Research Article

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Analysis of Residual Solvents-Impurities by HS-GC-FID: Case of Seven Samples of Ciprofloxacin API

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ABSTRACT

Organic volatile impurities known as residual solvents might appear during the production of active pharmaceutical ingredients (APIs). Analysis of residual solvents in pharmaceutical products is necessary due to the potential risk they provide to human health due to their toxicity and unfavorable side effects, as well as the possibility that they may change the physicochemical properties of the pharmaceutical product.

The goal of this study was to analysis 29 residual solvents-impurities using Head Space Gas Chromatography with Flame Ionization Detector (HS-GC-FID) in seven Ciprofloxacin Hydrochloride API samples that were gathered from seven pharmaceutical companies located in Algeria. A flame-ionization detector and a silica column covered with a 1.8 m layer of phase G43 were both installed in the GC. Helium served as the carrier gas, having a split ratio of 1:5 and a linear velocity of 35 cm/s. The temperature of the column began at 40 °C and increased to 240 °C. The temperature of the injection was 140 °C, while the detector temperature was 250 °C. Twenty-nine organic solvents belong to classes 1 and 2 were analyzed in seven samples of Ciprofloxacin Hydrochloride API whose control is mandatory because of their carcinogenic and intrinsic toxicity. Only five solvents were identified wich are Hexane, Toluene, Acetonitrile, Methanol and Dichloromethane in the different samples. All samples collected satisfied the test of identification, so, the confirmation and the quantification procedures weren't realized. The HS-GC-FID technique used showed that the identified solvents differ from one sample to another of the same molecule. This showed that manufacturers didn't often use the same solvents to produce the same API, which justifies that residual organic solvent tests weren't usually mentioned in the specific monographs.

Keywords: Residual solvents, Solvents-impurties, Ciprofloxacin Hydrochloride, Fluoroquinolone, Antibiotic, HS-GC-FID

1. INTRODUCTION

Residual solvents are organic volatile impurities that can occur in the manufacture of Active Pharmaceutical Ingredients (APIs). They can also be present in the production of raw materials required for the production of pharmaceutical drug products [1]. Limits for the levels of residual solvents in pharmaceutical products have been set by the International Council for Harmonization of Technical Requirements for Pharmaceutical and Human Use (ICH) [2,3]. Three categories are used to classify residual solvents. Class 1 solvents should be avoided because they are either known or strongly suspected human carcinogens, environmental dangers, or both. Class 2 solvents have a threshold of toxicity that must be kept to the specified concentration in therapeutic products even though they are not genotoxic impurities. At a maximum concentration of 5000 ppm, class 3 solvents pose the least risk [4,5].

Pharmaceutical products require residual solvent analysis not only because they pose a risk to human health due to their toxicity and unfavorable side effects, but also because they may change the physicochemical properties of the product [6].

There are many analytical techniques available for residual solvent analysis, but gas chromatography (GC) is the most popular and selective one. Nevertheless, thermolabile or nonvolatile compounds are frequently included in pharmaceutical samples. These compounds may contaminate the chromatographic system or their chromatogram peak locations may interfere with the analyte's peak locations. Combining gas chromatographic analysis and headspace sampling can solve those issues because headspace sampling only analyzes the volatile components of the sample [7].

A fluoroquinolone antibiotic called Ciprofloxacin **(Figure 1)** is used to treat a variety of bacterial illnesses. This includes, among other things, infections of the bones and joints, the abdomen, some types of infectious diarrhea, the respiratory and skin tracts, typhoid fever, and the urinary tract [9].

Over 18 pharmaceutical companies in Algeria produce Ciprofloxacin [10]. This study's primary goal was to analyze 29 residual solvents-impurities using Head Space Gas Chromatography with Flame Ionization Detector (HS-GC-FID) in seven samples of Ciprofloxacin Hydrochloride API that were gathered from seven pharmaceutical companies located in Algeria.

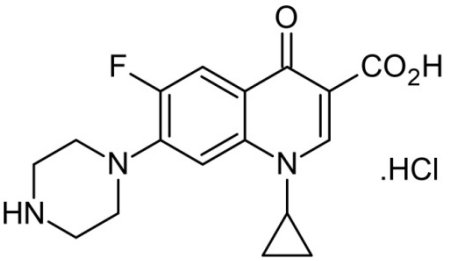

Figure 1. Chemical structure of Ciprofloxacin Hydrochloride [11].

2. MATERIALS AND METHODS

Samples Collection

Using the Algerian drug nomenclature dated the 31 December 2014, seven samples of Ciprofloxacin Hydrochloride raw material were obtained from seven pharmaceutical industries situated in Algeria [10]. The samples were collected between April 1, 2015, and December 31, 2016. Together with the raw material, the compendium also includes the following essential details (origin, supplier/manufacturer, expiration date, analytical certificate, synthesis route, Drug Master File, etc.) [12;15]. The samples were not past their expiration dates, and we named them C1, C2, C3, C4, C5, C6, and C7. They were examined just before their expiration date while being kept at ambient temperature, shielded from light and humidity. We didn't get all the necessary information for certain samples.

Apparatus

We employed a gas chromatograph (GC-2010 Plus-Shimadzu, Japan) coupled to flame ionization detector (FID) and headspace extraction sampler "HS" (Auto sampler AOC-5000 Plus-Shimadzu, Japan) to analyze the residual solvents. Capillary column (MEGA-624 Fast) of fused silica covered with a crosslinked mixture of 6 % polycyanopropylphenylsiloxane and 94 % poly (dimethyl) siloxane (w: 30 m, Ø: 0.32 mm ID, film thickness: 1.80 µm) and headspace vials with 20 mL volume and their stoppers in polytetrafluoroethylene (PTFE).

A pH meter (Mettler Toledo, USA) was used to measure the pH of solutions, an ultrasonic bath (Elmasonic S 130 H, Germany) was used to dissolve the samples, and an analytical balance (Kern ALS-200-

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4N, Germany) was used to weigh the materials.

Reagents and Chemicals

Dimethyl sulfoxide (99.5%) was obtained from Riedel-de Haën, France, and the standard solutions of USP Class 1, USP Class 2 Mix A, and USP Class 2 Mix B residual solvents used for peak identification were purchased from Restek (Bellefonte, USA).

Composition of Residual Solvents Standard Solutions:

- **Class 1_USP (10-50 mg/mL):** benzene, carbon tetrachloride, 1,1-dichloroethene, 1,1,1-trichloroethane, and 1,2-dichloroethane;
- **Class 2_USP_Mix A (0,35-19,4 mg/mL):** acetonitrile, toluene, 1,4-dioxane, ethylbenzene, p-xylene, m-xylene, isopropylbenzene, o-xylene, chlorobenzene; cyclohexane, methylcyclohexane, trans-1,2-dichloroethene, tetrahydrofuran, methanol and dichloromethane;
- **Class 2_USP_Mix B (50-290 µg/mL):** 2-hexanone, pyridine, n-hexane, nitromethane, chloroform, 1,2-dimethoxyethane, trichlorethylene and tetralin.

Standard solutions preparation

Stock solution for standard class 1: prepared from USP_Class 1 residual solvents at 10^{-5} mL/mL concentration.

Solution of Class 1 standard: prepared from **Standard stock solution for class 1** at 0.2 mL/mL concentration in headspace vial.

Stock solution A of class 2 standard: prepared from Class 2_Mixture A of USP residual solvents at 10^{-2} mL/mL concentration in headspace vial.

Stock solution B of class 2 standard : prepared from Class 2_Mixture B of USP residual solvents at 10^{-2} mL/mL concentration in headspace vial.

Standard solution of class 2 mixture A: prepared from stock solution A of class 2 standard at 0.5 mL/mL concentration in headspace vial.

Standard solution of class 2 mixture B: prepared from stock solution B of class 2 standard at 5 mL/mL concentration in headspace vial [16;18].

Preparation of test solutions

Test stock solution: prepared from each Ciprofloxacin Hydrochloride sample at 10 mg/mL concentration in headspace vial.

Test solution: prepared from test stock solution at 5 mL/mL concentration in headspace vial [16;18].

Preparation of suitability solution

Solution of class 1 system suitability: prepared from stock solution of class 1 standard at 0.2 mL/mL concentration in headspace vial [16;18].

Identification by Procedure A

The headspace operating parameters were set at syringe temperature of 80-90 °C, equilibration time of 60 min, equilibration temperature of 80 °C, transferline temperature of 85 °C, pressurization time greater than or equal to 60 S, injection volume of 1 mL and helium carrier gas at an appropriate pressure [16;18].

- If, in the chromatogram of the solution to be examined, there not appear any peak corresponding to one of the peaks of the chromatograms obtained with the standard solutions of class 1, class 2_Mix A or class 2_Mix B, the substance examined satisfies the test;
- If, in the chromatogram of the solution to be examined, there appears one (or more) peak(s) corresponding to one of the peaks of the chromatograms obtained with the standard solutions of class 1, class 2_Mix A, class 2_Mix B, having a surface area smaller than that of the standard(s), the substance examined satisfies the test;
- If, in the chromatogram obtained of the solution to be examined, there appears one (or more) peak(s) corresponding to one of the peaks of the chromatograms obtained with the standard solutions of class 1, class 2_Mix A or class 2_Mix B, having an area greater than or equal to that of the standard(s), the confirmation procedure B will be used to verify the peak(s) identity.

3. RESULTS AND DISCUSSION

The typical chromatograms supplied with the standard solutions of residual SCR solvents and the chromatograms obtained with the standard solutions are equivalent, which allowed us to identify the peaks corresponding to the solvents of each class with their retention times respectively.

Identification by Procedure A

System Compliance

Figure 2 shows the chromatograms obtained with standard solution of Class 1 and system suitability solution Class 1 and figure 3 shows the typical chromatogram of standard solution of Class 1_USP. The figures 4, 5 show the obtained chromatogram and the typical chromatogram of the standard solution of Class 2_Mix A. Figure 6 and 7 show the obtained chromatogram and the typical chromatogram of standard solution of Class 2_Mix B.

To compare figure 2 compare to figure 3, only the elution order was taken into account when determining the retention times of the peaks.

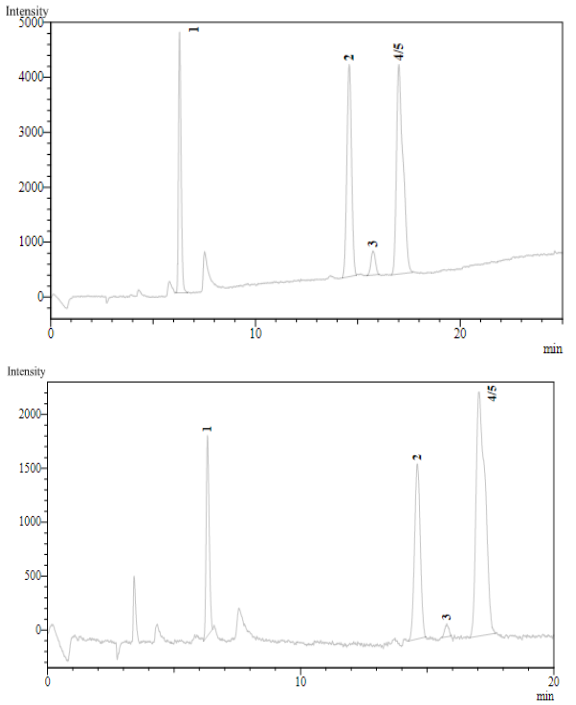

Figure 2. Obtained chromatograms of standard solution Class 1 and solution of Class 1 system suitability.

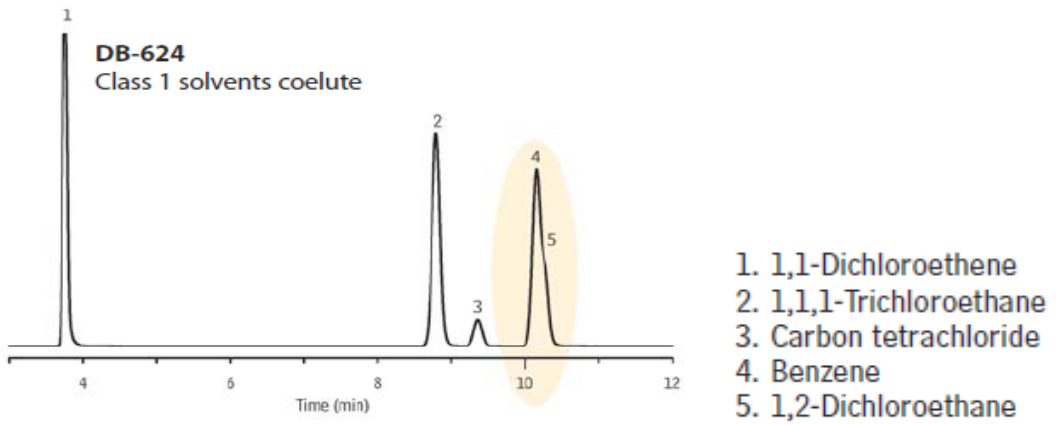

Figure 3. Typical chromatogram of Class 1_USP standard solution [19].

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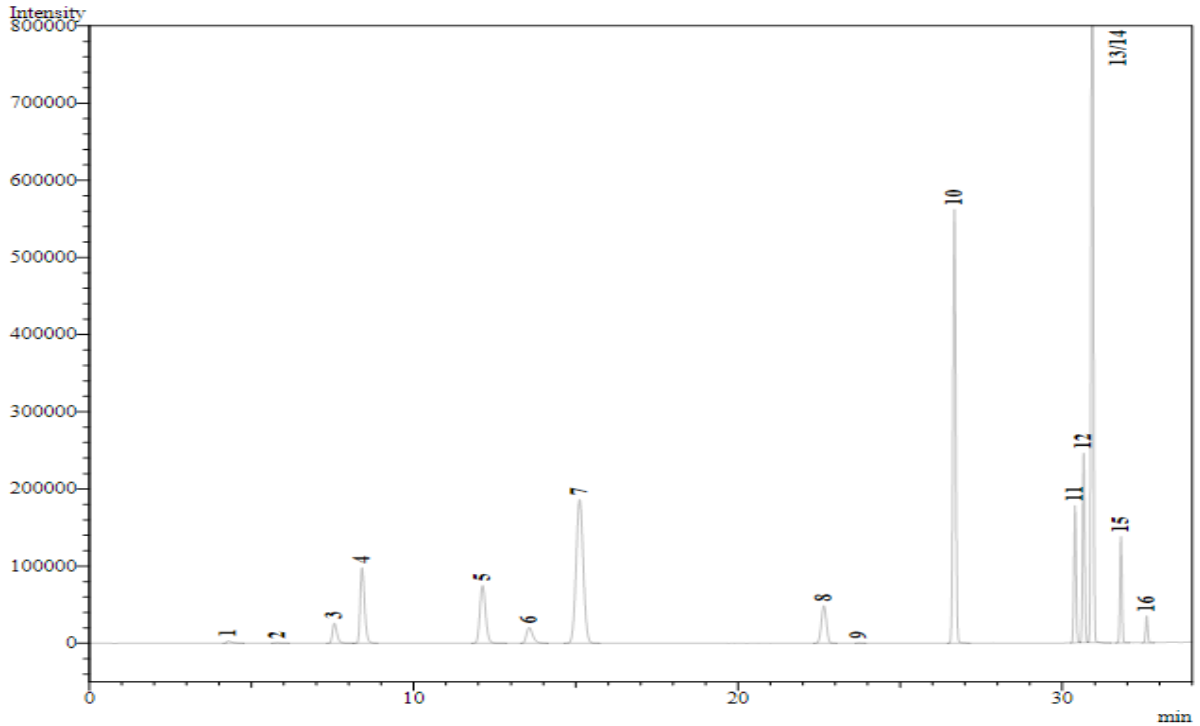

Figure 4. Obtained chromatogram of Class 2_Mix A standard solution.

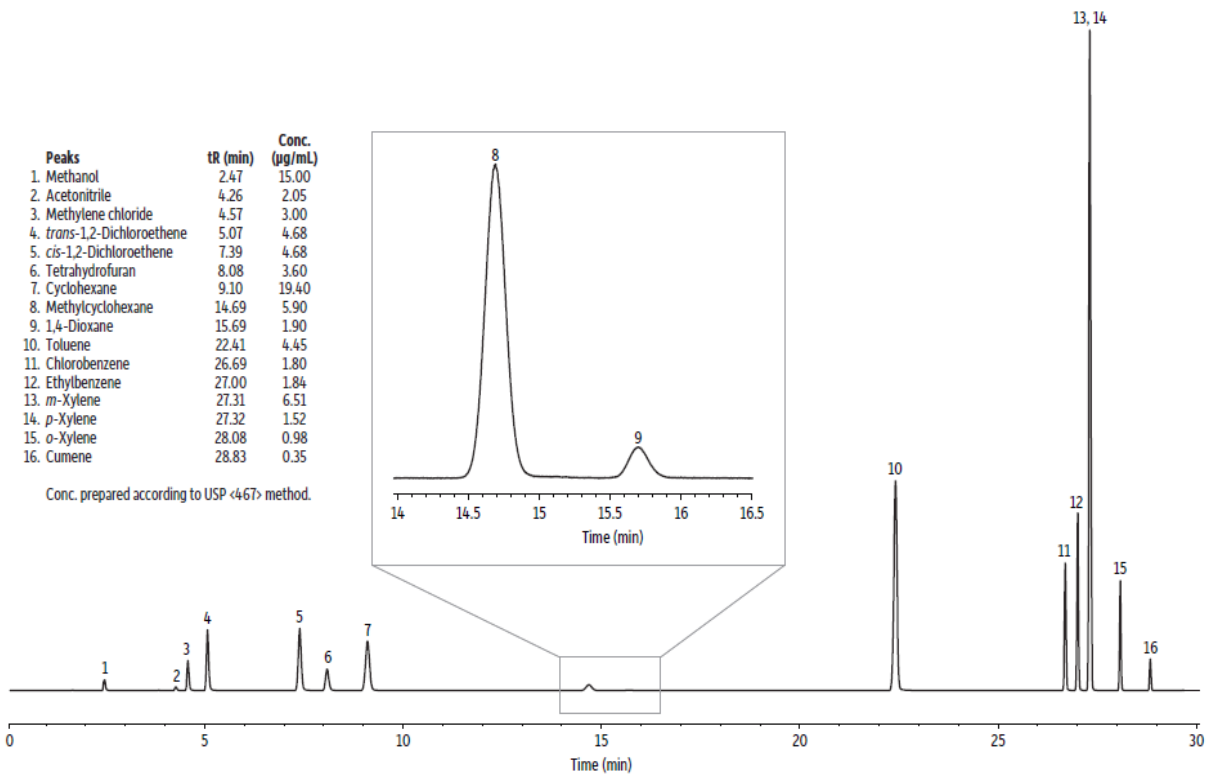

Figure 5. Typical chromatogram of Class 2_Mix A standard solution [20].

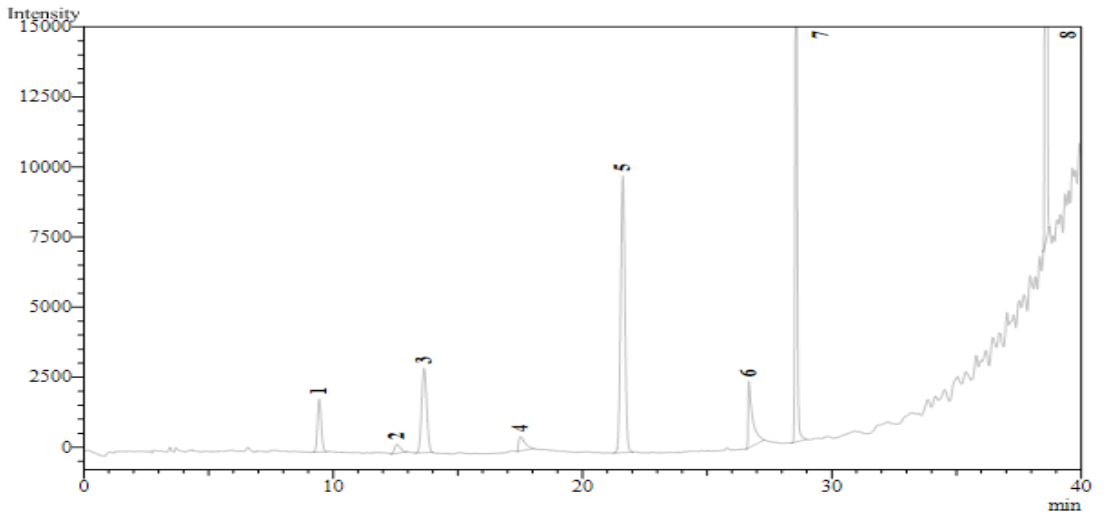

Figure 6. Obtained chromatogram of Class 2_Mix B standard solution.

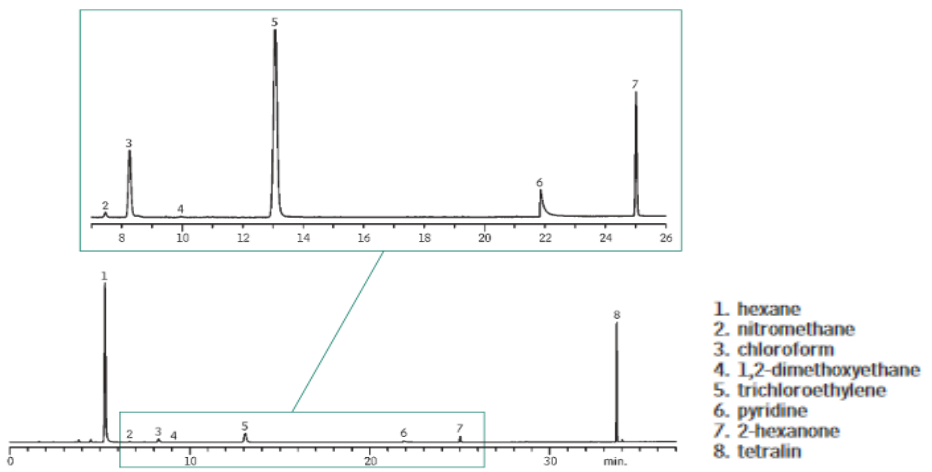

Figure 7. Typical chromatogram of Class 2_Mix B standard solution [21].

Identification of Solvents

The obtained chromatograms of (Class 1, Class 2_Mix A and Class 2_Mix B) standard solutions **(Figure 2, 4 and 6)** and the typical chromatograms supplied with standard solutions **(Figure 3, 5 and 7)** were comparable, which allowed us to identify the respective peaks corresponding to solvents of each class with their retention times.

Five Peaks of Class 1 Solvents*:*

- 1,1-Dichloroethene: 6.283 min;
- 1, 1,1-trichloroethane: 14.572 min;
- Tetrachloromethane: 15.775 min;
- Benzene: 17.007 min;
- 1,2-Dichloroethane: 17.007min.

Co-elution of Benzene and 1,2-dichloroethane which were eluted at the same retention time (TR: 17.007 min) **(Figure 2)**.

Sixteen Peaks of Class 2_Mix A Solvents:

- Methanol: 4.285 min;
- Acetonitrile: 5.800 min;
- Dichloromethane: 7.556 min;

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- trans-1,2-dichloroethene: 8.412 min;
- cis-1,2-Dichloroethene: 12.128 min;
- Tetrahydrofuran: 13.565 min;
- Cyclohexane: 15.12 min;
- Methylcyclohexane: 22.649 min;
- 1,4-Dioxane: 23.730 min;
- Toluene : 26.677 min ;
- Chlorobenzene : 30.394 min ;
- Ethylbenzene : 30.661 min ;
- m-Xylene : 30.927 min ;
- p-Xylene : 30.927 min ;
- o-Xylene : 31.811 min ;
- Isopropylbenzene (Cumene): 32.604 min.

Co-elution of m-Xylene and p-Xylene which were eluted at the same retention time (TR: 30.927 min) **(Figure 4)**.

Eight Peaks of Class 2_Mix B Solvents:

- n-Hexane: 9.444 min ;
- Nitromethane : 12.562 min ;
- Chloroform: 13.657 min;
- 1,2-Dimethoxyethane: 17.522 min;
- Trichloroethene: 21.631 min;
- Pyridine: 26.668 min;
- 2-Hexanone: 28.584 min;
- Tetralin: 38,596 min.

Signal-to-Noise Ratio

The signal-to-noise ratio of 1,1,1-trichloroethane peak was 8.82, which was greater than the limit required by the USP (at least 5). The signal-to-noise ratio of the following peaks: (1,1-dichloroethene, 1,1,1-trichloroethane, tetrachloromethane and benzene/1,2-dichloroethane) of system suitability solution were respectively: 8.98, 8.82, 3.07 and 10.09. These values were according to the standard required by the USP (minimum 3).

Resolution

The resolution between methylene chloride peak and acetonitrile peak was 6, value conform to the standard (at least 1.0). It was calculated by the software according to the resolution factor calculation formula.

Therefore, the system was, in compliance.

Analysis of Samples

Identification by Procedure A

The figures 8, 9, 10, 11, 12, 13 and 14 show the chromatograms obtained with the different test solutions of samples.

C1, C5, C6 and C7 samples: no peak detected corresponding to one of the obtained chromatograms of (Class 1 or Class 2_Mix A or Class 2_Mix B) standard solutions. So, C1, C5, C6 and C7 samples satisfied the test.

C2 sample: two peaks were appeared, Hexane and Toluene, they had respectively the following surfaces (2049 µV.min and 3096 µV.min) which were lower than those of the corresponding standards (7031 µV.min and 3311414 µV.min). So, C2 sample satisfied the test.

C3 sample: a single peak was detected, that of Acetonitrile, having an area of 5472 µV.min lower than that of the corresponding standard (6194 µV.min). So, C3 sample satisfied the test.

C4 sample: two peaks were appeared, Methanol and Dichloromethane, they had respectively the following areas (4499 µV.min and 9443 µV.min) which were lower than those of the corresponding standards

(33227 µV. min and 252054 µV.min). So, C4 sample

4. CONCLUSION

Twenty-nine organic solvents belong to classes 1 and 2 were analyzed in seven samples of Ciprofloxacin Hydrochloride API whose control is mandatory because of their carcinogenic and intrinsic toxicity. Only five solvents were identified which are Hexane, Toluene, Acetonitrile, Methanol and Dichloromethane in the different samples. All samples collected satisfied the test of identification, so, the confirmation and the quantification procedures weren't realized. The HS-GC-FID technique used showed that the identified solvents differ from one sample to another of the same molecule. This showed that manufacturers didn't often use the same solvents to

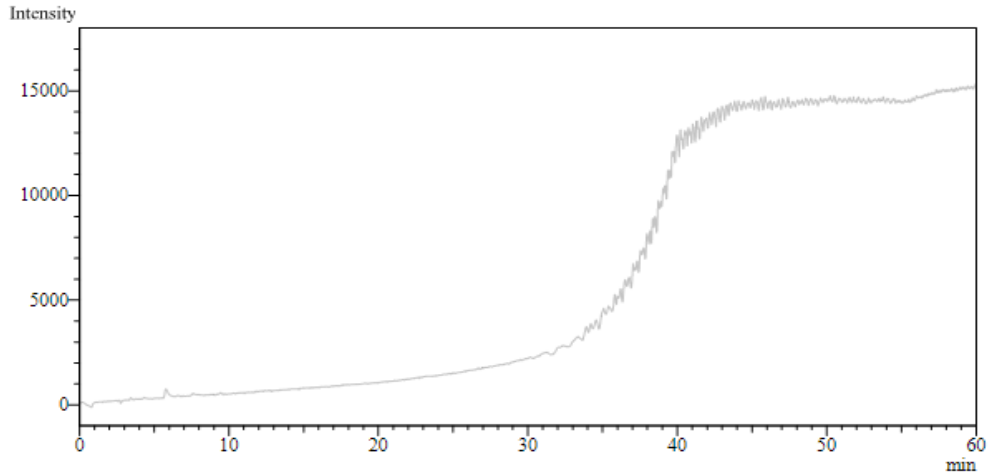

Figure 8. Chromatogram of C1 sample.

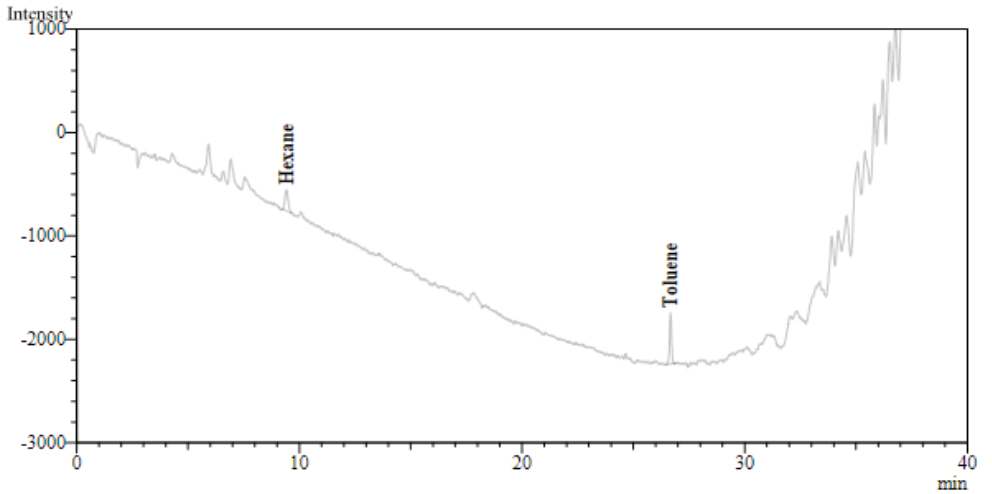

Figure 9. Chromatogram of C2 sample.

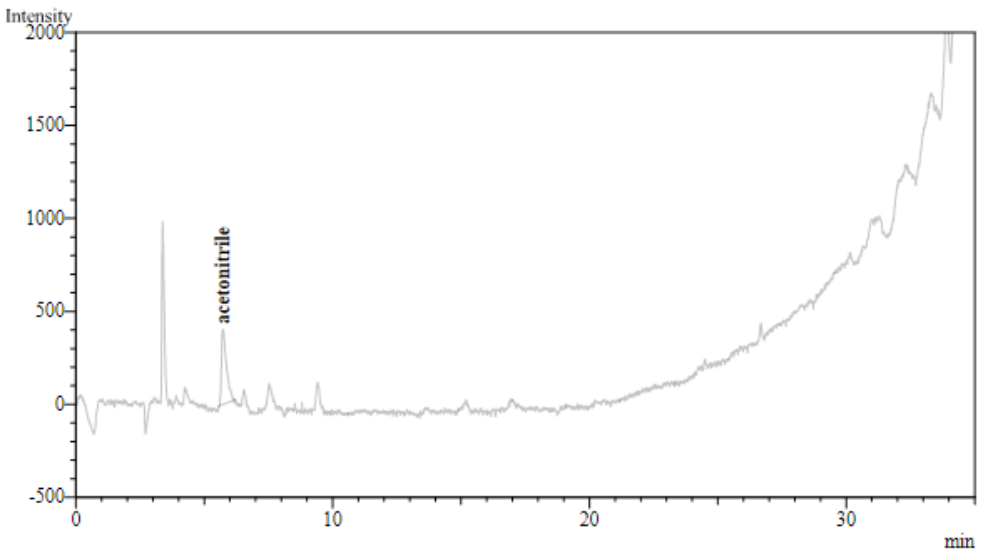

Figure 10. Chromatogram of C3 sample.

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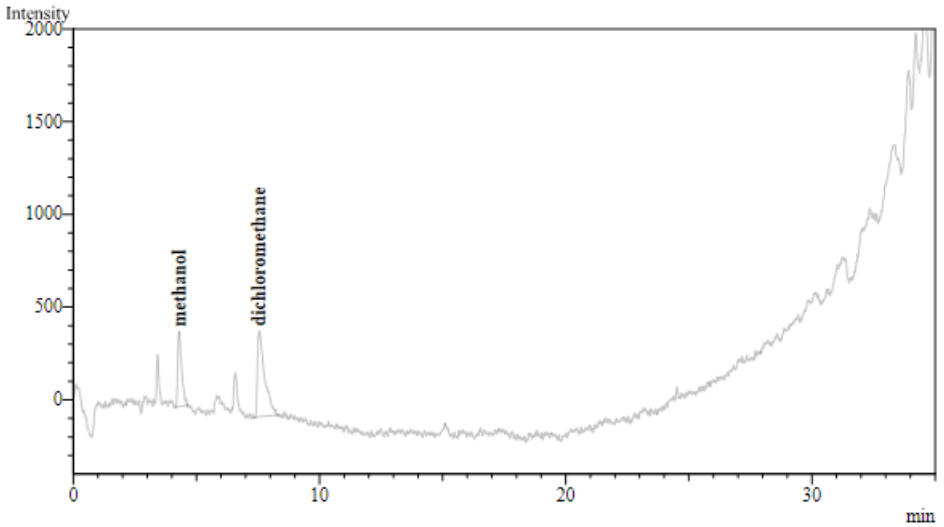

Figure 11. Chromatogram of C4 sample.

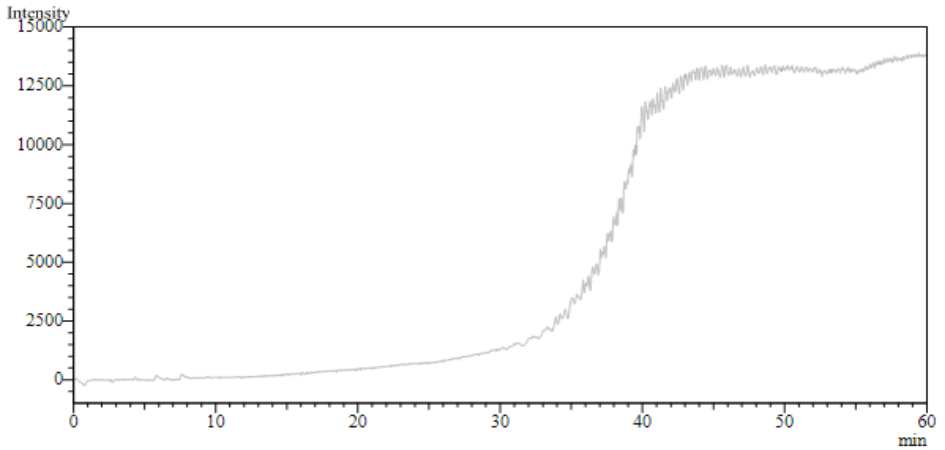

Figure 12. Chromatogram of C5 sample.

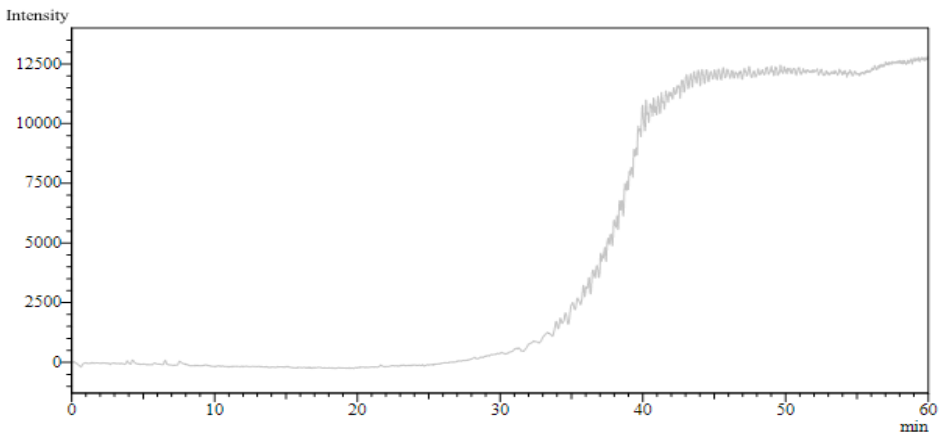

Figure 13. Chromatogram of C6 sample.

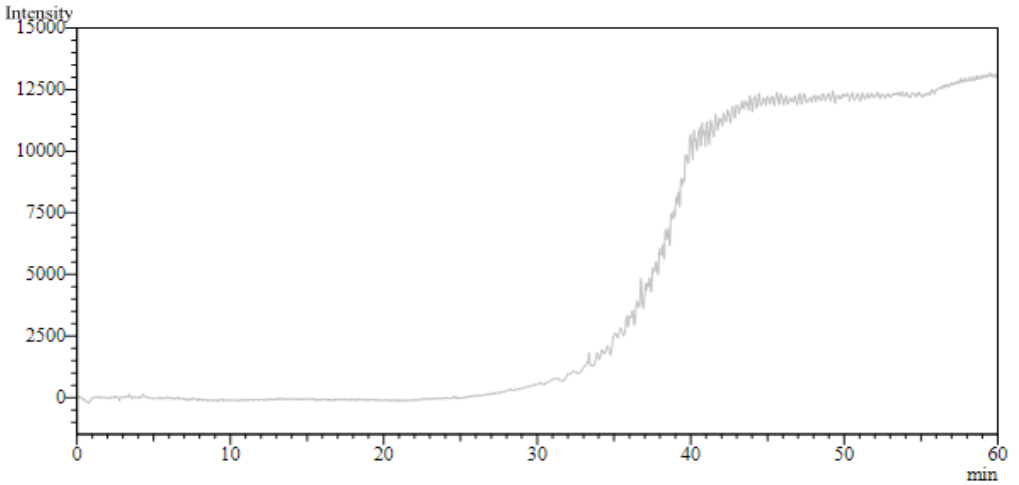

Figure 14. Chromatogram of C7 sample.

produce the same API, which justifies that residual organic solvent tests weren't usually mentioned in the specific monographs.

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Conflict of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

Statement of Researchers Contribution

Concept: M. D.; Design: M. D.; Supervision: M. D.; Resources: M. D.; Materials: M. D.; Data collection and/or processing: M. D.; Analysis and/or interpretation: M. D.; Literature search: M. D.; Writing manuscript: M. D.; Critical review: M. D.

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