1 **Development of competitive indirect ELISAs with flexible working range for the simple**

2 **quantification of melatonin in medicinal foods**

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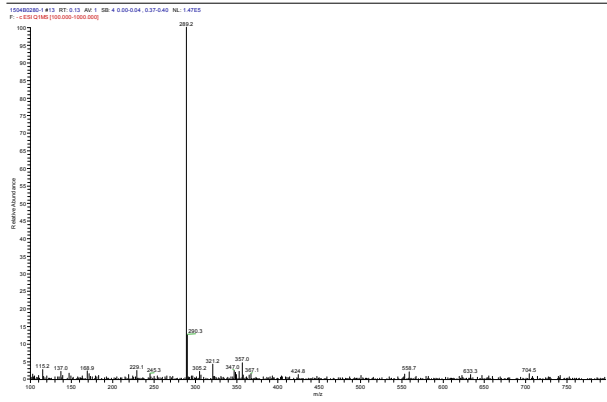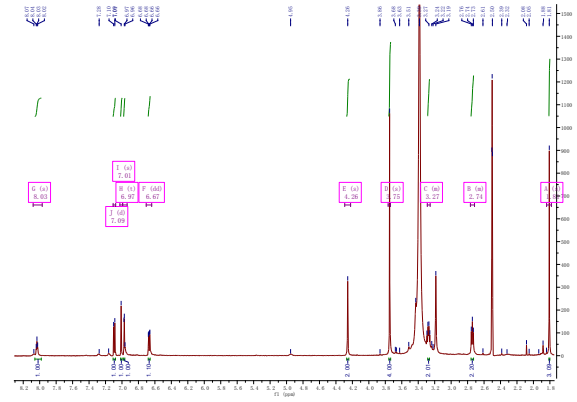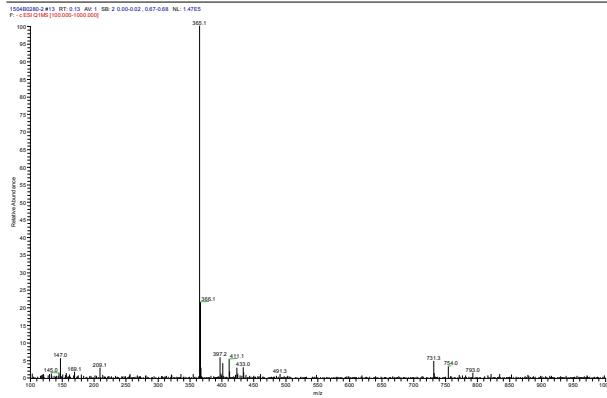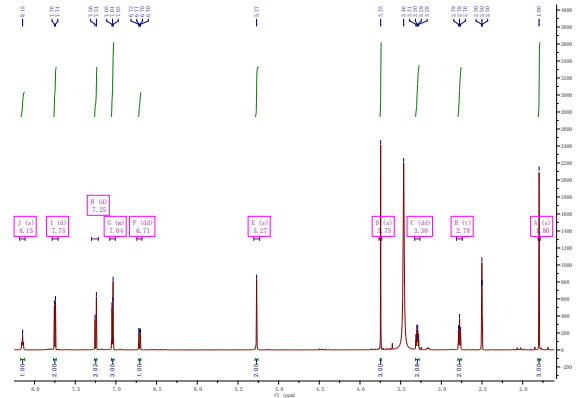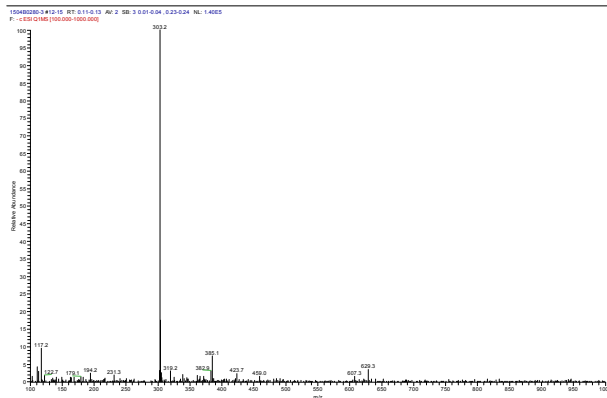
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Mass spectrogram of hapten A
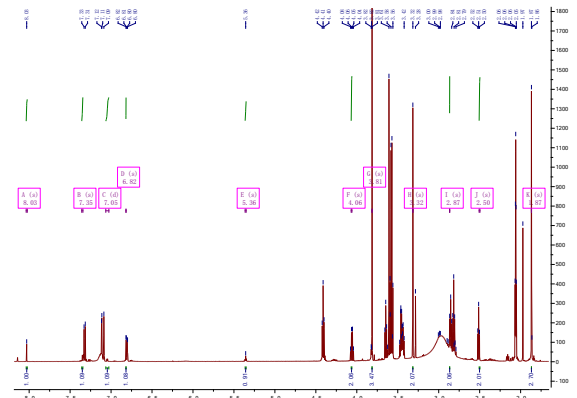
NMR spectrogram of hapten A

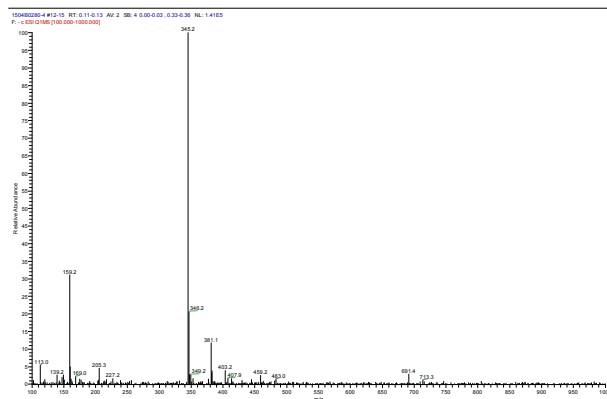Mass spectrogram of hapten B
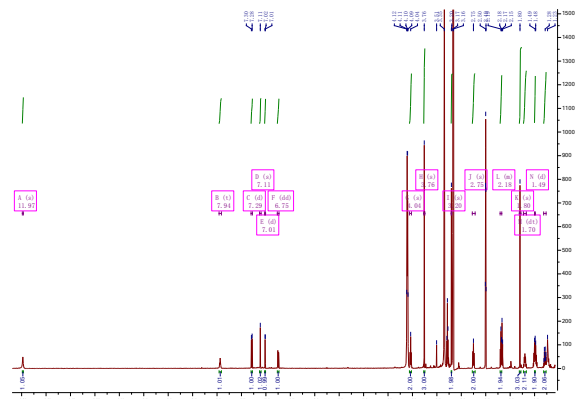
NMR spectrogram of hapten B

Mass spectrogram of hapten C

NMR spectrogram of hapten C

Mass spectrogram of hapten D

NMR spectrogram of hapten D

Figure S1. Mass and NMR spectrograms of MT haptens
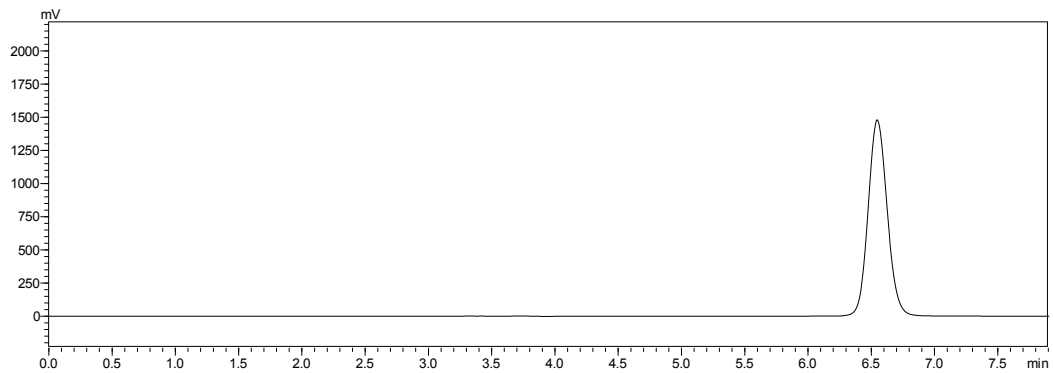
Figure S2. Chromatography of melatonin standard

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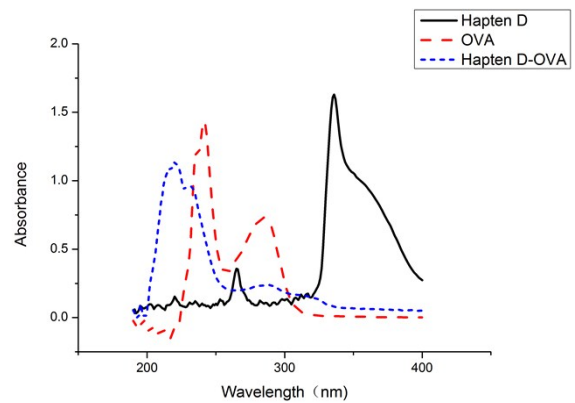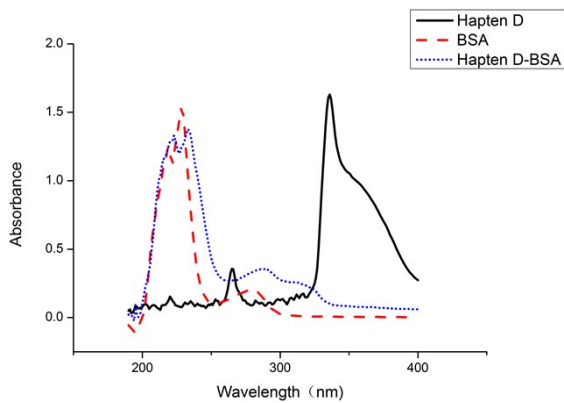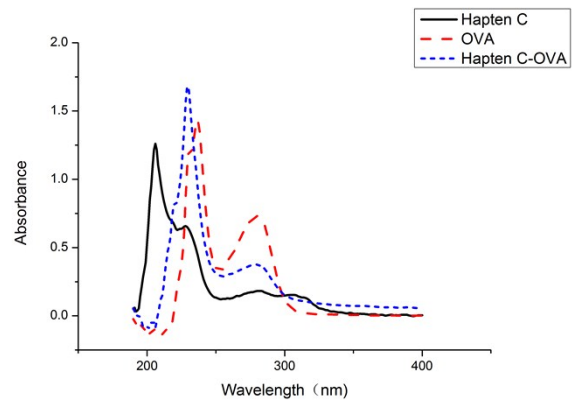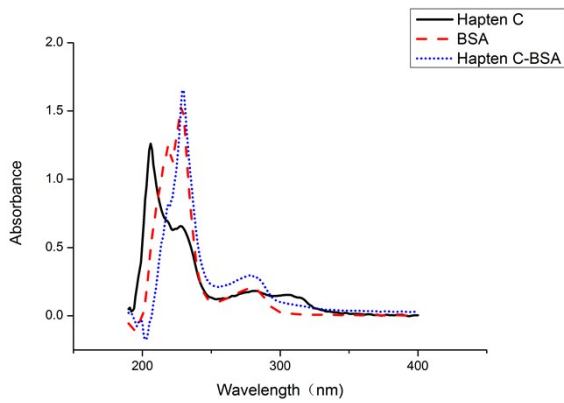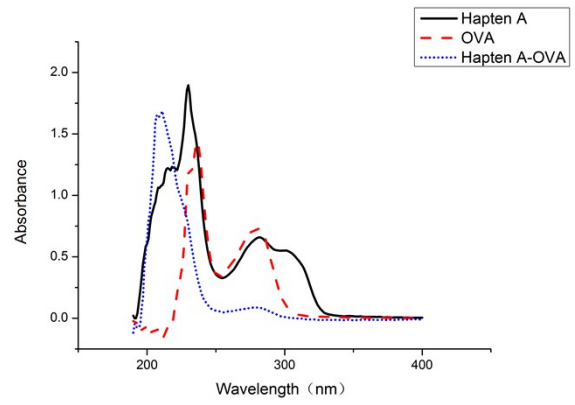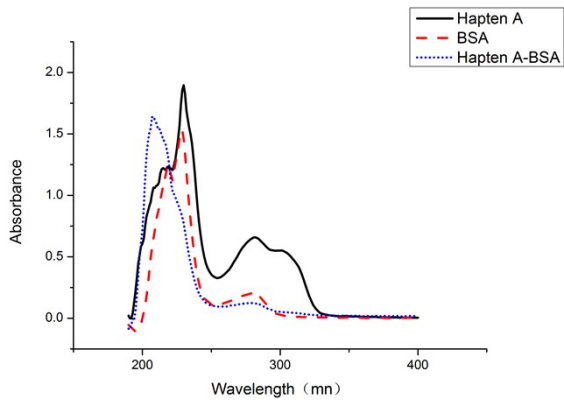
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75 Figure S3. Ultraviolet scanning of synthesized antigens
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$$y=25471x+20889$$
$$R^2=0.999$$
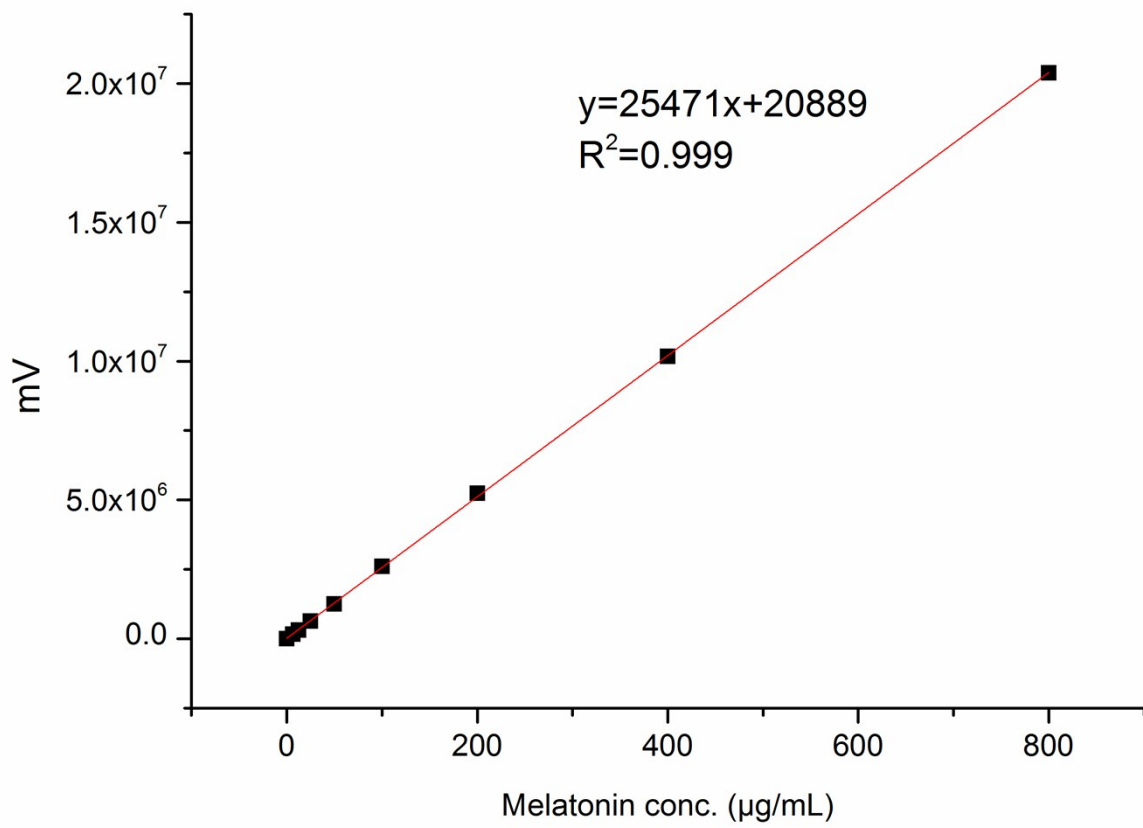
Figure S4. Calibration curve for histamine in LC–MS/MS.

107 Table S1. Effect of physicochemical parameters on heterologous ciELISA performance (n=3)

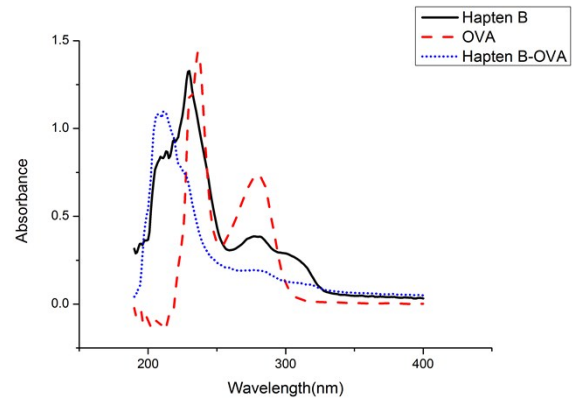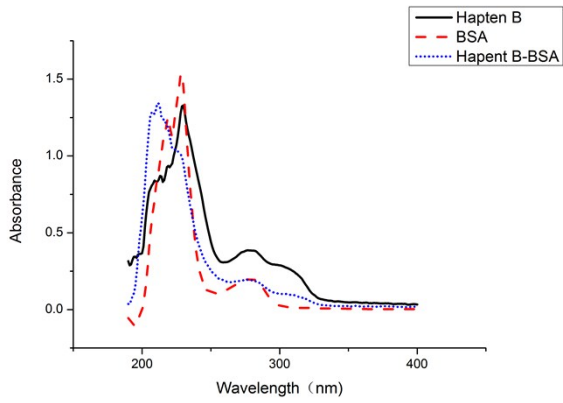| Parameters	A_{max}	IC_{50} (ng/mL)	A_{max}/IC_{50}
Coating concentration /antibody dilution			
2μg·mL^{-1}/1:16000	1.49	18.68	0.080
1μg·mL^{-1}/1:8000	1.15	25.61	0.045
0.5μg·mL^{-1}/1:4000	1.05	11.05	0.095
0.25μg·mL^{-1}/1:2000	1.42	8.07	0.176
0.125μg·mL^{-1}/1:1000	1.36	12.70	0.107
Ionic strength			
0.01M	1.42	4.62	0.308
0.02M	0.95	23.60	0.040
0.04M	0.78	5.47	0.143
0.08M	1.01	24.13	0.042
0.1M	1.32	6.24	0.211
Tween-20			
0	1.76	9.52	0.184
0.10%	1.43	26.04	0.055
0.20%	1.10	28.84	0.038
0.30%	0.78	54.61	0.014
0.40%	0.76	33.47	0.023
pH value			
5.4	1.45	7.13	0.204
6.4	1.38	10.68	0.129
7.4	1.03	19.27	0.053
8.4	1.16	15.46	0.075
9.4	1.22	9.39	0.130

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115 Table S2. Effect of physicochemical parameters on homologous ciELISA performance (n=3)

Parameters	A_{max}	IC_{50} (μg/mL)	A_{max}/ IC_{50}
Coating concentration /antibody dilution			
2μg·mL^{-1}/1:64000	2.17	24.19	0.019
1μg·mL^{-1}/1:32000	1.99	2.51	0.200
0.5μg·mL^{-1}/1:16000	1.97	1.64	0.311
0.25μg·mL^{-1}/1:16000	1.97	1.19	0.427
0.125μg·mL^{-1}/1:8000	1.89	2.93	0.180
Ionic strength			
0.01M	1.97	4.76	0.414
0.02M	1.72	3.55	0.484
0.04M	1.64	1.43	1.150
0.08M	1.69	0.35	4.832
0.1M	1.58	2.33	0.677
Tween-20			
0	1.91	6.44	0.297
0.10%	1.93	6.24	0.310
0.20%	1.93	8.41	0.230
0.30%	1.91	130.63	0.015
0.40%	2.16	10.68	0.202
pH value			
5.4	2.14	4.88	0.438
6.4	1.17	5.66	0.207
7.4	1.34	2.17	0.619
8.4	1.05	1.76	0.598
9.4	1.50	2.79	0.539

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Table S3. The RSD of inter-day of calibration curve (n=3)

Days	A_{max}	IC_{50} (μg/mL)
1	1.78	6.52
2	1.67	7.26
3	1.79	6.81
4	1.65	7.01
5	1.74	7.79
Mean±SD	1.73±0.06	7.08±0.48
RSD	3.60%	6.77%

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