Electronic Supplementary Information
Analytical Methods

Automatic solid-phase extraction by programmable flow injection coupled to chromatographic fluorimetric determination of fluoroquinolones

Patricia S. Peixoto[a], Eduarda M. P. Silva[a], Marcelo V. Osório[a], Luisa Barreiros[a,b], José L.F.C. Lima[a], Marcela A. Segundo[a,*]

[a] LAQV, REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, R Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

[b] NIIF, CISA, School of Allied Health Sciences, Polytechnic Institute of Porto, R Dr. António Bernardino de Almeida, 400 4200-072, Porto, Portugal

[*] Corresponding author.

Tel.: +351 220428676; Fax: +351 226093483

E-mail address: msegundo@ff.up.pt (M.A. Segundo)

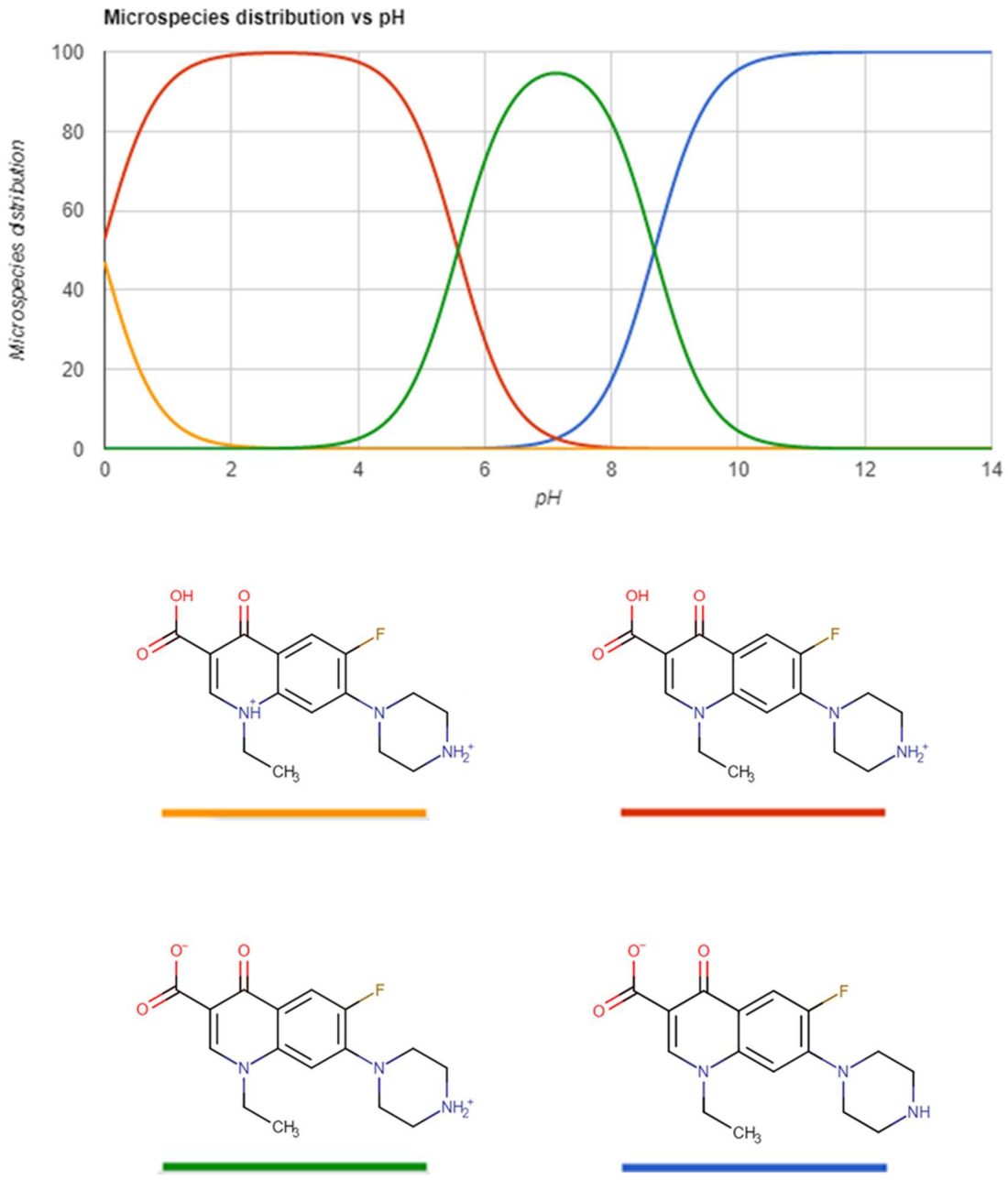

Figure S1. Norfloxacin structure featuring group ionization at different pH values. Calculations were performed using Chemicalize software (https://chemicalize.com/).

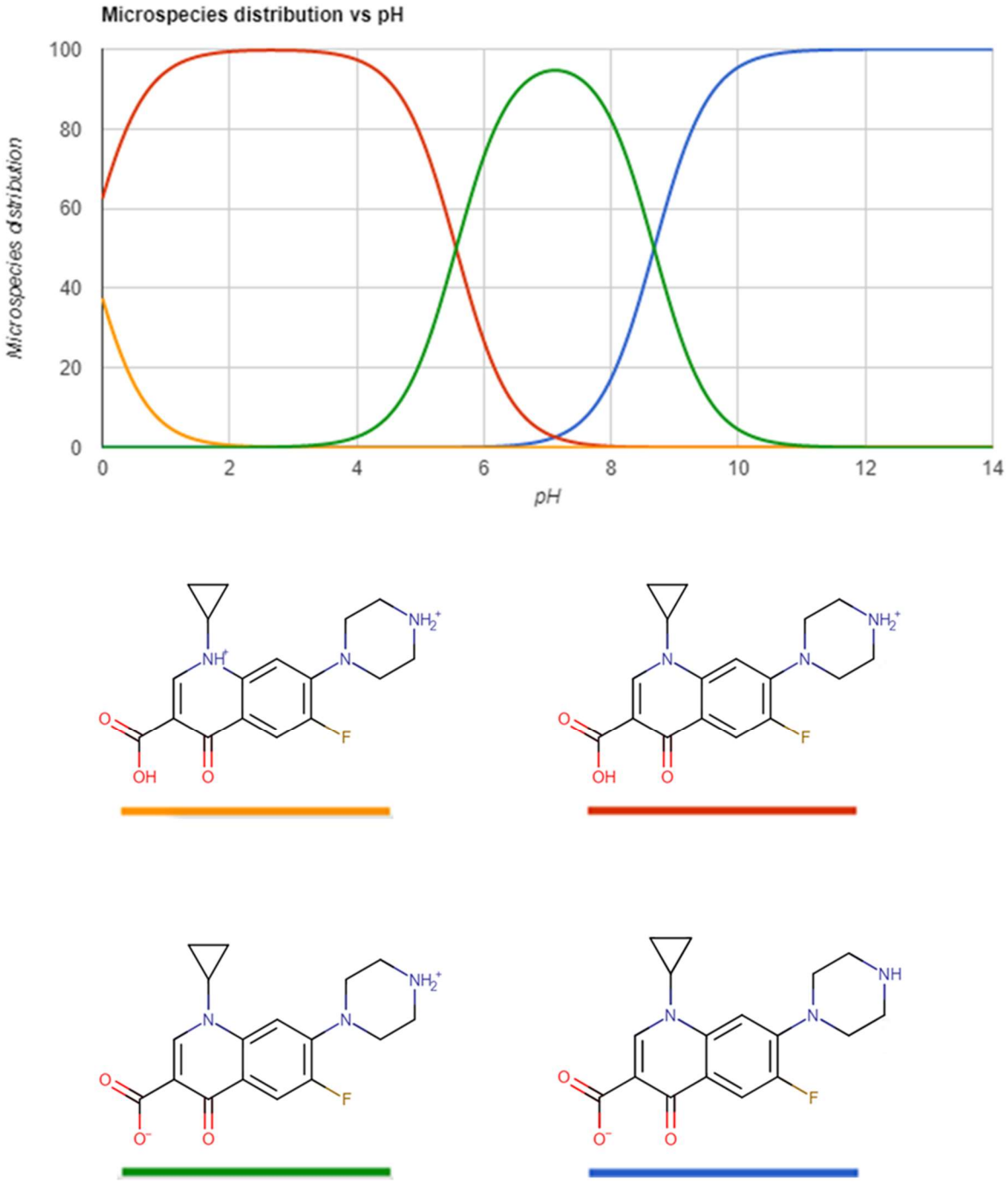

Figure S2. Ciprofloxacin structure featuring group ionization at different pH values. Calculations were performed using Chemicalize software (https://chemicalize.com/).

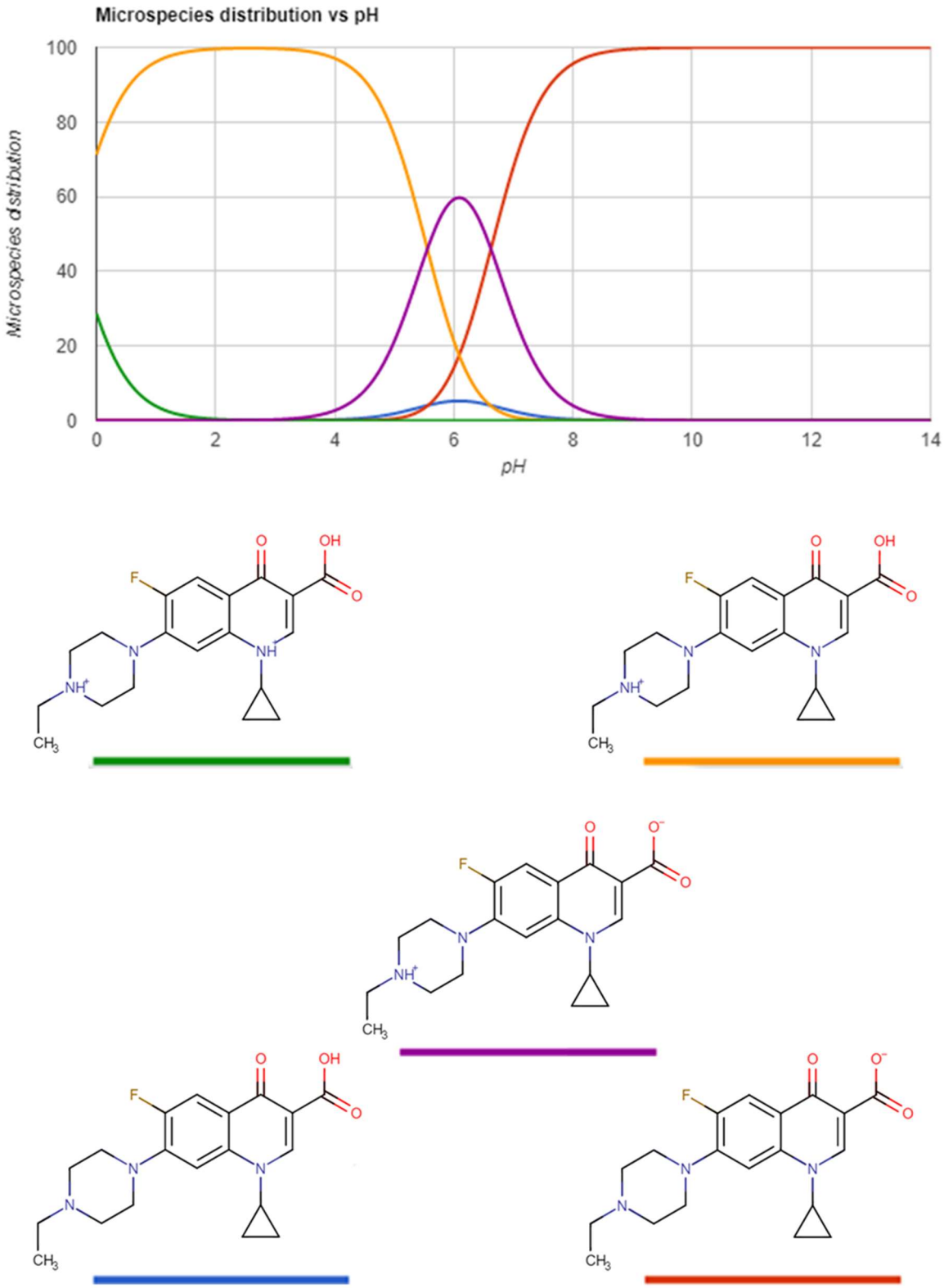

Figure S3. Enrofloxacin structure featuring group ionization at different pH values. Calculations were performed using Chemicalize software (https://chemicalize.com/).

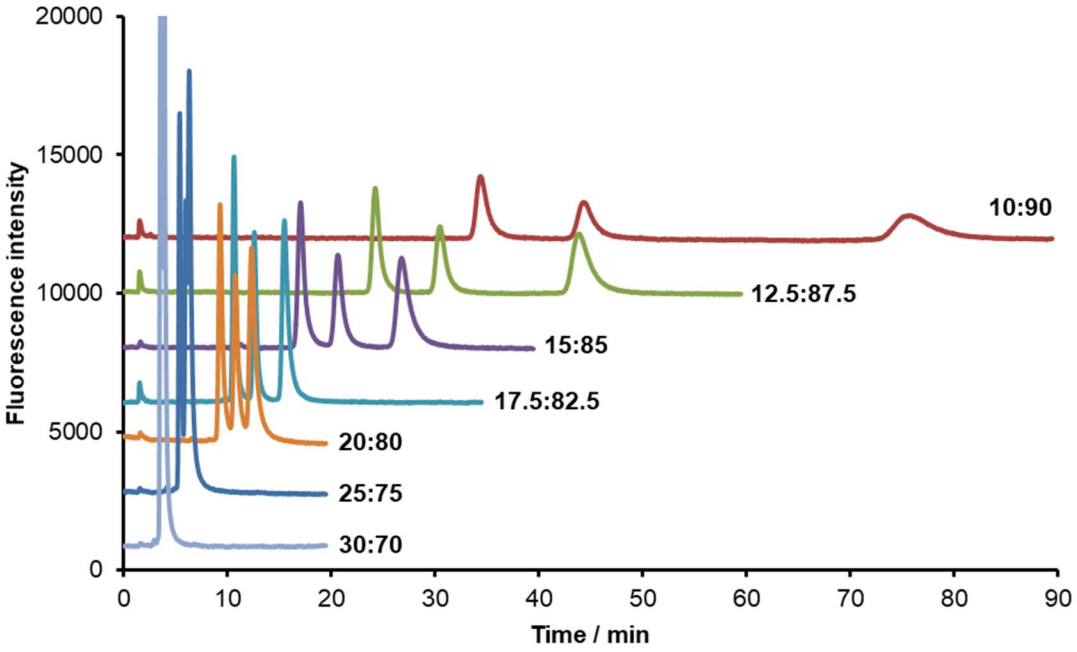

Figure S4. Effect of mobile phase composition on elution of norfloxacin (first peak), ciprofloxacin (second peak) and enrofloxacin (third peak) using different amounts of methanol-phosphoric acid (pH 3.0; 5.0 mM). Flow rate was kept at 1.0 mL min^{-1}, injection of 20 µL of 10 mg L^{-1} solution (in each fluoroquinolone).

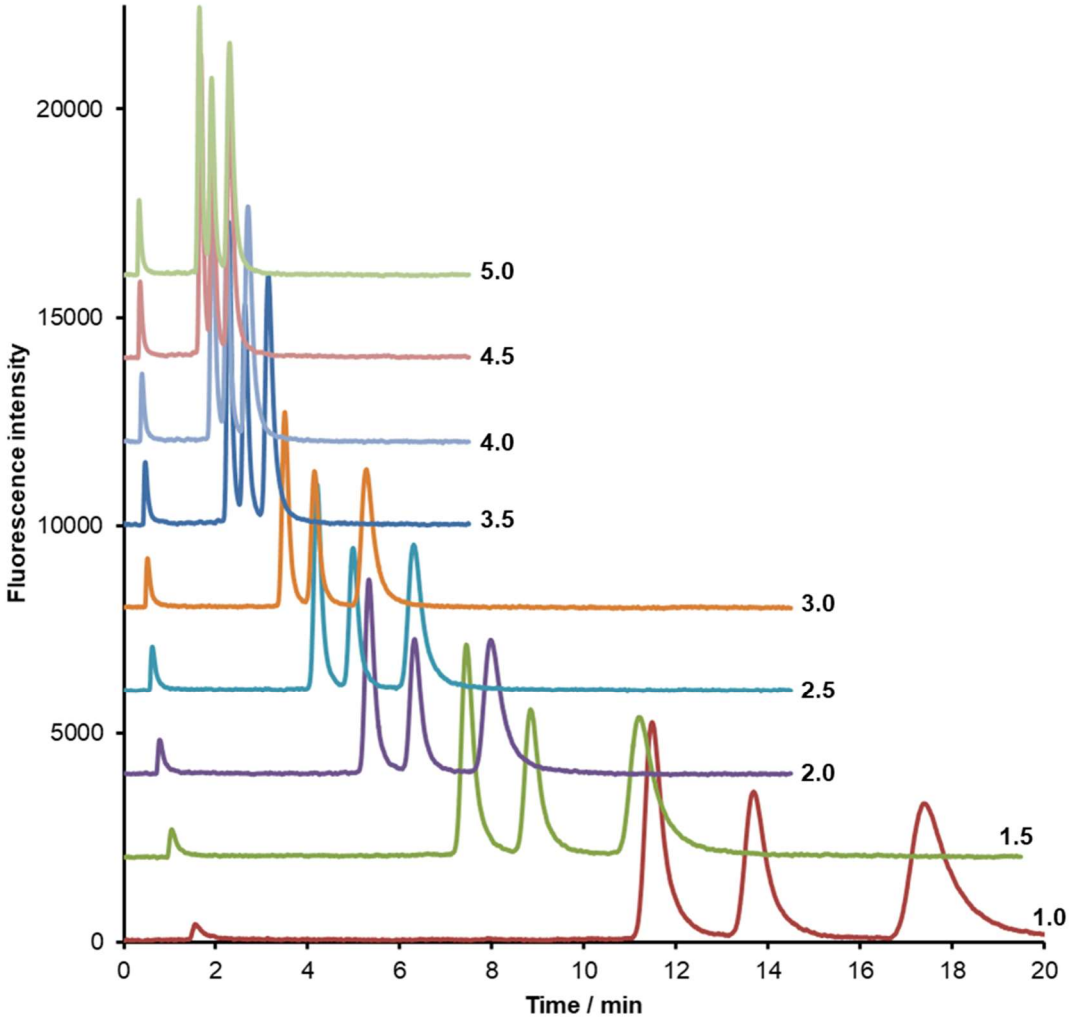

Figure S5. Effect of mobile phase flow rate on elution of norfloxacin (first peak), ciprofloxacin (second peak) and enrofloxacin (third peak). Mobile phase was methanol-phosphoric acid (pH 3.0; 5.0 mM) (17.5:82.5, v/v), for injection of 20 µL of 10 mg L^{-1} solution (in each fluoroquinolone).

Table S1. LOD and LOQ values for chromatographic and MSFIA-SPE procedures

	HPLC method[a] / μg L^{-1}		MSFIA-SPE-HPLC method[b] / ng L^{-1}	
	LOD	LOQ	LOD	LOQ
Norfloxacin	0.03	0.09	13	35
Ciprofloxacin	0.06	0.2	19	51
Enrofloxacin	0.04	0.1	6	17

[a] values for injection volume of 100 μL

[b] values for loading sample volume of 100 mL