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DETERMINATION OF QUINOLONES, MACROLIDES, SULFONAMIDES AND TETRACYCLINES IN HONEY QUECHERS SAMPLE PREPARATION AND UHPLC-MS/MS ANALYSIS

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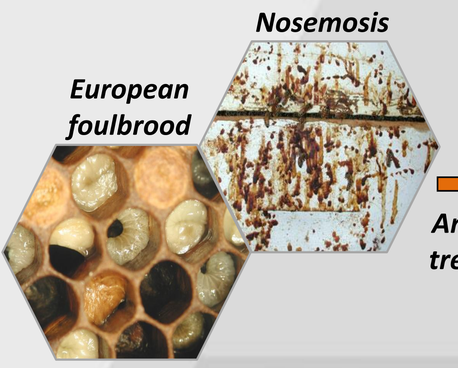
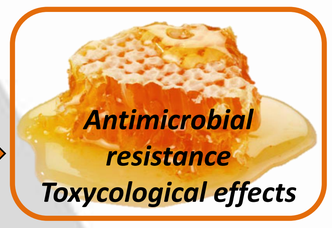
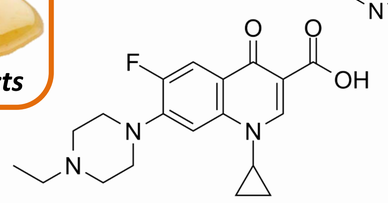
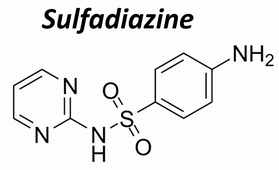
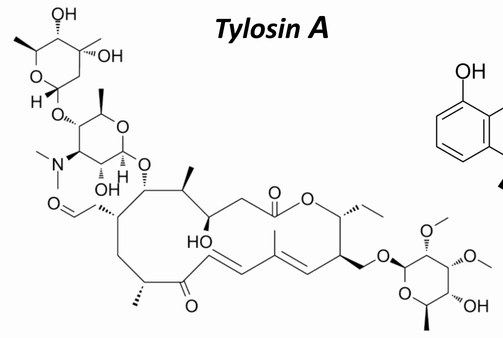
Monitoring the health of the beehive and timely prevention of any possible infection with various bacteria, mould, viruses or parasites is exceptionally important in beekeeping. Antibiotics may be found in honey originating from the environment and resulting from improper beekeeping practices. Enabling the detection of antibiotic residues in honey and suppressing the development of antibiotic resistance, require the development of a sensitive multi-class method for the determination of antibiotics. For the purpose of analysing honey, a screening and confirmatory method for the determination of 36 antibiotics was developed. The QUECHERS procedure and ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was selected to achieve high sensitivity and selectivity. Validation according to the new Commission Implementing Regulation (EU) 2021/808 included following performance characteristics: selectivity, trueness, precision, decision limit (CCα), detection capability (CCβ) and relative matrix effect. The method was validated in the measurement range from 1 to 20 µg/kg, where maximum trueness and acceptable coefficients of variation were achieved. For unauthorized pharmacologically active substances, the CCα was determined, ranging from 0.16 µg/kg for sulfaquinoxaline to 3.67 µg/kg for difloxacin. The high matrix influence of floral and chestnut honey indicated the need for quantitative analysis by using the matrix calibration curve.

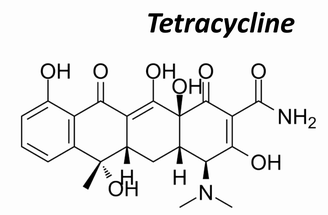












QUECHERS Sample preparation

2g honey ← Addition of internal standard mixture/ matrix spike for QC

10 ml of 1% acetic acid in ACN/H2O (v/v, 80:20)

Vortex 10 min

Addition of 4 g of Na_2SO_4 and 1 g of NaCl

Vortex, centrifuge at 4500rpm

6 ml of ACN aliquot added to Bond Elut dSPE salts

Vortex, centrifuge at 4500 rpm

4 ml of supernatant and 50 µl of DMSO

Evaporation using N_2 at 40 °C

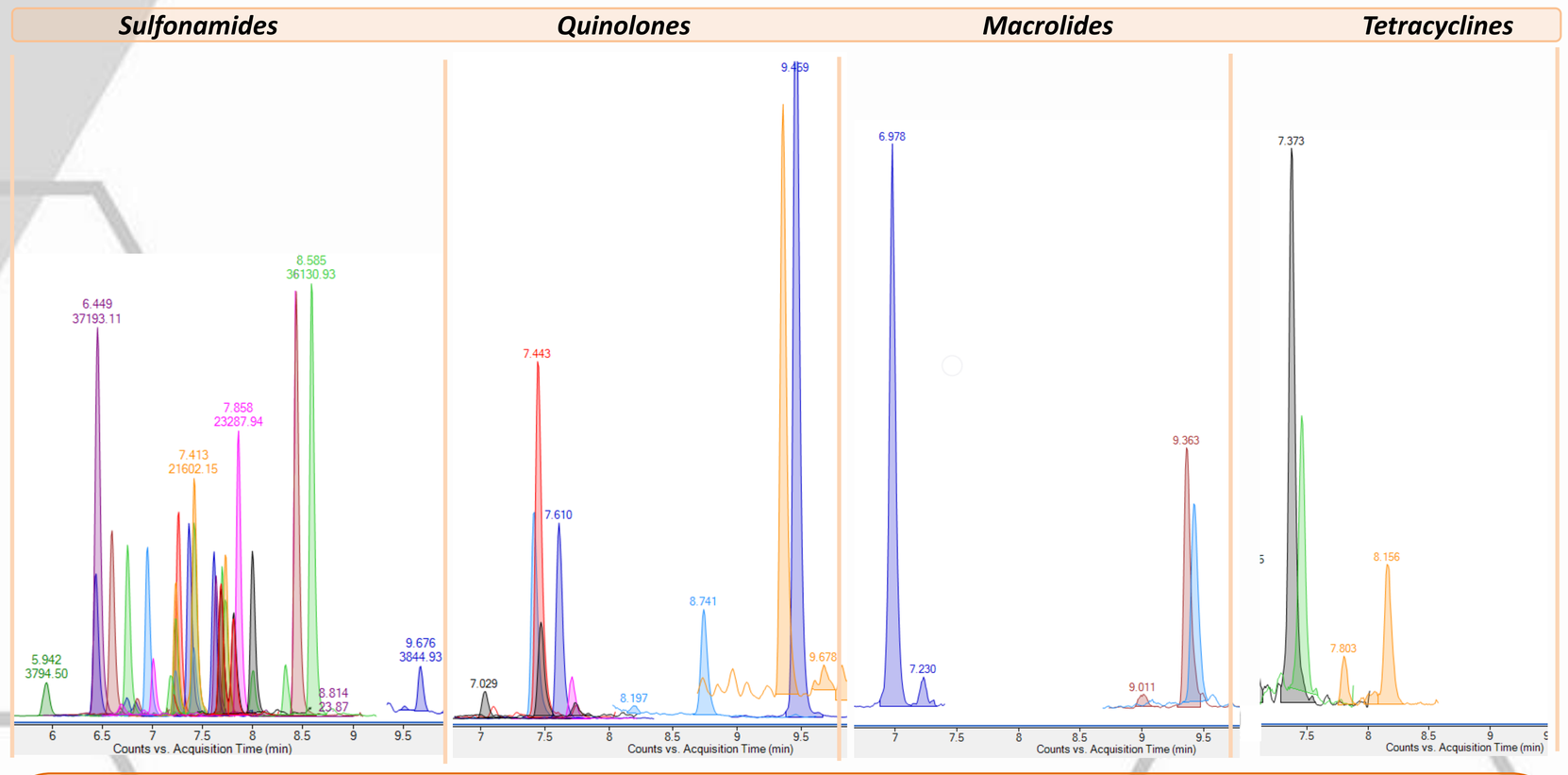
Dilution of dry residue in 0.5 ml of 2:8 ACN/H2O

Centrifuge at 10000 rpm, 4°C

Transfer extracts to the HPLC vials

UHPLC-MS/MS analysis

UHPLC-MS/MS chromatography



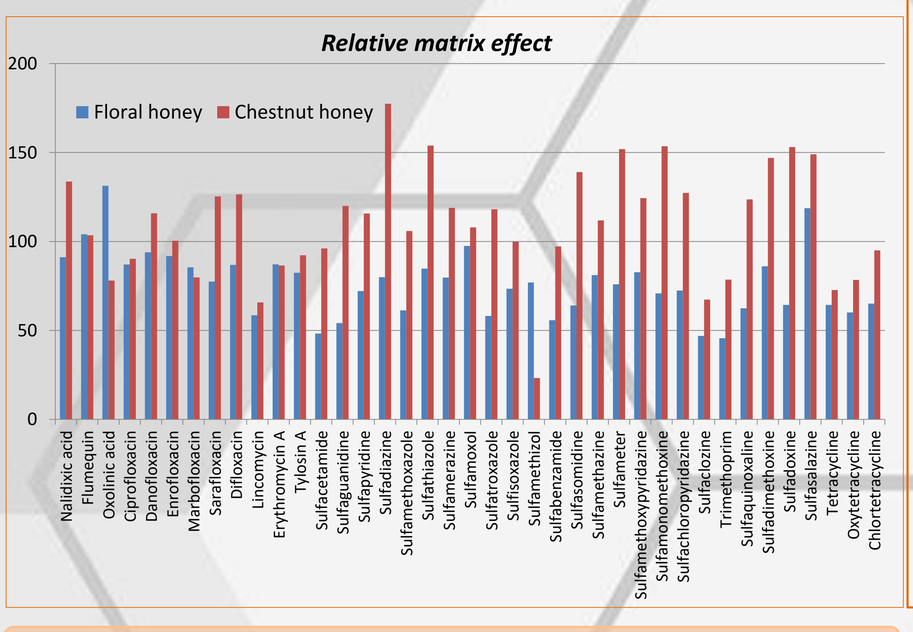
UHPLC-MS/MS PARAMETERS

- UHPLC Agilent Technology 1290 Infinity II, Triple Quad LC/MS 6470
- Chromatography: Flow 0,4 ml/min, thermostated at 40°C, Acquity UPLC HSS T3 column (1.8 µm, 2.1x150 mm)
- Autosampler: Injection volume 10 µl, thermostated at 10°C
- MS/MS parameters- positive ionisation mode: Gas temperature 150°C, Gas flow 11 l/min, Nebulizer 35 psi, Sheath gas temperature 300°C, Sheath gas flow 6 l/min, Capillary voltage 4000V, Nozzle voltage 500V

Time (min)	MFA (0.1% Formic acid)	MFB (MeOH)
0	100	0
3	100	0
10	5	95
13	5	95
13.5	100	0
17	100	0

Validation CIR (EU) 2021/808

According to Commission Implementing Regulation (EU) 2021/808 all validation parameters complied with the set requirements. Validation parameters were calculated using validation software for comprehensive in-house validation InterVal Plus® and it consists of the following experiments: linearity, specificity/selectivity, repeatability and inter-laboratory reproducibility (Rel sR), decision limit (CCα) and detection capability (CCβ). Relative matrix effect was tested using matrix extracts fortified after extraction and compared with a standard solution. Significant matrix effects were noticed during the analysis of different species of honey samples fortified at CCα concentration.



Analyte	Calibration interval µg/kg	CCα µg/kg	CCß µg/kg	Recovery [%] at CCα	Rel sR [%] at CCα	Analyte	Calibration interval µg/kg	CCα µg/kg	CCß µg/kg	Recovery [%] at CCα	Rel sR [%] at CCα
Fluoroquinolones						**Sulfonamides**					
CIPRO	1.0 - 20	1.34	1.86	96.6	9.9	SBZ	0.1 - 20	0.22	0.3	100.4	12.4
DAN	1.0 - 20	1.12	1.57	98.7	7.7	SAC	0.1 - 20	0.18	0.25	103.4	10.9
DIFLOX	1.0 - 20	3.67	5.6	101.2	16.4	SCP	0.1 - 20	0.23	0.32	100.8	12.6
ENR	1.0 - 20	2.53	3.73	104.1	12.3	SDZ	0.1 - 20	0.13	0.16	100.6	5.8
FLU	1.0 - 20	2.08	3.06	102.5	11.4	SDM	0.1 - 20	0.13	0.16	99.6	5.7
MARB	1.0 - 20	2.16	3.12	99.7	12.3	SDX	0.1 - 20	0.17	0.22	99.3	9.4
NALA	0.1 - 20	0.29	0.43	100.1	15.1	SGN	0.1 - 20	0.7	1.2	99.9	13.1
OXOA	0.75 - 20	1.15	1.6	102.7	7.8	SMR	0.1 - 20	0.27	0.39	100.2	14.3
SAR	0.25 - 20	0.61	1.11	100.2	18.4	SMZ	0.1 - 20	0.23	0.32	100.9	12.5
Macrolides and lincosamides						SMZOL	0.1 - 20	0.18	0.24	99.5	10.6
TYL	0.1 - 20	0.54	0.76	101	13	SMP	0.1 - 20	0.2	0.28	100.4	11.6
ERY	0.1 - 20	0.31	0.46	99.8	15.3	SMX	0.25 - 20	0.45	0.58	100.6	9.6
LINC	1.0 - 20	1.83	2.53	100.5	18.5	SMM	0.1 - 20	0.25	0.36	100.2	13.5
Tetracyclines						SMOX	0.1 - 20	0.48	0.76	100	17.8
TTC	0.25 - 20	0.51	0.7	101.9	11.9	SPYR	0.1 - 20	0.23	0.33	100.4	12.9
CLTTC	0.5 - 20	1.04	1.58	101.2	13.6	SQX	0.1 - 20	0.16	0.21	100.7	8.6
OXYTTC	0.5 - 20	0.83	1.18	98.6	10.3	SSL	1.0 - 20	3.26	5.07	94.2	18.7
						STZ	0.1 - 20	0.17	0.22	100.4	9.1
						STROX	0.1 - 20	0.23	0.31	99.6	12.8
						SSOM	0.25 - 20	0.3	0.34	98.2	4.1
						TMP	0.1 - 20	0.38	0.58	100.1	16.5

Conclusion

The proposed screening and confirmation method based on the UHPLC-MS/MS technique was successfully developed and validated for screening and confirmation of quinolones, macrolides, lincosamides, sulphonamides, and tetracyclines. The QUECHERS extraction procedure allows for fast and specific extraction of the selected analytes, ensuring high sensitivity of the method and ability to determine residues in the range from 0.1 to 20 µg/kg. In the routine use of the methodology, the results of the analyses shall be considered non-compliant when equal to or above the decision limit for confirmation (CCα).

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