Synthesis of Novel Haptens and Development of an Enzyme-Linked Immunosorbent Assay for the Quantification of Histamine in Foods

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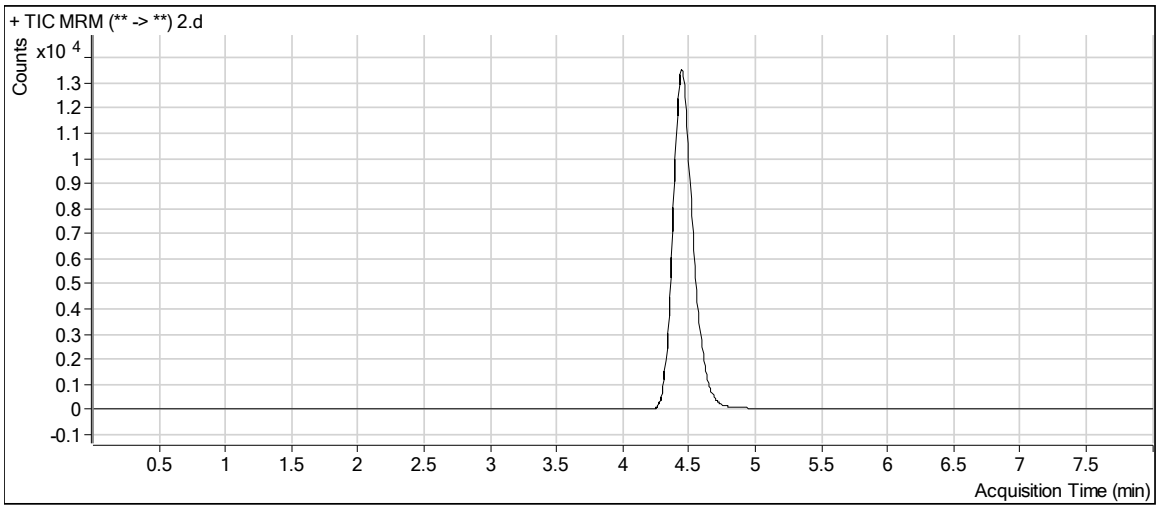
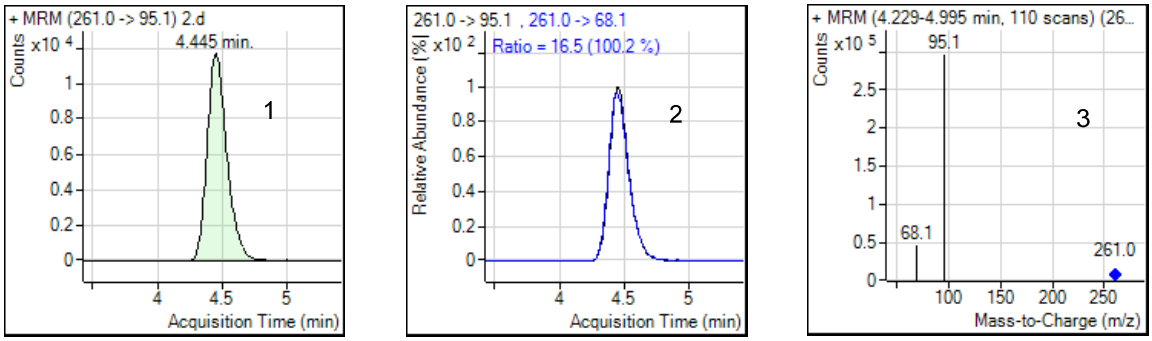


(A)



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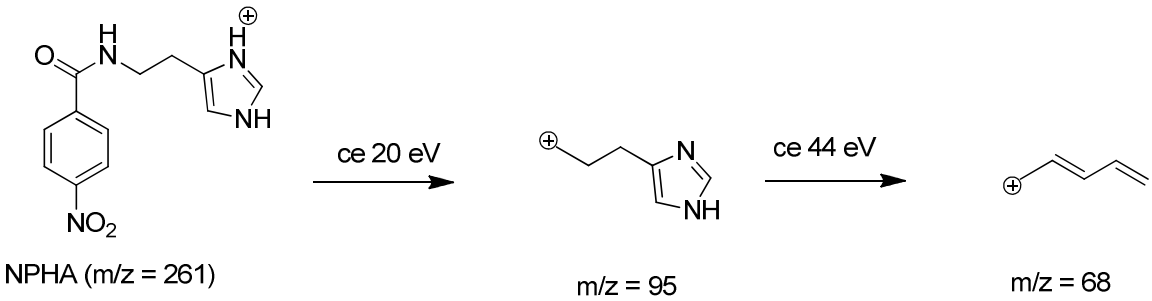

(C)

Figure S1. Total ion chromatography of the histamine standard (A, 10 ng/mL) and mass spectrometry of NPHA (B1, quantitation MRM; B2, confirmation MRM and B3, mass spectrum). The *m/z* 261.0 peak in B3 represents NPHA's parent ion; *m/z* 95.1 peak, the quantitative ion; and *m/z* 68.1, a qualitative ion. Fragmentation pathway of NPHA (C, ce = collision energy)

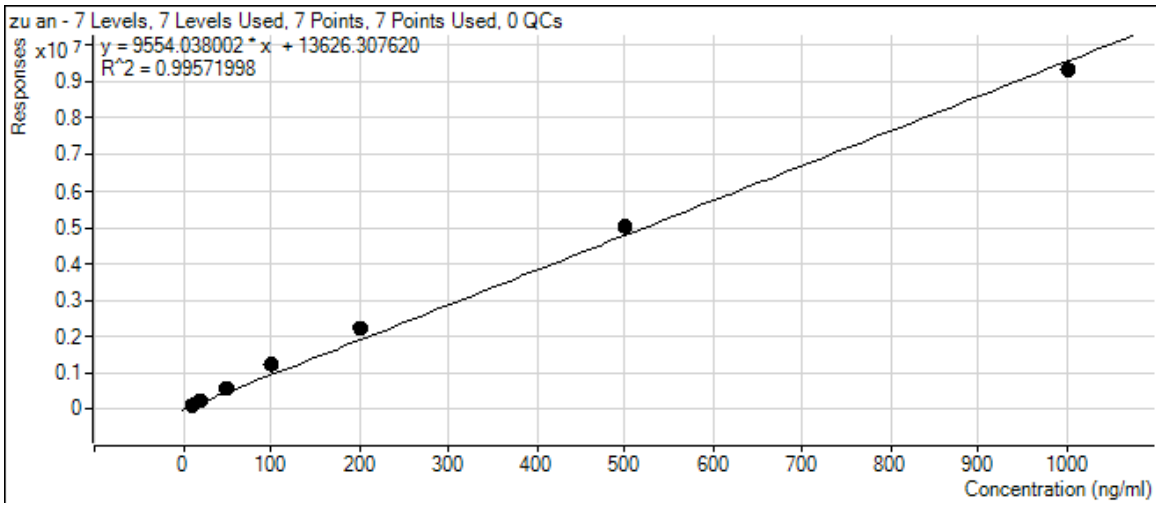

Figure S2. Calibration curve for histamine in LC–MS/MS.

Table S1. Effect of physicochemical parameters on ciELISA performance (n = 3)[a].

Parameters	Amax (mean ± SD)	IC_{50} (ng/mL)[b] (mean ± SD)	Amax/IC_{50} (mean ± SD)
Coating concentration (ng/mL)/ antibody dilution			
2000/1:32000	1.54 ± 0.013	26.7 ± 2.53	0.0576 ± 0.0014
1000/1:16000	1.63 ± 0.015	24.4 ± 2.25	0.0668 ± 0.0016
500/1:8000	1.37 ± 0.011	22.3 ± 2.31	0.0614 ± 0.0013
250/1:4000	1.24 ± 0.009	10.5 ± 1.74	0.118 ± 0.0021
125/1:2000	1.44 ± 0.010	7.8 ± 1.32	0.185 ± 0.0027
62.5/1k	1.57 ± 0.012	8.9 ± 1.57	0.176 ± 0.0031
PO_4^{3-} concentration (mmol/L)			
10	1.16 ± 0.008	6.84 ± 1.24	0.169 ± 0.0026
20	1.65 ± 0.014	8.02 ± 1.45	0.205 ± 0.0032
50	1.89 ± 0.015	9.82 ± 1.52	0.192 ± 0.0024
100	1.93 ± 0.017	10.09 ± 1.67	0.191 ± 0.0026
Tween-20 (%)			
0	1.65 ± 0.012	13.6 ± 2.03	0.121 ± 0.0021
0.02	1.52 ± 0.013	9.7 ± 1.56	0.157 ± 0.0023
0.05	1.41 ± 0.011	6.9 ± 1.12	0.204 ± 0.0018
0.10	1.34 ± 0.013	7.8 ± 1.26	0.172 ± 0.0026
0.20	1.26 ± 0.008	12.3 ± 1.57	0.102 ± 0.0015
pH value			
6.6	1.37 ± 0.007	8.4 ± 1.21	0.163 ± 0.0023
7.4	1.45 ± 0.009	6.4 ± 1.18	0.227 ± 0.0031
8.2	1.62 ± 0.011	9.5 ± 1.32	0.171 ± 0.0027
8.5	1.54 ± 0.012	13.5 ± 1.54	0.114 ± 0.0019

[a] SD,standard deviation.
[b] Using standard NPHA as competitor.