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Electronic Supporting Information

Application of core-satellite polydopamine-coated Fe_3O_4 nanoparticles-hollow porous molecularly imprinted polymer combined with HPLC-MS/MS for quantification of macrolide antibiotics

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EXPERIMENTAL SECTION

Reagents. Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), dopamine hydrochloride (DA), trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), ethylene glycol ($(CH_2OH)_2$), dehydrate anhydrous sodium acetate (CH_3COONa), trihydroxymethylaminomethane (Tris), 2-morpholinoethanesulfonic acid (MES), tetraethoxysilicane (TEOS), Potassium phosphate dibasic anhydrous (K_2HPO_4), ammonium fluoride (NH_4F), cetyltrimethylammonium bromide (CTAB), N-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), methacrylic acid (MAA), 2,2'-Azobis(2-methylpropionitrile) (AIBN) and ethylene glycol dimethacrylate (EGDMA) were purchased from Aladdin Industrial Corporation (Shanghai, China).

Preparation of MCM-41 particles. The optimal experiment conditions as follows: NH_4F (1.5 g, 40.5 mmoL) and CTAB (0.91 g, 2.5 mmoL) were dissolved in 250 mL of ultrapure water and heated up to 80 °C. Under magnetic stirring (1000 rpm), TEOS (4.5 mL, 4.21 g) was added dropwise into the solution mentioned above that was proceeded under constant stirring for 6 h. After centrifugation at 10,000 rpm and repeated washing by water and ethanol, the solid product was dried via freeze-drying technique. To remove the excess surfactant template (CTAB), the product was refluxed at 80 °C for 24 h in the solution contains 200 mL ethanol and 8 mL of hydrochloric acid (37%). This procedure was repeated several times to make sure that the CTAB were completely removed. The resulting MCM-41 were centrifuged and washed with ultrapure water and dried for further use.

Preparation of HPMIPs. The HPMIPs was prepared as follows. SPI (211 mg, 0.25 mmoL) and MAA (107 µL, 1.0 mmoL) were dissolved in 16 mL of acetonitrile and 4.0 mL of methanol. This mixture was mixed with ultrasonic for 30 min for preparation of preassembly solution. After adding as-prepared MCM-41 (0.5 g), EDMA (0.75 mL, 5.0 mmoL) and AIBN (0.15 g), the solution was deoxygenated thoroughly with argon gas for 15 min. The reaction was allowed to proceed for 24 h at 60 °C under 1000 rpm. After polymerization, the surface imprinted polymers (MMIPs) were washed with 30 mL of 10% (v/v) HF and ethanol solution was used to soak the MMIPs. The mixture was vortexed for 5 min and kept static for another 12 h to remove MCM-41 matrix. Then, HPMIPs were washed the template molecules away by 30 mL of eluent (methanol/acetic acid, 8:1, v/v) with ultrasound at 100 w for 48 h (renewed the eluent every 8 h). The resultant mixture was separated by centrifugation at 4000 rpm for 10 min and then washed with ethanol at least five times. Finally, the HPMIPs were dried under vacuum at 60 °C for 24 h.

The preparation of the corresponding hollow porous non-imprinted polymers (HPNIPs) was same to the procedures above except in the absence of SPI as template molecule.

Milk sample preparation. 2 mL of milk sample was accurately transferred into a 10 mL polypropylene centrifuge tube and fortified with 100 µL of the working solution at an appropriate concentration. After adding 5 mL of acetonitrile, the mixture

was vortexed for 2 min and centrifuged at 10000 rpm for 10 min. Acetonitrile was selected as extraction solvent because it could precipitate protein and extract less fat compare with methanol. Then the obtained supernatant was transferred into a 10 mL polypropylene centrifuge tube and concentrated about 0.2 mL on a rotary evaporator at 50 °C. The concentrated solution was diluted to 5 mL with K_2HPO_4 buffer (20 mM, pH 8.0) for further MDSPE clean-up.

RESULTS and DISCUSSION

Adsorption isotherm. Put 5 mg of Fe_3O_4@PDA-HPMIPs/Fe_3O_4@PDA-HPNIPs directly into 25 mL conical flask containing 10 mL of SPI aqueous solution with concentration ranging from 1 to 100 μg·mL^{-1}. The suspensions were sealed and oscillated for 24 h at 25 ^{o}C by a shaker to attain equilibrium binding for SPI. After separating by the external magnetic field, the remaining amount of SPI in the aqueous solution was measured by HPLC-MS/MS. the equilibrium concentrations Q (mg·g^{-1}) of SPI were calculated based on the following equation：

$$Q = \frac{(C - C_t)V}{m}$$

Where C (mg·mL^{-1}) and C_t (mg·mL^{-1}) is the initial and final SPI concentration, respectively. V (mL) is the sample volume and m (g) is the mass of coating.

Adsorption kinetic. Adsorption kinetic study was carried out as follows. 5 mg of Fe_3O_4@PDA-HPMIPs/Fe_3O_4@PDA-HPNIPs were put into 10.0 mL SPI aqueous solutions with 25 μg·mL^{-1}. The systems were oscillated at 25 ^{o}C and 100 μL of solution was taken out at the times of 2, 5, 10, 15, 20, 30 and 40 min to measure the SPI concentration with HPLC-MS/MS method.

Table S1. MRM parameters for the monitored macrolides

Analyte	MW	Precursor ion (m/z)	Product ion (m/z)	Dwell time (ms)	Fragment (V)	CE (eV)
Azithromycin	748.51	375.4	591.4*	200	115	10
			158.0	200	115	10
Spiramycin	842.51	422.3	174.1*	200	110	15
			101.2	200	110	10
Tilmicosin	868.57	435.4	696.4*	200	115	15
			174.0	200	115	25
Clarithromycin	747.48	748.5	158.1*	200	150	30
			591.4	200	150	25
Josamycin	827.47	828.5	174.1*	200	160	35
			229.4	200	160	30
Roxithromycin	836.52	837.6	679.4*	200	160	15
			158.1	200	160	15
Tylosin	915.52	916.5	174.2*	200	150	55
			774.4	200	150	50

* transitions used for quantitation.

Table S2. The data of Fe_3O_4@PDA, Fe_3O_4@PDA-HPNIPs and Fe_3O_4@PDA- HPMIPs materials

Material	C (%)	N (%)	Q_{max} (μmoL·g^{-1})	Surface area (m^2·g^{-1})
Fe_3O_4@PDA	15.5	2.5	/	/
Fe_3O_4@PDA-HPNIPs(Cycle 2)	/	/	32.9	22.9
Fe_3O_4@PDA-HPMIPs (Cycle 1)	16.4	2.2	65.6	/
Fe_3O_4@PDA-HPMIPs (Cycle 2)	33.1	1.9	103.6	47.8
Fe_3O_4@PDA-HPMIPs (Cycle 3)	39.8	1.7	116.7	/

Table S3. The recovery and precision of the proposed method

Compound	Spiked level	Intra-day (n=6)		Inter-day (n=3)	
	($\mu g \cdot kg^{-1}$)	Recovery (%)	RSD	Recovery (%)	RSD
	1	98.7	6.9	94.3	12.1
Azithromycin	4	93.1	10.7	93.1	11.0
	40	99.1	5.4	97.4	5.8
	1	94.5	11.3	89.6	11.0
Spiramycin	4	86.6	7.2	92.2	12.1
	40	96.7	7.5	94.3	7.6
	1	93.3	12.0	91.2	6.3
Tilmicosin	4	86.8	11.1	86.7	8.7
	40	94.0	3.8	94.2	4.4
	1	88.2	4.9	84.2	9.3
Clarithromycin	4	117	10.3	115	9.2
	40	113	6.9	112	7.0
	1	94.4	9.2	90.4	7.5
Josamycin	4	99.9	8.7	97.5	8.1
	40	116	6.6	114	6.4
	1	91.8	10.8	88.8	8.6
Roxithromycin	4	103	9.8	101	9.5
	40	103	7.6	99.8	7.3
	1	85.5	10.2	84.9	8.2
Tylosin	4	94.2	9.6	91.0	10.8
	40	92.5	8.7	93.3	8.5

Table S4. pKa (acid dissociation constant), log D (distribution constant) and log P (partition constant) values of the selected MACs

Analytes	pKa							log P	log D (pH=3)	log D (pH=9)
Tylosin	14.97	14.39	13.43	12.95	12.45	8.43		2.32	-1.18	2.21
Spiramycin	14.76	13.88	13.12	12.53	9.33	8.44		2.50	-4.50	1.92
Tilmicosin	14.67	13.75	13.14	12.55	10.16	8.55		4.19	-2.81	2.87
Josamycin	13.82	12.71	8.51	-1.33				3.22	-0.28	3.09
Azithromycin	14.52	13.95	13.33	12.90	12.43	9.57	8.91	2.44	-4.56	1.55
Clarithromycin	14.48	13.41	12.94	12.46	9.00			3.24	-0.26	2.94
Roxithromycin	14.02	13.61	13.08	13.06	12.83	9.08	2.29	3.00	-0.58	2.66

Note: This data comes from https://www.chemaxon.com.

Table S5. Result of the proposed method for real honey samples

Actual samples	AZI ($\mu g \cdot kg^{-1}$)	SPI ($\mu g \cdot kg^{-1}$)	TILM ($\mu g \cdot kg^{-1}$)	CLA ($\mu g \cdot kg^{-1}$)	JOS ($\mu g \cdot kg^{-1}$)	ROX ($\mu g \cdot kg^{-1}$)	TYL ($\mu g \cdot kg^{-1}$)
Sample 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sample 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21
Sample 3	n.d	0.49	n.d	n.d	n.d	n.d	1.5
Sample 4	n.d	0.72	n.d	n.d	n.d.	n.d	2.5
Sample 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table S6. Validation results of the detected macrolides in the milk sample

Sample matrix	Compound	Linear range (μg·kg^{-1})	R^2	Spiked level (μg·kg^{-1})	Recovery (%, n=3)	LOD (μg·kg^{-1})
Milk	Azithromycin	0.4-40	0.9978	5	96.1	0.065
				25	93.0	
	Spiramycin	0.4-40	0.9949	5	97.1	0.009
				25	118.4	
	Tilmicosin	0.4-40	0.9976	5	80.7	0.057
				25	101.3	
	Clarithromycin	0.4-40	0.9950	5	89.3	0.027
				25	96.8	
	Josamycin	0.4-40	0.9953	5	100.4	0.012
				25	91.8	
	Roxithromycin	0.4-40	0.9962	5	87.4	0.012
				25	94.3	
	Tylosin	0.4-40	0.9988	5	100.9	0.030
				25	95.6	

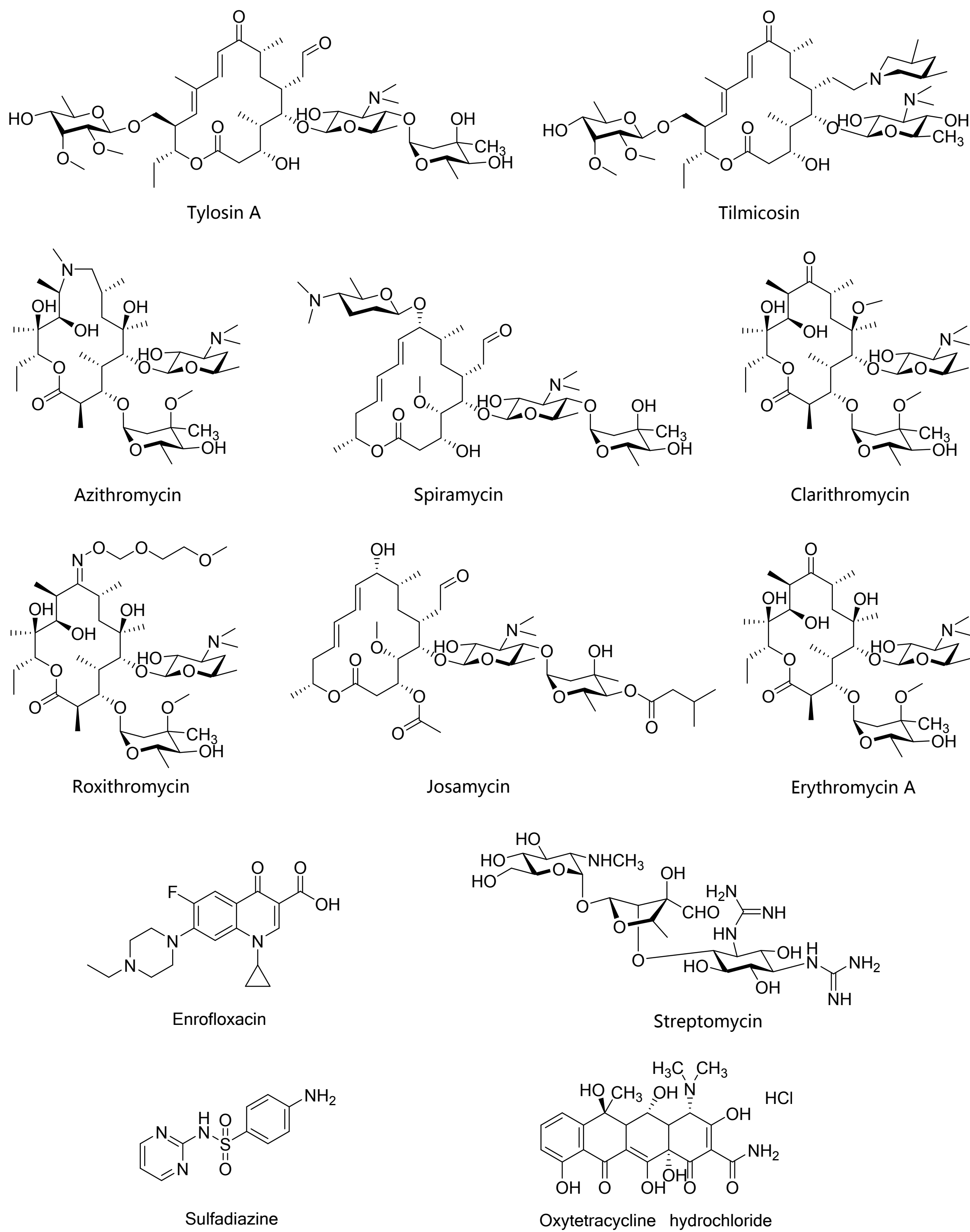

Figure S1. Chemical structures of macrolide antibiotics, oxytetracycline hydrochloride, streptomycin, sulfadiazine and enrofloxacin.

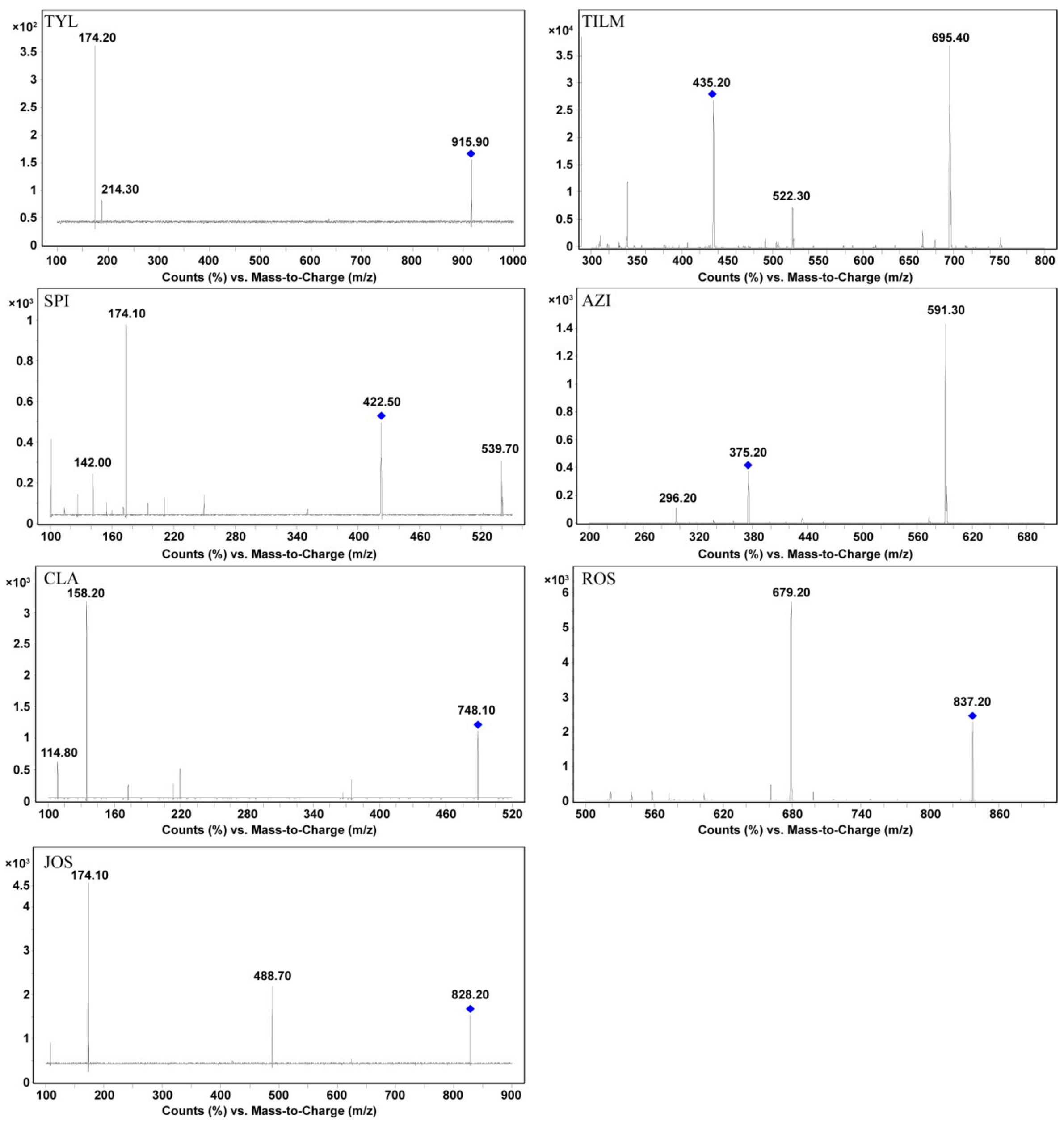

Figure S2. ESI^+ mass spectrum of seven model analytes.

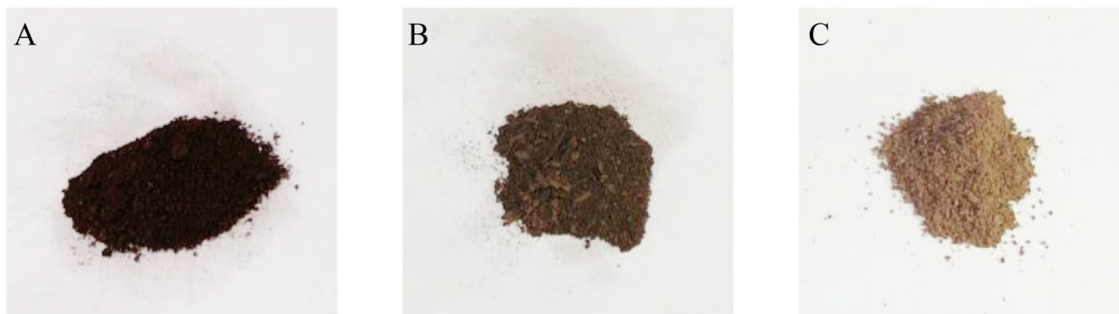

Figure S3. Photos of Fe_3O_4@PDA and Fe_3O_4@PDA-HPMIPs. (A) Fe_3O_4@PDA, (B) Fe_3O_4@PDA-HPMIPs (Cycle 1), (C) Fe_3O_4@PDA-HPMIPs (Cycle 3).

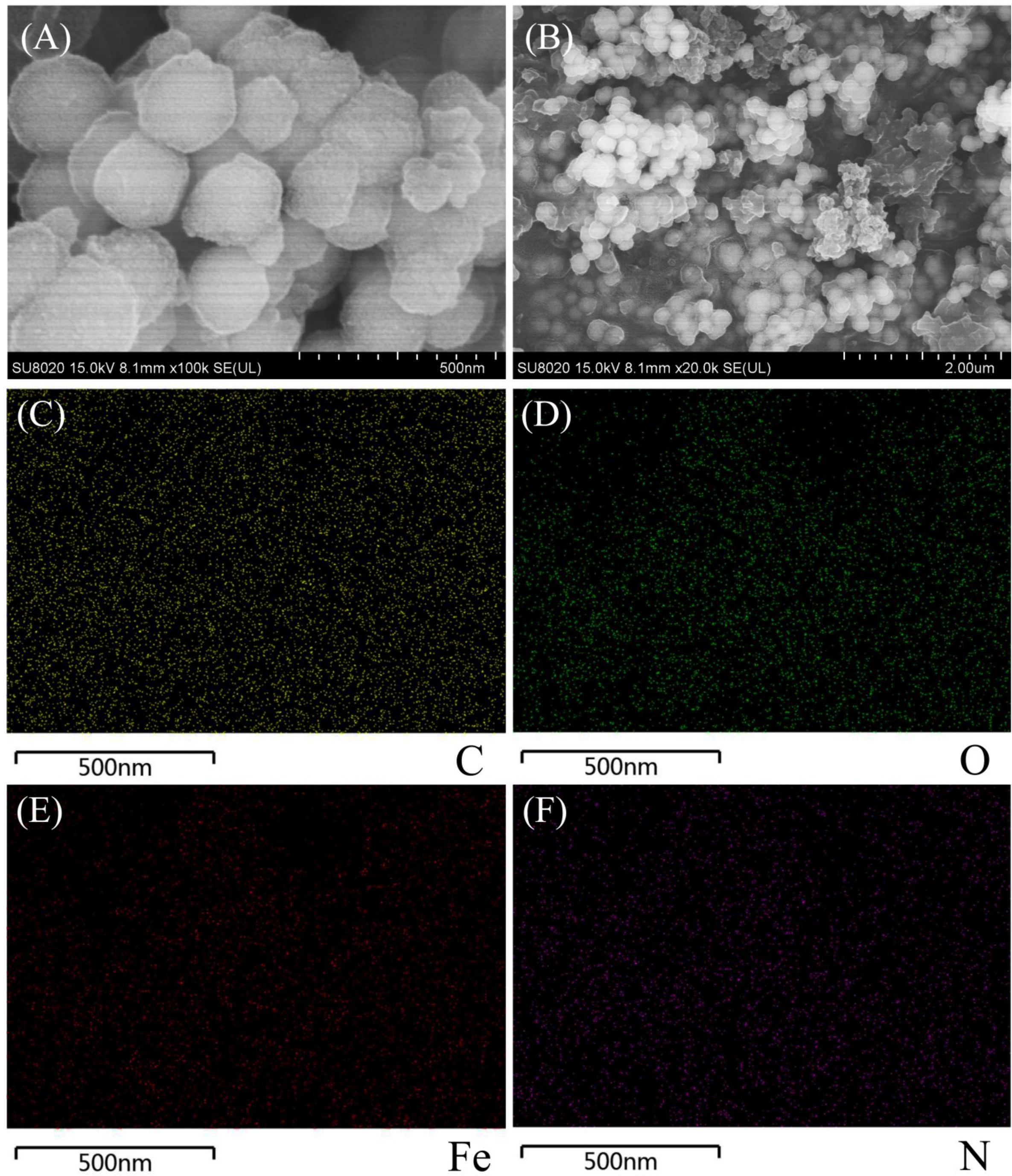

Figure S4. SEM images of Fe_3O_4@PDA (A) and Fe_3O_4@PDA-HPMIPs (B). (C-F) EDX elemental mapping images of C, O, Fe and N of Fe_3O_4@PDA-HPMIPs, respectively.

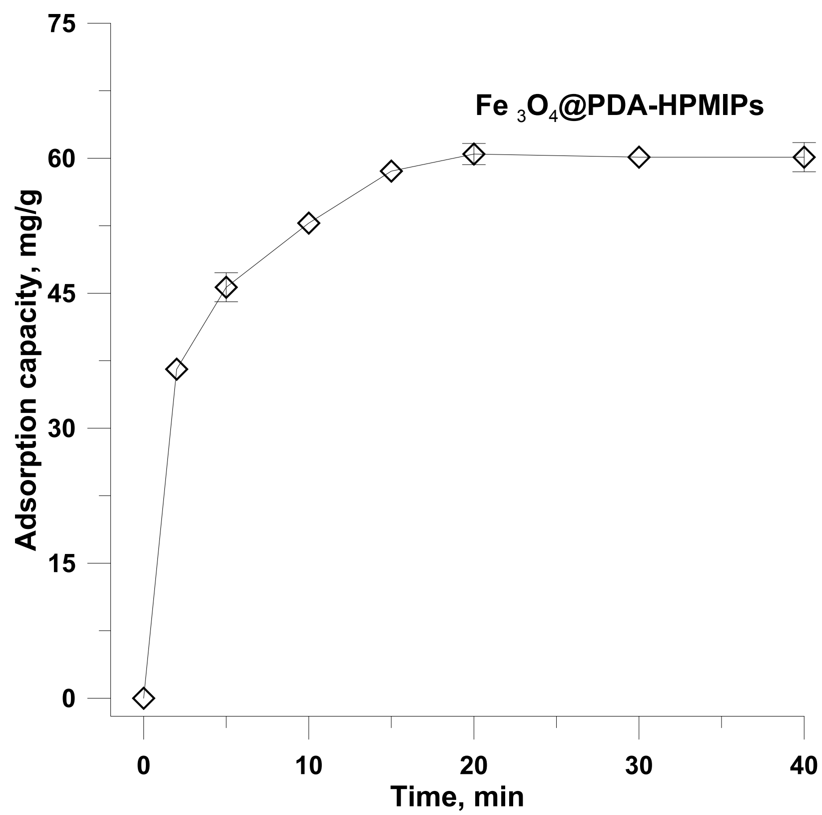

Figure S5. Kinetic adsorption curves of Fe_3O_4@PDA-HPMIPs for SPI.

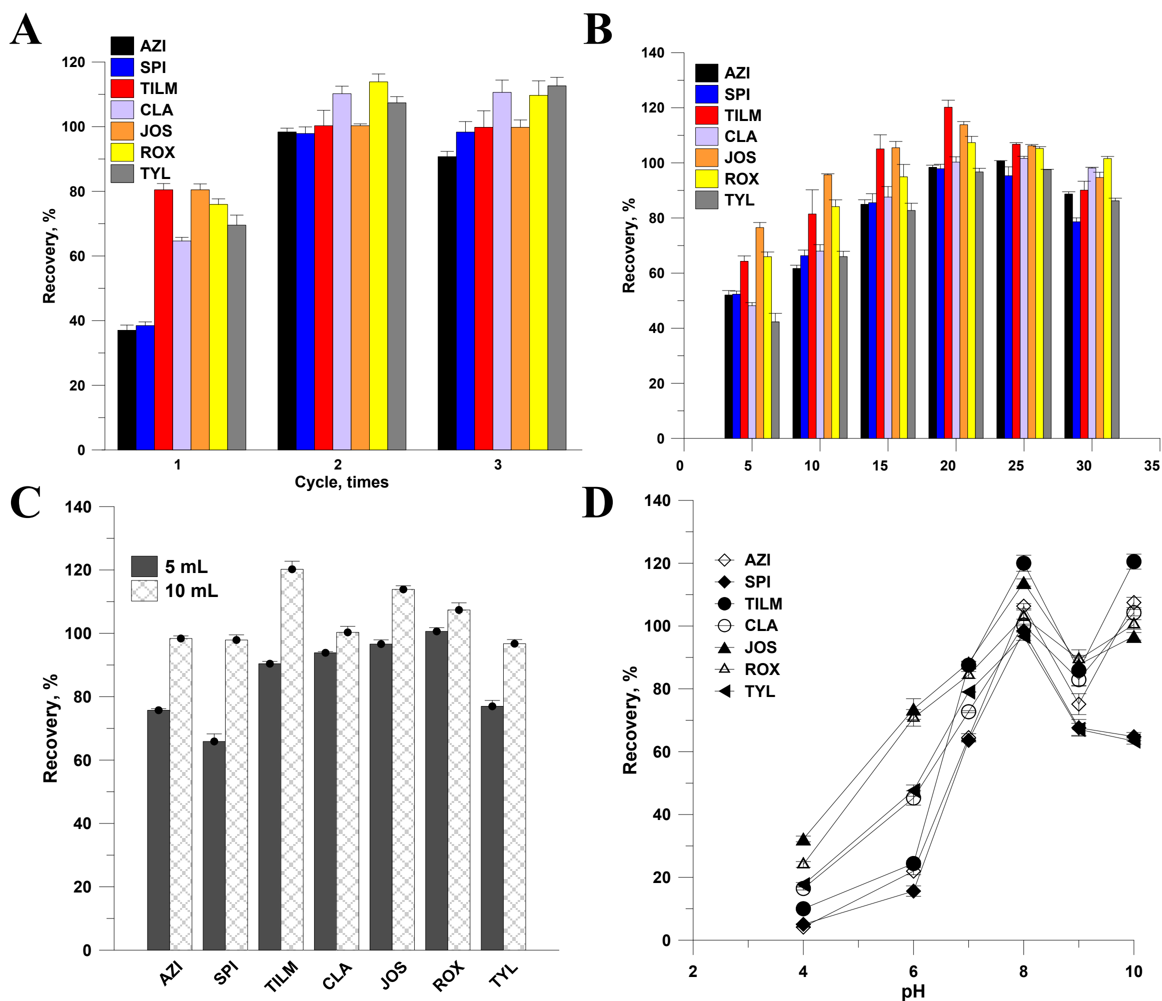

Figure S6. (A) Effect of the sorbent type: 1 Fe_3O_4@PDA-HPMIPs (cycle 1), 2 Fe_3O_4@PDA-HPMIPs (cycle 2) and 3 Fe_3O_4@PDA-HPMIPs (cycle 3). (B) Effect of the amount of sorbent (5-30 mg). (C) Effect of extraction volume (5 mL and 10 mL). (D) Effect of pH: 4.0, 6.0, 7.0, 8.0, 9.0, 10.0.

(n=3; spiking level=40 μg·kg^{-1})

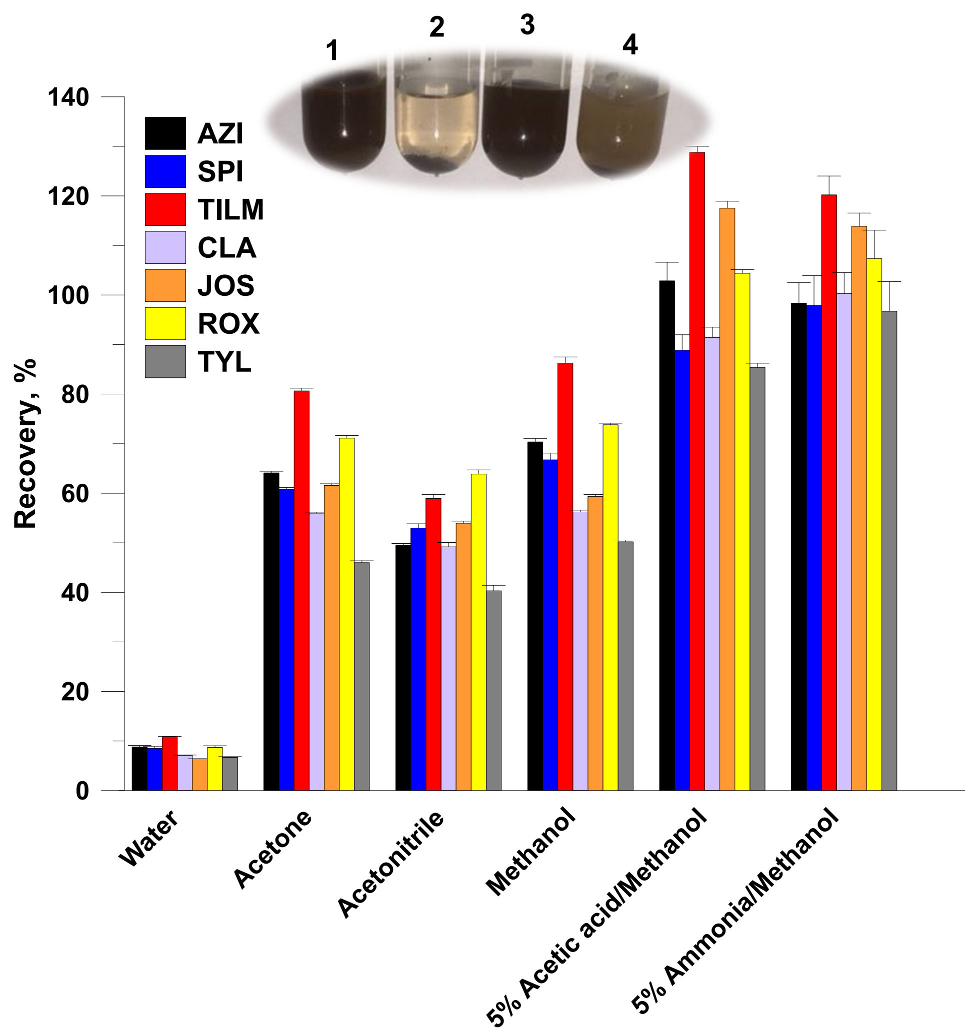

Figure S7. Effects of desorption solvent on the extraction efficiency. Inset: Dispersity of the Fe_3O_4@PDA-HPMIP in different desorption solvent (1, methanol; 2, acetonitrile; 3, water; 4, acetone).

(n=3; spiking level=40 μg·kg^{-1})

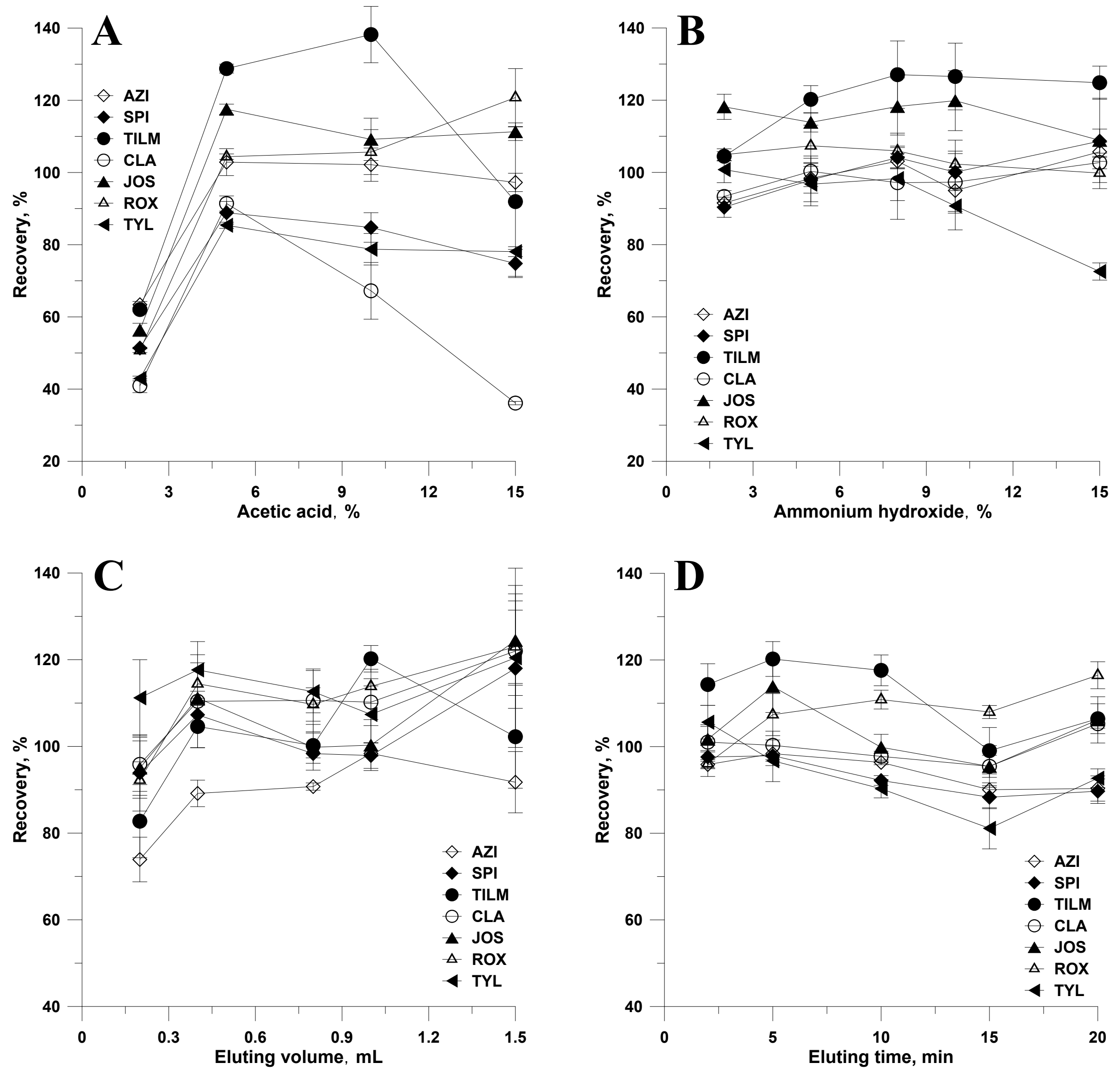

Figure S8. Effect of several parameters on the extraction efficiency. (A) Effects of acetic acid content in desorption solvent. (B) Effects of ammonium hydroxide content in desorption solvent. (C) Effect of the eluting volume. (D) Effect of the eluting time.

(n=3; spiking level=40 $\mu g \cdot kg^{-1}$)

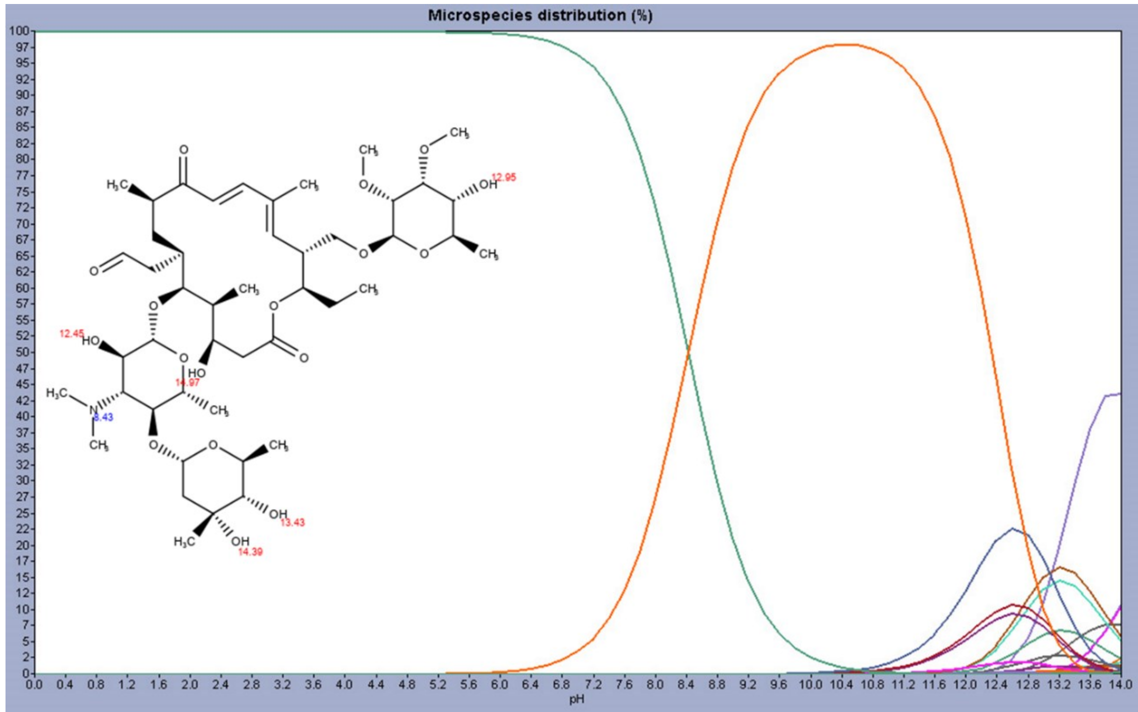

Figure S9. Acid dissociation constant of tylosin.

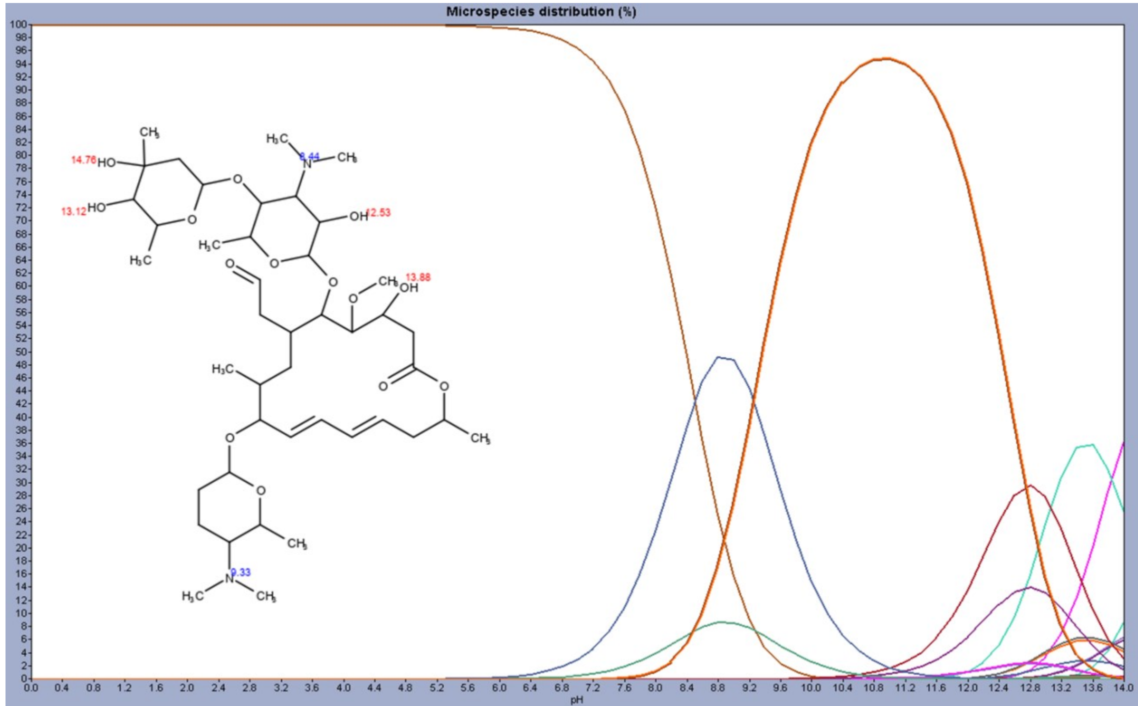

Figure S10. Acid dissociation constant of spiramycin.

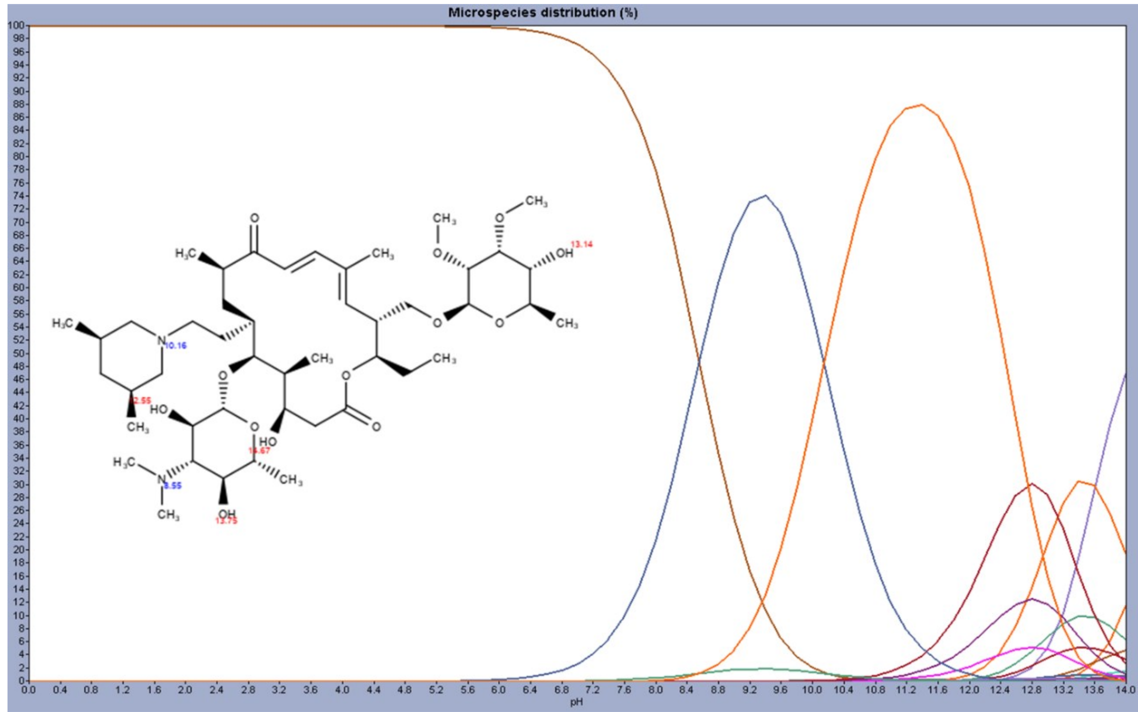

Figure S11. Acid dissociation constant of tilmicosin.

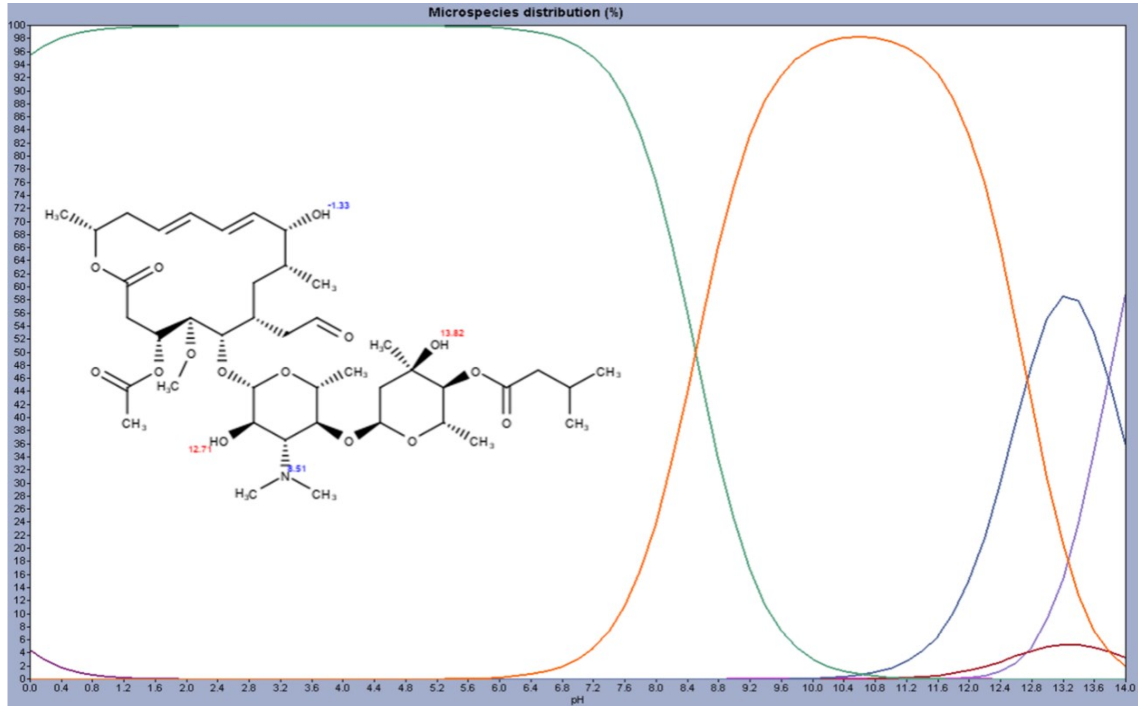

Figure S12. Acid dissociation constant of josamycin.

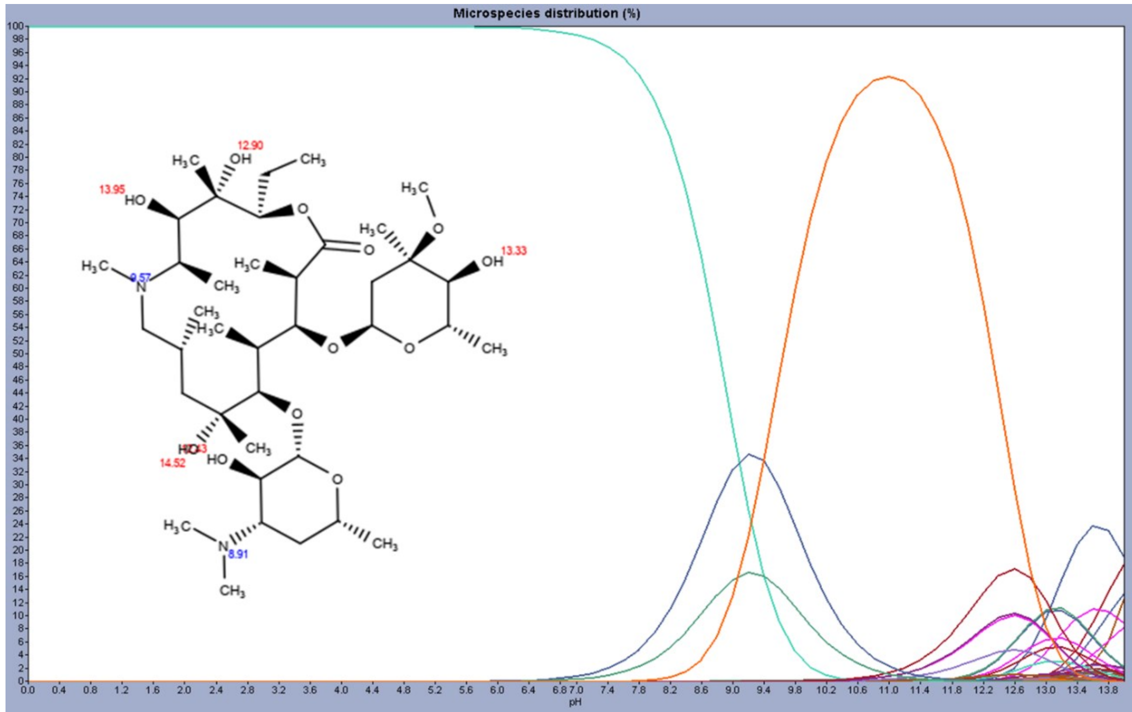

Figure S13. Acid dissociation constant of azithromycin.

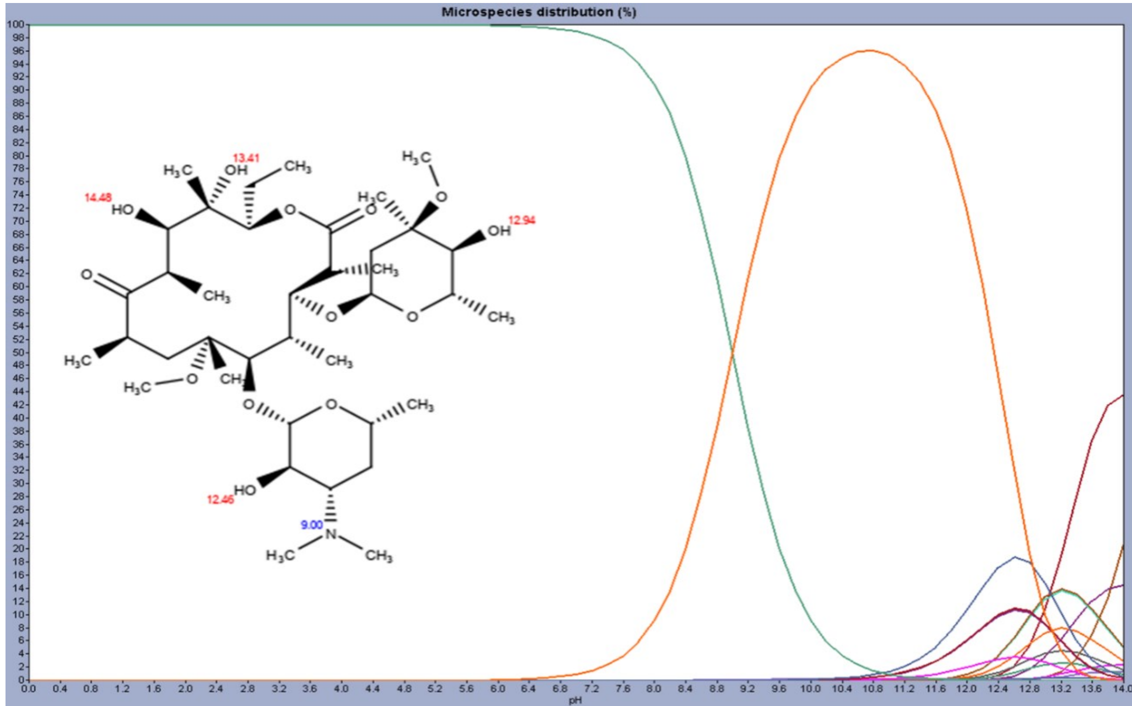

Figure S14. Acid dissociation constant of clarithromycin.