Supplemental File 2. Main differences between the optimized and the non-optimized protocol for TAA, AA and DHAA extraction and quantification. The samples used were the same in both cases

Section	Step	Optimized protocol	Non-optimized protocol
Reagent and solution preparation	Extraction solution	8% acetic acid (v/v), 1% MPA (w/v), 1 mM EDTA	5% acetic acid (v/v), 3% MPA (w/v)
	Reducing solution	40 mM DTT in 0.5 M Tris pH 9.0	30 mM DTT in dH_2O
	Sulphuric acid (H_2SO_4)	0.4 M	-
	Hydrochloric acid (HCl)	2M	5M
	Stock AA standard preparation	Solvent: Ultrapure water pH 2.0 acidified by 98-100% formic acid	Solvent: dH_2O
	AA calibration curve	5 points (0.5, 2.5, 5, 10, 25 $\mu g\ mL^{-1}$) Solvent: Ultrapure water pH 2.0 acidified by 98-100% formic acid	6 points (10, 20, 40, 60, 80, 100 $\mu g\ mL^{-1}$) Solvent: 1.5% MPA
Extraction of AA and DHAA	Extraction solution volume (mL)	5	3
	Mixing	Vortex 5 s; orbital shaker (2000 rpm) 10 min at room temperature	Vortex 1 min
	Sonication (ultrasound bath)	10 min at room temperature	No
	Centrifugation (4,000 x *g*)	10 min at 4 °C	20 min at room temperature
	Filtration	0.22-µm regenerated cellulose filter	0.45-µm regenerated cellulose filter
DHAA reduction to AA	Incubation at room temperature	30 min	5 min
	Stop reaction	200 µL of 0.4 M H_2SO_4	No
Determination	Instrument	UPLC: Acquity H-Class	HPLC: Hewlett Packard 1050
	Detector	PDA eλ Detector λabs for AA=245 nm	eλ Detector λabs for AA=265 nm
	Column	Acquity UPLC HSS T3 (150 mm x 2.1 mm x 1.8 µm)	HPLC C18 Tracer column (250 mm x 4 mm x 5 µm)
	Channels	A: CH_3OH; B/Wash: H_2O:CH_3OH (50:50 v:v); C: Ultrapure water pH 2.0 with formic acid; D/Seal Wash	No
	Mobile phase	0.3 $ml{\cdot}min^{-1}$ of 2%A + 98%C (isocratic mode)	1 $mL\ min^{-1}$ of KH_2PO_4 30 mM adjusted pH 3.0 HCl 5M (isocratic mode)
	Column temperature	30 ºC	Room temperature
	Autosampler temperature	5 ºC	No (room temperature)
	Injection volume (µl)	5	20
	AA retention time (min)	1,874	2,980
	Total running time (min)	3	7