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Generic ciprofloxacin tablets contain the stated amount of drug and different impurity profiles: A ^{19}F, ^{1}H and DOSY NMR analysis

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Abstract

The objective of this study was to control the purity of 16 commercial formulations of ciprofloxacin tablets purchased in different countries or via the Internet using ^{19}F and ^{1}H nuclear magnetic resonance (NMR). Twelve out of the sixteen commercial formulations of ciprofloxacin measured by ^{19}F NMR contain the active ingredient within $100 \pm 5\%$ of stated concentration. Three formulations have a lower ciprofloxacin content between 90 and 95% and one shows a higher concentration superior to 105%. The impurity profile was characterised using ^{19}F and ^{1}H NMR, and is characteristic of the manufacturer. Four to twelve fluorinated impurities among them fluoride ion and two already known compounds were detected and quantified in the sixteen formulations analysed by ^{19}F NMR. Two other non-fluorinated impurities were observed in the seven formulations analysed with ^{1}H NMR. The total content of impurities as well as their individual levels are in agreement with those reported previously in the few studies devoted to ciprofloxacin purity. However, all the formulations do not comply with the limits for impurities given in the ciprofloxacin monograph of the European Pharmacopeia. Finally, a "signature" of the formulations was obtained with Diffusion-Ordered SpectroscopY (DOSY) ^{1}H NMR which allowed the characterisation of some excipients present in the formulations studied.

Keywords: ^{19}F NMR; ^{1}H NMR; DOSY ^{1}H NMR; Ciprofloxacin; Impurities

1. Introduction

Medicines need to be safe, effective, and of good quality in order to produce the desired effect. Generic drugs allow access to affordable treatment for people living in poor countries. Also, in wealthier countries, when prices of medicines are high and price differentials between identical products exist, there is a greater incentive for the consumer to seek medicines outside the normal supply system. If one buys a drug via the Internet, he never knows where it has been manufactured and if quality controls have been done on the medicine.

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Fig. 1) is an essential synthetic antibiotic belonging to a group called fluoroquinolones as it bears a fluorine atom at position 6 of the 4-quinolone nucleus. It has a broad spectrum of antimicrobial activity and remains effective in a wide variety of indications.

There is a strong interest in determining fluoroquinolone purity for the purpose of pharmaceutical quality control. The published analytical methods for the analysis of ciprofloxacin and its impurities in pharmaceutical formulations are based on HPLC, which is also recommended by European and US Pharmacopoeias [1–4], capillary electrophoresis [5,6] and HP TLC [7].

Fardella et al. [8] applied ^{1}H and ^{19}F NMR spectroscopy to assay three widely used fluoroquinolones (pefloxacin, norfloxacin, and ofloxacin) in some commercial preparations. They showed that the method is accurate and precise, and provides a rapid and specific procedure with little sample preparation. Taking advantage of the resolving power of ^{1}H NMR, an analytical procedure to directly measure ciprofloxacin in the presence of metronidazol and ampicillin without separation steps has been reported by Reinscheid [9]. Only the assay of the active principle ciprofloxacin was described in the two papers from Fardella et al. and Reinscheid [8,9].

We decided to conduct a study to control the purity of ciprofloxacin tablets purchased in different countries or via the Internet. Indeed, under-concentration of the antibiotic may have an impact on clinical response and lead to the selection of

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resistant bacteria in patients. We were also interested in the impurity profiles and their quantitation. We thus analysed 16 commercial formulations of ciprofloxacin tablets using ^{19}F and ^{1}H nuclear magnetic resonance (NMR). We also used Diffusion-Ordered ^{1}H NMR SpectroscopY (^{1}H NMR DOSY) to compare some of these formulations in terms of excipients in order to get a "spectral signature" of the formulation and its manufacturer. To the best of our knowledge, this is the first study that thoroughly checked the quality of numerous pharmaceutical formulations of ciprofloxacin.

2. Experimental

2.1. Reagents

Pure ciprofloxacin hydrochloride was purchased from European Pharmacopoeia (Strasbourg, France). Impurities certified reference standard (CRS) (Fig. 1) A (fluoroquinolonic acid, 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid), B (1-cyclopropyl-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid), C (ethylenediamine analog, 7-[(2-aminoethyl)-amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-6-(1-piperazinyl)-3-quinoline carboxylic acid), and D (7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-3-quinoline carboxylic acid) were obtained from the WHO Collaborating Center (Stockholm, Sweden). Chromium (III) acetylacetonate ($Cr(acac)_3$) was obtained from Aldrich (Sigma–Aldrich, Saint-Quentin Fallavier, France), 3-chloro-4-fluoroaniline from Avocado (Heysham, England), 1,3-dichloro-4-fluorobenzene, and 2,4-dichloro-5-fluoroacetophenone from AlfaAesar (Heysham, England).

2.2. Commercial formulations of ciprofloxacin

Sixteen ciprofloxacin commercial formulations were analysed, one being the brand formulation from BAYER (Ciflox®), the other fifteen being generic drugs from different countries (Table 1). The amount of ciprofloxacin in the various formulations was 250 mg (number **1**–**10**) or 500 mg (number **11**–**16**). All samples, as received, were stored in the dark at ambient temperature and humidity. They were all analysed within expiry dates.

2.3. Treatment of samples

2.3.1. For ^{19}F NMR analysis

According to the procedure described by Novakovic et al. [7], a 250 mg tablet was powdered and transferred to a 100 mL

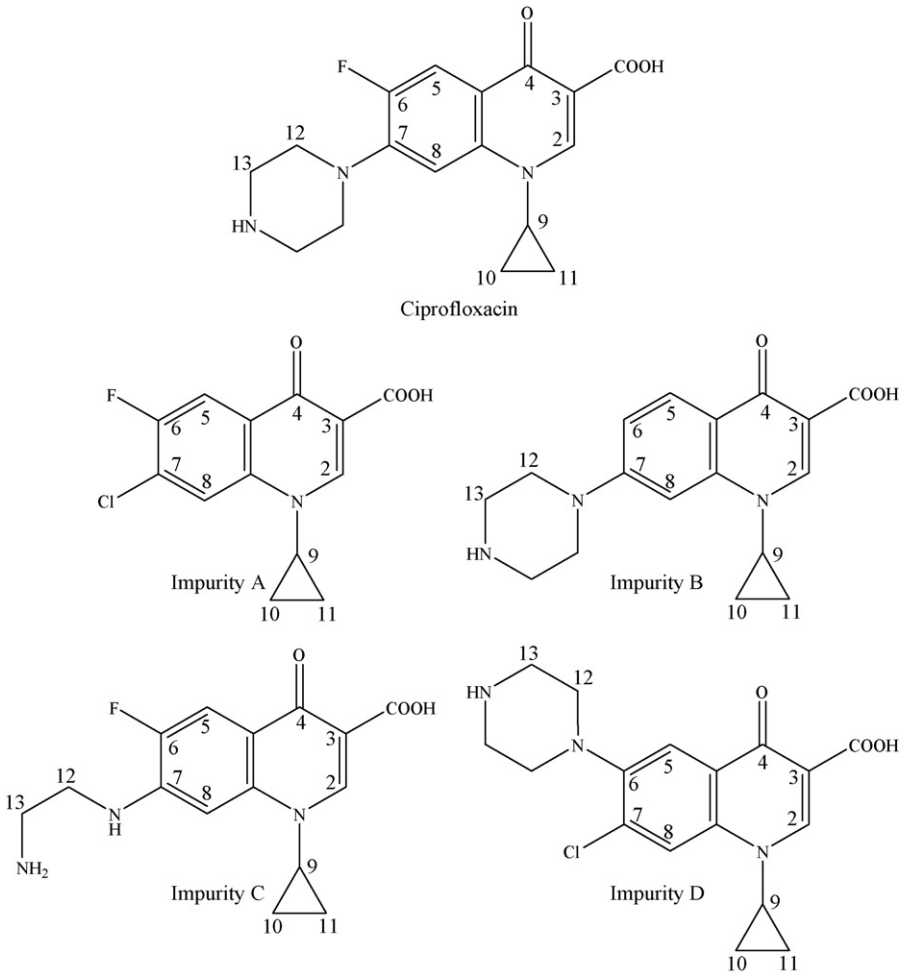

Fig. 1. Structure of ciprofloxacin and its main impurities.

Table 1
Ciprofloxacin commercial formulations analysed in this study

Formulation name	Batch number	Expiry date	Manufacturer name	Country of manufacturing
1 Ciflox 250 mg	DR19	04/2009	Bayer	Germany
2 Ciprofloxacine Biogaran 250 mg	81931	06/2006	Delta Limited	Island
3 Cipflox 250	T-013/1	04/2005	Pacific Pharmaceuticals Ltd.	New Zealand
4 Ciprocin 250	8	09/2007	Racha Lab	Syria
5 Sipro 250	1135	03/2006	Asia pharmaceutical industries	Syria
6 Ceproz-250	001CB	07/2006	Elsaad Pharmaceuticals	Syria
7 Ciprofloxacine USP 250 mg	MF-207	08/2007	Micro Nova Pharmaceuticals Limited	India
8 Ciprofloxacin hydrochloride tablets USP	21214	11/2005	FDC Limited	India
9 Medociprin 250	A8K029	10/2007	Medochemie Ltd.	Cyprus
10 Neocip-250	MP3181	10/2006	Okasa Ltd.	India
11 Ciprofloxacin 500 mg	CIF-043	06/2007	Brown & Burk Pharmaceutical Ltd.	India
12 Ciplox-500	G45073	09/2007	Cipla Ltd.	India
13 Medociprin 500	A8K032	10/2007	Medochemie Ltd.	Cyprus
14 Cuminol® 500 mg	06050507	06/2008	Gedeon Richter	Romania
15 Cifran 500 mg	1454183	10/2007	Ranbaxy Laboratories Limited	India
16 Ciprinol 500 mg	N08056	09/2009	KRKA	Slovenia

volumetric flask. The content was dissolved in about 50 ml of water under magnetic stirring during 15 min and sonicated for 10 min. The suspension was then diluted to 100 mL and an aliquot (3 mL) was centrifuged (10 min, 3000 rpm). The supernatant was doped with 2.5 mg of $Cr(acac)_3$ and its pH measured prior to NMR analysis. The pH of the solutions from the different formulations was 4.75 ± 0.25. We checked that these experimental conditions led to a complete extraction of ciprofloxacin from the formulation. The residual pellet was washed twice with 5 mL H_2O and the supernatants were assayed for ciprofloxacin. The first one contained 0.15% and the second 0.05% of nominal ciprofloxacin.

For the determination of impurities, an analogous procedure was used except that the final volume was 30 mL in order to enhance their concentration in the solution analyzed and so the signal-to-noise ratio in the ^{19}F NMR spectra. The pH of the solutions from the different formulations was 4.4–4.5. To ensure that all the impurities were extracted in 30 mL, the pellet from two experiments was washed twice with 5 mL H_2O and pooled supernatants were analyzed with ^{19}F NMR. No impurity was detected in the two samples analysed.

For a correct quantitation of fluoride ion (F^-), the pH was adjusted to 5.0–5.2 which allows separating the tiny F^- signal from the huge resonance of ciprofloxacin that shifts upfield of ≈0.2 ppm.

500 mg tablets were treated in the same experimental conditions except that the final volume was 200 mL for the assay of ciprofloxacin and 60 mL for the determination of impurities.

For the assay of ciprofloxacin, 3–7 coated tablets from each formulation were analyzed, whereas 3 or 4 tablets from each formulation were used for the quantitation of impurities.

2.3.2. For ^1H NMR analysis

Each tablet was powdered and dissolved in 5 mL of D_2O under magnetic stirring during 15 min then sonicated for 10 min. The suspension was then centrifuged (10 min, 3000 rpm) and the supernatant pH measured prior to NMR analysis.

2.3.3. For DOSY NMR analysis

One tablet of the formulation **1**, **3**, **5**, **7**, or **11** was powdered and stirred with 5 mL of a mixture of CD_3CN/D_2O (80/20) during 30 min. The suspension was then sonicated for 10 min and centrifuged (10 min, 3000 rpm). The supernatant was analysed.

2.4. NMR analysis

2.4.1. ^{19}F NMR

^{19}F NMR spectra were recorded at 282.4 MHz with inverse-gated ^1H-decoupling on a Bruker WB-AM 300 spectrometer (Bruker SA, Wissembourg, France) using 10-mm diameter NMR tube. The recording conditions were: probe temperature, 25 °C; sweep width 29411 Hz; 32768 data points zero-filled to 65536; pulse width, 7 μs (flip angle ≈40°); pulse interval, 3.6 s; number of scans, 2500 for ciprofloxacin quantitation (≈150 min) and 15,000–18,000 for impurity determination (≈15–18 h); line broadening caused by exponential multiplication, 3 Hz. Phase and baseline corrections were done manually. The chemical shifts (δ) were reported relative to the resonance peak of CF_3COOH (5% w/v aqueous solution) used as external chemical shift reference ($\delta = 0$ ppm).

The concentration of fluorinated compounds were measured by comparing the expanded areas of their respective NMR signals with that of the external standard for quantification placed in a coaxial capillary, namely a solution of sodium parafluorobenzoate (FBEN) in D_2O doped at saturation (≈3 mmol L^{-1}) with $Cr(acac)_3$, the paramagnetic agent used to shorten its T_1 relaxation time. The apparent concentration of the FBEN reference (2.54×10^{-4} mol L^{-1}) was previously measured against solutions of CRS ciprofloxacin of known concentrations under the recording conditions described above. The areas were determined using Bruker WinNMR software. Each data is the mean of at least five integrations. To check that the NMR conditions used allowed an accurate quantitation of ciprofloxacin, its T_1

relaxation time was measured using the inversion-recovery method. A value of 0.2 s was found for an aqueous solution of ciprofloxacin (2.34×10^{-2} mol L^{-1}) doped at saturation with $Cr(acac)_3$.

The limit of detection with our spectrometer after 15–18 h recording is ≈2 μmol L^{-1} at a signal-to-noise (S/N) ratio of 3, the S/N ratio being [2.5(peak height/noise height measured peak-to-peak)]. The accuracy of the assay is ±2% for a concentration of 8×10^{-3} mol L^{-1} and <±4% for a concentration between 10^{-5} and 8×10^{-3} mol L^{-1}.

2.4.2. ^1H and DOSY NMR

^1H NMR experiments were performed on a Bruker AVANCE 500 spectrometer operating at 500.13 MHz equipped with a 5 mm proton cryoprobe on 600 μL samples. The spectra were recorded in D_2O with all chemical shifts (δ) referred to an internal trimethylsilylpropane sulfonic acid (TMPS) reference. Typical acquisition parameters for the ^1H NMR experiments were as follows: probe temperature, 25 °C; sweep width 10,000 Hz; 32768 data points zero-filled to 65536; pulse width, 3 μs (flip angle ≈35°); pulse interval, 3.6 s; number of scans, 128. Spectra were acquired with a classical water suppression sequence using selective irradiation for eliminating residual water signal from D_2O. The 2D NMR experiments (gCOSY, gHSQC, gHMBC) were acquired using standard Bruker sequences. The concentrations of the impurities were determined by comparing the area of a selected proton signal with that of the H2 of ciprofloxacin.

For DOSY ^1H NMR, stimulated echo bipolar gradient pulse experiments were used with a pulse delay of 3 ms after each gradient, a pulse field gradient length of 1 ms, and a diffusion delay of 100 ms. Sequence parameters were adapted in order to have the intensity of the H2 NMR signal of ciprofloxacin strongly decreased (at least divided by 50) at 95% of the full gradient strength. Forty experiments were recorded with gradient intensity linearly sampled from 5 to 95%. The gradient system had been calibrated to 52.19 G cm^{-1} at maximum intensity.

All data were processed using Gifa 5.2 software with the inverse Laplace Transform method using the Maximum Entropy algorithm (MaxEnt). The processing parameters were 2048 points along the Laplace spectrum diffusion axis and 20,000 MaxEnt iterations. The inverse Laplace Transform was computed only on the columns presenting a signal 32-times greater than the noise level of the experiment. DOSY spectra are presented with chemical shift on the horizontal axis and diffusion coefficients expressed in $\mu m^2\ s^{-1}$ on the vertical axis.

2.5. HPLC analysis

2.5.1. HPLC apparatus and chromatographic conditions

HPLC was carried out using a Waters 2695 Alliance model with a Waters 2996 diode array detector. The analytical column was a reversed-phase column Luna C18 (100 mm × 3 mm I.D.; 3-μm particle size; Phenomenex, UK). The column temperature was 30 °C. The mobile phase consisted of a volumetric mixture (87:13 v/v) of acetonitrile and a buffer solution (containing 2.45 g L^{-1} phosphoric acid, adjusted to pH 3.3 with triethylamine). The flow rate was 0.6 ml min^{-1} and volume injection was 10 μL. A detection wavelength of 279 nm was chosen for the chromatography according to the absorption spectrum of ciprofloxacin.

2.5.2. Analytical procedure

A calibration curve was constructed from the analysis of four solutions containing pure ciprofloxacin in a concentration range of 0.012–0.072 mg mL^{-1}. Each standard solution was injected in triplicate in the chromatographic system. The linearity ($R^2 > 0.999$) was evaluated by linear regression analysis, which was calculated by the least square regression method.

Solutions of ciprofloxacin tablets (formulations **1** and **9**) were identical to those prepared for the ^{19}F NMR assay. An aliquot was diluted 100-fold with mobile phase before injection. Three determinations were carried out for each solution.

3. Results

3.1. ^{19}F NMR analysis of ciprofloxacin tablets

The data of ciprofloxacin content measured by ^{19}F NMR are reported in Table 2. Twelve out of the sixteen commercial formulations of ciprofloxacin (**1**–**4**, **7**, **9**–**13**, and **15**–**16**) contain the active ingredient within 100 ± 5% of stated concentration. Three formulations have a lower ciprofloxacin content (**5**: 92.8%, **6**: 90.2%, and **8**: 91.0%) and one shows a higher concentration (**14**: 107.3%).

The intra-batch variability is correct as it is <5% for all the tablets except one and even <2% for five formulations. A greater variability was observed for formulation **11**. In one tablet coming from this manufacturer, only 232.0 mg of ciprofloxacin were found, which corresponds to 46.4% of the stated concentration (this value was not included in Table 2).

HPLC was used to cross-validate the data for the two formulations **1** and **9**. The ciprofloxacin contents found by this method were 98.3 ± 1.5% (99.3 ± 2.8% with ^{19}F NMR) for formulation **1** and 97.1 ± 1.0% (95.7 ± 1.4% with ^{19}F NMR) for formulation **9**, showing a good correlation between the two methods.

The profile of fluorinated impurities is characteristic for the manufacturer. ^{19}F NMR spectra of three ciprofloxacin formulations are shown in Fig. 2. Slight pH variations observed for each formulation (pH 4.40–4.50) have no incidence on the ^{19}F NMR chemical shifts. The fluorinated impurities found in each formulation are listed in Table 3. Their structure is unknown except for the signals at −43.7 ppm, −39.7 ppm, and −55.9 ppm that were assigned to F^- and impurities A and C, respectively, by spiking with the authentic standards. 3-Chloro-4-fluoroaniline, 1,3-dichloro-4-fluorobenzene, and 2,4-dichloro-5-fluoroacetophenone were described as by-products of ciprofloxacin synthesis [1]. Small amounts of these fluorinated compounds were added in a solution of the formulation **7** and detected, respectively, at −52.8 ppm, −43.0 ppm,

Table 2
Amount of ciprofloxacin found in 16 commercial formulations

Formulation	Concentration stated (mg/tablet)	Concentration found (mg/tablet)	%	*n*	S.D.
1 Ciflox® Bayer (Germany)	250	248.2	99.3[a]	3	2.8[a]
2 Ciprofloxacin Biogaran (Island)	250	240.8	96.3	4	1.6
3 Cipflox Pacific (New Zealand)	250	249.3	99.7	5	2.5
4 Ciprocin Racha (Syria)	250	249.4	99.8	5	1.5
5 Sipro Asia (Syria)	250	231.9	92.8	5	1.1
6 Ceproz Elsaad (Syria)	250	225.6	90.2	6	3.3
7 Ciprofloxacin Micro Nova (India)	250	248.1	99.3	5	2.8
8 Ciprofloxacin FDC (India)	250	227.4	91.0	7	1.9
9 Medociprin 250 Medochemie (Cyprus)	250	239.2	95.7[b]	5	1.4[b]
10 Neocip Okasa (India)	250	239.6	95.8	4	1.3
11 Ciprofloxacin Brown & Burk (India)	500	507.2	101.4	7	5.7
12 Ciplox Cipla (India)	500	487.2	97.4	5	4.6
13 Medociprin 500 Medochemie (Cyprus)	500	479.5	95.9	5	3.1
14 Cuminol Gedeon Richter (Romania)	500	536.4	107.3	5	3.2
15 Cifran Ranbaxy (India)	500	505.4	101.1	5	2.5
16 Ciprinol KRKA (Slovenia)	500	481.7	96.3	5	3.2

[a] A cross-validation assay by HPLC gave 98.3 ± 1.5%.
[b] A cross-validation assay by HPLC gave 97.1 ± 1.0%.

−41.3 ppm. None of these three fluorinated compounds was detected in the formulations studied.

The amounts of impurities A, C, F^-, and total fluorinated impurities found in all the formulations studied are reported in Table 4. The results are expressed in mol% relative to nominal ciprofloxacin. Total impurity content ranges from 0.3 to 0.8 mol%. Impurity C described as a degradation product of ciprofloxacin [7] is detected in all the formulations studied at a concentration ranging from 0.002 to 0.06 mol%, whereas the amount of impurity A, a by-product from the synthesis route, is higher (0.01–0.3 mol%). Only the formulation from Romania does not contain this impurity. For the determination of F^-, additional spectra were recorded at pH 5–5.2 in order to avoid the overlap of its ^{19}F NMR signal with that of ciprofloxacin. F^- was detected in 11 out of 16 formulations analysed at a low concentration ranging from 0.01 to 0.02 mol%.

3.2. ^1H NMR analysis of ciprofloxacin tablets

^1H NMR spectra of pure ciprofloxacin and impurities A, B, C, and D were recorded and 2D NMR spectra (gCOSY, gHSQC, gHMBC) were used to make assignments. Results are reported in Table 5. The proton H2 is the more deshielded signal for all compounds. In ciprofloxacin and impurities A and C, fluorine

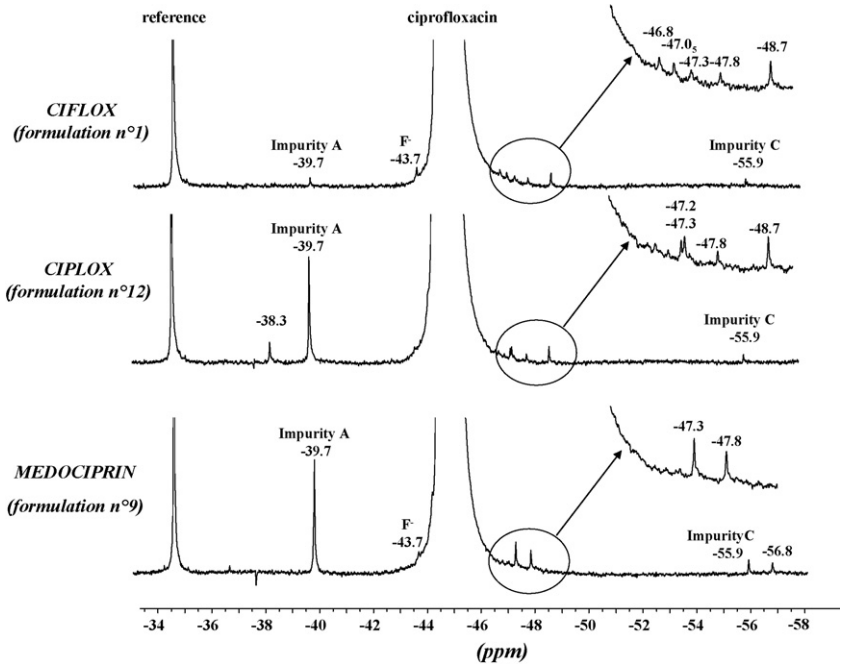

Fig. 2. ^{19}F NMR spectra of three commercial formulations of ciprofloxacin.

Table 3
Profile of fluorinated impurities in 16 ciprofloxacin formulations

Formulation	−38.3 ppm	Impurity A −39.7 ppm	F^- −43.7 ppm	−43.9 ppm	−44.2 ppm	−44.4 ppm	−44.6 ppm	−46.8 ppm	-47.0_5 ppm	−47.2 ppm[a]	−47.3 ppm[a]	−47.8 ppm	−48.7 ppm	Impurity C −55.9 ppm	−56.8 ppm
1 Ciflox® Bayer (Germany)		+	+			+	+	+	+	+		+	+	+	
2 Ciprofloxacin Biogaran (Island)		+				+	+							+	
3 Cipflox Pacific (New Zealand)		+	+		+	+	+							+	
4 Ciprocin Racha (Syria)		+		+	+	+	+		+	+				+	+
5 Sipro Asia (Syria)		+	+		+	+	+		+	+		+	+	+	+
6 Ceproz Elsaad (Syria)		+				+	+			+	+	+	+	+	
7 Ciprofloxacin Micro Nova (India)		+	+	+	+	+	+			+				+	+
8 Ciprofloxacin FDC (India)	+	+				+	+			+	+	+	+	+	+
9 Medociprin 250 Medochemie (Cyprus)		+	+			+	+			+		+		+	+
10 Neocip Okasa (India)	+	+	+			+	+			+	+	+	+	+	
11 Ciprofloxacin Brown & Burk (India)		+	+		+	+	+			+	+	+	+	+	+
12 Ciplox Cipla (India)	+	+	+	+	+	+	+			+	+	+	+	+	
13 Medociprin 500 Medochemie (Cyprus)		+	+		+	+	+					+		+	+
14 Cuminol Gedeon Richter (Romania)				+		+	+							+	
15 Cifran Ranbaxy (India)		+	+		+	+	+			+	+	+	+	+	
16 Ciprinol KRKA (Slovenia)		+	+		+	+	+		+	+	+			+	+

[a] When only one signal is present, it is difficult to attribute an unambiguous chemical shift.

Table 4
Amounts of fluorinated impurities in 16 ciprofloxacin tablets

Formulation[a]	F^- mol%[b]	Impurity A mol%[b]		Impurity C mol%[b]		Total impurities (including F^-, A and C) mol%[b]	
	Mean[c]	Mean[d]	S.D.	Mean[d]	S.D.	Mean[d]	S.D.
1 Ciflox® Bayer (Germany)	0.01	0.01	0.002	0.009	0.002	0.3	0.05
2 Ciprofloxacin Biogaran (Island)		0.04	0.001	0.002	0.003	0.3	0.07
3 Cipflox Pacific (New Zealand)	0.02	0.01	0.001	0.04	0.003	0.3	0.09
4 Ciprocin Racha (Syria)		0.2	0.004	0.02	0.005	0.3	0.006
5 Sipro Asia (Syria)	0.02	0.1	0.006	0.04	0.005	0.6	0.1
6 Ceproz Elsaad (Syria)		0.03	0.01	0.009	0.003	0.3	0.2
7 Ciprofloxacin Micro Nova (India)	0.02	0.2	0.02	0.02	0.005	0.6	0.3
8 Ciprofloxacin FDC (India)		0.3	0.01	0.01	0.01	0.4	0.2
9 Medociprin 250 Medochemie (Cyprus)	0.01	0.2	0.02	0.02	0.002	0.7	0.2
10 Neocip Okasa (India)	0.02	0.3	0.06	0.003	0.004	0.7	0.09
11 Ciprofloxacin Brown & Burk (India)	0.02	0.2	0.02	0.01	0.002	0.6	0.04
12 Ciplox Cipla (India)	n.q.[e]	0.2	0.009	0.008	0.003	0.6	0.2
13 Medociprin 500 Medochemie (Cyprus)	0.01	0.3	0.05	0.02	0.002	0.8	0.1
14 Cuminol Gedeon Richter (Romania)				0.05	0.006	0.4	0.05
15 Cifran Ranbaxy (India)	0.01	0.07	0.01	0.03	0.01	0.5	0.1
16 Ciprinol KRKA (Slovenia)	0.02	0.2	0.02	0.06	0.009	0.7	0.02

[a] The content of ciprofloxacin is 250 mg/tablet in formulations **1**–**10** and 500 mg in formulations **11**–**16**.
[b] Percentages are expressed in mol% relative to nominal ciprofloxacin.
[c] Only 2 tablets were analysed separately.
[d] 3–4 tablets were analysed separately.
[e] Signal too low to be quantified.

is present at the C6 position allowing to measure typical $^3J_{HF}$ coupling constant for H5 (12.9, 9.5 and 11.6 Hz, respectively) and lower $^4J_{HF}$ coupling constant for H8 (7.3, 6.5, and 7.1 Hz, respectively). A $^3J_{HH}$ coupling constant of 9.1 Hz is observed between H5 and H6 of impurity B, whereas no coupling was observed between aromatic protons for impurity D.

^{1}H NMR spectra of seven ciprofloxacin formulations (**3**, **7**, **8**, **9**, **11**, **12**, and **15**) were recorded in order to get the profile of fluorinated and non-fluorinated impurities. We have focused our study on the aromatic region (7–9 ppm) which is the region of the spectrum where the overlap of the signals is minimal. A model solution containing a mixture of the four impurities A, B, C, and D (concentration $\approx 3 \times 10^{-3}$ mol L^{-1}) was prepared step by step in D_2O at pH 5.0. Four ^{1}H NMR spectra (impurity B alone, impurities B+D, impurities B+D+C, and impurities B+D+C+A) were thus recorded. Compared to the spectra of the impurities alone at pH 5.0 or 8.1 (Table 5), the chemical shifts are different due to the pH for the impurity A or the concentration (according to a recent study on ciprofloxacin [10]). For example, the H2 signal of the impurity A appeared at a higher chemical shift than that of the impurity D (8.81 and 8.79 ppm, respectively) (Fig. 3).

By comparing a typical ^{1}H NMR spectrum of a ciprofloxacin formulation with that of the model solution containing impurities A, B, C, and D (Fig. 3), we also observed significant variations of the chemical shifts of the aromatic proton signals. The maximum shift (−0.4 ppm) was found for the H5 of impurity D. These variations are due to differences in concentration (as a result of aromatic interactions [10] between ciprofloxacin and impurities) and maybe also viscosity (as a result of the presence of various excipients in the pharmaceutical formulations). A correct attribution of the signals can thus only be done after spiking the formulation with authentic standards.

Due to numerous signal overlaps in the ^{1}H NMR spectra of the formulations, only one signal of each impurity can be used

Table 5
^{1}H NMR data of ciprofloxacin and its related impurities A, B, C, D

	Ciprofloxacin[a] δ (ppm), mutiplicity (J)	Impurity A[b] δ (ppm), mutiplicity (J)	Impurity B[a] δ (ppm), mutiplicity (J)	Impurity C[a] δ (ppm), mutiplicity (J)	Impurity D[a] δ (ppm), mutiplicity
H2	8.58 s	8.55 s	8.53 s	8.56 s	8.84 s
H5	7.40 d ($^3J_{HF}$ 12.9 Hz)	8.07 d ($^3J_{HF}$ 9.5 Hz)	7.88 d ($^3J_{HH}$ 9.1 Hz)	7.52 d ($^3J_{HF}$ 11.6 Hz)	7.97 s
H6			7.23 d ($^3J_{HH}$ 9.1 Hz)		
H8	7.48 d ($^4J_{HF}$ 7.3 Hz)	8.40 d ($^4J_{HF}$ 6.5 Hz)	7.29 s	7.11 d ($^4J_{HF}$ 7.1 Hz)	8.46 s
H9	3.70 m	3.68 m	3.65 m	3.69 m	3.77 m
H10-11	1.21 m–1.45 m	1.17 m–1.35 m	1.17 m–1.41 m	1.19 m–1.42 m	1.21 m–1.41 m
H12-13	3.55 m–3.64 m		3.50 m–3.79 m	3.40 m–3.75 m	3.46 m–3.55 m

[a] The concentrations were 2.3×10^{-2} mol L^{-1} for ciprofloxacin and 5.0×10^{-3} mol L^{-1} for impurities B, C, and D. The solutions were prepared in D_2O and were at pH 5.0 (observed pH reading not corrected for kinetic isotope effect).
[b] The impurity A being only very slightly soluble at pH 5, its ^{1}H NMR spectrum was recorded at pH 8.1 (concentration 3.1×10^{-3} mol L^{-1}).

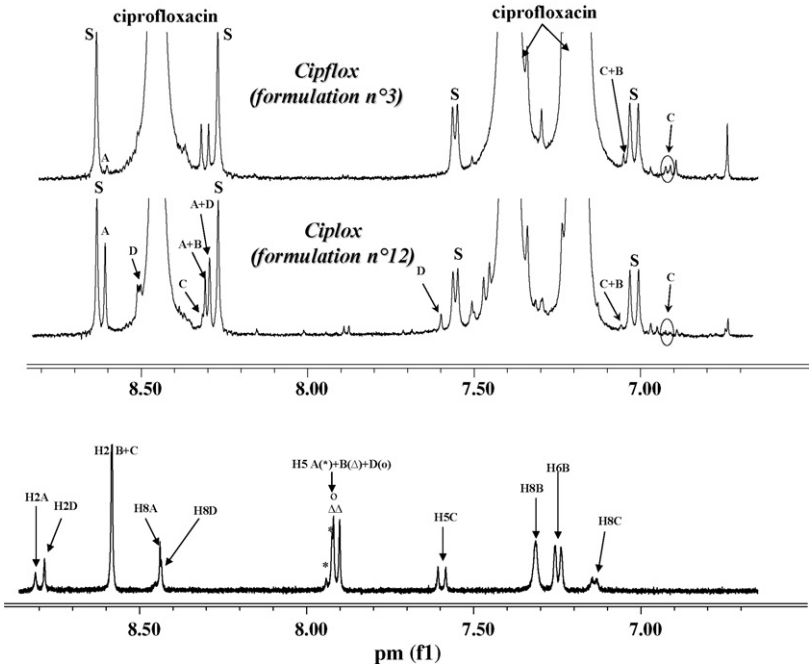

Fig. 3. ^{1}H NMR spectra of two commercial formulations of ciprofloxacin and a model solution containing impurities A, B, C, and D. S: satellite; A, B, C, D: signals of impurities A, B, C, and D.

for its assay. The chemical shift of the selected signal for each impurity in the seven formulations studied is reported in Table 6. Impurity A is easily quantified from its H2 singlet resonance, which is the most deshielded signal resonating at 8.6 ppm. The H8 doublet of impurity C gives a signal at 6.9 ppm. The characteristic signal of impurity D is the singlet of H5 at 7.6 ppm. To assign a specific signal to impurity B was more difficult as all its signals are overlapped with those from others impurities. However, impurity B was determined from the signal at 7.03 ppm. This signal is in fact the sum of one peak of the H5 doublet from impurity C and the H8 singlet from impurity B. So, to measure the amount of impurity B in the formulations, the half-area of the H8 doublet from impurity C was subtracted from the area of the signal at 7.03 ppm.

Table 6
Comparison of ^{19}F and ^{1}H NMR assays of impurities in generic ciprofloxacin tablets

Formulation	Impurity A				Impurity B		Impurity C				Impurity D	
	^{1}H NMR		^{19}F NMR		^{1}H NMR		^{1}H NMR		^{19}F NMR		^{1}H NMR	
	δ (ppm) H2, s[a]	mol%[b]	δ (ppm)	mol%[b]	δ (ppm) H8, s[a]	mol%[b]	δ (ppm), J H8, d[a]	mol%[b]	δ (ppm)	mol%[b]	δ (ppm) H5, s[a]	mol%[b]
3 Cipflox Pacific (New Zealand)	8.60	0.02	−39.76	0.01	n.d.[c]		6.92, J 7.2 Hz	0.04	−55.93	0.04	n.d.[c]	
7 Ciprofloxacin Micro Nova (India)	8.58	0.2	−39.79	0.2	n.d.[c]		n.d.[c]		−55.94	0.02	7.55	0.2
8 Ciprofloxacin FDC (India)	8.59	0.3	−39.81	0.3	n.d.[c]		n.d.[c]		−55.94	0.01	7.57	0.2
9 Medociprin 250 Medochemie (Cyprus)	8.59	0.2	−39.82	0.2	7.03	0.007	6.91, J 7.0 Hz	0.02	−55.94	0.02	n.d.[c]	
11 Ciprofloxacin Brown & Burk (India)	8.60	0.2	−39.68	0.2	n.d.[c]		n.d.[c]		−55.90	0.01	7.60	0.3
12 Ciplox Cipla (India)	8.61	0.2	−39.74	0.2	7.06	0.02	6.92, J 6.8 Hz	n.q.[c]	−55.91	0.008	7.60	0.04
15 Cifran Ranbaxy (India)	8.60	0.07	−39.76	0.07	n.d.[c]		n.d.[c]		−55.93	0.03	n.d.[c]	

[a] s: singlet; d: doublet ($^{4}J_{HF}$).

[b] Percentages are expressed in mol% relative to nominal ciprofloxacin for ^{19}F NMR assays (since absolute concentrations of impurities were determined by comparing the area of their signal to that of an external calibrated reference) or relative to the area of the H2 signal of ciprofloxacin for ^{1}H NMR assays (since no reference for quantitation was used).

[c] n.d.: non-detected; n.q.: signal too low to be quantified.

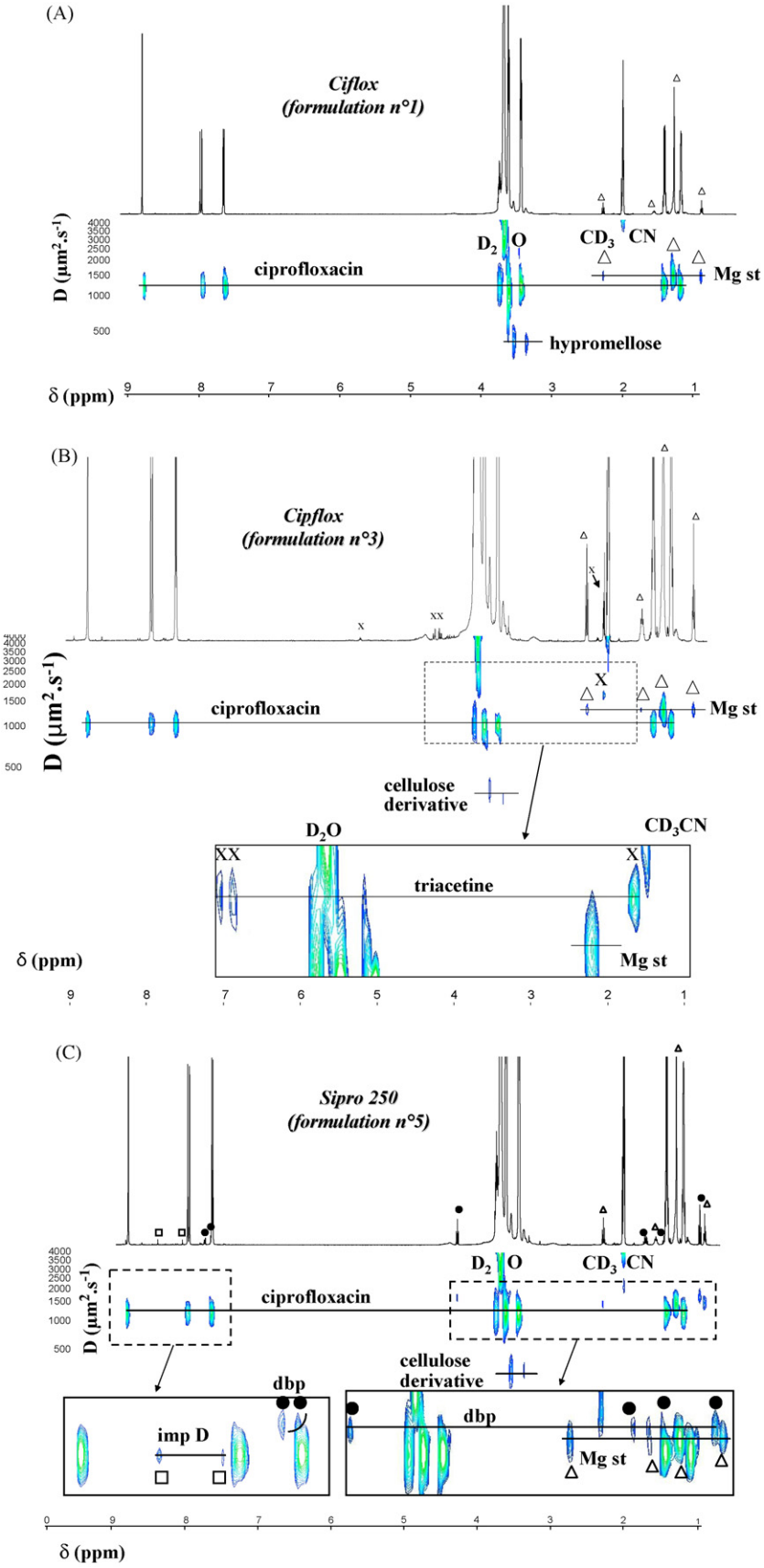

Fig. 4. DOSY ^{1}H NMR spectra of commercial formulations of ciprofloxacin (solvent CD_3CN/D_2O, 80/20). A: formulation **1**; B: formulation **3**; C: formulation **5**. Mg st (△): magnesium stearate; triacetine (x); impurity D (□); dbp (●): dibutyl phthalate.

Table 7
Self-diffusion coefficients[a] ($\mu m^2\ s^{-1}$) measured in the formulations studied with DOSY ^{1}H NMR

	Formulation **1**	Formulation **3**	Formulation **5**	Formulation **7**	Formulation **11**
Ciprofloxacin	1263 ± 40	1078 ± 18	1233 ± 9	1389 ± 29	1424 ± 30
Magnesium stearate	1545 ± 15	1363 ± 7	1565 ± 63	1788 ± 102	1647 ± 113
Hypromellose	435 ± 12				
Cellulose derivative		337 ± 40	332 ± 5	724 ± 25	679[b]
Dibutyl phatalate			1839 ± 107		
Triacetine		1659 ± 110			
Methyl paraben				2392 ± 168	2285 ± 16
Impurity D			1206 ± 17		

[a] The value of the self-diffusion coefficient was measured for each peak, and an average self-diffusion coefficient was determined for each formulation.
[b] Only one peak was observed.

The ^{1}H NMR quantitation of impurities A, B, C, and D is reported in Table 6. The non-fluorinated impurity B was only detected in formulations **9** and **12**, whereas the non-fluorinated impurity D was present in formulations **7**, **8**, **11**, and **12**. The amounts of the fluorinated impurity A measured with ^{1}H NMR are identical to those determined with ^{19}F NMR. The fluorinated impurity C could not be detected with ^{1}H NMR in formulations **7**, **8**, **11**, and **15**, whereas small amounts (≤0.03 mol%) were quantified by ^{19}F NMR.

3.3. ^{1}H DOSY NMR

Five formulations of ciprofloxacin (**1**, **3**, **5**, **7**, and **11**) were analysed with ^{1}H NMR DOSY and three DOSY spectra along with their corresponding 1D spectra are presented in Fig. 4. The peaks at 3.68 and 1.99 ppm correspond to the residual signals of water and acetonitrile, respectively. All the peaks of ciprofloxacin are lined up. The value of the self-diffusion coefficient was measured for each peak, and an average self-diffusion coefficient was determined for each formulation (Table 7). Several excipients could be observed depending on the formulation (Fig. 4 and Table 7). All the formulations contain the lubricant magnesium stearate that leads to four signals located at 0.89, 1.28, 1.56, and 2.28 ppm. They also contain a cellulose derivative, a tablet binder, giving two aligned signals at 3.37 and 3.54 ppm that is known to be hypromellose (hydroxypropylmethyl cellulose) for the brand formulation **1** but is unknown for the other formulations. Dibutyl phthalate (7.69, 4.28, 1.70, 1.42, and 0.95 ppm), a plasticizer, and impurity D (8.38 and 8.04 ppm) could be detected in the formulation **5**. The formulation **3** includes the hydrophilic plasticizer triacetine (glyceryl triacetate; 5.23, 4.23, 2.06, and 2.05 ppm). The antimicrobial preservative methylparaben (methyl 4-hydroxybenzoate; 7.88 and 6.89 ppm) was found in formulations **7** and **11** (data not shown). Glycerol that did not give observable DOSY peaks was identified in the 1D spectrum of formulation **11**.

4. Discussion

In a previous review [11], we demonstrated the validity of ^{19}F NMR for in vitro quantification of fluorinated drugs. The ^{19}F nucleus has favourable NMR characteristics: nuclear spin of 1/2, relatively narrow lines, 100% natural abundance, high sensitivity (83% that of proton), large chemical shift range (about 500 ppm), which minimises signal overlap. Provided that the ^{19}F NMR spectrum is acquired under conditions of full T_1 relaxation, it is possible to quantify the relative amounts of the different components in a mixture by measuring integrals of the fluorine peaks in the spectrum. The method is non-selective and unexpected substances are not overlooked during the investigation, since all low molecular weight molecules in solution (provided they bear the fluorine nucleus and are present at sufficient concentrations) are detected simultaneously in a single analysis. This contrasts with chromatography that usually requires some prior knowledge of the structure of unknown impurity in order to optimise chromatographic separation and detection.

It is reassuring that in this study all the formulations tested had ciprofloxacin concentrations measured by ^{19}F NMR within the specification of the US Pharmacopeia, which recommends that ciprofloxacin tablets should contain not less than 90% and not more than 110% of the labelled amount of the active ingredient [3]. Twelve out of the sixteen commercial formulations of ciprofloxacin contain the antibiotic within 100 ± 5% of stated concentration. Three formulations, two coming from Syria (formulations **5** and **6**) and one from India (formulation **8**), have lower ciprofloxacin amounts: 92.8 ± 1.1%, 90.2 ± 3.3%, 91.0 ± 1.9% of advertised concentrations, respectively, whereas the formulation from Romania (formulation **14**) shows a higher concentration (107.3 ± 3.2%). These data are in agreement with those reported by several authors who, for the validation of various analytical techniques, assayed ciprofloxacin in pharmaceutical formulations (tablets, capsules, injection, eye-drops) from different countries (Czech Republic, Austria, Brazil, Saudi Arabia, Jordan, Spain, China, India, and unknown) and found contents comprised between 94% and 107% of declared amount of ciprofloxacin [6,7,12–19]. However, our results differ from those of Weir et al. [20] who analyzed the content of 30 Indian generic ciprofloxacin eye drops by HPLC with fluorescence detection and found a great variability. The authors showed that 6 out of the 30 samples tested had ciprofloxacin concentrations lower than the standard advisory ranges (median—21.73%) and 24 higher (median + 19.42%).

From the ^{19}F and ^{1}H NMR spectra, it is obvious that the formulations do not present the same impurity profile (Figs. 2 and 3, Tables 3 and 6). This can be explained by differences in the

Table 8
Comparison of ciprofloxacin impurities in previous studies and in this study

	Altria and Chanter [5] Ciprofloxacin	Husain et al. [1] Commercial formulations[a]	Lacroix et al. [4] Ciprofloxacin raw materials[b]	Novakovic et al. [7] Cifloxinal® (PRO.MED.CS, Prague, Czech Republic)	Michalska et al. [6] Ciprofloxacin raw material		This study Commercial formulations
	CE (%)[c,d]	HPLC (%)	HPLC (%)[e]	HP TLC (%)[f]	HPLC (%)	CZE (%)[c]	NMR (%)
2,4-Dichloro-5-fluoroacetophenone		0.41 ± 0.007					n.d.[h]
Methyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-cyclopropylaminoacrylate		0.03 ± 0.0007					
3-Chloro-4-fluoroaniline		0.02 ± 0.0006					n.d.[h]
Impurity A		0.26 ± 0.005	0.03tr[g]/tr[g]/0.01		n.d.[h]	n.d.[h]	0–0.3
Impurity B					n.d.[h]	n.d.[h]	0.007/0.02
Impurity C			0.06/0.03/0.23	Detected but n.q.[h]	0.053 ± 0.0013	0.084 ± 0.0058	0.002–0.06
Impurity D			0.45/0.03/0.22/0.15	Detected but n.q.[h]	n.d.[h]	n.d.[h]	0.04–0.3
F^-							0.01–0.02
Unknown					0.093 ± 0.0016	0.096 ± 0.0152	0.07–0.5
Total impurities	0.68		1.00/0.55/0.65/0.27				0.3–1

[a] The number of formulations analysed, their name, origin and manufacturer were not indicated.
[b] Four samples of ciprofloxacin hydrochloride raw materials were analysed.
[c] CE: capillary electrophoresis; CZE: capillary zone electrophoresis.
[d] Six unidentified impurities were detected.
[e] Two HPLC methods were necessary to assay the impurities.
[f] Two unidentified impurities were also detected.
[g] tr: traces.
[h] n.d.: non-detected; n.q.: not quantified.

manufacturing process (route of synthesis, manufacturing equipment, contaminants and solvents used). Analysing impurity profile by NMR may thus provide a "spectral signature" of the manufacturing process. A comparison of impurity content is presented in Table 8. The values for total impurities are close to those reported by Lacroix et al. [4] and Altria and Chanter [5]. Impurities A, B, and D are by-products from the synthesis route and impurity C is a potential photolysis product [4]. Their levels are also in agreement with those reported by others authors (Table 8). However, all the formulations do not comply with the limits for impurities given in the ciprofloxacin monograph of the European Pharmacopeia, which authorizes a maximal content of 0.2% for impurities B, C, and D, 0.1% for any other impurity and 0.5% for total impurities [2]. If the contents of impurities B, C, and D are correct (except for impurity D in the indian formulation **11**), the level of impurity A is superior in one formulation from Syria (**4**), in all the formulations from India (**7**, **8**, **10**, **11**, and **12**) except one (**15**), and in the two formulations from Cyprus (**9** and **13**). Also, the percentage of total impurities is greater in several formulations (one from Syria (**5**), five from India (**7**, **8**, **10**, **11**, and **12**), the two from Cyprus (**9** and **13**) and that from Slovenia (**16**)).

This is the first study that reports the presence of F^- in pharmaceutical formulations of ciprofloxacin. It is easy to detect the signal of this compound with ^{19}F NMR, which is not the case with others techniques. Even if the level of F^- found was low (Table 4), it is nevertheless important to know it as the difference between safe and toxic concentrations in some human tissues is often small. Several analytical methods have been elaborated and developed for the determination of traces of F^-, such as spectrophotometric, potentiometric, chromatographic (ion chromatography and gas chromatography), radioanalytical methods and capillary zone electrophoresis [21]. A disadvantage of most of these methods is the need to develop complicated procedures particularly in complex matrices (preliminary separation, derivatisation, extraction), which is not the case with ^{19}F NMR that allows to get direct informations on all fluorinated compounds present in the sample, including F^-.

DOSY relies on differences in translation diffusion as a means to separate components in a solution mixture. The diffusion coefficient generally decreases with increasing molecular weight. The differences in the values of the diffusion coefficients (Table 7) are due to the various compositions of the formulations that resulted in media of different viscosity. In addition to ciprofloxacin, several excipients could be observed depending on the formulation studied (Fig. 4). In the brand formulation **1**, the lubricant magnesium stearate and the tablet binder hypromellose were detected whereas the other excipients, namely corn starch, microcrystalline cellulose, insoluble povidone, anhydrous colloidal silica and macrogol 4000, insoluble in the system of solvents employed (CD_3CN/D_2O), did not give ^1H NMR signals. The composition of the other tablets analysed was not known since no indication was given on the leaflet or there was no leaflet. However, the DOSY spectra clearly showed similarities and differences in the composition of the pharmaceutical formulations of ciprofloxacin, thus giving a signature of the manufacturer.

5. Conclusion

The results reported herein demonstrated that the quality of pharmaceutical formulations of ciprofloxacin sold in several countries or via the Internet is correct. Indeed, 12 out of the 16 formulations tested had ciprofloxacin concentrations within ±5% of advertised concentrations, 3 have concentrations between 90 and 95% and one superior to 105%. ^{19}F NMR is an attractive method to detect and assay fluorinated impurities present in the pharmaceutical formulations, including F^-. ^1H NMR allowed us to detect and quantify fluorinated and non-fluorinated impurities. With these two techniques, we could demonstrate that impurity profile and content were different in the various formulations analysed. DOSY NMR, which is now considerably easier to use thanks to improvements in spectrometer hardware and DOSY software, is a powerful tool for the analysis of complex mixtures.

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