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Royal jelly's phenolic profile via UPLC-VIP-HESI-TIMS-QTOF-MS: A thorough characterization following a multivariate optimization approach

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Introduction

A growing scientific interest in the analysis of royal jelly has been developed...

... due to its nutritional and financial significance.

Evidence relates its positive health impact to the high content of specific bioactive components, especially phenolic compounds[1].

Incorporation of VIP-HESI & TIMS in LC-HRMS workflows:

VIP-HESI

Due to the active exhaust
- Higher sensitivity & robustness
- Reduced source contamination & memory effects in source

Due to a moving vacuum-insulated probe
- Reduced thermal degradation of sensitive molecules [2]

TIMS

- Addition of a new dimension of separation
  4D- METABOLOMICS
- Additional identification confidence in wide-scope target screening [3]

Methods

Sample preparation

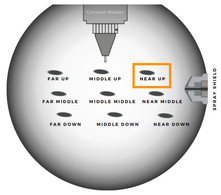
22 royal jelly samples from different regions across Greece → 0.1 g RJ sample → 4 mL MeOH:H_2O (80:20, v/v) → Ultrasonic extraction 15 min, rt → Centrifugation 10,000 rpm, 4°C, 15 min → Filter through RC filters, pore size 0.2 μm, diameter 15 mm → 1 mL supernatant → **LC-VIP HESI-HRMS**

- Instrumentation: UPLC-VIP-HESI-TIMS-QTOF-MS
- m/z range: 50-1100
- 1/k0 range: 0.40-1.40 Vs cm^{-2}
- Ionization mode: Negative
- Acquisition modes: bbCID & PASEF

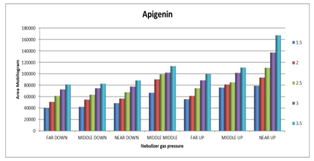
VIP-HESI Source Optimization

Step 1: Sprayer's position and Nebulizer gas pressure

➢ **9 Sprayer positions inside VIP-HESI**



Spiked royal jelly sample with 15 phenolic compounds
Equivalent results for all!



➢ **5 Nebulizer gas pressure values**
1.5, 2.0, 2.5, 3.0, 3.5 bars

The pressure of the nebulizer gas ↑ the intensities of all the analytes.

Near positions give higher peak areas than respective Far positions.

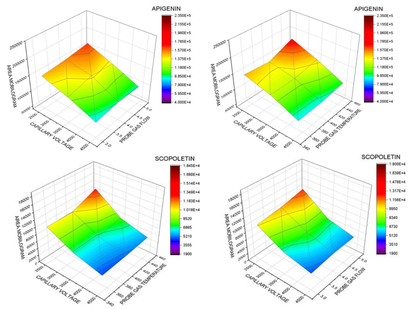
Up positions give higher peak areas than respective Down positions.

Step 2: Multivariate optimization of five VIP-HESI parameters

A: Capillary voltage*
B: Dry gas flow
C: Dry gas temperature
D: Probe gas temperature*
E: Probe gas flow*

*Statistically Significant Parameters

Optimized Parameters:
Capillary voltage: 2500V
Dry gas flow: 10 L min^{-1}
Dry gas temperature: 230 °C
Probe gas flow: 5 L min^{-1}
Probe gas temperature: 450°C



Method Validation

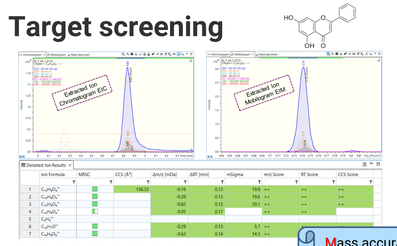
- ✓ Linearity
- ✓ Sensitivity
- ✓ Matrix Effect
- ✓ Accuracy
- ✓ Precision

Fit for purpose for 55 phenolic compounds

	R^2	LOD (μg g^{-1})	LOQ (μg g^{-1})	ME%	Recovery% (0.25 μg g^{-1})	Recovery% (1.0 μg g^{-1})	Recovery% (5.0 μg g^{-1})
Minimum value	0.98	0.00051	0.0016	-121	80	86	89
Maximum value	0.9992	0.10	0.31	88	101	109	106

	RSD% Repeatability (0.25 μg g^{-1})	RSD% Repeatability (1.0 μg g^{-1})	RSD% Repeatability (5.0 μg g^{-1})	RSD% Intermediate Precision (0.25 μg g^{-1})	RSD% Intermediate Precision (1.0 μg g^{-1})	RSD% Intermediate Precision (5.0 μg g^{-1})
Minimum value	1.0	0.64	1.1	1.3	0.87	1.2
Maximum value	5.9	6.7	5.7	10	12	19

Target screening



Database of 62 compounds
- phenolic acids
- lignans
- stilbenes
- phenolic lipids
- flavonoids

ASQ
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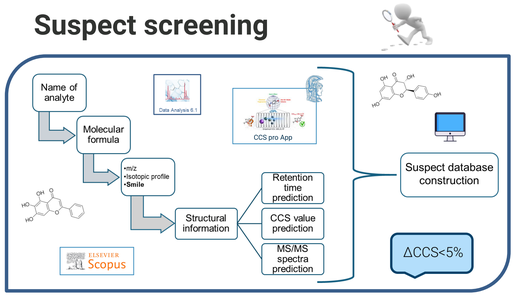
A total of **35 compounds** were identified and quantified in royal jelly samples.

Mass accuracy (Δm/z<2mDa)
Retention time (ΔRT±0.2 min)
Sigma values (mSigma <50)
Qualifier ions
CCS value (ΔCCS<2%)

Analyte	C MIN (μg g^{-1})	C MAX (μg g^{-1})	MEAN (μg g^{-1})	MEDIAN (μg g^{-1})	No of samples detected
Apigenin	0.10	1.2	0.34	0.26	22/22
Chrysin	0.11	5.7	1.1	0.62	22/22
Ferulic acid	<LOD	8.6	1.3	0.52	15/22
Galangin	0.21	2.3	0.66	0.61	22/22
Luteolin	0.44	4.7	1.3	1.6	22/22
Naringenin	<LOD	0.79	0.15	0.91	21/22
Pinocembrin	0.15	7.4	1.2	0.38	22/22
Quercetin	0.67	10	2.8	0.68	22/22
Sakuranetin	0.18	14	2.0	0.50	22/22

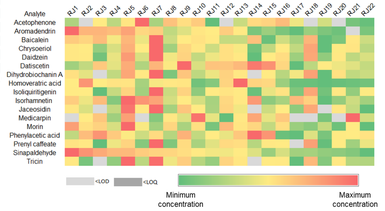
- 18/35 analytes such as Apigenin, Chrysin, Galangin were present in all samples.
- Quercetin had the highest mean concentration. (2.8 μg g^{-1})

Suspect screening



Database of 130 phenolic compounds

Identification of 85 phenolic compounds



Conclusions

- Seven different parameters were studied and optimized for the first time to facilitate the determination of various phenolic compounds in bee product matrices.
- The optimum parameters of VIP-HESI enhanced the sensitivity of the source, while the addition of TIMS increased the identification confidence.
- Target and suspect screening resulted in the finger-printing of phenolic compounds of Greek royal jelly.

Future perspectives

This methodology can be extended to assess factors essential to **guarantee royal jelly's quality, establish authenticity, and detect fraud cases in samples** safe guarding its health promoting properties and allowing beekeepers to expand their businesses obtaining highly competitive prices for their natural product.

Scan me!

timsTOF / food authenticity

References

[1]Giampieri, F.; Quiles, J. L.; Cianciosi, D.; Forbes-Hernández, T. Y.; Orantes-Bermejo, F. J.; Alvarez-Suarez, J. M.; Battino, M. Bee Products: An Emblematic Example of Underutilized Sources of Bioactive Compounds. *J. Agric. Food Chem.* **2022**, *70* (23), 6833–6848.
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[3]Ridgeway, M. E.; Lubeck, M.; Jordens, J.; Mann, M.; Park, M. A. Trapped Ion Mobility Spectrometry: A Short Review. *International Journal of Mass Spectrometry* **2018**, *425*, 22–35.