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ORIGINAL ARTICLE

# Analysis of Drug-Related Impurties by HPLC in Ciprofloxacin Hydrochloride Raw Material

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## ABSTRACT

**Objectives:** In this study, we report the quality control results of drug-related impurity analysis of seven raw materials of ciprofloxacin hydrochloride marketed in Algeria.

**Materials and Methods:** According to the European Pharmacopoeia (Eur. Ph.), high-performance liquid chromatography (HPLC) was used to analyze (B, C, D and E) impurities, while thin layer chromatography (TLC) used to control impurity A.

**Results:** HPLC analysis showed that the C1, C2, C3, C4, and C6 samples have individual contents of specified impurities (B, C, D, E), unspecified and the total of all present impurities conform to norms. The C5 sample contains a very high content (0.579%) of impurity C, which is a photodegradation product and the impurities total (0.625%) exceeding limit, while C7 sample has a slightly higher content (0.118%) of unspecified impurity. The control solution of impurity A was not migrated in all developed TLC plates, so the system is not compliant, for this reason, an HPLC analysis protocol was developed.

**Conclusion:** The results showed that impurity A content conformed in all samples except for the C6 sample, which has equal content to the limit. Therefore, we recommend revising the detecting technique of impurity A by TLC in the Eur. Ph. or replacing it with a more sensitive technique such as HPLC.

**Key words:** Drug-related impurities, specified, HPLC, TLC, ciprofloxacin hydrochloride

## INTRODUCTION

The identification and quantification of impurities in raw materials is critical to ensure effective and safe treatment. So, impurity control is a key component and a big challenge in the pharmaceutical industry.[1,2] Impurities relate to starting materials, by-products, breakdown products or polymorphs. They can appear at active pharmaceutical ingredient (APIs) production level as well as during or after the formulation process. Their concentrations may change upon storage of the product.[2,3]

Chemical determination of related impurities in APIs is important because a long exposure at low concentrations, can have undesirable side effects or toxicity and/or may interfere with the drug's activity.[3,4] There are no toxicity studies for the majority of impurities, so impurity analysis is a critical step in quality control.[5,6] Therefore, specific requirements for impurities are set by the regulatory authorities.[6,7]

Ciprofloxacin hydrochloride (CPF HCl) (Figure 1) is a synthetic antibiotic that is part of the list of essential drugs established by the World Health Organization (WHO), manufactured by several generic laboratories in Algeria, their high rate of prescription by clinicians thanks to their numerous indications in the different infections (gynecological, urinary, digestive, and respiratory, *etc.*). CPF HCl has several associated impurities, which are well described and defined in European Pharmacopoeia (Eur. Ph.) 8$^{th}$ edition. The specified impurities are A, B, C, D, and E, which are individually cited and limited by a specific acceptance criterion, while the impurity F is not specified that is present but limited by an overall acceptance criterion.[8] According to Eur. Ph., impurities B, C, D, and E are searched by high-performance

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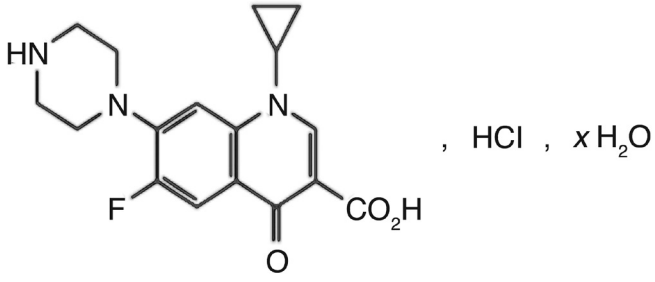



**Figure 1.** Chemical structure of CPF HCl[8]

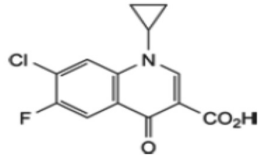
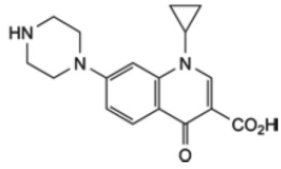
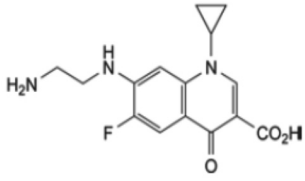
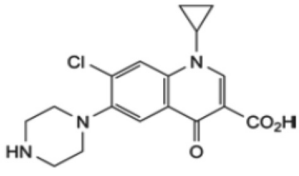
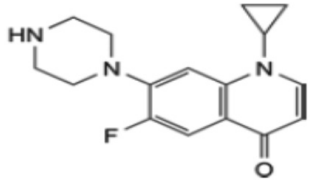
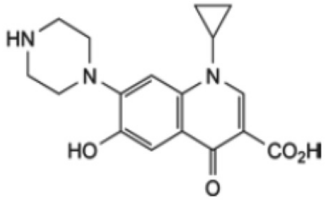
liquid chromatography (HPLC), while impurity A by thin layer chromatography (TLC) (Table 1).

In this paper, we analyzed and evaluated the drug-related impurities of seven samples of CPF HCl APIs marketed in Algeria using HPLC.

## MATERIALS AND METHODS

Seven samples of CPF HCl were collected from pharmaceutical producers located in Algeria.[9] They are labeled as follows: C1, C2, C3, C4, C5, C6, and C7 (Table 2).

**Table 1. Related substances of CPF-HCl[7,8]**

| Origin | Impurity | Structure | Analysis method |
|---|---|---|---|
| Synthesis by-product | **Impurity A** (specified)<br>Fluoroquinolonic acid: 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid |  | TLC |
| Synthesis by-product | **Impurity B** (specified)<br>Defluorinated derivative: 1-Cyclopropyl-4-oxo-7- (piperazin-1-yl) -1, 4-dihydroquinoline-3-carboxylic acid |  | HPLC |
| Photodegradation product | **Impurity C** (specified)<br>Ethylenediamine derivative: 7 - [(2-aminoethyl) amino] -1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid |  | HPLC |
| Synthesis by-product | **Impurity D** (specified)<br>7-Chloro-1-cyclopropyl-4-oxo-6- (piperazin-1-yl) -1, 4-dihydroquinoline-3-carboxylic acid |  | HPLC |
| Degradation products resulting from decarboxylation | **Impurity E** (specified)<br>Dicarboxylic derivative: 1-cyclopropyl-6-fluoro-7- (piperazin-1-yl) quinolin-4 (1H)-one |  | HPLC |
| Hydroxylation product | **Impurity F** (unspecified)<br>1-Cyclopropyl-6-hydroxy-4-oxo-7- (piperazin-1-yl) -1, 4-dihydroquinoline-3-carboxylic acid |  | HPLC |

*Research and quantification of impurities B, C, D, and E by HPLC*

*Standards, reagents, and apparatus*

The standard impurities "CPF HCl for identification of SCR peaks (containing impurties B, C, D, and E)" were purchased from Eur. Ph. (Strasbourg, France). Acetonitrile (HPLC grade), triethylamine and phosphoric acid were produced by Sigma-Aldrich.[8]

An HPLC-ultraviolet (UV) device (Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC systems) equipped with an automatic injector and UV detector.

*Analysis protocol*

*Mobile phase:* Thirteen volumes of acetonitrile were mixed with 87 volumes of phosphoric acid at 2.45 g/L.

*Test solution:* 25 mg of CPF HCl of each sample was dissolved in 50 mL of mobile phase.

*Control solution (c):* 1 mL of the test solution was diluted in 500 mL of mobile phase.

*Control solution (b):* 2.5 mg of CPF HCl for identification of SCR peaks is dissolved in 5 mL of mobile phase.

*Chromatographic conditions:* Temperature: 40°C; flow: 1.5 mL/min; injection volume: 50 µL of control solution (b) and (c); detection: 278 nm; column C18: 5 µm, 250 × 4.6 mm.[8]

*Research on impurity A by TLC*

*Standards, reagents, and apparatus*

The standard "impurity A of CPF SCR" was purchased from Eur. Ph. acetonitrile (HPLC grade), ammonia, dichloromethane, and methanol were obtained from Sigma-Aldrich. Silica gel plate F254 for TLC, chromatography tank, and UV lamp at 254 nm were from CAMAG.

*Procedure*

*Test solution:* 50 mg of CPF HCl was dissolved in 5 mL water.

*Control solution:* Impurity A standard (10 mg) is dissolved in a mixture (0.1 mL of diluted ammonia and 90 mL water), completed to 100 mL with water, and 2 mL is diluted in 10 mL of water.

*Mobile phase:* Acetonitrile, concentrated ammonia, methanol, and methylene chloride (10:20:40:40 V/ V/ V/ V); the deposit volume: 5 µL.

*Development:* At the bottom of the chromatography tank, a container of 50 mL concentrated ammonia was deposited. The vessel was closed, and the plate was exposed to ammonia vapors for 15 min. The plate was developed on ¾; drying in air and exanimated under UV at 254 nm.

*Limits*

The sample is compliant, if the impurity A spot is not more intense than main spot of the control solution (0.2%).[10]

*Research and quantification of impurity A by HPLC*

*Analysis protocol*

*Mobile phase:* Acetonitrile (50 volumes) were mixed with 50 volumes of phosphoric acid at 2.45 g/L.

*Standard stock solution:* Standard (5 mg) is dissolved in 50 mL of mobile phase.

To determine the maximum absorption of impurity A, the standard solution was scanned in UV over a range of 200 to 400 nm.

*The establishment of the calibration curve:* Five dilutions were prepared from the standard stock solution (0.1 mg/mL) (Table 3).

*Test solution:* CPF HCl (50 mg) was dissolved in 5 mL of water.

*Chromatographic conditions:* Temperature: 25.9°C, flow: 1.5 mL/min, injection volume: 20 µL, detection at 260 nm, column C18: 5 µm, 150 × 4.6 mm.

**Table 2. Collection of CPF HCl raw material from local producers**

| Sample | Local producer | Batch number | Expiration date | Manufacturer/supplier |
|---|---|---|---|---|
| C1 | Lab C1 | A004801 | 04/2017 | Unknown |
| C2 | Lab C2 | CIC 0074 | 01/2017 | Baselux (Spain) |
| C3 | Lab C3 | CICA 4066 | 12/2019 | Chemo (Swiss) |
| C4 | Lab C4 | 10271610 | 07/2018 | Dr. Reddy's Laboratories (India) |
| C5 | Lab C5 | 120801 | 08/2016 | Pharmaceutical Co. Ltd. (China) |
| C6 | Lab C6 | KOFA0062 | 03/2017 | Dr. Reddy's Laboratories (India) |
| C7 | Lab C7 | 0251103F | 07/2019 | Unknown |

**Table 3. Dilution range of the calibration curve**

| | 1st Dilution | 2nd Dilution | 3rd Dilution | 4th Dilution | 5th Dilution |
|---|---|---|---|---|---|
| Stock solution (mL) | 0.5 | 1 | 1.5 | 2 | 2.5 |
| Solvent (mL) | 9.5 | 9 | 8.5 | 8 | 7.5 |
| Diluted solution (%) | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 |

*System compliance:* Linearity of the calibration curve with a correlation coefficient greater than 0.990. The symmetry factor of the impurity (A) peak must be between 0.8 and 1.5.

*Identification of impurity A:* Its retention time.

*Results expression*

The impurity A content of each sample is expressed by extrapolating its area on the calibration curve: y = a X + b

y: Impurity (A) area, X: Impurity (A) concentration (%)

Calculus formula of impurity A content

$$\textbf{Impurity A content}\ (\%) = \frac{(\text{Peak Area} - b)}{a} \times \frac{50}{\text{Wieght (mg)}}$$

In this study, there was no statistical data analysis.

## RESULTS AND DISCUSSION

*Research and quantification of impurities B, C, D, and E by HPLC*

*System compliance*

Figures 2 and 3 showed the obtained chromatogram of control solution (b) and typical chromatogram.

These two chromatograms were superimposable and comparable, which enabled us to identify the CPF HCl main peak and impurity E, B, C, and D peaks corresponding.

Retention time (RT) of CPF HCl is 8.962, a value close to that required by Eur Ph that must be at about 9 min. The RT obtained for each impurity (E, B, C, and D) is respectively (3.547 min, 5.977 min, 6.650 min and 11.855 min). All these values are close to those given in the standard chromatogram or calculated from RRT (RT_impurity E: 3.58 min, RT_impurity B: 5.377 min, RT_impurity C: 6.273 min, RT_impurity D: 10.754 min).

The resolution between peaks of impurity B and C is 3, value complies with the required standard (at least 1.3). The symmetry factor of the CPF HCl peak is 1.4, conforming to Eur. Ph. standard (between 0.8 and 1.5). The symmetry factor of peaks belonging to impurities E, B, C, and D (1.16, 1.31, 1.30, and 1.17). All these values were conformed. Therefore, the system compliance is validated.

*Sample analysis*

Obtained chromatograms of the sample analysis were presented in Figures 4-6, and 7. Table 4 presents individual contents of (B, C, D, E, unspecified) impurity, and the impurities total.

According to the Eur. Ph. standards, the individual content of impurities B, C, and D must be less than or equal to 0.2%, impurity E, less than or equal to 0.3% and unspecified impurity less than or equal to 0.1%. Any other impurity with individual content less than or equal to 0.05% (exclusion limit) shall not be taken into consideration. The impurities total content shall not exceed 0.5%.

Samples C1, C2, C3, C4, and C6 have individual content of specified impurities B, C, D, and E or unspecified, and the impurities total in the required standards.

C5 sample contains very high content (0.579%) of impurity C compared to the limit, and a total (0.625%) exceeding the norm. This explains that the sample has degraded in impurity C, which is a photodegradation product despite having been well preserved. This result is consistent since the sample was analyzed in date close to its expiry date (August 2016) or it degraded during handling.

C7 sample has individual content of unspecified impurity (known structure such as impurity for unknown structure) equal to 0.118%, slightly higher than the general acceptance criterion and a total in the norm.

*Research on impurity A by TLC*

Figure 8 shows the TLC plates revelation under UV lamp. The first plate revealed 4 main spots corresponding to test solutions of C1, C2, C3, and C4 samples and no spot of the control solution appeared. The second plate revealed three main spots corresponding to the test solution of C5, C6, and C7 samples and no spot of the control solution appeared. Because of the absence of control migration, a third plate was prepared in

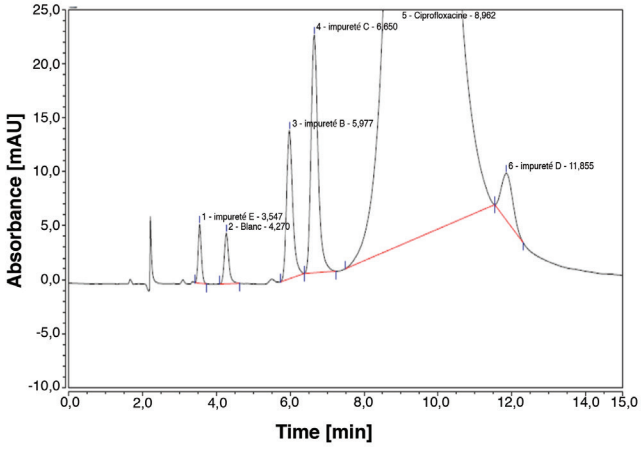



**Figure 2.** Chromatogram of control solution

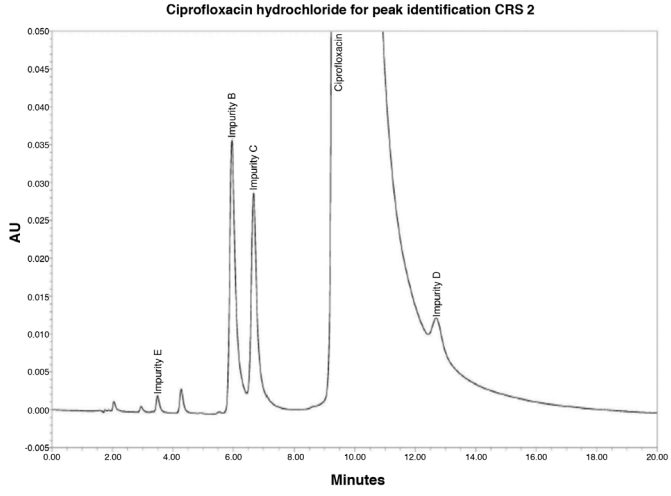



**Figure 3.** Typical chromatogram

which the stock control solution (0.1 mg/mL) was deposited but still has not been migrated.

TLC was re-tested several times, while using

- New reagents to prepare the mobile phase (plate 3);
- New plates silica gel F254 for TLC (plate 3);
- Second control solution prepared from the first vial (plate 4);
- The third control solution was prepared from a second vial of impurity standard (plate 5).

The control was not migrated in all developed TLC plates, so the system is not compliant. For this reason, an HPLC analysis protocol for impurity A was developed.

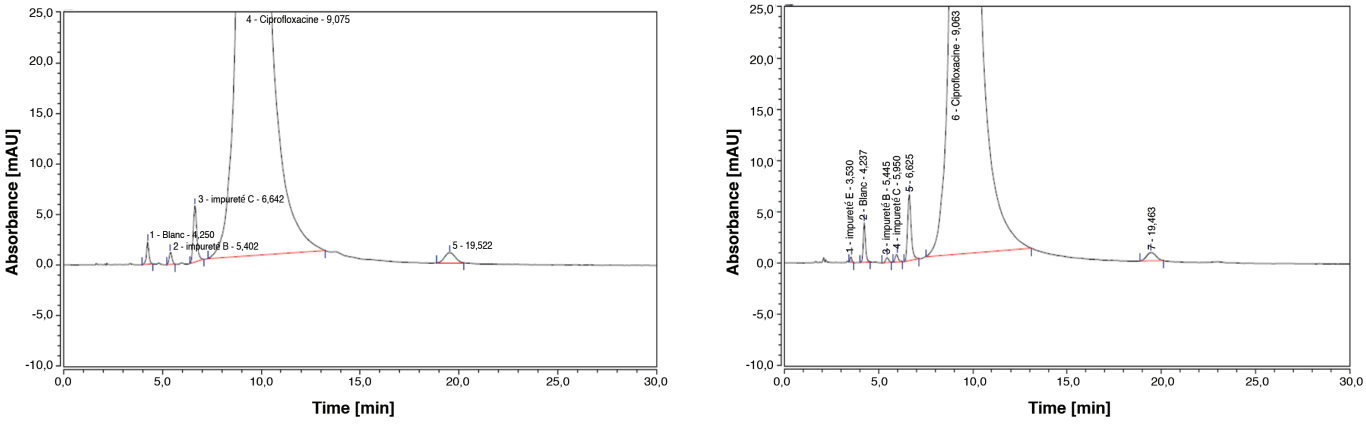



**Figure 4.** Chromatograms of samples C1 and C2

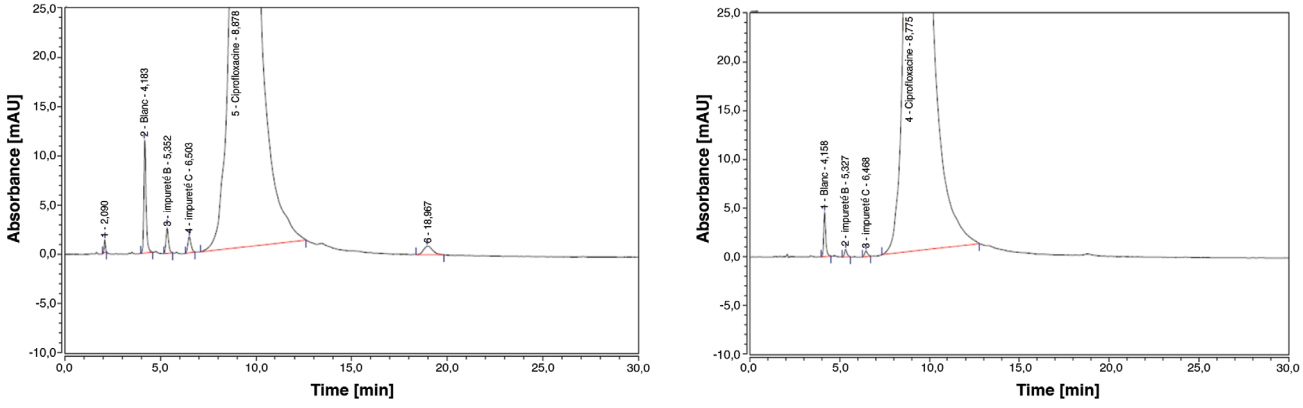



**Figure 5.** Chromatograms of C3 and C4 samples

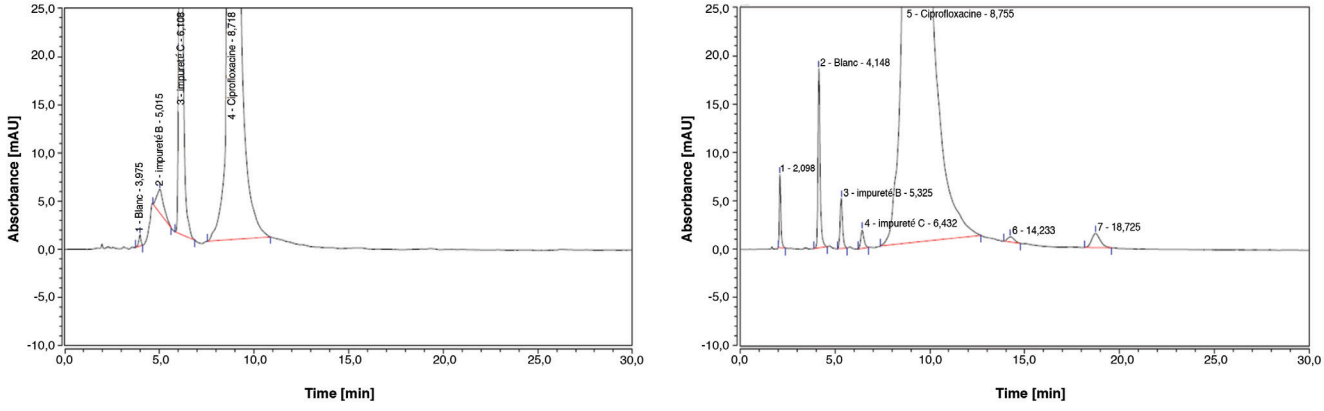



**Figure 6.** Chromatograms of samples C5 and C6

*Research and quantification of impurity A by HPLC*

*Maximum absorption of the standard solution*

Figure 9 shows the absorption spectrum of impurity A in UV.

*System compliance*

The chromatograms obtained with various standard solutions of the calibration range are shown in Figures 10 and 11.

The maximum absorption of impurity A is 260 nm and its RT is 3.208 min. The symmetry factor of impurity A peak was 1.40, conforming to the norm. The correlation coefficient of the calibration curve is 0.997, which shows that the curve linearity is validated. Therefore, the system is compliant.

*Sample analysis*

Figures 12 and 13 show the obtained chromatograms of all sample analysis. Table 5 shows the individual contents of impurity A.

According to Eur. Ph., the individual content of impurity A must be less than 0.2%.

Impurity A was not detected in the C2 sample, while C1, C3, C4, C5, and C7 had a content conform but C6 had content equal to the limit.

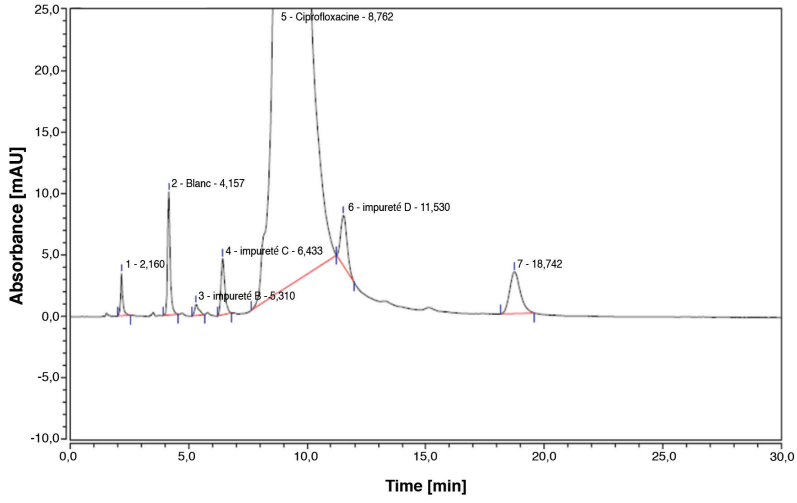



**Figure 7.** Chromatogram of sample C7

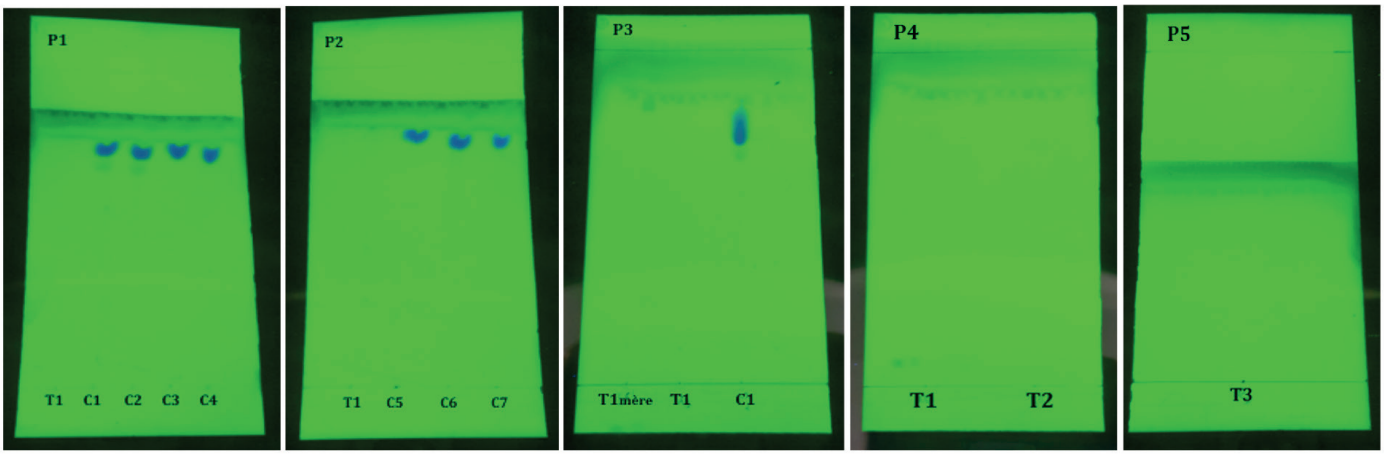



**Figure 8.** TLC plates revealated under UV lamp

T1: 1st control solution (0.02 mg/mL) prepared from the first vial

T1 stock: 1st stock control solution (0.1 mg/mL) prepared from the first vial

T2: 2nd control solution (0.02 mg/mL) prepared from the first vial

T3: 3rd control solution (0.02 mg/mL) prepared from a second standard vial

Table 4. Individual content of (B, C, D, E, and unspecified) impurity and the impurities total

| Ciprofloxacin hydrochloride sample | Impurity | Impurity area (mAU-min) | Control (c) area (mAU-min) | Control (c) weight (mg) | Dilution factor control (c) | Control concenration (µg/mL) | Theoric concenration of control (c) (µg/mL) | Theoric concenration of control (c) (%) | Correction factor | Real concenration control (c) (%) | Individual content of impurity (%) | Impurities total (%) | Norms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | Imp B | 0.202 | 3.375 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 0.7 | 0.202 | 0.008 | 0.087 | Imp B ≤0.2<br>Imp C ≤0.2<br>Imp D ≤0.2<br>Imp E ≤0.3<br>Imp unspf ≤0.1<br>Imp total ≤0.5<br>Exculsion limit: 0.05 |
| | Imp C | 1.189 | 3.375 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 0.6 | 0.202 | 0.043 | | |
| | Imp D | ND | 3.375 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 1.4 | 0.202 | ND | | |
| | Imp E | ND | 3.375 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 6.7 | 0.202 | ND | | |
| | Imp unspf 1 | 0.606 | 3.375 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 1 | 0.202 | 0.036 | | |
| C2 | Imp B | 0.086 | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 0.7 | 0.203 | 0.003 | 0.136 | |
| | Imp C | 0.137 | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 0.6 | 0.203 | 0.005 | | |
| | Imp D | ND | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 1.4 | 0.203 | ND | | |
| | Imp E | 0.052 | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 6.7 | 0.203 | 0.020 | | |
| | Imp unspf 1 | 1.404 | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 1 | 0.203 | 0.081 | | |
| | Imp unspf 2 | 0.465 | 3.514 | 50.7 | 0.00002 | 1.014 | 1 | 0.2 | 1 | 0.203 | 0.027 | | |
| C3 | Imp B | 0.411 | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.7 | 0.201 | 0.018 | 0.070 | |
| | Imp C | 0.424 | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.6 | 0.201 | 0.016 | | |
| | Imp D | ND | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 1.4 | 0.201 | ND | | |
| | Imp E | ND | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 6.7 | 0.201 | ND | | |
| | Imp unspf 1 | 0.093 | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 1 | 0.201 | 0.006 | | |
| | Imp unspf 2 | 0.473 | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 1 | 0.201 | 0.030 | | |
| C4 | Imp B | 0.126 | 3.559 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.7 | 0.201 | 0.005 | 0.010 | |
| | Imp C | 0.153 | 3.559 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.6 | 0.201 | 0.005 | | |
| | Imp D | ND | 3.559 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 1.4 | 0.201 | ND | | |
| | Imp E | ND | 3.559 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 6.7 | 0.201 | ND | | |
| C5 | Imp B | 1.038 | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.7 | 0.201 | 0.046 | 0.625 | |
| | Imp C | **15.342** | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 0.6 | 0.201 | **0.579** | | |
| | Imp D | ND | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 1.4 | 0.201 | ND | | |
| | Imp E | ND | 3.190 | 50.2 | 0.00002 | 1.004 | 1 | 0.2 | 6.7 | 0.201 | ND | | |

**Table 4. Continued**

| Ciprofloxacin hydrochloride sample | Impurity | Impurity area (mAU-min) | Control (c) area (mAU-min) | Control (c) weight (mg) | Dilution factor control (c) | Control concenration (µg/mL) | Theoric concenration of control (c) (µg/mL) | Theoric concenration of control (c) (%) | Correction factor | Real concenration control (c) (%) | Individual content of impurity (%) | Impurities total (%) | Norms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C6 | Imp B | 0.775 | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 0.7 | 0.200 | 0.033 | 0.147 | Imp B ≤0.2<br>Imp C ≤0.2<br>Imp D ≤0.2<br>Imp E ≤0.3<br>Imp unspf ≤0.1<br>Imp total ≤0.5<br>Exculsion limit: 0.05 |
| | Imp C | 0.366 | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 0.6 | 0.200 | 0.013 | | |
| | Imp D | ND | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 1.4 | 0.200 | ND | | |
| | Imp E | ND | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 6.7 | 0.200 | ND | | |
| | Imp unspf 1 | 0.664 | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 1 | 0.200 | 0.040 | | |
| | Imp unspf 2 | 0.221 | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 1 | 0.200 | 0.013 | | |
| | Imp unspf 3 | 0.763 | 3.284 | 50.0 | 0.00002 | 1 | 1 | 0.2 | 1 | 0.200 | 0.046 | | |
| C7 | Imp B | 0.189 | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 0.7 | 0.202 | 0.009 | 0.302 | |
| | Imp C | 0.861 | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 0.6 | 0.202 | 0.034 | | |
| | Imp D | 1.287 | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 1.4 | 0.202 | 0.119 | | |
| | Imp E | ND | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 6.7 | 0.202 | ND | | |
| | Imp unspf 1 | 0.349 | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 1 | 0.202 | 0.023 | | |
| | Imp unspf 2 | 1.790 | 3.071 | 50.5 | 0.00002 | 1.01 | 1 | 0.2 | 1 | 0.202 | 0.118 | | |

Imp: Impurity, Unspf: Unspecified, ND: Not detected

**Table 5. Individual content of impurity A**

| Ciprofloxacin hydrochloride sample | Injection number | Weight (mg) | Peak area | a | b | Impurity content (%) | Average (%) | Norm (%) |
|---|---|---|---|---|---|---|---|---|
| C1 | 1<br>2 | 50.15<br>50.15 | 2.412<br>2.440 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.01<br>0.01 | 0.01 | Impurity A <0.2 |
| C2 | 1<br>2 | 50.20<br>50.20 | ND<br>ND | 119.4887<br>119.4887 | 0.84257<br>0.84257 | ND<br>ND | ND | |
| C3 | 1<br>2 | 50.00<br>50.00 | 20.457<br>20.471 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.16<br>0.16 | 0.16 | |
| C4 | 1<br>2 | 50.10<br>50.10 | 1.536<br>1.529 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.01<br>0.01 | 0.01 | |
| C5 | 1<br>2 | 50.25<br>50.25 | 5.496<br>5.460 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.04<br>0.04 | 0.04 | |
| C6 | 1<br>2 | 50.20<br>50.20 | 24.431<br>24.516 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.20<br>0.20 | 0.20 | |
| C7 | 1<br>2 | 50.20<br>50.20 | 18.704<br>18.772 | 119.4887<br>119.4887 | 0.84257<br>0.84257 | 0.15<br>0.15 | 0.15 | |

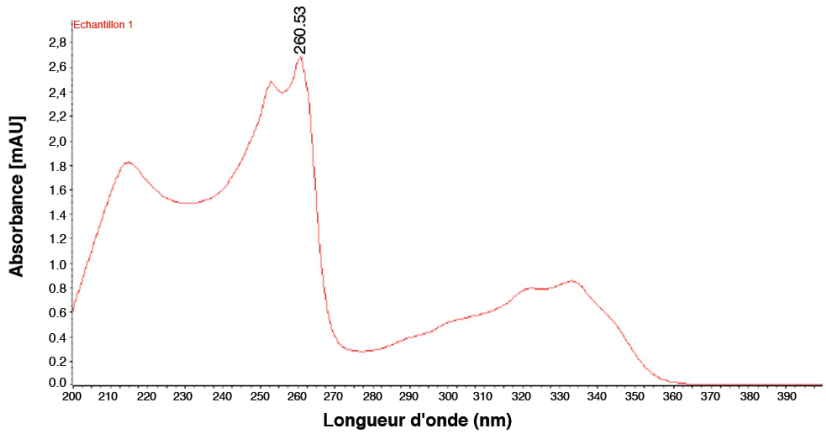



**Figure 9.** Absorption spectrum of impurity A in UV

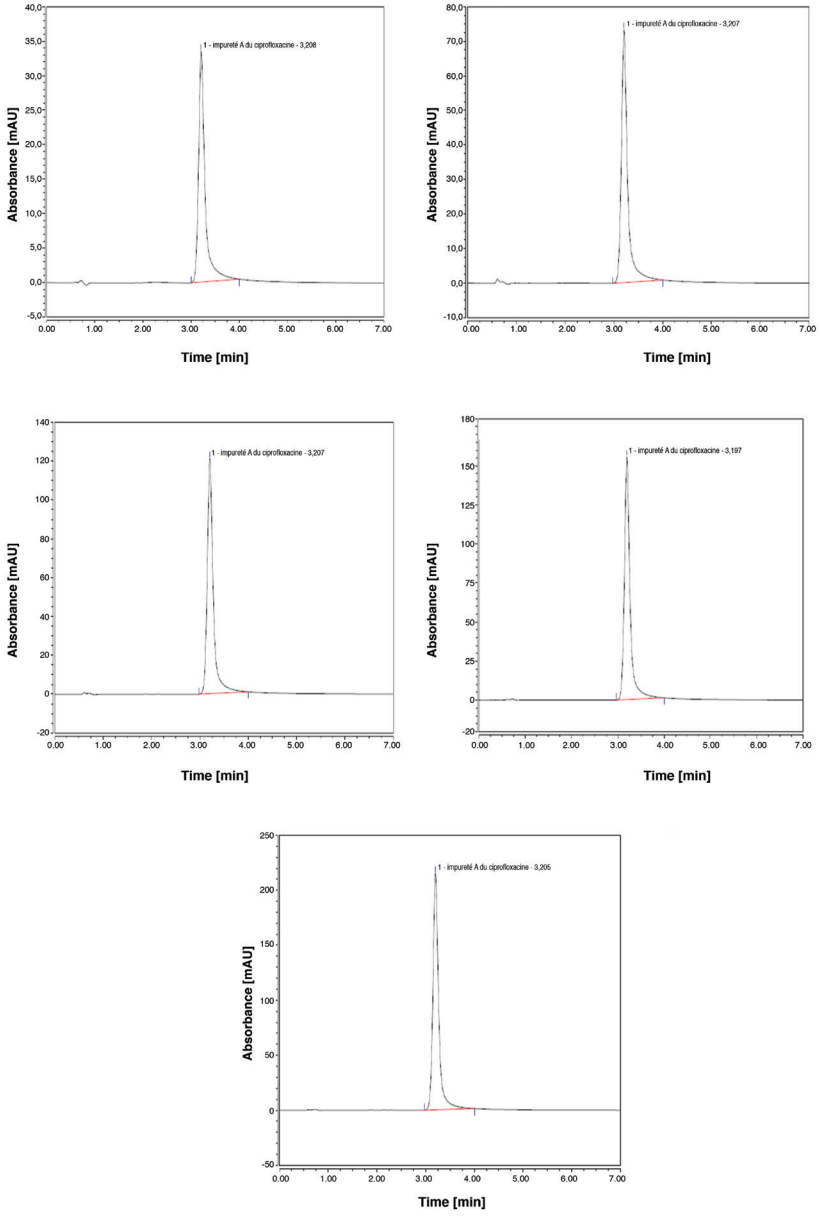



**Figure 10.** Chromatograms of standard solution at 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%, respectively

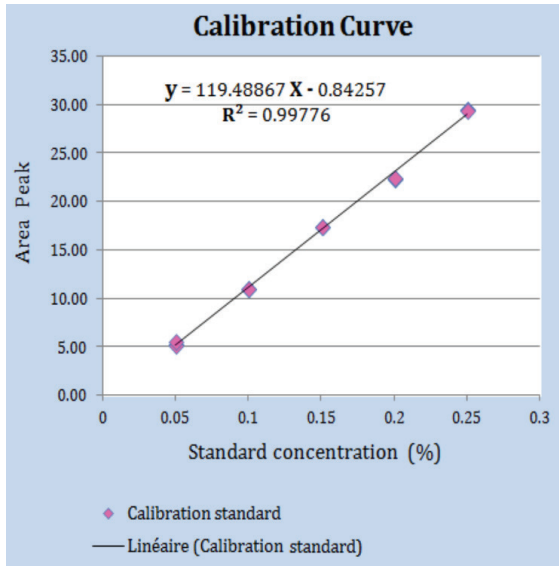



**Figure 11**. Calibration curve of standard solution

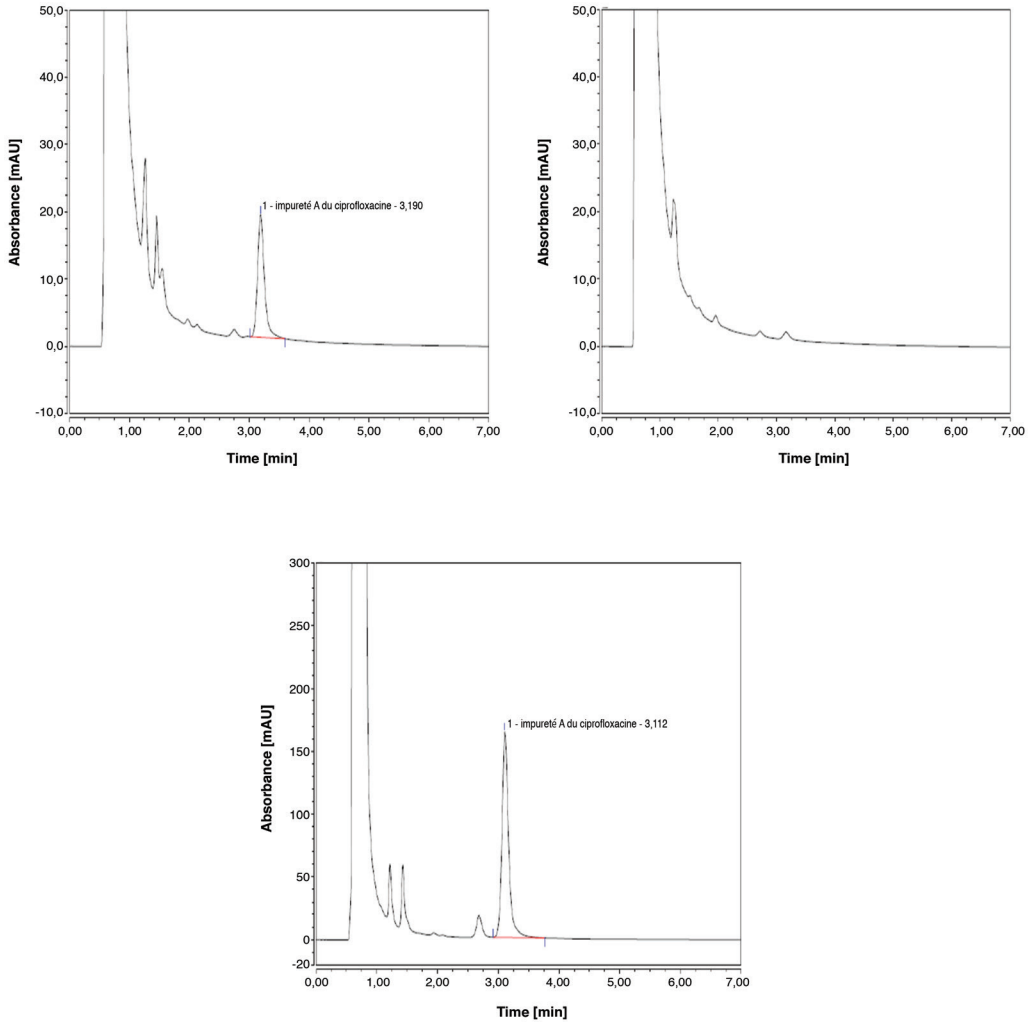



**Figure 12**. Chromatogram of C1 samples, C2 and C3

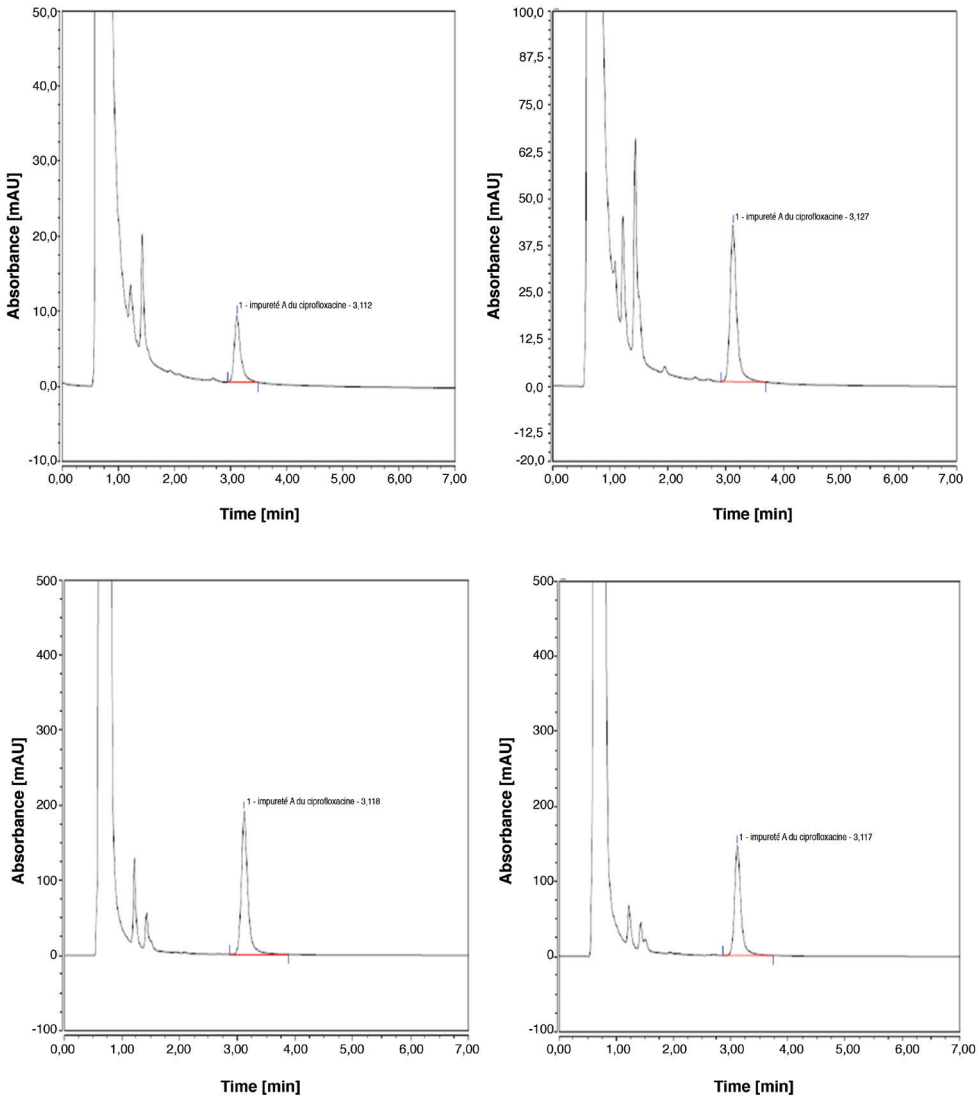



**Figure 13**. Chromatograms of C4, C5, C6, and C7

## CONCLUSION

The specified and unspecified impurities (A, B, C, D, and E) was precisely determined in seven samples of CPF_HCl by HPLC. The C1, C2, C3, C4, and C6 samples have individual contents of specified impurities (B, C, D, and E), unspecified and the total of all present impurities conforms to norms. The C5 sample contains very high content of impurity C, which is a photodegradation product and the impurities total exceeding limit, while sample C7 has a slightly higher content of unspecified impurity. Impurity A content is conformed in all samples except for the C6 sample, which has equal content to the limit. According to the detecting technique of impurity A by TLC in the Eur. Ph., the control solution was not migrated, so we recommend revising this method or replacing it with a more sensitive technique such as HPLC.

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*Ethics*

**Ethics Committee Approval:** Not applicable.

**Informed Consent:** Not applicable.

**Peer-review:** Externally peer-reviewed.

*Authorship Contributions*

Concept: D.M., H.T., Design: D.M., Data Collection or Processing: D.M., N.H., K.F.E.H., Analysis or Interpretation: D.M., K.F.E.H., N.H., N.H.Z., Literature Search: D.M., Writing: D.M.

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