Analysis of 29 Residual Solvents-Impurities in Five Samples of Fluconazole API by Head Space Gas Chromatography with Flame Ionization Detector.

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ABSTRACT
The main objective of this work was to analysis 29 residual solvents-impurities by Head Space Gas Chromatography with Flame Ionization Detector (HS-GC-FID) in five samples of Fluconazole API, collected from five pharmaceutical industries installed in Algeria. The GC was equipped with a flame-ionization detector and silica column coated with 1.8 µm layer of phase G43. The carrier gas was helium with a linear velocity of 35 cm/s and a split ratio of 1:5. The column temperature was 40 °C then it rised to 240 °C. The injection temperature was 140 °C and that of detector was 250 °C. Twenty-nine organic solvents belong to classes 1 and 2 were analyzed in five samples of Fluconazole API whose control is mandatory because of their carcinogenic and intrinsic toxicity. Only four solvents were identified wich are Toluene, Dichloromethane, Methanol and Acetonitrile in the different analyzed samples and the Toluene was quantified in F3 sample. All samples collected satisfied the test except F3 sample which contained a slight excess of Toluene estimated of 6 ppm. This slight excess showed that F3 sample wasn’t well purified and this may be due to the difficulty of solvents complete removal.
1. INTRODUCTION

Nowadays, the impurities present a serious public health problem, threatening the safety and the efficacy of medical treatment [1]. Since the Valsartan withdrawal on July 2018 due to a carcinogenic impurity denominate N-nitrosodimethylamine (N-NDMA) [2-5], the impurities control has become mandatory for all pharmaceutical products [6]. An impurity is defined as an excess chemical component that remains with active pharmaceutical ingredient (API), excipient or final product. It becomes toxic or carcinogenic to humans if its content exceeds the safety limits set by health authorities [7]. The International Conference on Harmonization (ICH) classified impurities according to their nature and origin in three categories, Organic related-impurities, Elemental-impurities and Residual solvents-impurities [8].

The residual solvents-impurities are defined as residual solvents which come from organic or inorganic liquids used during manufacturing processes including crystallization and purification stages [9]. It should be noted that is very difficult to completely eliminate them from the processes and they are classified into four classes:

- **Class 1 solvents**: they are human carcinogens to avoid in APIs and excipients manufacturing which are Benzene, Tetrachloromethane, Dichloroethane, Dichloroethene and Trichloroethane;

- **Class 2 solvents**: they are non-genotoxic animal carcinogens can cause irreversible toxic effects such as neurotoxicity or teratogenicity, their use is limited which are Methanol, Acetonitrile, Dichloromethane, trans-dichloroethene, cis-Dichloroethene, Tetrahydrofuran, Cyclohexane, Methylcyclohexane, Dioxane, Toluene, Chlorobenzene, Ethylbenzene, m-Xylene, p-Xylene, o-Xylene, Isopropylbenzene, n-Hexane, Nitromethane, Chloroform, Dimethoxyethane, Trichloroethene, Pyridine, Hexanone and Tetralin;

- **Class 3 solvents**: they have a low toxic potential for humans and they are limited to 0.5 % (no exposure limit is required) which are Acetic acid, Acetone, Anisole, 1-Butanol, 2-Butanol, Butyl acetate, Tert-Butylmethyl ether, Cumene, Dimethyl sulfoxide, Ethanol, Ethyl acetate, Ethyl ether, Ethyl formate, Formic acid, Heptane, Isobutyl acetate, Isopropyl acetate, Methyl acetate, 3-Methyl-1-butanol, Methyl ethyl ketone, Methyl isobutyl ketone, 2-Methyl-1-propanol, Pentane, 1-Pentanol, 1-Propanol, 2-Propanol and Propyl acetate;

- **Class 4 solvents**: their toxicological data are lacking which are 1,1-Diethoxypropane, 1,1-Dimethoxyethane, 2,2-Dimethoxypropane, Isooctane, Isopropyl ether, Methyl isopropyl ketone, Methyltetrahydrofuran, Petroleum ether, Trichloroacetic acid and Trifluoroacetic acid [9].

It is very difficult to remove these solvents completely from drug manufacturing processes; however their toxicity is generally known, which makes it very easy to choose the appropriate control methods [10].
The chapters and guidelines relating to residual solvents include three guidelines:

- Guideline ICH (Q3C): Guidelines for residual solvents;
- General monographs of the European Pharmacopeia (Eur Ph): Chapter 5.4. Residual solvents and Chapter 2.4.24. Identification and control of residual solvents;

Fluconazole is a systemic antifungal drug, belonging to the synthetic imidazole family [14], that is used to prevent and treat a variety of fungal and yeast infections like cutaneous-mucous candidiasis and in mycoses linked to AIDS [15]. In Algeria, it is produced by more than 13 pharmaceutical producers [16]. The main objective of this work was to analysis 29 residual solvents-impurities by Head Space Gas Chromatography with Flame Ionization Detector (HS-GC-FID) in five samples of Fluconazole API, collected from five pharmaceutical industries installed in Algeria.

2. RESEARCH METHOD

2.1. Collection of samples

Five samples of Fluconazole API were collected from five pharmaceutical industries installed in Algeria by referring to the Algerian drug nomenclature dated the 31st December 2014 [16, 28-30]. The samples are collected during the period from 1st April 2015 to 31st December 2016. The compendium covers not only the API but also the following necessary information (origin, supplier/manufacturer, expiration date, analysis certificate, synthesis route, Drug Master File, etc.) [17-20, 27-30]. The samples weren’t expired and we labeled them as follows: F1, F2, F3, F4 and F5. They were stored at room temperature, protected from light and humidity and analyzed prior to their expiration date. For some samples, we did not receive all the necessary informations.

2.2 Equipments

Please note that the first paragraph of a section or subsection is not indented. The first paragraphs that follows a table, figure, equation etc. does not have an indent, either.

Subsequent paragraphs, however, are indented. For residual solvents-impurities analysis, we used 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). 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).
Other instruments in this research included analytical balance (Kern ALS-200-4N, Germany) was used for weighing materials, pH meter (Mettler Toledo, USA) was used to check the pH of solutions and an ultrasonic bath (Elmasonic S 130 H, Germany) was used to dissolve the samples.

2.3. Chemicals and reagents

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) and Dimethyl sulfoxide (99.5 %) was procured from Riedel-de Haën, France.

Composition of residual solvents standard solutions:

- **Class 1_USP (10-50 mg/mL)**: 1,1-dichloroethene, 1,1,1-trichloroethane, Carbon tetrachloride, Benzene and 1,2-Dichloroethane;

- **Class 2_USP_Mix A (0,35-19,4 mg/mL)**: Cyclohexane, Methylcyclohexane, trans-1,2-dichloroethene, Tetrahydrofuran, Methanol, Dichloromethane, cis-1,2-dichloroethene, Acetonitrile, Toluene, 1,4-Dioxane, Ethylbenzene, p-Xylene, m-Xylene, Isopropylbenzene, o-Xylene and Chlorobenzene;

- **Class 2_USP_Mix B (50-290 μg/mL)**: n-Hexane, Nitromethane, Chloroform, 1,2-Dimethoxyethane, Trichlorethylene, Pyridine, 2-hexanone and tetralin.

Preparation of standard solutions

**Class 1 standard stock solution**: prepared from USP_Class 1 residual solvents at $10^{-5}$ mL/mL concentration.

**Class 1 standard solution**: prepared from Class 1 standard stock solution at 0.2 mL/mL concentration in headspace vial.

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

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

**Class 2 mixture A standard solution**: prepared from Class 2 standard stock solution A at 0.5 mL/mL concentration in headspace vial.

**Class 2 mixture B standard solution**: prepared from Class 2 standard stock solution B at 5 mL/mL concentration in headspace vial [21-23, 27].

Preparation of test solutions

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

**Test solution**: prepared from test stock solution at 5 mL/mL concentration in headspace vial [21-23,29].

Preparation of suitability solution
Class 1 system suitability solution: prepared from Class 1 standard stock solution at 0.2 mL/mL concentration in headspace vial [21-23].

Identification by procedure A

The headspace operating parameters were set at equilibration temperature of 80 °C, equilibration time of 60 min, transfer-line temperature of 85 °C, syringe temperature of 80-90 °C, pressurization time greater than or equal to 60 S, injection volume of 1 mL and helium carrier gas at an appropriate pressure [21-23, 27].

Quantification of toluene in F3 sample by procedure C

The chromatographic and headspace conditions were set in the same way as the identification by procedure A.

Toluene standard stock solution (44.5 ppm): prepared from USP_toluene standard at 44.5 ppm concentration.

Toluene standard solution: prepared from Toluene standard stock solution at 0.2 mL/mL concentration.

Spiked test solution of F3 sample: 1 mL of toluene standard stock solution was added to 5 mL of test stock solution of F3 sample in headspace vial [21-23, 29].

Calculation of the Toluene residual solvent amount in F3 sample by the following formula:

\[
\text{Residual Solvant Content (ppm)} = 5 \times \frac{C \times (\mu g/mL)}{W \times (g)} \times \frac{A1}{(A2 - A1)}
\]

C: Concentration of Toluene standard stock solution (μg/mL)
W: Sample weight (g)
A1: Toluene peak area in the test solution
A2: Toluene peak area in the spiked test solution

3. RESULTS AND ANALYSIS

3.1. Identification by procedure A

3.1.1. Compliance of system

The fig. 1 shows the obtained chromatograms of Class 1 standard solution and Class 1 system suitability solution and figure 2 shows the typical chromatogram of Class 1_USP standard solution. The fig. 3 and 4 show the obtained chromatogram and the typical chromatogram of Class 2_Mix A standard solution. The figure 5 and 6 show the obtained chromatogram and the typical chromatogram of Class 2_Mix B standard solution.
**Fig. 1.** Obtained chromatograms of Class 1 standard and Class 1 system suitability solutions.

**Fig. 2.** Typical chromatogram of Class 1_USP standard solution [24].

**Fig. 3.** Obtained chromatogram of Class 2_Mix A standard solution.
Fig. 4. Typical chromatogram of Class 2_Mix A standard solution [25].

Fig. 5. Obtained chromatogram of Class 2_Mix B standard solution.

Fig. 6. Typical chromatogram of Class 2_Mix B standard solution [26].
3.1.2 Identification of solvents

The obtained chromatograms of the standard solutions (Class 1, Class 2_Mix A and Class 2_Mix B) (Fig.1, 3 and 5) and the typical chromatograms supplied with standard solutions (Fig. 2, 4 and 6) 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: 17.007 min.

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

Sixteen peaks of Class 2_Mix A solvents:
- Methanol: 4.285 min;
- Acetonitrile: 5.800 min;
- Dichloromethane: 7.556 min;
- 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) (Fig. 3).

Eight peaks of Class 2_Mix B solvents:
- n-Hexane: 9.444 min;
- Nitromethane: 12.562 min;
- Chloroform: 13.657 min;
- 1,2-Dimethoxyethene: 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 acetonitrile peak and methylene chloride peak was 6, value conform to the standard (at least 1.0).

In conclusion, the system was compliant.

3.1.3. Analysis of samples

Identification by procedure A

The fig. 7, 8, 9, 10 and 11 show the obtained chromatograms of test solutions of F1, F2, F3, F4 and F5 samples.

F1 sample: two peaks were appeared, Dichloromethane and Toluene (Fig. 7), they had respectively the following surfaces (9900 µV.min and 1703 µV.min) which were lower than those of the corresponding standards (252054 µV.min and 3311414 µV.min). So, F1 sample satisfied the test.

F2 sample: no peak detected corresponding to one of the obtained chromatograms of standard solutions (Class 1 or Class 2_Mix A or Class 2_Mix B) (Fig. 8). So, F2 sample satisfied the test.

F3 sample: only one peak was detected, that of Toluene (Fig. 9), having a surface area of 3339135 µV.min, greater than that of the corresponding standard (3311414 µV.min). So F3 sample didn’t satisfy the test. Confirmation and quantification of Toluene was mandatory.

F4 sample: only one peak was detected, that of Toluene (Fig. 10), having a surface area of 100679 µV.min, lower than that of the corresponding standard (3311414 µV.min). So, F4 sample satisfied the test.

F5 sample: three peaks were appeared, Methanol, Acetonitrile and Dichloromethane (Figure 11) having respectively the following areas (3205 µV.min, 5604 µV.min and 9171 µV.min) which were lower than those the corresponding standards (33227 µV.min, 6194 µV.min and 252054 µV.min). Therefore, F5 sample satisfied the test.
Fig. 7. Chromatogram of F1 sample.

Fig. 8. Chromatogram of F2 sample.

Fig. 9. Chromatogram of F3 sample.
Fig. 10. Chromatogram of F4 sample.

Figure 11. Chromatogram of F5 sample.

Quantification of Toluene in F3 sample by procedure C

The fig. 12, 13 and 14 show the obtained chromatograms of Toluene standard solution, F3 sample solution and F3 spiked sample solution.

The Toluene content in F3 sample was estimated at 896 ppm, this value was greater than the allowed limit of 890 ppm (Table 1). Knowing that Toluene was used as solvent in synthesis route or purification process of Fluconazole API, this excess in Toluene showed that this sample had not been well purified, however it should be remembered that organic solvents are not always easy to eliminate.
Fig. 12. Chromatogram of Toluene standard solution.

Fig. 13. Chromatogram of F3 sample solution.

Fig. 14. Chromatogram of F3 spiked sample solution.
Table 1. Toluene content in F3 sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Weight (g)</th>
<th>Toluene Concentration (µg/mL)</th>
<th>Toluene Area in Test Solution A1 (µV.min)</th>
<th>Toluene Area in Spiked Test Solution A2 (µV.min)</th>
<th>Toluene Content (ppm)</th>
<th>Allowed Limit (ppm)</th>
</tr>
</thead>
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<tr>
<td>F3</td>
<td>0,2504</td>
<td>44,5</td>
<td>3339120</td>
<td>6650539</td>
<td>896,01</td>
<td>890</td>
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</table>

**Conclusion**

Twenty-nine organic solvents belong to classes 1 and 2 were analyzed in five samples of Fluconazole API whose control is mandatory because of their carcinogenic and intrinsic toxicity. Only four solvents were identified which are Toluene, Dichloromethane, Methanol and Acetonitrile in the different samples and the Toluene was quantified in F3 sample. All samples collected satisfied the test except F3 sample, which contained a slight excess of Toluene, estimated of 6 ppm. This slight excess showed that F3 sample was not well purified and this may be due to the difficulty of solvents complete removal. 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 did not often use the same solvents to produce the same API, which justifies that residual organic solvent tests were not usually mentioned in the specific monographs.

**Disclosure of interest**

The authors declare that they have no competing interests.

**Author’s contribution**


**Acknowledgment**

The principal author gratefully acknowledges the staff of WanyLab laboratory and Hikma pharmaceuticals for carryout of this research work and all pharmaceutical industries for providing us with Fluconazole API samples in particular: Saidal-Medea, Mérinal, CPCM Pharma, Inpha-Medis, and Hikma Pharmaceuticals.
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