Supplementary material

- Validation of the HPLC isocratic method: Calibration and validation solution preparation

  - ✓ Calibration standard solutions preparation (CS)

20mg of ciprofloxacin hydrochloride monohydrate RS and 20 mg of metronidazole base RS from Tokyo Chemical Industry (Belgium) were weighed in a 50 mL volumetric flask. These reference substances were dissolved with 20 mL of diluent (0.025 M orthophosphoric acid buffer adjusted to pH 2 with triethylamine/methanol in 75/25 v/v). The resulting solution was shaken for 10 minutes in an ultrasonic bath to reach the complete dissolution. The volumetric flask was then filled up with the diluent and shaken mechanically. The obtained stock solution contained 400 µg/mL of ciprofloxacin RS and 400 µg/mL of metronidazole RS.

Different dilutions of this stock solution have been made in order to obtain calibration solutions of the following concentrations:

- Level 1 (50%): 8µg/mL of Ciprofloxacin RS and 8µg/mL of Metronidazole RS;
- Level 3 (100%): 16µg/mL of Ciprofloxacin RS and 16µg/mL of Metronidazole RS;
- Level 5 (150%): 24µg/mL of Ciprofloxacin RS and 24µg/mL of Metronidazole RS.

  - ✓ Validation standard solutions preparation (VS)

The validation solutions were prepared to mimic as closely as possible the composition of ciprofloxacin and metronidazole tablets available on the Cameroonian market. Individual solutions were prepared using a mixture of powdered ciprofloxacin RS, powdered metronidazole RS and powdered excipients mixture (constituting the matrix). This mixture was composed of Starch (28.10%), croscarmellose (18.20%), povidone (14.80%), hypromellose (14.15%), polyethylene glycol 20000 (7.70%), lactose (8.85%), and sodium starch glycolate (8.20%). to mimic the composition of the real samples available on the Cameroonian market.

Individual weighing was done in 50 mL volumetric flasks, in order to obtain the following concentration levels:

- Level 1 (50%): 20mg of Ciprofloxacin RS + 20mg of Metronidazole RS+110mg of excipients mixture;
- Level 2 (75%): 30mg of Ciprofloxacin RS + 30mg of Metronidazole RS +90mg of excipients mixture;
- Level 3 (100%): 40mg of Ciprofloxacin RS + 40mg of Metronidazole RS +70mg of excipients mixture;
- Level 4 (125%): 50mg of Ciprofloxacin RS + 50mg de Metronidazole RS + 50mg of excipients mixture;
- Level 5 (150%): 60mg of Ciprofloxacin RS + 60mg de Metronidazole RS + 30mg of excipients mixture.

These powder mixtures were dissolved with 20 mL of diluent (0.025M ortho phosphoric acid buffer adjusted to pH 2 with triethylamine/methanol in 75/25 ratio). The obtained solutions were shaken for 10 minutes in an ultrasonic bath. The volumetric flasks were then filled up with the diluent and shaken mechanically. Stock solutions were obtained at the following concentrations for Ciprofloxacin RS and Metronidazole RS:

- Level 1 (50%): 400µg/mL of Ciprofloxacin RS and 400µg/mL of Metronidazole RS;
- Level 2 (75%): 600µg/mL of Ciprofloxacin RS and 600µg/mL of Metronidazole RS;
- Level 3 (100%): 800µg/mL of Ciprofloxacin RS and 800µg/mL of Metronidazole RS;
- Level 4 (125%): 1000µg/mL of Ciprofloxacin RS and 1000µg/mL of Metronidazole RS;
- Level 5 (150%): 1200µg/mL of Ciprofloxacin RS and 1200µg/mL of Metronidazole RS.

After dilution, the final solutions were obtained at the following concentrations:

- Level 1 (50%): 8µg/mL of Ciprofloxacin RS and 8µg/mL of Metronidazole RS;
- Level 2 (75%): 12µg/mL of Ciprofloxacin RS and 12µg/mL of Metronidazole RS;
- Level 3 (100%): 16µg/mL of Ciprofloxacin RS and 16µg/mL of Metronidazole RS;

- Level 4 (125%): 20µg/mL of Ciprofloxacin RS and 20µg/mL of Metronidazole RS;
- Level 5 (150%): 24µg/mL of Ciprofloxacin RS and 24µg/mL of Metronidazole RS.

3 replicates were prepared for each concentration level (5 concentration levels for the validation solutions and 3 concentration levels for the calibration solutions). The final solutions were filtered through a 0.45µ PTFE syringe filter system and then brought to HPLC for analysis.

✓ Sample solutions preparation

Three tablets were powdered and a quantity of powder corresponding to 40mg of Ciprofloxacin or Metronidazole was weighed into a volumetric flask and diluted with the diluent to obtain a target concentration of 16µg/mL. The final solutions were filtered through a 0.45µ PTFE syringe filter system and then brought to HPLC for analysis. Three replicates were performed per sample.

Table S1: Chromatographic parameters

Chromatographic parameters	USP Method (Ciprofloxacin)	Adapted method validated
Stationary phase	4.6*250mm C18, 5µ	X-BRIDGE 2.1*100mm C18, 3.5µ
Mobile phase	0.025M orthophosphoric acid adjusted to pH3 with Triethylamine /Acetonitrile (87/13) (v/v)	0.025M orthophosphoric acid adjusted to pH3 with Triethylamine and Methanol (75:25) (v/v)
Diluent	0.025M orthophosphoric acid adjusted to pH2 with Triethylamine /Acetonitrile (87/13) (v/v)	0.025M orthophosphoric acid adjusted to pH2 with Triethylamine and Methanol (75:25) (v/v)
Oven temperature	30°C	30°C
Flow	1.5mL/min	0.3mL/min
Injection volume	10µL	5µL
Wavelength	278nm (Ciprofloxacin)	278nm (Ciprofloxacin), 254nm (Metronidazole)
Analysed API	Ciprofloxacin	Ciprofloxacin and Metronidazole

Table S2 : Validation parameters

Validation parameters	LPAC (Liège)	LANACOME (Yaoundé)
Number of series	6	3
Number of replicates	3	3
Number of concentration levels	5	5
Number of operators	2	1
Apparatus	HPLC Alliance DAD Waters (Waters, Eschborn, Germany)	HPLC DAD Agilent (Agilent Technologies, Santa Clara, CA, USA)

Table S3: Validation data in LPAC

	Concentration levels (µg/ml)	Ciprofloxacin	Metronidazole
Trueness: relative bias (%)	8	-0.28	-1.26
	12	-1.09	-0.66
	16	-1.26	-1.28
	20	-0.66	-1.06
	24	-0.99	-1.07
Intra-assay precision: repeatability (RSD%) /Between assay precision (RSD%)	8	1.47/2.41	0.90/2.22
	12	0.68/1.31	0.80/1.11
	16	0.99/1.92	1.08/1.75
	20	0.97/1.96	1.08/1.92
	24	0.99/2.23	1.03/1.91
Accuracy: relative β-expectation limits (%)	8	[-5.95 , 5.38]	[-6.93 , 4.42]
	12	[-4.280 , 2.088]	[-3.13 , 1.82]
	16	[-5.97 , 3.46]	[-5.39 , 2.83]
	20	[-5.52 , 4.20]	[-5.66 , 3.55]
	24	[-6.60 , 4.61]	[-5.69 , 3.55]
Linearity	Slope	0.9891	0.9889
	Intercept	0.0283	0.0075
	R^2	0.9966	0.9972
Dosing range		(8.098 - 24.07)	(8.14 - 24.13)
Type of model: linear regression			

Table S4: Validation data in LANACOME

	Concentration levels (µg/ml)	Ciprofloxacin	Metronidazole
Trueness: relative bias (%)	8	-0.46	-0.20
	12	-0.78	-0.08
	16	-0.30	-0.59
	20	0.66	-0.53
	24	0.75	-0.38
Intra-assay precision: repeatability (RSD%) /Between assay precision (RSD%)	8	0.37/0.52	0.77/0.77
	12	0.61/0.67	0.44/0.48
	16	1.28/1.31	0.81/0.97
	20	0.39/0.78	0.43/0.54
	24	0.45/0.61	0.25/0.34
Accuracy: relative β-expectation limits (%)	8	[-1.86 , 0.94]	[-1.87 , 1.46]
	12	[-2.30 , 0.75]	[-1.18 , 1.02]
	16	[-3.17 , 2.57]	[-2.91 , 1.73]
	20	[-2.01 , 3.34]	[-1.90 , 0.84]
	24	[-0.86 , 2.36]	[-1.30 , 0.54]
Linearity	Slope	1.0170	0.9938
	Intercept	-0.2392	0.0363
	R^2	0.9994	0.9997
Dosing range		(8.07 - 24.08)	(8.23 - 24.20)
Type of model: linear regression			

Table S5: List of samples by stated manufacturer and compliance with assay and disintegration tests

Stated continent of origin	Stated country of origin	Manufacturing company code	INN	Number of batches	N samples	N compliant	N Non-compliant disintegration	N Non-compliant assay (HPLC)
Africa	Cameroon	A	Ciprofloxacin	5	11	11	0	0
			Metronidazole	15	40	40	0	0
		B	Ciprofloxacin	1	4	4	0	0
	Senegal	C	Metronidazole	11	12	12	0	0
	Morocco	D	Metronidazole	4	5	5	0	0
	Nigeria	E	Ciprofloxacin	1	1	0	0	1
		F	Ciprofloxacin	3	3	0	0	3
America	USA	G	Ciprofloxacin	2	5	5	0	0
Europe	Germany	H	Ciprofloxacin	6	7	7	0	0
		I	Ciprofloxacin	3	11	11	0	0
			Metronidazole	1	3	3	0	0
	Austria	J	Metronidazole	1	3	3	0	0
		K	Ciprofloxacin	1	1	1	0	0
	Spain	L	Ciprofloxacin	1	1	1	0	0
	France	M	Metronidazole	9	18	18	0	0
	United Kingdom	N	Ciprofloxacin	1	3	3	0	0
		O	Ciprofloxacin	2	5	5	0	0
		P	Ciprofloxacin	1	1	0	1	1
			Metronidazole	1	2	2	0	0
		Q	Ciprofloxacin	1	2	2	0	0
		R	Ciprofloxacin	1	8	3	5	0
		S	Ciprofloxacin	1	1	1	0	0
Asia	China	T	Metronidazole	2	4	4	0	0
		U	Ciprofloxacin	4	4	4	0	0
		V	Metronidazole	1	6	6	0	0
		W	Ciprofloxacin	1	1	1	0	0
		X	Ciprofloxacin	1	1	1	0	0
		Y	Metronidazole	1	1	1	0	0
		Z	Metronidazole	1	7	7	0	0
			Ciprofloxacin	1	1	1	0	0
		AA	Ciprofloxacin	2	4	4	0	0
			Ciprofloxacin	2	2	2	0	0
			Metronidazole	3	4	4	0	0
		AB	Ciprofloxacin	1	1	1	0	0
		AC	Ciprofloxacin	1	1	1	0	0
	India	AD	Metronidazole	2	3	3	0	0
		AE	Ciprofloxacin	1	4	4	0	0
			Metronidazole	1	7	2	5	0
		AF	Ciprofloxacin	3	6	6	0	0
		AG (1)	Ciprofloxacin	1	1	1	0	0
		AG (2)	Ciprofloxacin	1	1	1	0	0
		AH	Ciprofloxacin	4	4	4	0	0
		AI	Ciprofloxacin	4	8	8	0	0

		AJ	Ciprofloxacin	1	1	1	0	0
		AK	Ciprofloxacin	2	2	2	0	0
		AL	Ciprofloxacin	1	1	1	0	0
		AM	Ciprofloxacin	3	3	3	0	0
		AN	Ciprofloxacin	1	1	1	0	0
		AO	Ciprofloxacin	2	7	7	0	0
		AP	Ciprofloxacin	1	1	1	0	0
		AQ	Ciprofloxacin	1	1	1	0	0
		AR	Ciprofloxacin	2	2	2	0	0
			Metronidazole	1	1	0	1	0
		AS	Ciprofloxacin	4	5	5	0	0
			Metronidazole	4	6	6	0	0
		AT	Ciprofloxacin	1	1	0	0	1
		AU	Ciprofloxacin	1	2	2	0	0
		AV	Ciprofloxacin	3	4	4	0	0
		AW	Ciprofloxacin	4	6	6	0	0
			Ciprofloxacin	1	2	2	0	0
		AX	Ciprofloxacin	1	1	1	0	0
			Metronidazole	1	7	6	1	0
	Turkey	AY	Ciprofloxacin	3	4	4	0	0
Unknown	Unknown	AZ	Ciprofloxacin	1	2	2	0	0
			Metronidazole	3	13	13	0	0
			Ciprofloxacin	1	1	1	0	0
Total				**153**	**292**	**274**	**13**	**6**

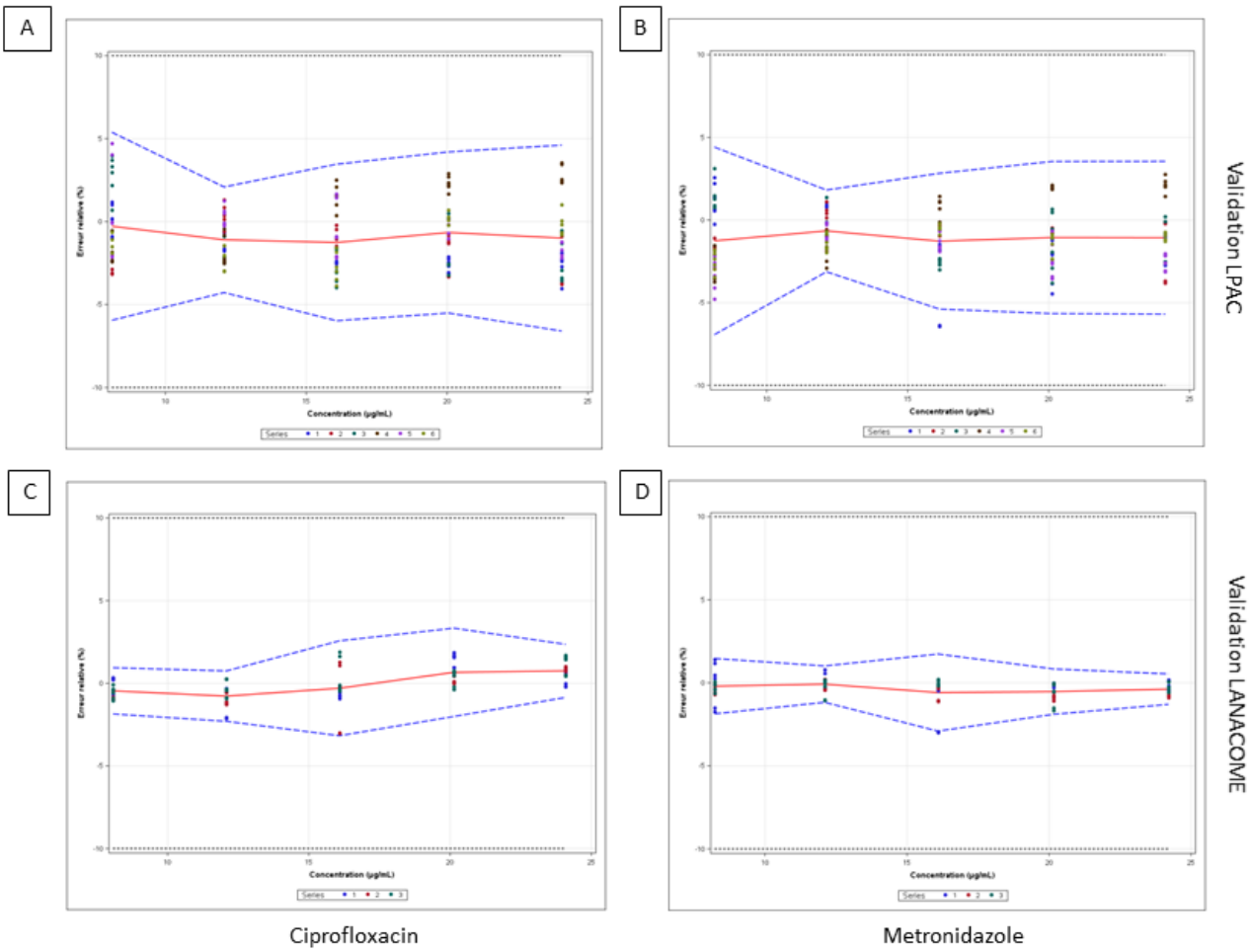

Figure S1: A) and B) Accuracy profiles of Ciprofloxacin and metronidazole respectively, obtained at LPAC, C) et D) Accuracy profiles of Ciprofloxacin and metronidazole respectively, obtained at LANACOME.

Legend: The plain red line is the relative bias, the dashed blue lines are the β-expectation tolerance limits, and the dashed black-lines represent the acceptance limits. The dots represent the relative error of the results and are plotted with respect to their targeted concentration.

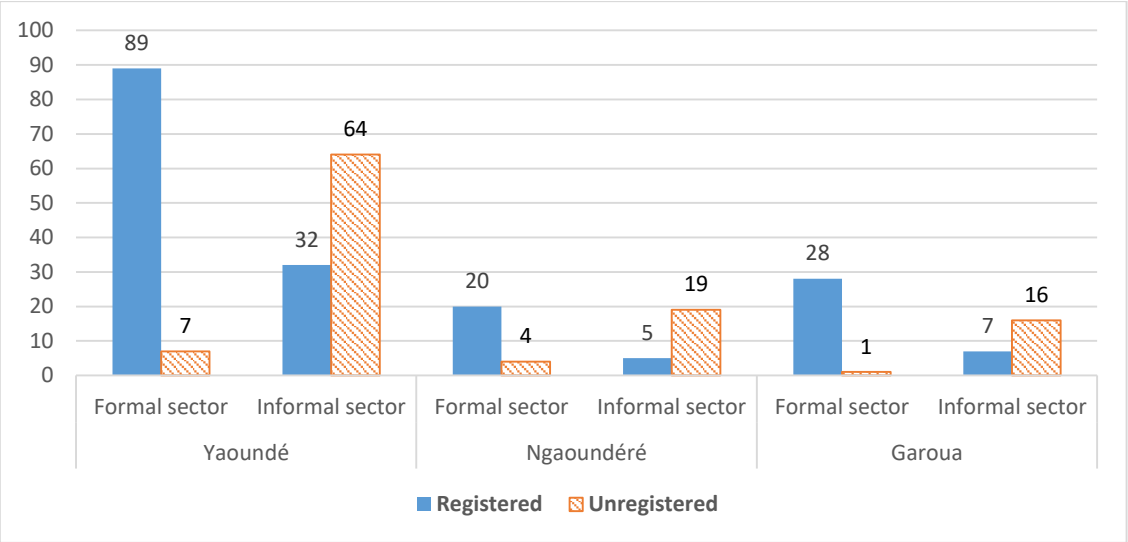

Figure S2: Distribution of samples according to their registration status and city of collection

Figure S3: Pictures of samples presenting irregularities on visual inspection test.

<u>Legend</u>: (a): Ciprofloxacin sample repackaged, (b): Lack of information about manufacturing laboratory either on primary and secondary packaging, (c): Presence of stains on some tablets, (d): Presence of a half tablet in an intact blister, (e): Internal packaging cut in two parts, (f): Sample having a piece of sticky paper with a batch number, manufacturing and expiry dates corresponding to those on the blister (BN:MP8658, Manufacturing date: 12/2018, Expiry date: 11/2022), pasted over the printed batch number, manufacturing, and expiry dates different from the first ones on the secondary packaging (BN:MP9208, Manufacturing date: 03/2019, Expiry date:02/2023).

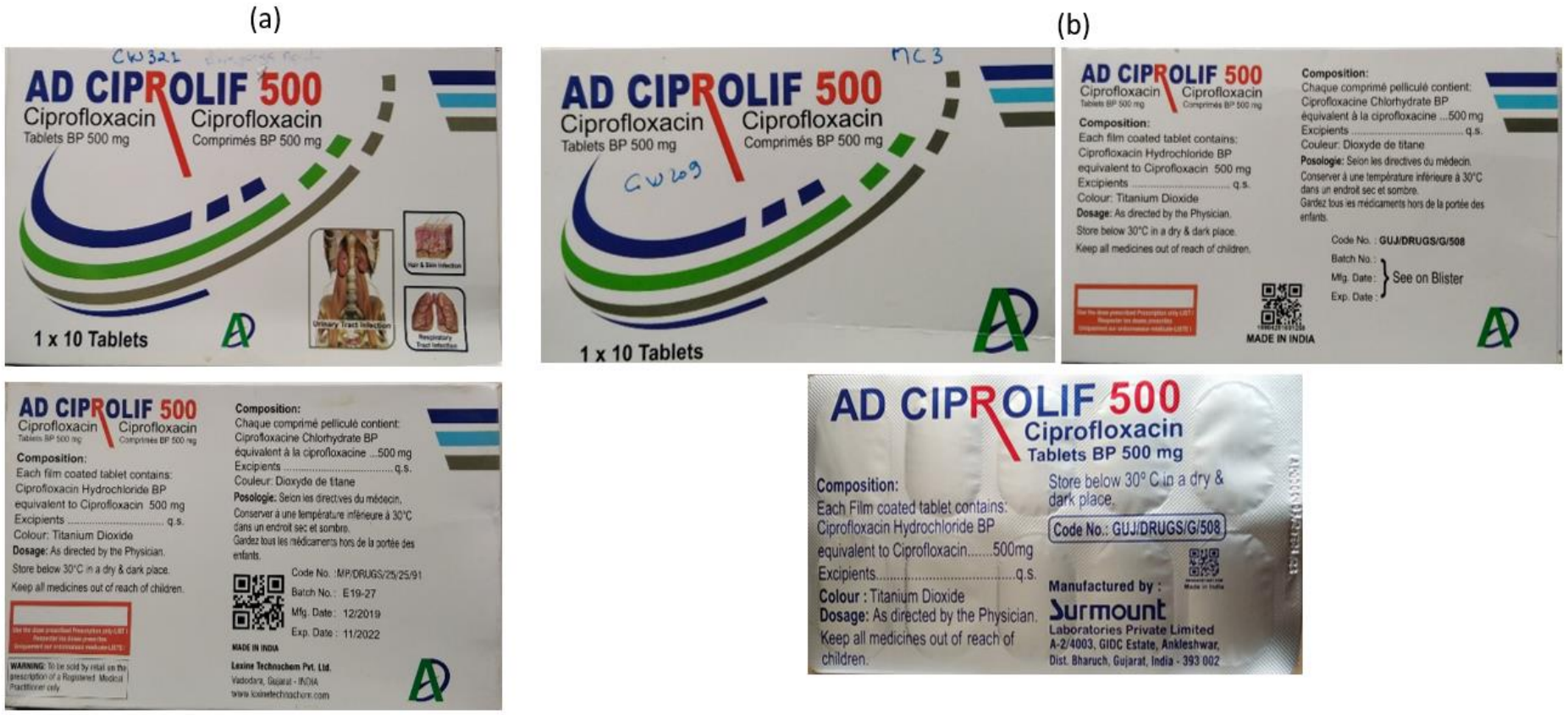

Figure S4: Pictures of samples presenting same brand names with different manufacturing companies.
Legend: (a) had as stated manufacturing company Lexine Technochem and (b) Surmount Laboratories

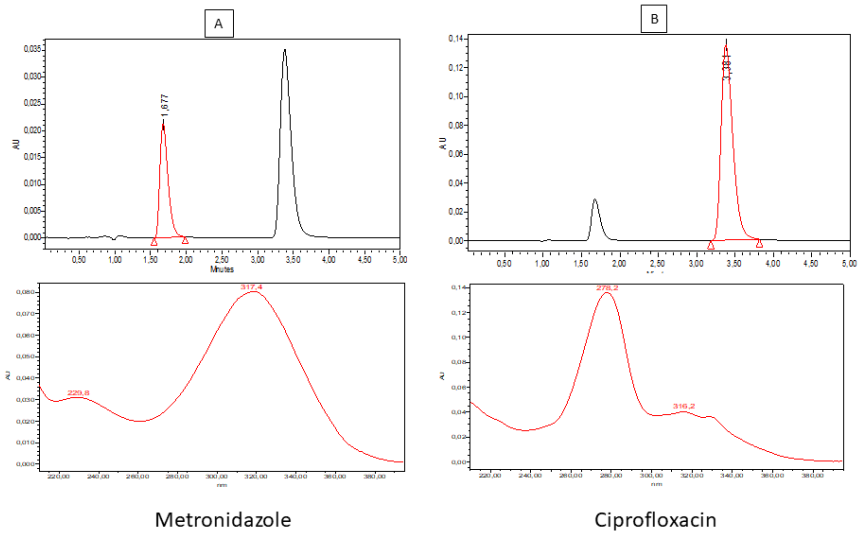

Figure S5: A) and B) Chromatograms of a mixture of ciprofloxacin CRS and metronidazole CRS.

Legend: A was recorded at 254 nm (metronidazole, retention time=1.7min), and B at 278 nm (ciprofloxacin, retention time=3.4min).

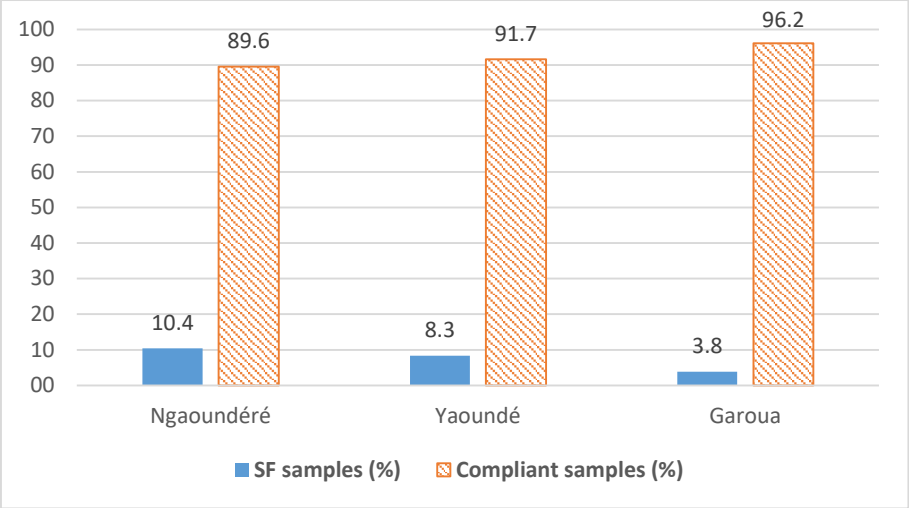

Figure S6: Prevalence of SF samples according to the city of sampling

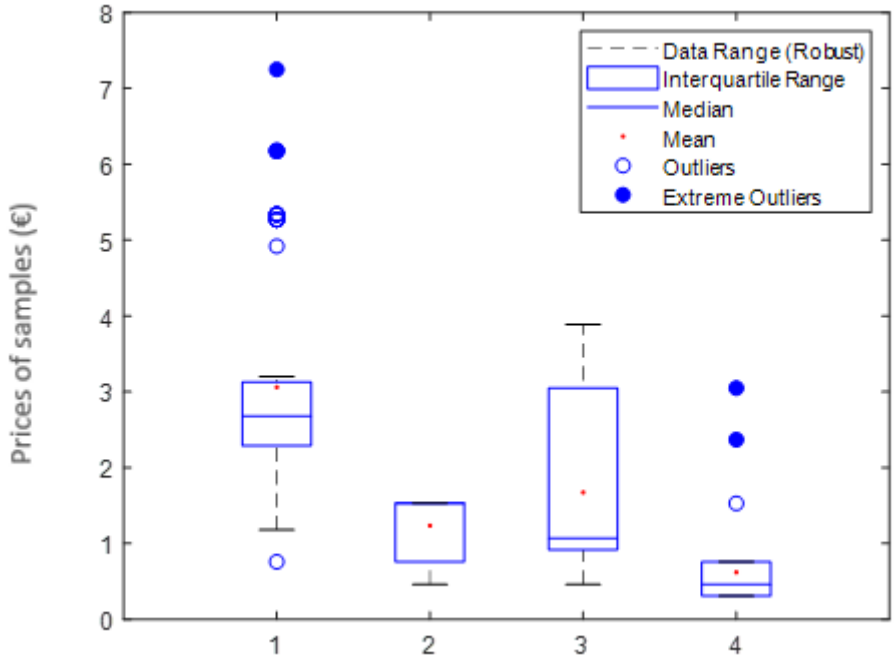

Figure S7: Boxplot of sample's price according to API and sampling sector.
Legend: 1=Ciprofloxacin prices in the formal sector; 2=Ciprofloxacin prices in the informal sector; 3=Metronidazole prices in the formal sector; 4=Metronidazole prices in the informal sector; €=Euros