



Targeted quantitation of 28 antibiotics in honey

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INTRODUCTION

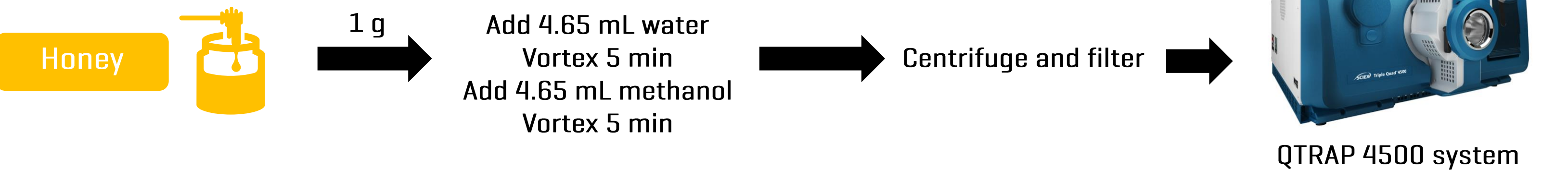
Honey is a complex natural product containing bioactive compounds such as sugars, enzymes and proteins, organic acids and polyphenols.^{1,2} Many of these compounds are associated with honey’s broad health benefits, including antioxidant, anti-inflammatory, antimicrobial and antiviral properties.^{1,2} However, the use of antibiotics in apiculture can lead to residue contamination in honey, posing potential health risks to consumers.

Antibiotic use in beekeeping has been prohibited in several regions, including the European Union, under Commission Regulation [EU] No. 37/2010. Given these regulatory demands, selective and sensitive analytical methods for monitoring antibiotics in honey are needed. The European Union Reference Laboratories [EURL] updated the Minimum Method Performance Requirements [MMPRs] in 2020 for several antibiotic classes in food; however, api-products such as honey are excluded due to a zero-tolerance policy for antibiotic residues.

This work describes the analysis of 28 antibiotics in honey across seven classes using a simple dilution-based sample preparation procedure. Apparent recovery, absolute recovery and matrix effect were evaluated without the use of any internal standards, demonstrating the method’s suitability for routine residue analysis.

MATERIALS AND METHODS

Sample preparation



Chromatographic conditions

Time [min]	Flow [mL/min]	%A	%B
0	0.4	95	5
9.0	0.4	30	70
12.0	0.4	2	98
13.0	0.4	2	98
13.1	0.4	95	5
15.0	0.4	95	5

MPA: Water with 5mM ammonium formate and 0.1% [v/v] formic acid
MPB: Acetonitrile with 0.2% [v/v] formic acid
Analytical column: Phenomenex Kinetex Phenyl Hexyl [100 x 3 mm, 2.6 µm 10 µL
Injection volume: 10 µL
Flow rate: 0.4 mL/min

Mass spectrometry conditions

Parameter	Value	Compound	Class	MRM	Polarity	DP [V]	CE [V]	CXP [V]
Polarity	+/-	Amoxicillin	β-lactam	366.2 > 349.1	+	40	15	14
Ion source gas 1	65 psi	Ampicillin	β-lactam	350.2 > 106.0	+	35	35	10
Ion source gas 2	50 psi	Benzyl penicillin	β-lactam	335.2 > 176.0	+	60	30	12
Curtain gas	35 psi	Chloramphenicol	Amphenicol	320.9 > 151.9	-	-55	-20	-11
Source temperature	550°C	Thiamphenicol	Amphenicol	354.0 > 184.9	-	-65	-25	-13
Ion spray voltage	4500 V	Florfenicol	Amphenicol	357.6 > 337.9	-	-55	-15	-13
CAD gas	9 psi	Erythromycin	Macrolide	734.8 > 576.3	+	30	30	30
		Tylosin	Macrolide	916.5 > 772.2	+	50	50	10
		Lincomycin	Macrolide	407.3 > 126.1	+	35	30	8
		Clindamycin	Macrolide	425.2 > 126.0	+	80	55	12
		Furazolidone	Nitroimidazole	226.2 > 139.0	+	75	20	4
		Furaltadone	Nitroimidazole	325.2 > 252.1	+	65	25	10
		Nitrofurazone	Nitroimidazole	199.2 > 182.1	+	55	15	10
		Oxytetracycline	Tetracycline	461.2 > 426.1	+	65	25	18
		Chlortetracycline	Tetracycline	479.2 > 444.1	+	45	30	14
		Tetracycline	Tetracycline	445.3 > 410.1	+	45	30	16
		Sulfadiazine	Sulfonamide	251.2 > 156.0	+	60	20	10
		Sulfamethazine	Sulfonamide	279.2 > 186.0	+	60	25	12
		Sulfadimethoxine	Sulfonamide	311.2 > 156.0	+	80	30	10
		Sulfadoxine	Sulfonamide	311.1 > 156.0	+	65	25	12
		Sulfapyridine	Sulfonamide	250.2 > 155.9	+	70	25	10
		Sulfamethoxazole	Sulfonamide	254.2 > 155.9	+	65	20	8
		Sulfamethoxy pyridazine	Sulfonamide	281.2 > 156.0	+	70	25	16
		Sulfathiazole	Sulfonamide	256.1 < 1560.0	+	70	20	8
		Trimethoprim	Sulfonamide	291.2 > 230.2	+	70	35	4
		Enrofloxacin	Fluoroquinolone	360.3 > 342.2	+	65	30	6
		Ciprofloxacin	Fluoroquinolone	332.2 > 314.1	+	75	30	12
		Norfloxacin	Fluoroquinolone	320.2 > 302.1	+	80	30	16

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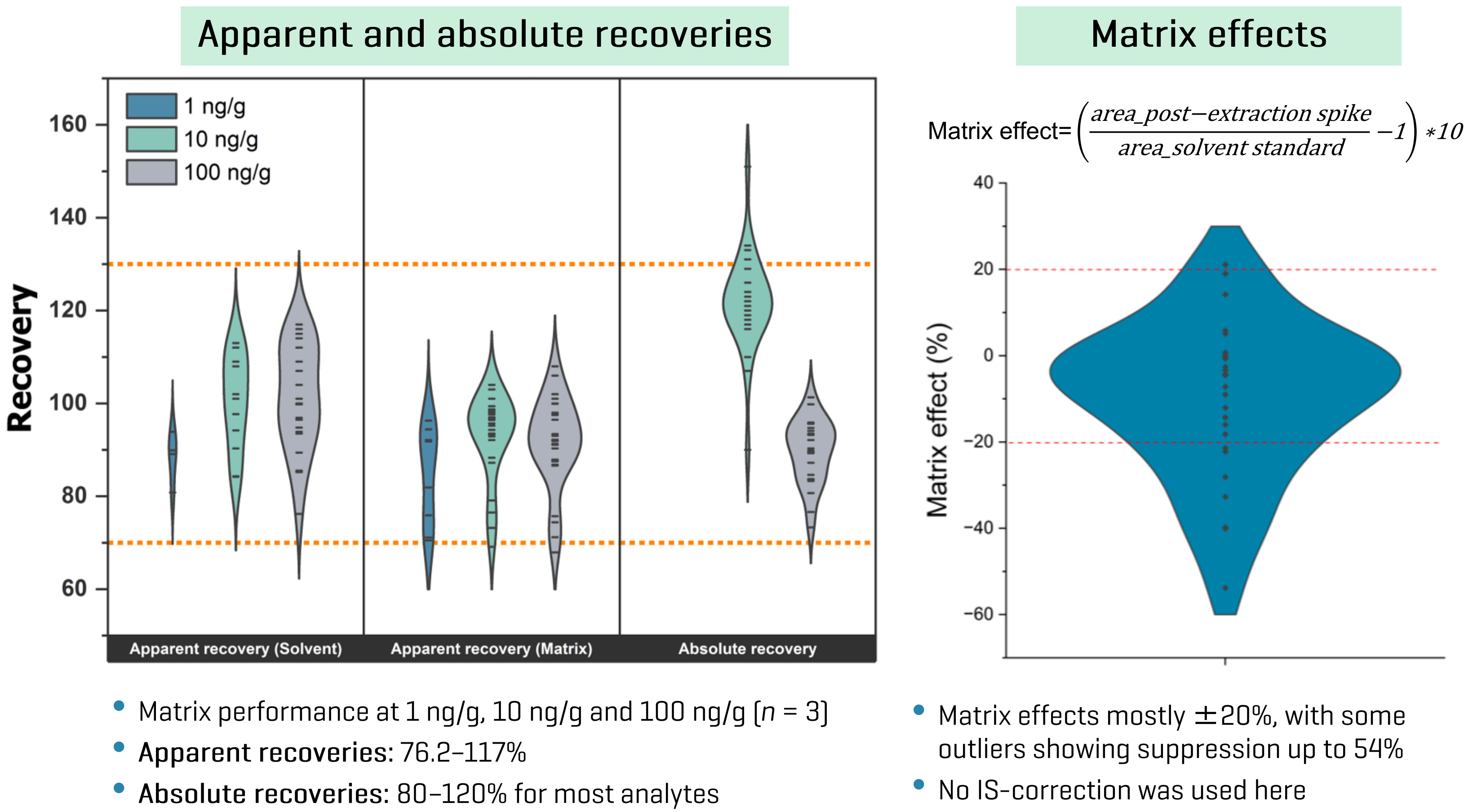
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SENSITIVITY PERFORMANCE IN SOLVENT AND MATRIX-MATCHED STANDARDS

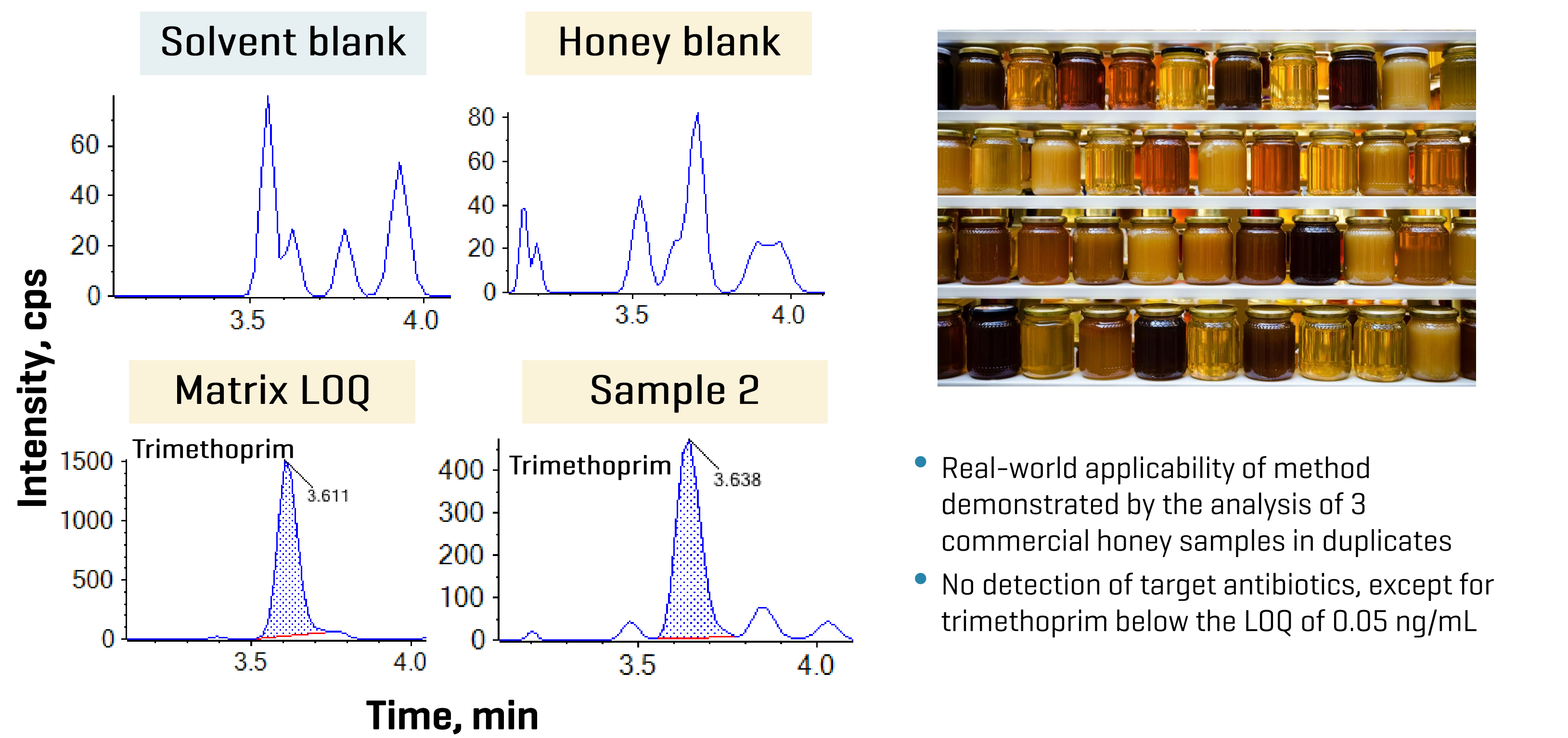
Compound	Solvent			Matrix-matched		
	LOQ (ng/mL)	Mean accuracy [%CV]	r ²	LOQ (ng/g)	Mean accuracy [%CV]	r ²
Erythromycin	0.05	120 [18]	0.997	0.5	111 [7.3]	0.998
Chlortetracycline	0.5	112 [16]	0.996	10	94.2 [8.9]	0.997
Enrofloxacin	0.05	128 [2.4]	0.997	0.5	97.9 [17]	0.999
Norfloxacin	0.1	91.0 [8.9]	0.997	1.0	82.1 [3.1]	0.992
Ciprofloxacin	0.1	83.1 [18]	0.996	1.0	76.7 [7.9]	0.996
Clindamycin	1.0	90.5 [13]	0.994	5.0	73.8 [7.0]	0.994
Lincomycin	0.05	89.1 [5.9]	0.999	1.0	85.5 [7.3]	0.998
Tylosin	0.25	84.3 [14]	0.998	5.0	93.0 [4.8]	0.998
Trimethoprim	0.1	96.9 [2.6]	0.998	0.5	76.3 [15]	0.997
Sulfadoxine	0.025	73.8 [12]	0.997	0.25	81.0 [9.5]	0.997
Sulfadiazine	0.05	116 [8.6]	0.999	1.0	82.5 [17]	0.997
Sulfathiazole	0.25	75.6 [13]	0.997	1.0	83.3 [8.6]	0.994
Sulfamethazine	0.05	115 [8.5]	0.999	0.5	92.2 [12]	0.998
Sulfamethoxy pyridazine	0.05	96.1 [10]	0.997	0.5	90.3 [18]	0.997
Sulfamethoxazole	0.05	98.5 [19]	0.996	1.0	82.0 [14]	0.997
Sulfadimethoxine	0.05	104 [15]	0.998	0.5	118 [12]	0.998
Sulfapyridine	0.05	90.1 [20]	0.997	0.5	80.8 [11]	0.994
Benzyl penicillin	2.5	101 [9.1]	0.996	50	99.1 [9.0]	0.997
Amoxicillin	5.0	92.1 [16]	0.994	50	104 [15]	0.998
Ampicillin	0.25	104 [6.3]	0.999	2.5	100 [4.7]	0.999
Oxytetracycline	1.0	114 [7.7]	0.995	10	90.9 [1.7]	0.996
Tetracycline	0.5	110 [4.7]	0.999	5.0	98.5 [11]	0.999
Furaltadone	0.025	111 [8.6]	0.998	1.0	92.9 [7.9]	0.998
Furazolidone	0.25	81.8 [13]	0.997	0.5	73.9 [17]	0.996
Nitrofurazone	1.0	85.8 [9.1]	0.992	50	94.5 [12]	0.992
Chloramphenicol	0.1	94.9 [16]	0.996	1.0	72.0 [3.6]	0.994
Thiamphenicol	0.25	81.5 [16]	0.993	2.5	114 [11]	0.994
Florfenicol	0.25	92.2 [20]	0.998	2.5	76.0 [7.0]	0.995

- Linearity of r² ≥0.991 for all analytes
- In-vial solvent and matrix LOQs 0.025–5 ng/mL, but <0.5 ng/mL for most analytes
- Back-calculated in-sample LOQs ranged from 0.25–50 ng/g
- LOQ based on accuracy (±30%), %CV <20% (n = 3), S/N ≥10, ion ratios (±30%)

QUANTITATIVE PERFORMANCE IN MATRIX SPIKES



METHOD APPLICABILITY IN COMMERCIAL HONEY



TRADEMARKS/LICENSING

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