How to deal with sweet matrix? – a method for LC-MS/MS analysis of antibiotics in honey, regardless of its kind

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Honey is a valuable product, often used in medicine and as a dietary ingredient. All of its sweetness disappears when it reaches the laboratory as a matrix for LC-MS/MS analysis, due to the great diversity of components content (over 180) between each, which undobtly affects its properties.

Data were acquired on QTRAP 5500+ (SCIEX) mass spectrometer coupled with ExionAC LC (SCIEX) and processed with SciexOS 2.2 software. The LC-MS/MS analysis was performed in positive ionization sMRM mode during reversed phase 12-min separation on Fortis H2O C18 chromatographic column. Mobile phases consisted of water, acetonitrile and formic acid additive.

Present results concern the determination of 57 antibiotics residues belonging to different groups, such as Sulfonamides (16) Fluorochinones (8) Macrolides (6) Tetracyclines (6) Cefalosporines (5) Chinolines (2) Aminocyclitols (2) Diterpens and Diaminpyrimidines in a single unified sample preparation procedure followed by LC-MS/MS analysis.

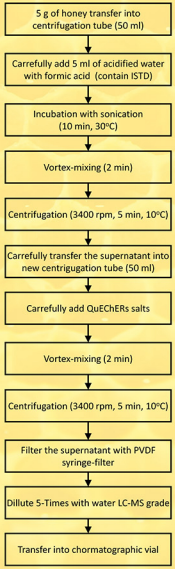

Fig 1. Sample preparation procedure

Table 1. The results of method validation

Analyte	Group	Linearity range [ug/kg]	R	CV [%]	Analyte	Group	Linearity range [ug/kg]	R	CV [%]
Dicloxacillin	Beta lactams	0.5-500	0.99932	5.11	Sulfaguanidine	Sulfonamides	0.5-500	0.99516	12.91
Oxacillin		0.5-500	0.99714	5.09	Sulfacetamide		0.5-500	0.99690	4.03
Cloxacillin		0.5-500	0.99749	5.64	Sulfdiazine		0.5-500	0.99658	6.91
Nafcillin		0.5-200	0.99719	19.07	Sulfathiazole		0.5-500	0.99732	6.69
Penicillin V		0.5-500	0.99870	7.67	Sulfpyridine		0.5-200	0.99873	4.66
Penicillin G		10-200	0.99853	15.30	Sulfamerazine		0.5-500	0.99724	6.84
Ceftiofur		0.5-500	0.99946	11.31	Sulfamethazine		0.5-500	0.99631	5.08
Cefazolin		0.5-500	0.99718	8.69	Sulfamethoxypyridazine		0.5-200	0.99904	4.51
Ampicillin		0.5-500	0.99901	9.37	Sulfamonomethoxine		0.5-500	0.99810	5.99
Amoxicillin		10-500	0.99735	13.29	Sulfchloropyridazine		0.5-500	0.99703	4.03
Flumequine	Fluoroquinolones	0.5-200	0.99914	7.62	Sulfadoxine		0.5-200	0.99905	5.07
Difloxacin		0.5-500	0.99873	5.38	Sulfamethoxazole		0.5-500	0.99561	3.42
Sarafloxacin		0.5-500	0.99742	5.57	Sulfisoxazole		0.5-500	0.99653	4.63
Enrofloxacin		0.5-500	0.99774	4.86	Sulfchloropyrazine		0.5-500	0.99758	10.82
Danofloxacin		0.5-500	0.99823	14.20	Sulfachinoxaline		0.5-500	0.99556	4.21
Ciprofloxacin		0.5-500	0.99892	13.77	Sulfadimethoxine		0.5-500	0.99527	4.69
Norfloxacin		0.5-500	0.99849	11.49	Cefoperazone	Cephalosporins	10-500	0.99770	16.53
Marbofloxacin		0.5-500	0.99847	7.10	Cefalonium		20-500	0.99777	19.12
Doxycycline	Tetracyclines	0.5-500	0.99753	12.99	Cefalexin		0.5-500	0.99522	14.7
Chlorotetracycline		0.5-500	0.99755	17.02	Cefquinome		20-500	0.99559	11.62
4-epi-chlorotetracycline		0.5-500	0.99916	17.27	Cefapirin		0.5-500	0.99933	12.53
4-epi-tetracycline		0.5-500	0.99666	12.11	Josamycin	Macrolides	0.5-200	0.99848	11.08
Oxytetracycline		10-500	0.99857	14.84	Tylosin		0.5-500	0.99916	9.17
4-epi-oxy-tetracycline		0.5-500	0.99809	15.70	Erythromycin		0.5-500	0.99561	19.86
Tetracycline		0.5-500	0.99610	14.74	Tilmicosin		0.5-500	0.99596	6.23
Nalidixic acid	Chinolines	0.5-200	0.99881	5.29	Spiramycin		0.5-500	0.99866	9.08
Oxolinic Acid		0.5-500	0.99625	6.50	Tulathromycin		0.5-500	0.99559	16.81
Tiamulin	Diterpenes	0.5-200	0.99512	7.71	Trimethoprim	Diaminopyrimidines	0.5-500	0.99773	8.29
Lincomycin	Aminocyclitols	0.5-500	0.99724	13.72					

Validation of the method met the criteria of linearity (R>0.995) while maintaining a wide range of concentrations, and a reproducibility determined in 10 different matrices at CV<20%

Tab 2. Ion source parameters

	POS
CUR	35
CAD	9
IS	3500
TEM	550
GS1	45
GS2	40

Tab 3. sMRM scan parameters

Parameter	Value
Cycle time	0.4
MRM window	30
Settling time	0
Pause	5

Tab 4. General LC-MS parameters

Stop time:	12.00 min	
Flow:	0.5000 mL/min	
Time (min)	Flow (ml/min)	B Conc (%)
1	0.5	5
8	0.5	90
8.1	0.5	100
10	0.5	100
10.1	0.5	5
11	0.5	5
Compressibility settings (GPa):		
Mobile phase A	Wate	0.45
Mobile phase B	Acetonitrile	1.2
Injection		10 µL
Sampling speed:		5 µL/s
Use cooler temperature:		Yes
Cooler temperature:		8 °C
Column Oven		
Oven temperature:		40 °C

The effective analytical procedure for the determination of 57 antibiotics residues from different honey samples in one sample preparation procedure and one LC-MS/MS run.