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Research Paper

Determination of Elemental Impurities Ciprofloxacin hydrochloride and Fluconazole via ICP-OES

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Abstract

In this paper, we report the results of quality control based in elemental impurities determination of 12 raw materials samples of Ciprofloxacin hydrochloride and Fluconazole intended for the manufacture of their generic products marketed in Algeria. These samples were supplied by different pharmaceuticals companies. Heavy metals limit test and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) were used to control and quantify the elemental impurities in these samples. The limit tests results showed that all samples were conformers except F5 sample. The ICP-OES analysis showed that the content of each element is normal in all samples except Cobalt content is greater than the limit required in C4 sample. The highlighting of certain elemental impurties exceeds safety standards confirm that heavy metals test is neither specific nor exact, so it's recommended to apply the new procedures based on safety data which are well described in the USP new chapters or the Eur Ph general chapter.

Keywords: Elemental Impurities; ICP-OES; Ciprofloxacin Hydrochloride; Fluconazole

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1. Introduction

Nowadays, impurities present a serious health problem, threatening the therapeutic efficacy of medicines. They are classified as organic impurities, elemental impurities and residual solvents. Elemental impurities can arise from several sources, either they have been intentionally introduced into the synthesis route, such as catalysts, salting-out method, solvent used in the extraction process or they may be present as contaminants from interaction with equipment [1]. In some cases, elements cannot be completely removed from Active Pharmaceutical Ingredients (APIs) after synthesis. Therefore, they are detectable in the raw material or commercial product but the maximum tolerable amount of these impurities should be considered [2]. They can be classified into three classes on the basis of their toxicity (based on the respective permitted daily exposure) and likelihood of occurrence in the drug product. It is important to highlight that all elements which were used in the production of APIs are considered as intentionally added and should be evaluated independently from the route of administration and respective class [2]. For several years, the standard method for elemental impurties control has been the "heavy metals limit test", which proved to be limited and does not cover metals with the highest toxic potential. For this reason, discussions have been held within the expert groups of the different pharmacopoeias and within the ICH framework to develop more appropriate harmonized procedures based on safety data for the elemental impurties control [3, 4].

In this study, we will control and analysis the elemental impurities by Heavy Metals Limit Test and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) in the raw materials of Ciprofloxacin hydrochloride and Fluconazole intended for the manufacture of their generic products marketed in Algeria.

2. Material and Methods

2.1. Sampling

Samples of an antibiotic and antifungal raw materials called Ciprofloxacin hydrochloride and Fluconazole were obtained by soliciting all producers of their commercial products installed in Algeria by referring to the Algerian drug nomenclature dated the 31st December 2014 [5]. The samples are collected during the period from 1st April 2015 to 31st December 2016. The compendium covers not only the raw material but also the following necessary information (origin, supplier / manufacturer, expiration date, analysis bulletin, synthesis route, Drug Master File.....etc). Seven samples of Ciprofloxacin hydrochloride and five of Fluconazole were collected among approximately 27 producers. They weren't expired and we labeled them as follows: C1, C2, C3, C4, C5, C6, C7 and F1, F2, F3, F4, F5. For some samples, we didn't receive all the necessary information (Table 1). All of them were stored at room temperature, protected from light and humidity and analyzed prior to their expiration date.

Table 1: Raw material collection from local producers.

Sample	Local Producer	Batch number	Expiration Date	Manufacturer-S
		Ciprofloxacin hydrochloride		
C1	Lab C1	A004801	04/2017	/
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 Labo
C5	Lab C5	120801	08/2016	Pharmaceutical (
C6	Lab C6	KOFA0062	03/2017	Dr Reddy's Labo
C7	Lab C7	0251103F	07/2019	/
		Fluconazole		
F1	Labo F1	FLP 0270313	03/2017	Synergene Activ
F2	Labo F2	FLU_1411026	11/2019	Granules India L
F3	Labo F3	FL0A 4005	04/2019	Quimica Sintetic
F4	Labo F4	FLP 1021012	09/2017	Synergene Activ (India)
F5	Labo F5	20021067	05/2018	Mylan Laborator

2.2 Heavy metals limit test

The heavy metals detected by various tests described in the general method are those which precipitate at pH 3.5 as colored sulphides, the precipitating agent used is thioacetamide. The comparison is carried out with a control containing a known amount of Pb, so the total content of "heavy metals" is expressed as Pb.

They were determinate in Ciprofloxacin hydrochloride according to the Method II of the United State Pharmacopoeia (USP 38 NF 33) and in Fluconazole according to the limit test B of the European Pharmacopoeia (Eur Ph) 8th edition.

2.2.1. Standard solutions, reagents, apparatus and procedure

1. USP Method II [6]

Solutions prepared: pH 3.5 buffer solution, lead nitrate stock solution (1000 ppm), standard lead solution (10µg of Pb/ml), 6N hydrochloric acid, 6N ammonium hydroxide, 1N acetic acid and thioacetamide reagent.

Apparatus: muffle furnace (Nabertherm L15/11/B170) and Sand bath (Harry Gestigkeit GMBH).

Samples processing: each Ciprofloxacin hydrochloride sample is treated as follows:

Test solution: 1 g of Ciprofloxacin hydrochloride was placed in a crucible, a sufficient quantity of concentrated sulfuric acid was added to moisten it, then placed in sand bath at low temperature until the acid was completely evaporated and finally calcined in muffle furnace at 600 ° C for 2 hours until complete carbonization. To the carbonized mass, we added 2 mL of nitric acid, 5 drops of sulfuric acid then heated in sand bath until the total fumes disappeared and finally a second calcination at 600 ° C for 2 hours. After cooling the crucible, adding 4 mL of 6N HCl then evaporating in sand bath until total dryness for 15 min. Adding a drop of concentrated HCl, 10 mL of hot water and allowed to react for 2 min. The pH of the solution was adjusted with 6N ammonia until an alkaline solution was obtained. The obtained solution was diluted with water to 25 mL then adjusted to pH between 3.0 and 4.0 with 1N acetic acid. A second dilution with water to 40 mL and finally put it in screw tube.

Control solution: 2 mL of standard Lead solution (20 µg of Pb) was diluted with 23 mL of water. The pH was adjusted with acetic acid to value between 3.0 and 4.0. A second dilution to 40 mL with water and finally put it in screw tube.

Solutions treatment (Control, Test)

To each screw tube, 2 mL of pH 3.5 buffer solution was added followed by 1.2 mL of thioacetamide reagent, dilution with water to 50 mL, mixed and finally allowed to stand for 2 min. A color comparison between control and test solutions was carried out.

Limit: the sample contains at most 20 ppm of heavy metals if the color of test solution is less intense than that of control solution.

2. Limit test B of the Eur Ph [7]

Solutions prepared: solvent (mixture of 15 water volumes and 85 methanol volumes), pH 3.5 buffer solution, thioacetamide solution 40 g / L, thioacetamide reagent and Lead solution (1 ppm).

Samples processing: each Fluconazole sample was treated as follows:

Test solution: 2 g of Fluconazole dissolved in 20 mL of solvent, 12 mL of this solution was taken.

Control solution: mixture of 10 mL of Lead solution 1ppm and 2 mL of test solution.

Blank solution: 10 mL of solvent and 2 mL of test solution.

To each solution, 2 mL of pH 3.5 buffer solution and 1.2 mL of thioacetamide reagent were added and

mixed immediately. The solutions were examined 2 min afterwards.
For some samples, the test was difficult to evaluate, solutions filtration on membrane filter (pore diameter 3 µm) was carried out and comparison of obtained spots was performed.
System compliance: control solution showed a slight brown coloration compared to the blank solution.
Limit: sample contains at most 10 ppm of heavy metals if the brown color of test solution is not more intense than that of control solution.

2.3 ICP-EOS Analysis
2.3.1. Main elements added intentionally in route synthesis
For some samples, we relied on the synthesis process provided to detect intentionally added elements, but for others unfortunately we couldn't get access [8, 9, 10 and 11]. The added elements are: Co, Ag, Cu, Sn and Cr for Ciprofloxacin Hydrochloride and Ag, Cu, Sn and Cr for Fluconazole [12, 13, 14 and 15].

2.3.2. Elements to be researcher
10 elements were analyzed according to the requirements and the availability of standards elements:
Class 1 elements: they are significantly toxic in all administration routes, their evaluation is obligatory for the following four elements: Cd, Pb, As and Hg.
Class 2A elements: they are toxic and must be evaluated for the following three elements: Co, Ni and V. Because of lack of standard availability, Vanadium couldn't be analyzed.
Class 2B elements: their evaluation is obligatory only if an element of this class has been added intentionally. According to the synthesis processes, Ag was the only element of this class that has been added intentionally.
Class 3 elements: they have a relatively low toxicity; their evaluation is dependent on the administration route. Ciprofloxacin Hydrochloride is just intended for the formulation of oral form, the evaluation of class 3 elements is not obligatory, even if they had been added intentionally. Fluconazole is intended for the formulation of parenteral and oral forms, so the evaluation of Cu, Sn and Cr is obligatory.
Class 4 elements: they aren't included in the risk assessment because of their low toxicity.

2.3.3. Standard solutions, reagents and apparatus
Standard single-element solutions (1000 ppm) were used in analysis: Cd, Pb, As, Hg, Co, Ni, Ag, Cu, Sn, Cr and Nitric acid was used as reagent. They were procured from Sigma- Aldrich. The water used to prepare the solutions was distillated, deionized and then purified using a Milli-Q system (Millipore, Billerica).
An ICP-OES device (Thermo Scientific iCAP 7000 Series Inductively Coupled Plasma Spectrometer) provides low cost multi-element analysis for measuring trace elements in a diverse sample range. It's driven by the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software and the Element Finder plug-in which reduces method development time and removes the need for wavelength selection by the user.

2.3.4. Analysis protocol [16]
Solvent (0.5% acidified water) was prepared with 5 mL of concentrated nitric acid in 1L of purified water. Standard multi-element stock solution (1mg /L): 100 µL was taken from each standard single-element solution (1000 ppm) in a 100 mL vial and completed with acidified water.
Establishment of the calibration curve: five dilutions covering the range of desired concentrations were prepared from the standard stock solution in 50 mL vial (Table 2).
Test solution: 250 mg of each sample was dissolved in mixture of 100 mL of acidified water and 0.5 mL of concentrated nitric acid and heating at 250 °C for 2 h. The solution obtained was filtered by a membrane (0.45 µm). **Table 2**: Dilution range of the calibration curve

	1st Dilution	2nd Dilution	3rd Dilution	4th Dilution	5th Dilution
Stock solution Volume (mL)	0.15	0.3	0.45	0.6	0.75
Permitted water (mL)	49.85	49.7	49.55	49.4	49.25
Final Concentration (ppm)	0.003	0.006	0.009	0.012	0.015

2.3.5. Samples analysis and results expression: the samples were analysis after the standard solutions have been read. The results are given directly by the software (Qtegra). Calculus Formula of the elements concentration (ppm) in each sample

$$\textbf{Element concentration (ppm)} = \frac{\text{Concentration ppm}}{\text{Sample weight (mg)}} \times 250\ (\text{mg})$$

2.3.6. Limits [17]
According to the new chapter (232) USP 40, the limit concentrations of the inhalation route wasn't taken into account because Ciprofloxacin Hydrochloride was intended just for the oral form manufacture and Fluconazole for oral and parenteral forms.

3. Results and Discussion
3.1. Heavy metals limit test

Figure 1: Method II results

Ciprofloxacin hydrochloride samples: all the test solutions exhibit less intense coloration than that of control solution. This shows that all samples are conformers and contain maximum 20 ppm of heavy metals.

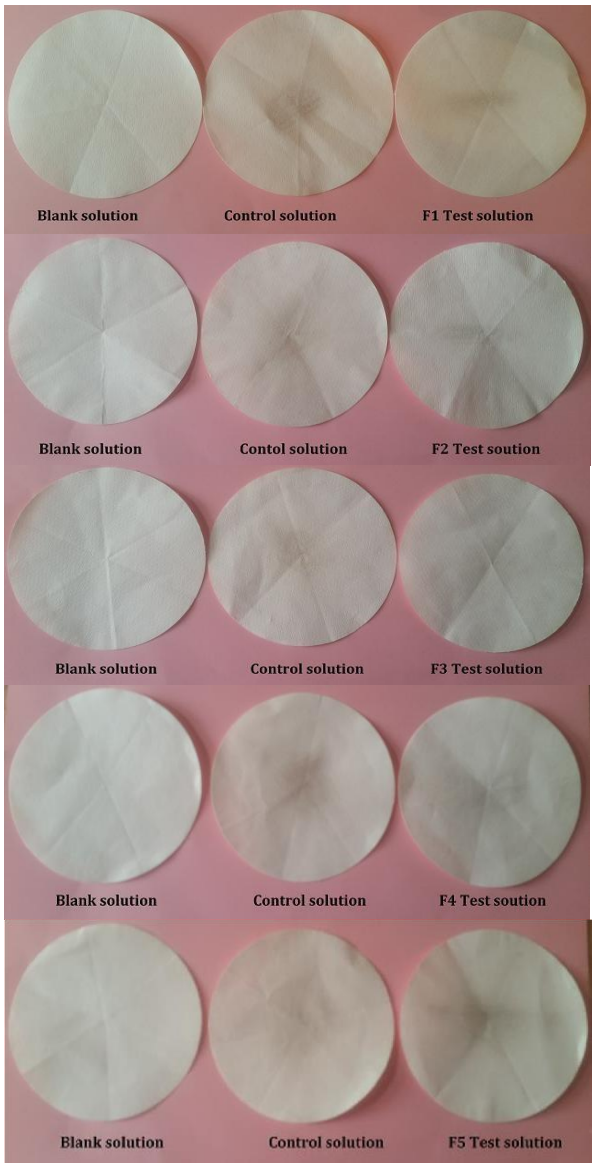

Figure 2: Limit test B results

Fluconazole samples: all control solutions exhibit a slightly brown spot compared to the blank solutions. So the system is compliant.

3.2. ICP-EOS Analysis

3.2.1. Calibration curves

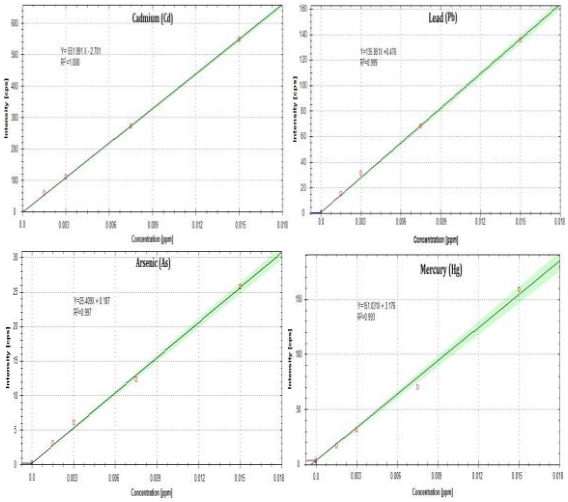

Figure 3: Calibration curves for class 1 elements

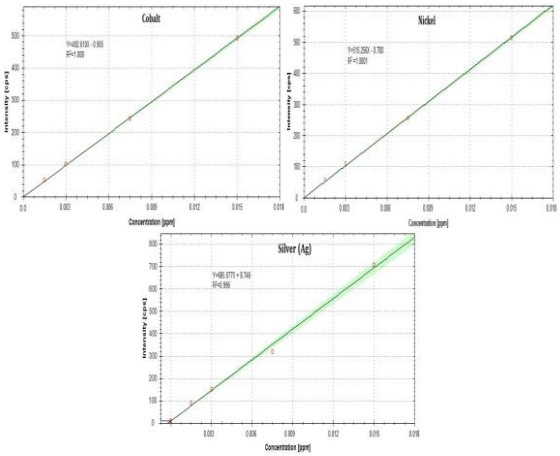

Figure 4: Calibration curves for class 2 elements

Stains comparison (Test solution and control solution)

The brown spot obtained with the test solution of samples (F1, F2, F3 and F4) was less intense than that obtained with the corresponding control solution. This shows that these samples are conformers and contain maximum 10 ppm of heavy metals.

The brown spot obtained with the test solution of sample F5 was more intense than that obtained with the corresponding control solution. This shows that this sample is not-compliant.

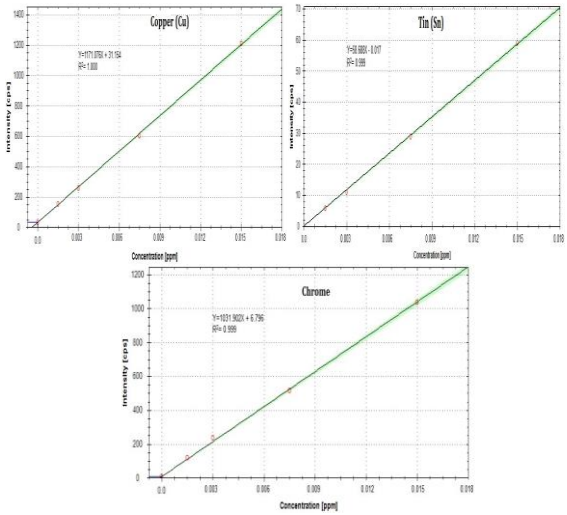

Figure 5: Calibration curves for class 3 elements

3.2.2. Elemental impurities content of each sample

Table 3: Elemental impurities content (ppm) of Ciprofloxacin hydrochloride samples

Element (ppm)	Class	C1	C2	C3	C4	C5	C6	C7	Limit (ppm) (Oral route)
Cd	1	0.008	0.006	0.005	0.006	0.006	0.005	0.005	0.5
Pb	1	0	0.002	0	0	0.008	0.02	0.008	0.5
As	1	0.024	0.031	0.031	0.003	0.005	0.006	0.003	1.5
Hg	1	0	0	0	0	0	0	0	3
Co	2A	0	0	0.001	**6.5**	0	0.003	0	5
Ni	2A	0	0	0.003	0	0	0	0.001	20
Ag	2B	0.008	0	0	0	0	0	0.01	15
Cu	3	0	0	0	0	0.007	0.019	0	300
Sn	3	0.002	0	0.017	0	0.007	0.017	0.006	600
Cr	3	0	0.002	0.006	0	0.006	0.008	0.006	1100

Table 4: Elemental impurities content (ppm) of Fluconazole samples

Element (ppm)	Class	F1	F2	F3	F4	F5	Limit (ppm) (Oral)	Limit (ppm) (Prenteral)
Cd	1	0.006	0.008	0.004	0.005	0.006	0.5	0.2
Pb	1	0	0.011	0.009	0	0.009	0.5	0.5
As	1	0	0.009	0.022	0.009	0.008	1.5	1.5
Hg	1	0	0	0	0	0	3	0.3
Co	2A	0.001	0	0.003	0.002	0	5	0.5
Ni	2A	0	0	0	0	0	20	2
Ag	2B	0.019	0	0	0.008	0.005	15	1
Cu	3	0	0	0	0	0.002	300	30
Sn	3	0.003	0	0.01	0.013	0.007	600	60
Cr	3	0	0	0.002	0.017	0.005	1100	110

All calibration curves have a correlation coefficient (R^2) greater than 0.990, so the curves linearity is validated. To take into account the possible error margin, the variation coefficient is calculated for all samples with values less than 2%, so the measurement seems correct.

The elemental impurties contents of each sample were discussed and compared to the limits of oral route for Ciprofloxacin Hydrochloride and to the limits of oral and parenteral route for Fluconazole.

Ciprofloxacin hydrochloride samples: the content of each element (Cd, Pb, As, Hg, Ni, Ag, Cu, Sn and Cr) is in the norm in all samples except Cobalt content is greater than the limit required in C4 sample (6,5 ppm) (Table 3). **Fluconazole samples:** the content of each element (Cd, Pb, As, Hg, Co, Ni, Ag, Cu, Sn and Cr) is normal in all samples (Table 4).

4. Conclusion

Ten elements were accurately determined in 12 samples of Ciprofloxacin Hydrochloride and Fluconazole APIs by ICP-EOS. The content of each element is normal in all samples except Cobalt content is greater than the limit required in C4 sample. The limit tests showed that all samples are conformers except F5 sample. It isn't surprising that we found these results, which confirm that many manufacturers use the traditional method of heavy metals analysis, which is a colorimetric limit test; it doesn't cover metals that toxic potential is highest; therefore it's neither specific nor exact. So, it's recommended to apply the new procedures based on safety data for the control of this impurties such as the use of more modern and sensitive analytical technologies like ICP-MS and ICP-OES which are well described with the limits allowed in the new chapters (232) and (233) of the USP or the general chapter 2.4.20 of the Eur Ph.

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