Supporting information for:

Cucurbit[8]uril-based potentiometric sensor coupled to HPLC for determination of tetracycline residues in milk samples

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Experimental section

Potentiometric measurements. All experiments were carried out at room temperature of 22±1°C. To calibrate each tetracycline-selective electrode, the dynamic potentiometric response was obtained at increasing concentrations of tetracycline antibiotics solution and then, the corresponding logarithmic concentrations *(c_{TC})* were plotted versus the steady-state EMF value (ionic strength adjusted with 0.05 M of oxalic acid solution). The data was fitted to the Nernst equation to obtain the sensitivity (slope) and intercept [49]. The limit of detection (LOD) of the potentiometric electrodes was calculated as the concentration related to the cross point between the extrapolation of the lines defining the nonresponsive range and linear-response range of the electrode [49]. The response time was determined as the time that elapses between the instant at which the concentration of the target TC is changed in the solution and the first instant at which the measured EMF of the electrochemical cell becomes equal to 95% of the signal at a steady-state [49].

Tables

Table S1. Linear fittings (EMF response in mV *versus* logarithmic concentration) for the tetracycline-selective electrode (ISM B) in batch and flow conditions.

Calibration parameter		OTC	TTC	CTC	DXC
Slope[a], mV dec^{-1}					
	Batch	56.2±0.9	53.8±2.2	63.4±2.1	59.0±0.1
	Flow	11.2±0.3	24.9±5.8	34.7±6.2	21.0±7.0
LRR, M					
	Batch	6.0×10^{-6} – 1.0×10^{-3}	1.0×10^{-6} – 1.0×10^{-3}	3.0×10^{-7} – 1.0×10^{-3}	3.0×10^{-7} – 1.0×10^{-3}
	Flow	1.0×10^{-5} – 1.0×10^{-4}	1.0×10^{-5} – 1.0×10^{-4}	1.0×10^{-5} – 1.0×10^{-4}	1.0×10^{-5} – 1.0×10^{-4}
LOD[a,b], M					
	Batch	$(2.5\pm0.0)\times10^{-6}$	$(4.3\pm0.5)\times10^{-7}$	$(1.5\pm1.3)\times10^{-7}$	$(1.8\pm0.7)\times10^{-7}$
	Flow	$(7.5\pm0.2)\times10^{-6}$	$(5.2\pm0.2)\times10^{-6}$	$(8.2\pm0.1)\times10^{-6}$	$(7.1\pm1.5)\times10^{-6}$

[a] Standard deviation value was calculated from three subsequent calibrations of the same electrode

[b] Based on conventional definition used in potentiometry [5]

LRR: Linear response range; LOD: Limit of detection.

Table S2. Limits of detection (calculated by IUPAC recommendation [49]) of the miniaturized TC-selective electrodes prepared with different amount of MWCNTs.

Calibration parameter	ISM	MWCNTs (wt%)	OTC	TTC	CTC	DXC
LOD (IUPAC)[c], M						
	B	0.0	7.5×10^{-6}	5.2×10^{-6}	8.2×10^{-6}	7.1×10^{-6}
	C	1.0	5.5×10^{-6}	1.9×10^{-6}	7.5×10^{-6}	7.9×10^{-6}
	D	2.0	5.2×10^{-6}	3.4×10^{-6}	7.9×10^{-6}	7.7×10^{-6}

Table S3. Chromatographic parameters of the target tetracycline antibiotics using the proposed HPLC-potentiometric method.

Analyte	t_R	N_{eff}	k	α	R_s	T_f
Oxytetracycline	9.3	5900	8.0			1.4
				1.4	6.3	
Tetracycline	12.9	6121	11.5			1.6
				1.6	13.8	
Chlortetracycline	21.2	48926	18.3			1.5
				1.2	8.2	
Doxycycline	24.7	44519	21.4			1.4

t_R, retention time, in minutes; N_{eff}, effective plate number; k, retention factor; α, separation factor, between every two successive peaks; R_s, peak resolution, between every two successive peaks; T_f, tailing factor.

Table S4. Chromatographic parameters of the target tetracycline antibiotics using the HPLC-UV method.

Analyte	t_R	N_{eff}	k	α	R_s	T_f
Oxytetracycline	9.3	8221	8.0			1.2
				1.4	7.4	
Tetracycline	12.9	8233	11.5			1.1
				1.6	16.0	
Chlortetracycline	21.2	67084	18.3			1.1
				1.2	9.0	
Doxycycline	24.7	47146	21.4			1.1

t_R, retention time, in minutes; N_{eff}, effective plate number; k, retention factor; α, separation factor, between every two successive peaks; R_s, peak resolution, between every two successive peaks; T_f, tailing factor.

Table S5. Analytical figures of merit obtained with the proposed HPLC method using the UV detector (355 nm).

Validation parameters	OTC	TTC	CTC	DXC
Calibration curve	Peak area (U.A. x s) vs [TC] μM			
Linear range, M	$1.0x10^{-8}$–$1.0x10^{-5}$	$1.0x10^{-8}$–$1.0x10^{-5}$	$1.0x10^{-7}$–$1.0x10^{-8}$	$1.0x10^{-7}$–$1.0x10^{-8}$
R^2	0.9995±0.0002	0.9994±0.0001	0.9992±0.0007	0.9977±0.0031
Slope	0.067±0.007	0.081±0.009	0.034±0.004	0.047±0.008
Intercept	0.001±.0.006	0.001±0.008	0.000±0.003	-0.004±0.004
LOD, M (μg L^{-1})	$3.0x10^{-9}$ (1.4)	$3.0x10^{-9}$ (1.3)	$3.0x10^{-9}$ (1.4)	$3.0x10^{-9}$ (1.3)
LOQ, M (μg L^{-1})	$1.0x10^{-8}$ (4.6)	$1.0x10^{-8}$ (4.4)	$1.0x10^{-8}$ (4.8)	$1.0x10^{-8}$ (4.4)
Precision (RSD%)				
		Intra-day		
3.0 x 10^{-7} M	4.4	5.4	3.8	8.2
1.0 x 10^{-6} M	3.7	3.8	4.2	4.0
1.0 x 10^{-5} M	2.9	3.3	3.8	4.2
		Inter-day		
3.0 x 10^{-7} M	6.1	11.5	10.8	9.4
1.0 x 10^{-6} M	7.5	5.3	19.1	14.3
1.0 x 10^{-5} M	4.0	2.6	1.2	11.4

Table S6. Results obtained for determination of tetracycline antibiotics in spiked milk samples by HPLC using the proposed potentiometric detector compared with the UV detector (*n=3* for each concentration).

Analytes		Samples								
		UHT skimmed milk			UHT semi-skimmed milk			Fresh semi-skimmed milk		
	Added µg L^{-1}	HPLC-POT	HPLC-UV	p-value[a]	HPLC-POT	HPLC-UV	p-value[a]	HPLC-POT	HPLC-UV	p-value[a]
OTC	50	<LOQ	46.7±1.4	–	<LOQ	52.9±2.8	–	<LOQ	49.0±2.0	–
	100	100.1±3.2	102.3±3.6	0.478	103.3±4.9	101.3±3.5	0.607	98.0±6.9	98.0±1.0	0.990
	200	204.6±10.7	193.8±0.6	0.223	178.0±6.2	179.9±3.5	0.677	180.4±15.0	177.1±15.2	0.802
TTC	50	49.1±4.2	48.4±0.8	0.806	50.1±1.4	48.2±1.7	0.231	51.9±1.6	49.4±1.2	0.097
	100	91.0±6.1	96.1±2.4	0.247	87.7±5.3	86.1±3.6	0.678	84.1±3.2	81.6±0.7	0.258
	200	174.3±16.8	163.3±3.3	0.383	194.4±8.6	197.4±4.2	0.617	183.1±13.9	183.6±3.5	0.952
CTC	50	46.9±3.0	41.3±2.7	0.074	47.6±1.2	48.3±4.0	0.778	49.2±3.0	52.4±2.7	0.144
	100	88.5±7.9	96.3±0.7	0.231	88.7±2.0	85.7±1.1	0.083	103.9±4.5	101.8±0.5	0.510
	200	197.0±15.4	195.7±2.4	0.901	192.4±5.6	188.1±3.6	0.331	185.4±11.5	179.2±2.3	0.456
DXC	50	45.9±3.2	52.3±3.8	0.087	48.5±2.1	51.9±2.0	0.111	44.1±1.9	45.2±1.6	0.503
	100	95.6±2.0	97.0±0.7	0.319	108.5±2.1	111.5±1.0	0.087	88.1±3.1	85.4±1.4	0.242
	200	190.1±12.2	184.7±4.6	0.513	162.7±6.8	165.2±4.4	0.624	177.4±10.7	171.7±2.4	0.459

N.D. Not detected.

[a] p-value calculated from the bilateral t-test with 95% confidence level.

Table S7. Comparison of the proposed method with other published methods for determination of tetracycline antibiotics in milk.

Analytes	Extraction method	Sample volume	Instrumental technique	Analysis time	LOD/LOQ	PF	Intra-day RSD%	Recovery %	Ref.
CTC, DXC, MTC, TTC	IL-DLLME	5 mL	HPLC-DAD	27 min	0.12-0.45/- μg L^{-1}	25-98	0.6-1.2	75.8-109.2	[69]
CTC, OTC, TTC	MSPD	0.18 g	HPLC-MS/MS	18 min	0.2-0.3/0.7-1.1 μg kg^{-1}	N.G.	3.8-6.9	84.7-92.8	[71]
CTC, DMC, DXC, OTC, TTC	dSPE	3 g	UHPLC-HRMS	> 30 min	-/25 μg kg^{-1}	N.G.	9-19	101-109	[12]
CTC, DXC	Prime HLB- SPE	1 g	HPLC-MS/MS	> 60 min	0.5-1.0/5.0-10.0 μg kg^{-1}	N.G.	5.1-9.5	70.3-102.4	[13]
CTC, DXC, OTC, TTC	RACNTs-SPE	0.5 mL	HPLC-DAD	12 min	7.5-13.2/25.0-44.0 μg L^{-1}	N.G.	3.9-17.6	47.0-59.0	[72]
CTC, DMC, OTC, TTC,	Mag-SPE	10 mL	HPLC-MS/MS		8.1-83.2/17.4-182.7 ng L^{-1}	50	1.1-5.4	87.0-101.8	[11]
TTC	Mag-SPE	5 mL	HPLC-DAD	> 30 min	3.5/9.8 μg L^{-1}	100	3.8	94.6-105.4	[65]
CTC, DXC, OTC, TTC	Mag-SPE	1 mL	HPLC-DAD	> 45 min	40/50 μg L^{-1}	4	1.1-2.2	87.8-107.5	[66]
DXC, OTC, TTC	Mag-SPE-DLLME	1 mL	HPLC-UV	> 60 min	1.8-2.9/6.1-9.8 μg L^{-1}	66.7	0.1-6.9	70.6-121.5	[70]
DXC, CTC, OTC, TTC	HLB- SPE	5 g	HPLC-MS/MS	> 60 min	1-10/50 μg kg^{-1}	10	1.8-8.3	74.4-101	[67]
CTC, OTC, TTC	HLB-SPE	5 mL	HPLC-DAD	> 60 min	20/60 μg L^{-1}	10	2.7-4.9	83-112	[68]
CTC, DXC, OTC, TTC	Prime HLB- SPE	1 mL	HPLC-POT	> 60 min	13.3-46.0/44.4-92.1 μg L^{-1}	Not applied	0.5-11.2	81.3-108.5	This work

CTC – chlortetracycline; DAD – diode-array detector; DLLME – dispersive liquid liquid microextraction; DMC – demeclocycline; dSPE – dispersive solid phase extraction; DXC – doxycycline; HRMS – high resolution mass spectrometry; IL – ionic liquid; MSPD – matrix solid phase dispersion; MagSPE – Magnetic solid phase extraction; MS/MS - tandem mass spectrometric detector; MSPD – matrix solid phase dispersion; MTC – methacycline; N.G. not given; OTC – oxytetracycline; PF – pre-concentration; POT – potentiometric detector; RACNTs – restricted accesses carbon nanotubes; SPE – solid phase extraction; TTC – tetracycline; UV – ultraviolet detector.

Figures

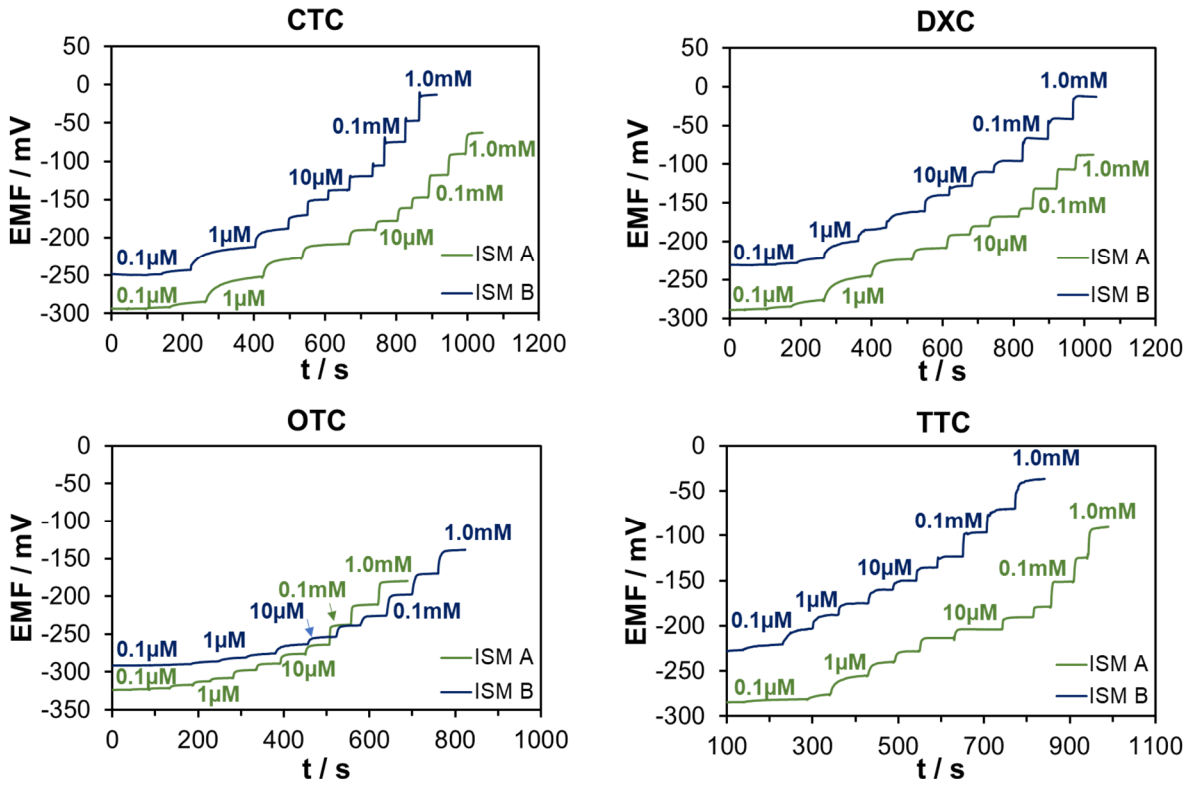


Figure S1. Dynamic potentiometric responses for ion-selective electrodes prepared without (ISM A) and with CB[8] (ISM B) in batch conditions at the increasing concentration of chlortetracycline (CTC), doxycycline (DXC), oxytetracycline (OTC), and tetracycline (TTC).

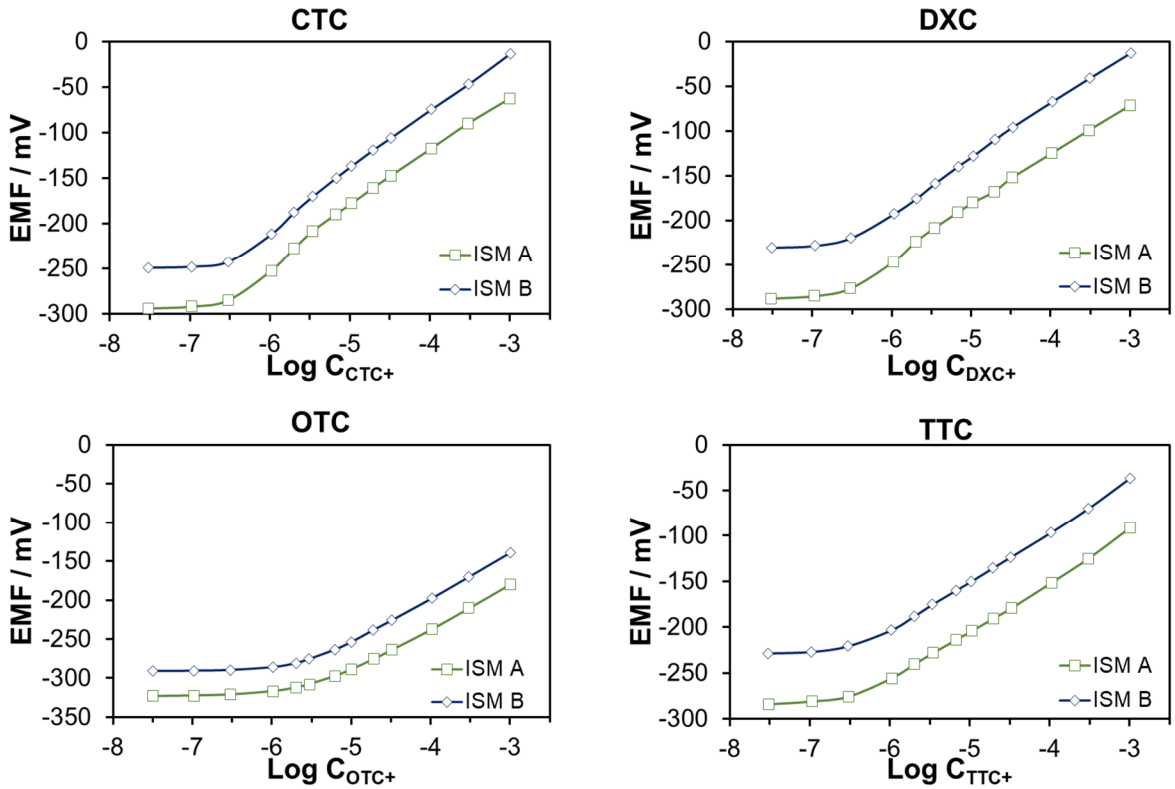


Figure S2. Calibration curves of EMF responses of CTC, DXC, OTC, and TTC obtained with the ion-selective electrodes prepared without (ISM A) and with CB[8] (ISM B) in batch conditions.

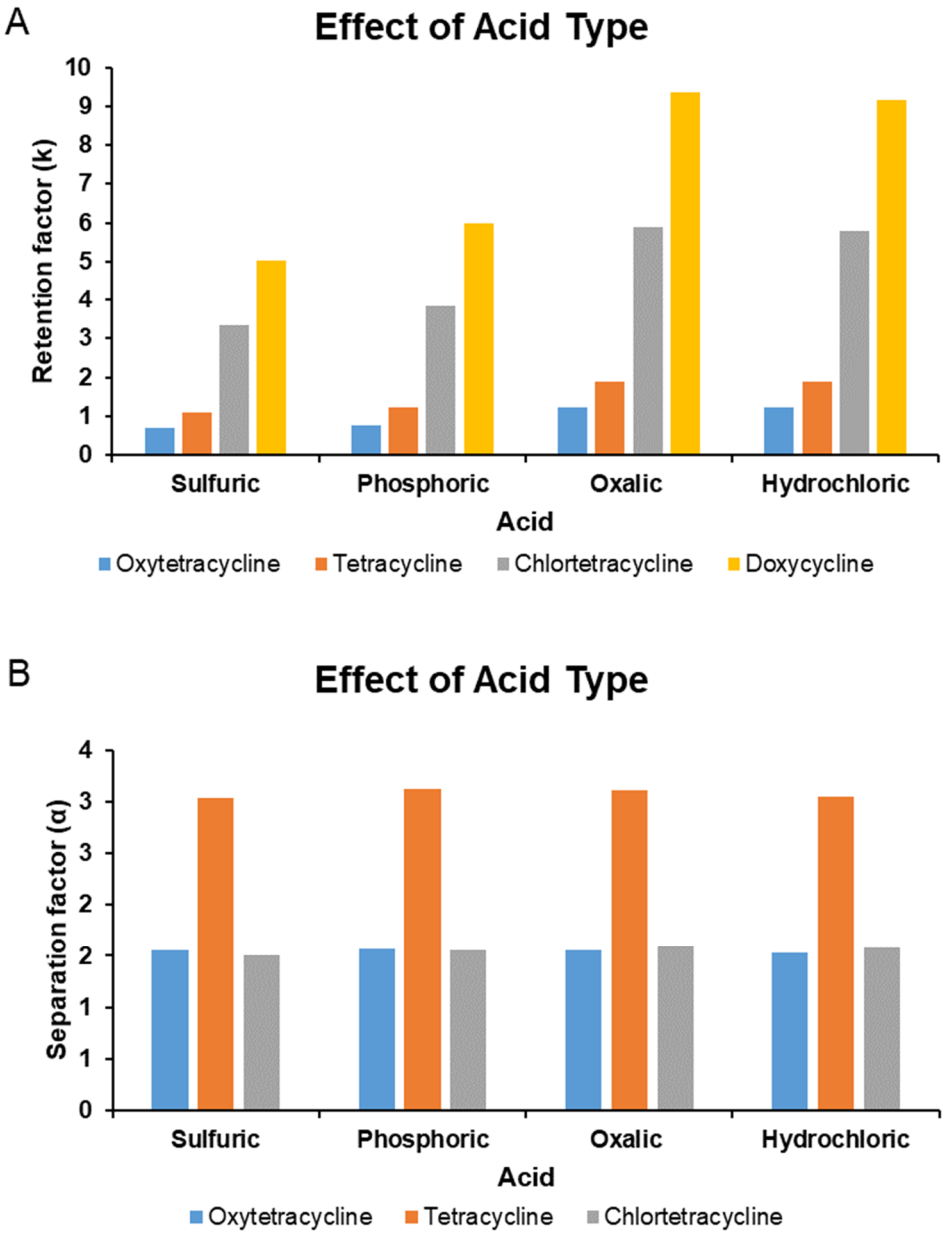


Figure S3. Effect of the acidifying agent of the mobile phase in the (A) retention and (B) separation factors.

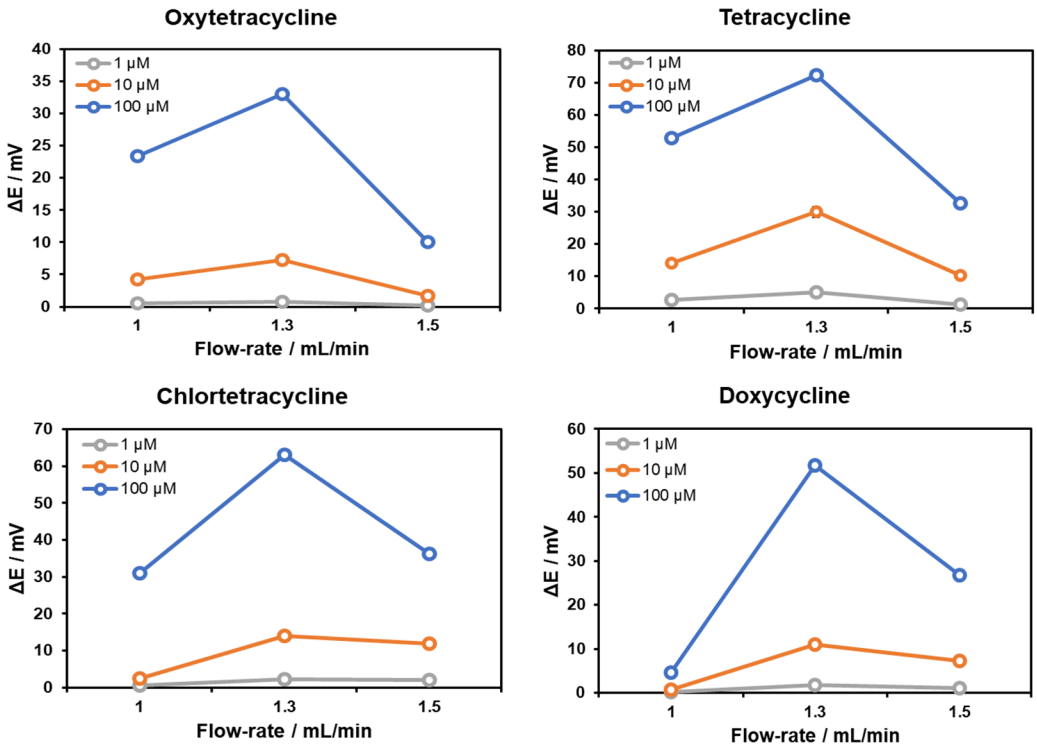
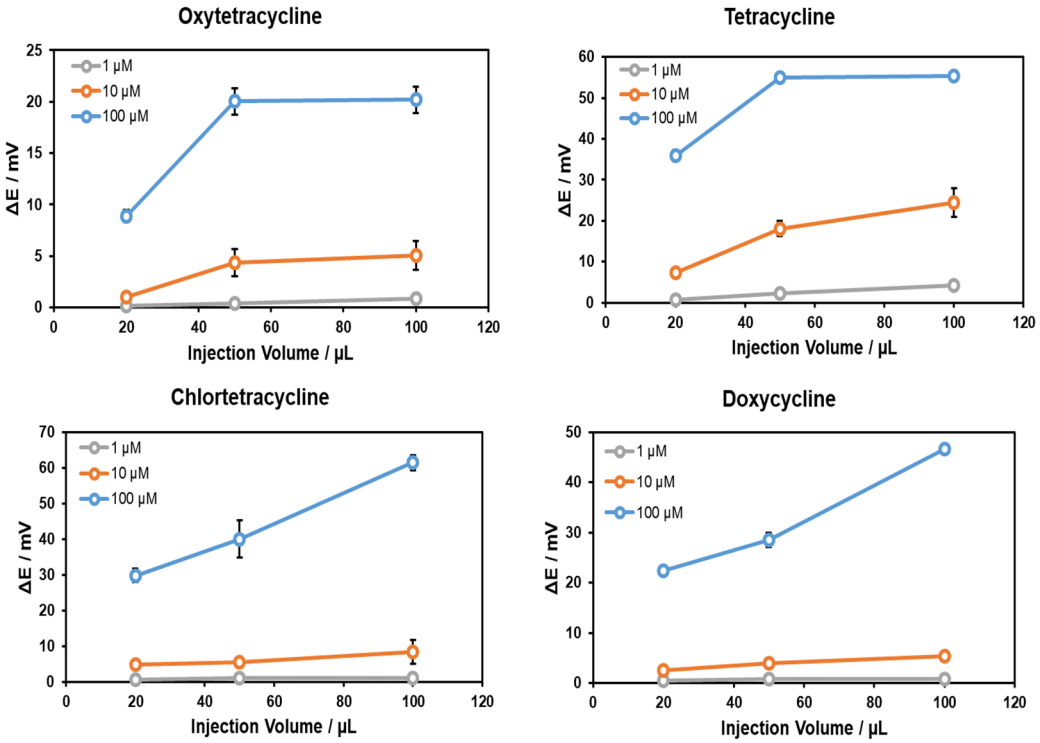


Figure S4. Detector performance (peak heights in mV) for standard solutions of four tetracycline antibiotics at 1.0, 10.0, and 100.0 µM as a function of the mobile phase flow rate.

Gradient elution: solvent A – 0.005 M H_2SO_4:ACN (90:10, v/v) and solvent B – 0.005 M H_2SO_4:ACN (80:20, v/v); Column: Luna 5 µm C8(2), 150 x 4.6 mm I.D. (Phenomenex); Injection volume: 100 µL.



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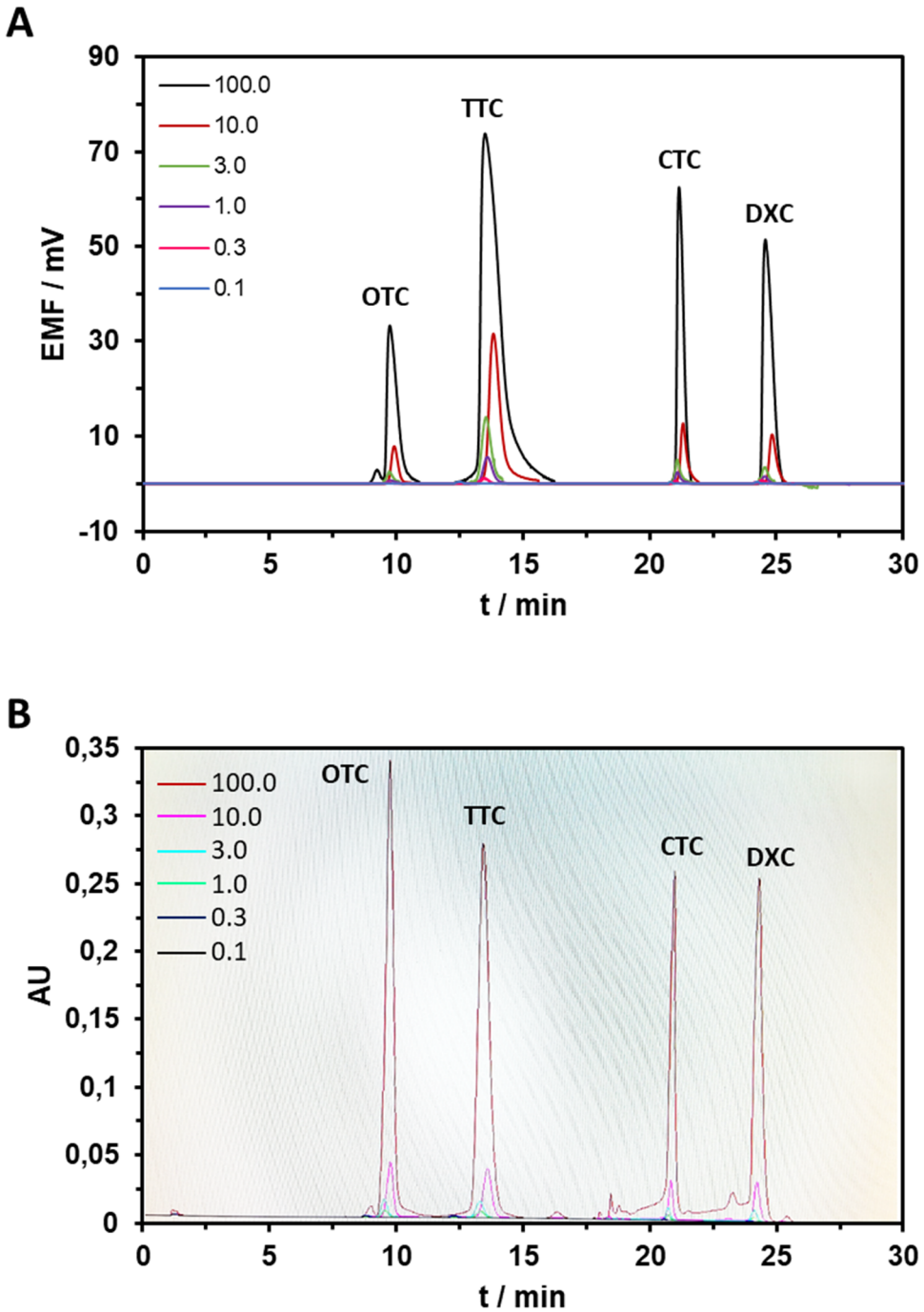

Figure S6. Chromatograms at increasing concentrations (0.1, 0.3, 1.0, 3.0, 10.0, 100.0 μM) of tetracycline antibiotics obtained with the proposed tetracycline potentiometric detector (A) and the UV detector (wavelength 355 nm) in HPLC.

Gradient elution: solvent A – 0.005 M H_2SO_4:ACN (90:10, v/v) and solvent B – 0.005 M H_2SO_4:ACN (80:20, v/v); Column: Luna 5 μm C8(2), 150 x 4.6 mm I.D. (Phenomenex); Flow rate: 1.3 mL min^{-1}.

Chlortetracycline (CTC), doxycycline (DXC), oxytetracycline (OTC), and tetracycline (TTC).