

Supplementary Material

Fast chromatographic determination of free amino acids in bee pollen

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Table S1. Summary of trueness studies for some of the studied amino acids.

Amino acid	GC-MS			HPLC-MS		
	Mean (%) \pm RSD (%)			Mean (%) \pm RSD (%)		
	Low	Medium	High	Low	Medium	High
ALA	99 \pm 8	103 \pm 13	100 \pm 7	95 \pm 10	98 \pm 7	102 \pm 8
GLU	92 \pm 12	95 \pm 8	87 \pm 10	90 \pm 8	97 \pm 14	95 \pm 11
LEU	102 \pm 8	97 \pm 4	99 \pm 7	98 \pm 6	104 \pm 4	99 \pm 8
PRO	91 \pm 7	94 \pm 9	96 \pm 6	94 \pm 12	92 \pm 3	95 \pm 7
TRP	88 \pm 11	86 \pm 8	87 \pm 5	92 \pm 9	90 \pm 3	93 \pm 9
VAL	85 \pm 5	88 \pm 6	91 \pm 2	90 \pm 6	88 \pm 10	85 \pm 8

Low- LOQ (see Tables 1 and 2); Medium QC-50 nmol/mL; High QC-200 nmol/mL.

Table S2.. Weights of the five principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5
ALA	0.236057	-0.036155	0.177358	0.311309	0.339895
GLY	0.314397	-0.031592	0.056109	0.055225	0.109943
VAL	0.303928	-0.100335	-0.177942	0.012101	0.143828
LEU	0.262050	-0.263823	0.072527	0.183980	0.030612
ILE	0.275516	-0.206456	-0.137586	0.141888	-0.122005
THR	0.309164	-0.167850	-0.006714	-0.040024	-0.074947
GABA	0.234345	0.165024	0.029122	-0.362413	0.311457
SER	0.214260	-0.101585	0.284065	-0.252636	-0.336496
PRO	-0.251365	-0.064490	0.285289	-0.064884	0.361294
ASN	0.169649	0.311969	-0.063674	-0.374812	0.189562
ASP	0.184648	0.388299	-0.047240	0.169538	-0.101457
MET	0.134758	-0.079638	0.511498	-0.185445	-0.239149
HYP	-0.019473	0.314684	0.288880	0.132603	-0.100750
GLU	0.261909	-0.186448	-0.199916	0.174555	-0.168466
PHE	0.099522	0.383165	0.228584	0.298679	-0.107669
GLN	0.254724	0.078593	-0.089529	-0.401106	0.242718
LYS	0.322003	0.106982	-0.038615	0.068153	-0.031016
HIS	0.129948	0.428577	0.080410	0.175902	-0.005519
TYR	0.059226	-0.220286	0.500309	-0.098823	-0.013847
TRP	0.002956	-0.143675	0.191574	0.312750	0.525214

Table S3. Eigenvalues of the correlation matrix.

Principal component	Eigenvalue	Difference	Proportion	Accumulated variability (%)
1	8.99183530	4.9626684	0.4496	44.96
2	4.0291669	1.7987107	0.2015	65.11
3	2.2304562	0.4620516	0.1115	76.26
4	1.7684046	0.4078791	0.0884	85.10
5	1.3605255		0.0680	91.90

Table S4. Number of observations and percentage classified in each group using a quadratic discriminant analysis.

Origin	AP	COM	Total
AP	4	0	7
%	100.00	0.00	100.00
COM	0	8	8
%	0.00	100.00	100.00
Total	4	8	12
	33.33	66.67	100.00

AP, samples from experimental apiaries; COM, commercial samples.

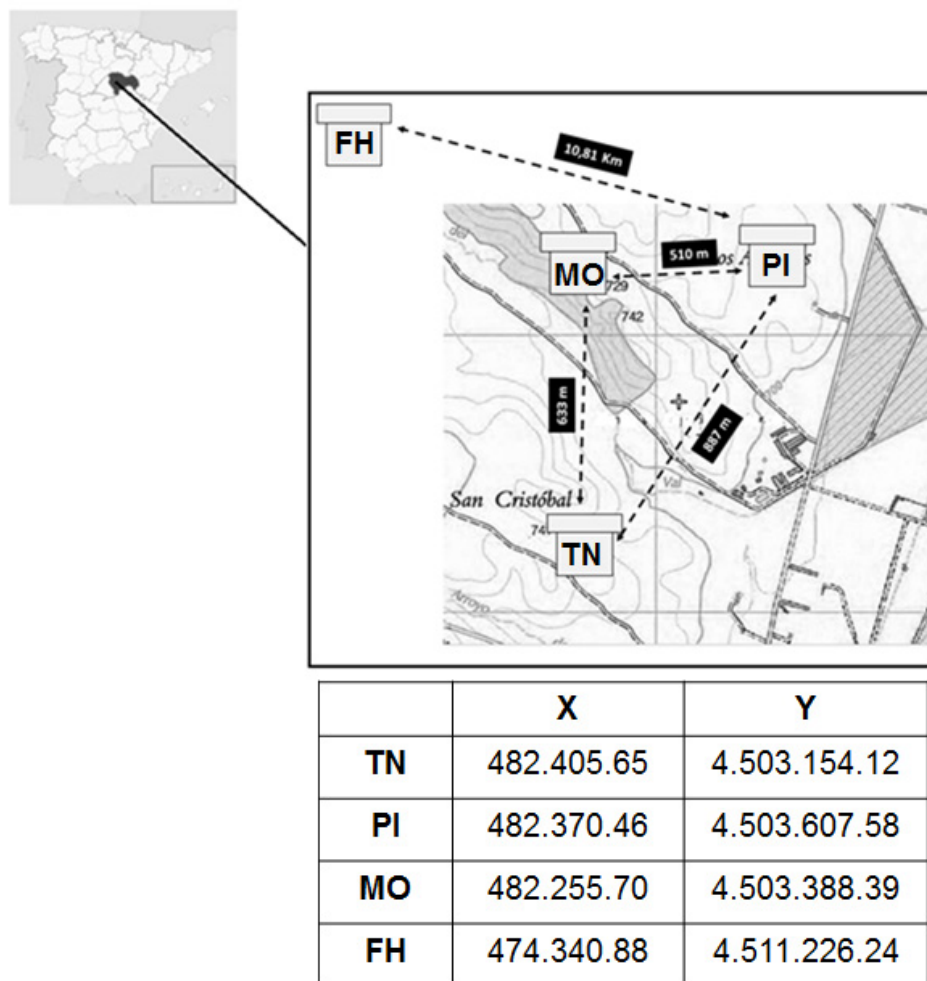


Figure S1.- Location and Global Positioning System (GPS) data of the apiaries (Fuentelahiguera, FH; Pistacho, PI; Monte, MO; Tio Natalio, TN). Adapted from Foods, 11, Ares, A. M., Tapia, J. A., González-Porto, A. V., Higes, M., Martín-Hernández, R, Bernal, J., Glucosinolates as markers of the origin and harvesting period for discrimination of bee pollen by UPLC-MS/MS, 1446, 2022, with permission from MDPI.

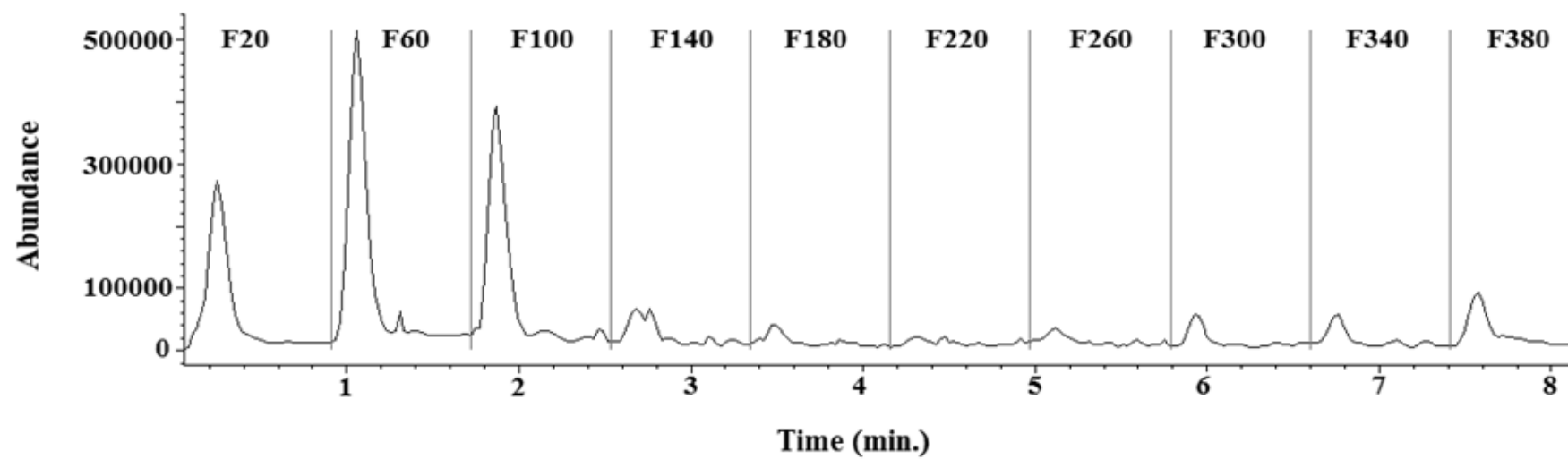


Figure S2. Results of the flow injection analysis of a GLN standard solution for optimizing the value of the fragmentor voltage (20-380V; F20-F380).

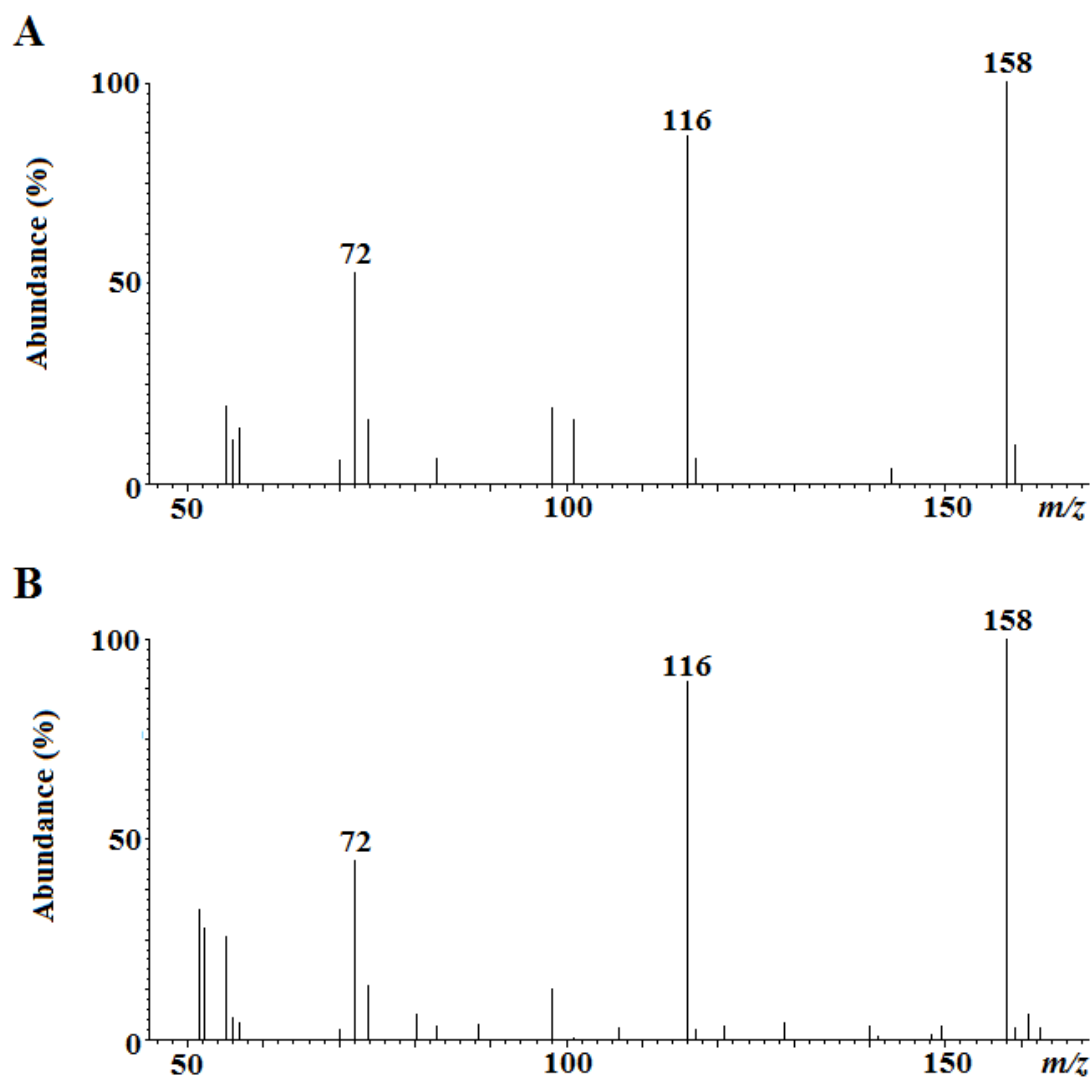


Figure S3. MS spectra of VAL in (A) solvent and (B) matrix-matched standards. The GC-MS conditions are summarized in subsection 2.4.1.

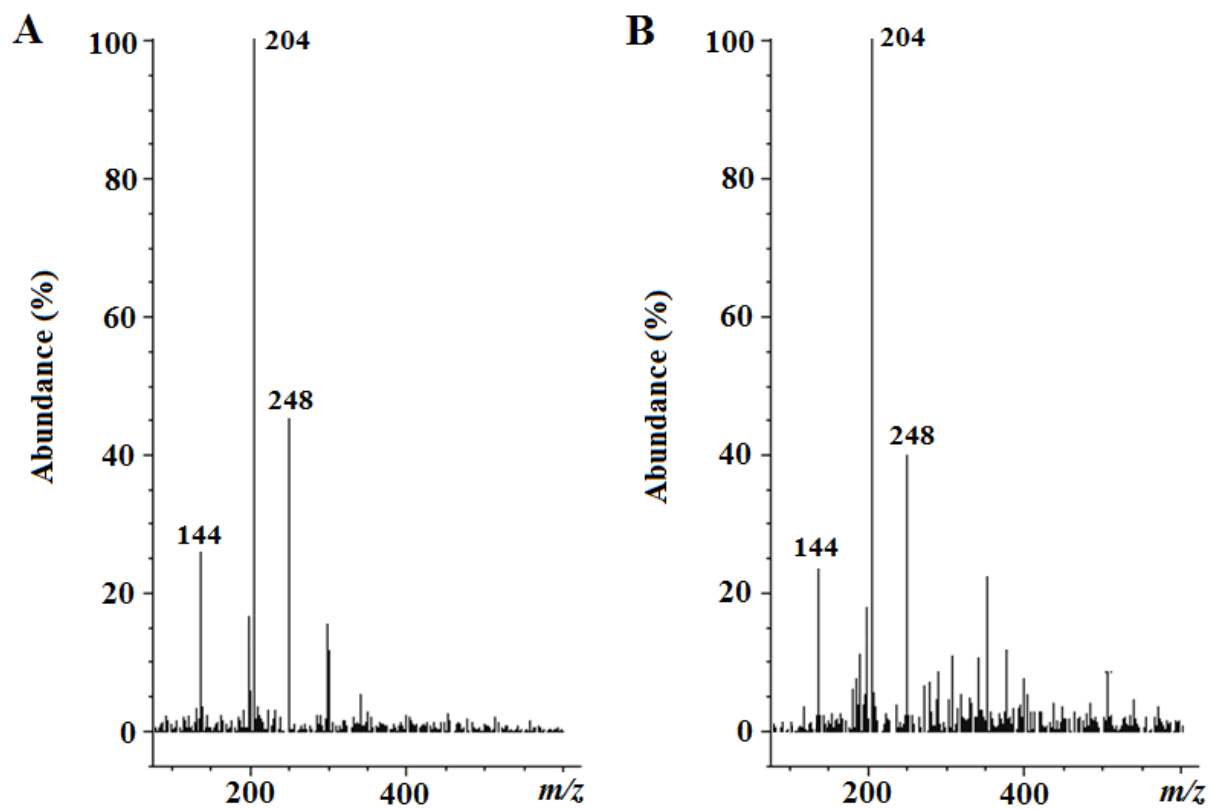


Figure S4. MS spectra of GLY in (A) solvent and (B) matrix-matched standards. The HPLC-MS conditions are summarized in subsection 2.4.2.