Detection of serotonin, melatonin and their metabolites in honey

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Supplementary Materials

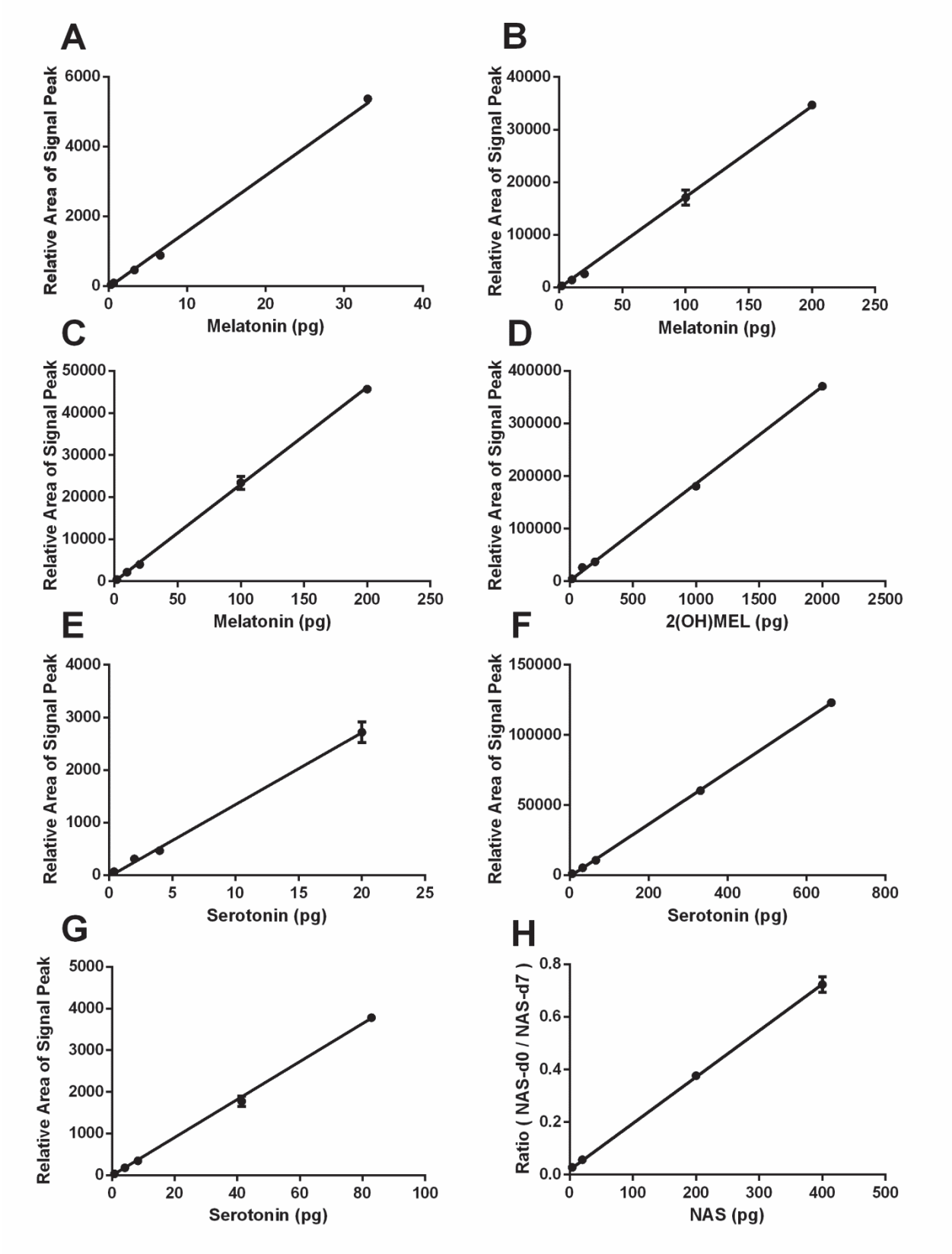

Figure S1. Standard curves for melatonin and its derivatives used for quantification. The peak area was calculated using Waters MassLynx™ Software with EIC of A, Melatonin, *m/z* = 174.1 $[M+H-NH_2CH_3CO]^+$; B, Melatonin, *m/z* = 233.1 $[M+H]^+$; C, Melatonin, *m/z* = 255.1 $[M+Na]^+$; D, 2(OH)MEL, *m/z* = 249.1 $[M+H]^+$; E, Serotonin for commercial honey sample 1, *m/z* = 160.1 $[M+H-NH_3]^+$; F, Serotonin for Australian honey and Polish commercial honey, *m/z* = 160.1 $[M+H-NH_3]^+$; G, Serotonin for commercial honey sample 2 and Polish natural honey, *m/z* = 160.1 $[M+H-NH_3]^+$; H, NAS, *m/z* = 160.1 $[M+H-NH_2CH_3CO]^+$ and NAS-d7, *m/z* = 164.1 $[M+H-NH_2CH_3CO]^+$

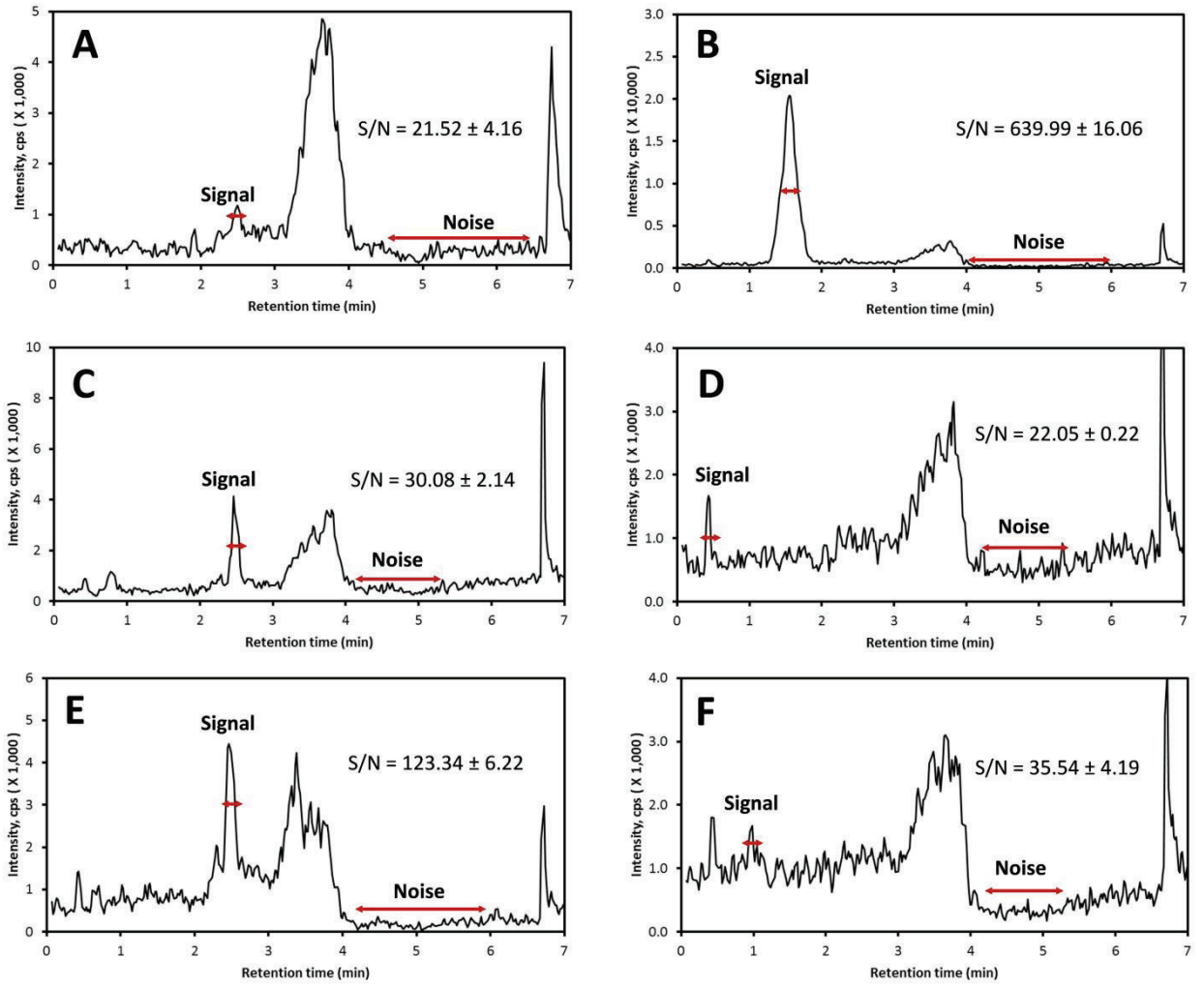

Figure S2. The limits of quantification (LOQ) were determined by calculating the ratio of signal/noise (S/N) using Waters MassLynx™ Software with EIC of A, 0.33 pg Melatonin, *m/z* = 174.1 $[M+H-NH_2CH_3CO]^+$; B,

20 pg 2(OH)MEL, *m/z* = 249.1 $[M+H]^+$; C, 2 pg Melatonin, *m/z* = 233.1 $[M+H]^+$; D, 0.4 pg Serotonin, *m/z* = 160.1 $[M+H-NH_3]^+$; E, 2 pg Melatonin, *m/z* = 255.1 $[M+Na]^+$; F, 0.4 pg NAS, *m/z* = 160.1 $[M+H-NH_2CH_3CO]^+$.

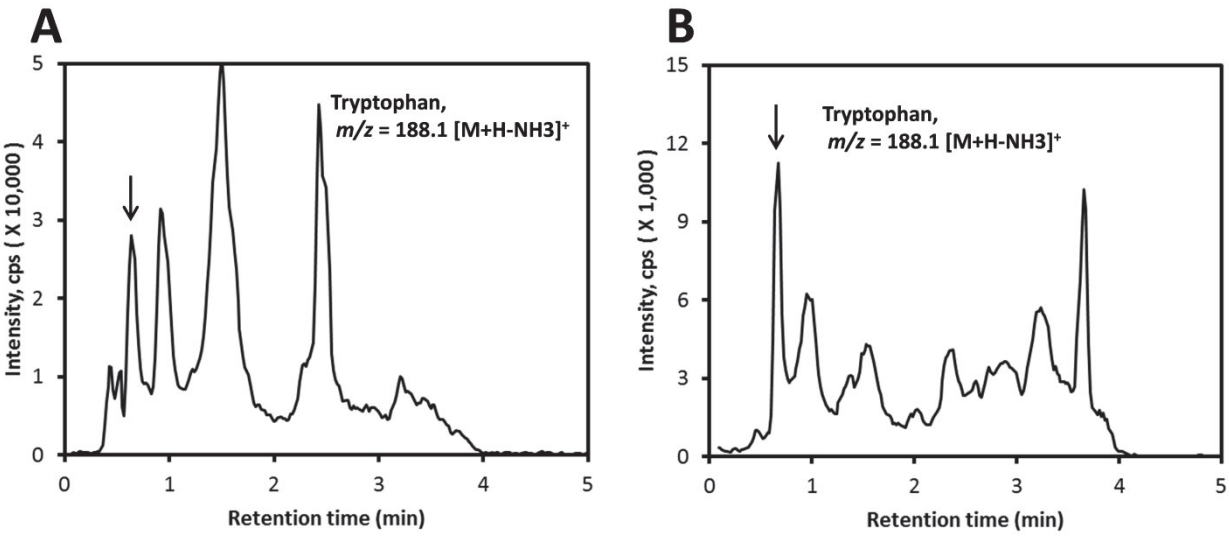

Figure S3. Detection of tryptophan in honey. A, Tryptophan in Australian honey; B, Tryptophan in commercial honey sample 1.

Table S1. Recoveries of standard melatonin by methylene chloride and ethyl acetate extraction procedurs. Melatonin was added to water:honey mixture and samples were extracted with methylene chloride or ethyl acetate as described in the Methods, then analyzed by LC-MS.

Samples	Recovery (%)		
	m/z = 233.1 $[M+H]^+$	*m/z* = 255.1 $[M+Na]^+$	*m/z* = 174.1 $[M+H-NH_2CH_3CO]^+$
Melatonin Standard	100	100	100
Methylene chloride extraction	77.17 ± 9.24	74.71 ± 0.68	87.10 ± 6.56
Ethyl acetate extraction	58.38 ± 4.21	62.34 ± 0.74	66.26 ± 4.07

Table S2. Recoveries of standard serotonin by methylene chloride and ethyl acetate extraction procedures. Serotonin was added to water:honey mixture and samples were extracted with methylene chloride or ethyl acetate as describes in the Methods, then analyzed by LC-MS.

Samples	Recovery (%) *m/z* = 160.1 $[M+H-NH_3]^+$
Serotonin Standard	100
Methylene chloride extraction	15.20 ± 0.31
Ethyl acetate extraction	133.69 ± 3.72

Table S3. Recoveries of standard NAS by methylene chloride and ethyl acetate extraction procedures. Serotonin was added to water:honey mixture and samples were extracted with methylene chloride or ethyl acetate as describes in the Methods, then analyzed by LC-MS.

Samples	Recovery (%) *m/z* = 160.1 $[M+H-NH_2CH_3CO]^+$
NAS Standard	100
Methylene Chloride extraction	17.95 ± 0.49
Ethyl Acetate extraction	82.05 ± 5.02