metabolites.

Xevo TQs parameters				
Desolvation gas	Nitrogen		**Cone gas flow**	150 L h^{-1}
Desolvation gas flow	900 L h^{-1}		**Source temperature**	100 °C
Desolvation temperature	350 °C		**Capillary voltage**	2.0 kV
MRM – ESI +				
Compound ID	**Parent (m/z)**	**Cone voltage (V)**	**Daughter (m/z)**	**Collision energy (eV)**
ACh	147	14	87	12
			58	32
DA	137	14	119	18
			91	20
GABA	104	40	87	7
			69	10
Epi	184	21	166	7
			107	20
5-HT	160	56	132	14
			115	24
NE	152	14	135	14
			107	14
Glu	148	30	84	10
			56	10
His	112	8	95	12
			68	22
Nico	163	24	80	19
			84	17
Coti	177	30	98	20
			80	20

Supplementary Table ST2. Quality parameters obtained by LC-MS/MS for the target neurotransmitters. CC: concentration range for calibration curve, r^2: regression coefficient; IDL: instrumental detection limit; MDL: method detection limit; RSD: relative standard deviation

Target metabolite	CC	r^2	IDL	MDL	Recovery ± RSD	Intra-day precision	Inter-day precision	Matrix effect ± RSD
	ng/mL		(pg)	(pg/larvae head)	%	%	%	%
ACh	0.005 – 0.5	0.9980	8.1	26.6	148 ± 9	5.0	8.0	74 ± 21
DA	0.005 – 2.5	0.9953	10.5	39.4	70 ± 16	4.4	6.8	144 ± 6
GABA	0.005 – 2.5	0.9973	1.1	3.6	94 ± 2	0.9	3.9	111 ± 5
Epi	0.005 – 2.5	0.9945	1.4	6.3	68 ± 8	4.9	19.5	110 ± 1
5-HT	0.005 – 2.5	0.9923	2.1	10.1	81 ± 6	5.9	16.7	128 ± 7
NE	0.005 – 2.5	0.9926	10.3	58.5	86 ± 7	2.7	8.8	145 ± 1
Glu	0.005 – 2.5	0.9990	8.6	33.9	60 ± 11	1.0	1.9	66 ± 14
His	0.005 – 2.5	0.9949	1.1	23.8	123 ± 8	3.0	6.3	83 ± 20