Tripolitana Medical Journal; 2023, Vol. 12, No. 1, pp. 13 - 18

Research Article

ISSN 2077-5628

Chemical and Microbial Quality Evaluation of Different Brands of Water for Injection Marketed in Libya

Omar A. Rbeida, Zaema A. Elbaroudi, Hadeel E. Alshebani and Taqwa R. Alabany

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya

Received 10 April 2023/ Accepted 28 June 2023

ABSTRACT

In this study, the quality and safety of three different brands of water for injection marketed in Libya has been evaluated for physical, chemical and microbiological specifications according to well recognized pharmacopoeia guidelines.

Samples were collected from different pharmacies and hospitals in Tripoli region and analyzed directly after collection. The Physical tests and appearance were normal for all samples. The chemical evaluation showed normal level of conductivity for all samples. Except S_3 it was higher than the limit 1.85 (± 0.07). The chloride and sulphates level were also higher in S_3 than the others (0.82 ppm ± 0.01), (0.25ppm ± 0.05) respectively.

The total dissolved solids were in accordance to normal level obtained for all samples. All the samples passed the microbial evaluation; the test of coliform was given no growth in all the samples including the expired control sample S4.

The deviation of some brands could be due to the lack of applied GMP practice during manufacturing procedures and other source of errors such as the container substance, presence of impurities and ionic interferants from water supply which influence the finished product of WFI. An awareness should be taken by the manufacturers to follow up GMP procedures during all steps of production and GLP in quality control testing.

Keywords- Water for injection; Quality Control; Microbial Contamination; Reverse Osmosis.

INTRODUCTION

*W*ater is an essential ingredient of various pharmaceutical preparations, it plays a pivotal role in pharmaceutical processing.[1]

Water for Injection is a clear, colorless, odorless liquid. It is sterile containing non dissolving substances, it is used for parenteral administration or mixed with powdered preparations to be ready for injection, it is, therefore, important that water should meet set of standards given in different official texts. Sterile Water for Injection according to USP (1231) is a sterile, non pyrogenic, solute-free preparation of distilled water for injection. Water for Injection contains no bacteriostatic, antimicrobial agent or added buffer and is intended only for single dose injection after admixture with an appropriate solute or solution.[2]

As parenteral formulations are administered directly to tissues and systemic circulations, they (including their vehicles) should not vary significantly from physiological pH.[3] The control of chemical purity of water for injection presents few major problems. The chemical instability of WFI after production and in dispensing places could be due to the effect of the container material which made either from glass or flexible container fabricated from a specially formulated non-plasticized film containing polypropylene and thermoplastic elastomers.[4] The critical issue is that of ensuring consistent microbiological quality with respect to removal of bacteria and their endotoxins.[5]

The United States pharmacopeia (USP) monograph of water for injection requires that water should meet all the requirements for purified water, therefore it must be obtained by a suitable process and purified by distilled or reverse osmosis, it should also meet the requirements of the bacterial endotoxin test and contains not more than 0.25 USP endotoxin units per ml using suitable means to minimize microbial growth (Table 1).

Distillation and reverse osmosis (RO) are the only acceptable method in the United States pharmacopeia for producing water for injection. However, in the bulk pharmaceutical and biotechnological industries, ultra-filtration is employed to minimize endotoxins in those drug substances that are administered parentally for some ophthalmic products, such as the ophthalmic irrigation.[8] Distillation has been shown to be effective and the most reliable method in removing endotoxins from contaminated water samples.[9]

Thus, the objective of this research is to evaluate the quality and safety of commercial water for injection marketed in Tripoli-capital region with respect to their endotoxin concentration, chemical impurities and influence of conditions of storage, transport and handling.

WFI considering compendial when it meets the standards set forth by the United States Pharmacopeia (USP) or the European Directorate for the Quality of Medicines and

@ Author to whom correspondence should be addressed: Dr. Omar A. Rbeida; Tel:00 218 91 515 4980; E-mail: kokali422011@gmail.com

Health Care (EDQM). On the other hand, Non-compendial WFI is water that does not meet the standards set forth by the USP, it may be used in the preparation of non-parenteral products or for other pharmaceutical applications.[10,11]

Compendial WFI is typically produced using a combination of distillation, reverse osmosis, and other purification technologies. The specific procedure used will vary depending on the manufacturer's equipment and capabilities.[12]

Risks of contaminated water for injection:

Preparation of water for injection passed by many steps that could prone contamination randomly or systematically due to either personal errors or technical errors. The possible contamination sources are resulted due to pretreatment process and impurities from the feed water, these can cause serious health hazards for patients. The major effect factor is due to the tonicity of water, WFI needs to be isotonic to be safe for injection and compatible with the body fluid isotonicity, injection of non isotonic WFI can cause red blood cells to shrink and break down as the body destroys the cells prematurely in hemolysis process, which lead to serious health problems for patients, including infections, emboli, and tissue damage and can be very severe, even leading to kidney.[13] Other possible side effects include infections and the spread of waterborne diseases, fungi, bacteria and other pathogens. In pharmaceutical manufacturing, contaminated water can even threaten the integrity, safety and effectiveness of drugs and solutions. Contamination of water for injection (WFI) according to USP can be classified into main types:

i) Microbial contamination:

WFI is primarily used in products or processes that come into direct contact with the bloodstream. It is therefore essential that the microorganism and their endotoxin levels be controlled and monitored as they can elicit a `pyrogenic response when introduced to the bloodstream. Therefore, the control of the microbiological quality of WFI is a high priority. Some types of microorganism may proliferate in water treatment components and in the distribution systems. It is crucial to minimize microbial contamination by proper design of the system, periodic sanitization, and by taking appropriate measures to prevent microbial proliferation. It can occur at any stage of production and storages. The USP (1231) limits the total viable aerobic count (TVAC) of WFI to 10 cfu/100 mL, USP also limits the presence of specific types of microorganisms in WFI. For example, WFI must be free of Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus.[14]

Pharmaceutical water are generally produced by continuous processes, water is likely to have been used well before definitive test results are available. Failure to meet a compendial specification would require investigating the impact and making a pass/fail decision on all product lots between the previous sampling's acceptable test result. Endotoxins are pyrogens produced by Gram-negative bacteria. WFI can be contaminated by endotoxins if the source water contains Gram-negative bacteria, or if the WFI comes into contact with equipment or surfaces that have been contaminated with endotoxins.

ii) Source of chemical contamination:

The chemical contamination however is less common, but can be more serious, as it can include toxic or harmful chemicals. Chemical contaminants can enter WFI from a variety of sources, such as leaching from containers or piping, or from cleaning and sanitizing agents. USP sets limits on a variety of chemical contaminants for WFI, including heavy metals, organic compounds, and endotoxins.[15]

iii Particulate contamination:

The presence of particulate in sterile water for injection is a serious concern for pharmaceutical manufacturers and safety of patients. Particulate matter can block blood vessels, risk of developing complications from particulate matter, such as emboli and tissue damage, in some cases, particulate matter can even lead to death.[16] The USP limits set to control the particulate in water for injection in small and large volume injections.[17]

WFI used in the manufacturing of parenteral drugs must contain no more than 10 particles per milliliter that are greater than 10 microns in size. However, despite the USP's strict limits, particulate matter can still be found in WFI, a number of studies have found that particulate matter is present in a significant percentage in WFI samples, the possible of potential sources of particulate matter in WFI, including airborne dust and fibers, waterborne microorganisms, metal particles from production equipment and rubber particles from gaskets and seals.

Particulate matter can enter WFI at any point during its production, storage, or handling. Despite these efforts, the risk of particulate in WFI remains a serious concern. However following GMP during manufacturing can help to prevent particulate contamination of WFI by using high-quality production equipment and materials, implementing strict sanitation and cleaning procedures, and regularly testing WFI for particulate matter.[17]

Quality defects of some WFI products

*T*he steps to obtain WFI products passed with different stages start from portable water to the end of sterile, clear and safe WFI products, these complicated steps can be a source of fetal errors unless very good QC regime set up to assess that GMP requirements were applied and strictly followed. Recent examples of FDA recall and self recall of some WFI produced companies, mainly due to shortage of following up GMP during manufacturing.[18] The use of the impacted product can be associated with potential adverse events such as anaphylaxis, fever, gastrointestinal disturbances, vein irritation, localized vein inflammation. Among the main defects is due to visible particulate.[20] Substandard WFI products and FDA warring due to GMP violations.[19]

MATERIALS AND METHODS

The experimental work based on the United States pharmacopeia monographs (USP), instrument was validated

and calibrated according to the manufacturing specifications. The laboratory measurements were carried out at QC laboratory of Al-Sadeem Company (Tripoli-Libya) the lab is certified ISO 17025.

Apparatus

1. Intelligent water analysis Modele DR 3900, Hach company, USA, used for analysis of (chloride, sulphate, nitrate) certified by EPA.

2. HQ40d, Hach company, United State, used to estimate (pH, conductivity, TDS) certified by EPA.

3. Memmert Incubator, Germany, used to estimate total coliform bacteria.

Chemicals and Reagent

Pure grade reagents were used, Bromocresol green, Methyl red, Sulfa ver, Nitra ver, ordant black, Mercuric thiocynate, sulfa ver 4 reagent (barium chloride, dihydrate (40-50 %), citric acid (50-60%) powder white purchased from Hach, USA.

Procedures of physical specifications testing

Samples and their packages were observed; their label, color and nature of content were noted. Date of expiration and volume labeled were registered. Testing of the clarity, odor, turbidity and absence of particulate were carried out in the same conditions of temperature, humidity and pressure. The pH was measured at room , temperature was determined using pH meter for the pH range of (5.0-7.0), pH meter calibrated using reference buffer solutions of pH (4-12) each 6 hr. interval.

Quality testing of microbiological contamination

i) Endotoxin Test

Endotoxin concentration of the samples was evaluated. The sample and reagent preparation were performed according to the manufacturer's specifications.[20] Hundred micro litters of each of the dilutions were dispersed into endotoxin free vials, mixed thoroughly for 30 seconds with a vortexer. Hundred microliters of reagent water were dispensed into endotoxin free vials in replicates. Hundred microliters of reconstituted limulus amoebocyte lysate were added to each vial. They were incubated again for 45 minutes at 37±1C°. Then hundred microliters of reconstituted chromogenic substrate solution were added to each vial, swirled gently avoiding foaming. They were incubated again for 6 minutes at 37±1C°.

Five hundred microliters of 25% acetic acid in endotoxin free water were added to the vials after incubation. The absorbance of each reaction vial was read at 545 nm.

ii) Determination of total coliform bacteria

The technique test using reference media (EC).[21] The medium contains two different chromogenic enzyme substrate: Magenta-Gal and X Gluc - Red and blue, 1.5 ml of water for injection was taken by syringes. EC media was added to water, Incubation time was 48 hour at temperature 37 C°.

Testing of chemical quality specifications

By using of HQ40d laboratory meter connecting with smart intellectual electrode that automatically recognizes the testing parameter used to determine the pH, conductivity and TDS as specified by the manufacture.

i) Measurement of total hardness

150 ml of sample has been taken in conical flask then we added to the conical flask, 2ml of puffer solution pH 10, 3 drops of mordant black indicator were added, titrated with 0.1 M of EDTA till color changed from pink to blue.[22]

Total hardness mg/l = (M) EDTA×V EDTA × M. wt ($CaCO_3$) ×1000/V sample (ml).

ii) Measurement of nitrate, sulphate and chlorides.

Reagents and procedures are specified by the manufacturer using UV intelligent water analysis DR3900 spectrophotometer. For Nitrate: 10ml of WFI mixed with Nitra Ver reagent (benzenesulfonic acid, 4-amino, benzoic acid, 2,5-dihydrox y,cadmium,phosphoric acid, potassium salt).[23] For sulphate. by using of sulfa ver 4 reagent (barium chloride, dihydrate (40-50 %), citric acid (50-60%) powder white.[24] Chlorides was determined using mixture of 0.8 ml mercuric thiocyanate solution + 0.4ml ferric ion solution.[25] In all cases the coloured solution was measured at visible ranges.

RESULTS

1. Evaluations of physical characteristics (Table 2).

2. The evaluation of chemical quality (Table 3).

Table 1: Combined QC important parameters for water for injection by USP, Eur.Ph and JP

Test	USP	Eur. Ph	JP
Conductivity	<1.3 uS/cm at 25° C	<1.3 uS/cm at 25° C	<1.3 uS/cm at 25° C
Total organic carbon	<500 ppb	<500 ppb	<0.5 mg/l
Bacteria	<10 CFU/100 ml	<10 CFU/100 ml	<10 CFU/100 ml
Endotoxin	<0.25 IU/ml	<0.25 IU/ml	<0.25 IU/ml

Table 2: Evaluation of physical spécifications for the studied samples

Test	S1	S2	S 3	S4
Volume	5ml	5ml	10ml	5ml
Container material	Plastic	Glass	Plastic	Plastic
Odour	Odorless	Odorless	Odorless	Odorless
Turbidity	Not turbid	Not turbid	Not turbid	Not turbid
Clarity	Clear	Clear	Clear	Clear
Transparency	Transparent	Transparent	Transparent	Transparent

Table 3: Results of chemical quality testing of the different WFI Brands according to USP (1231) specifications.

Test (Normal limit)	S1 (±SD)	S2 (±SD)	S3 (±SD)	S 4 (±SD)
Conductivity (<1.37) µS/cm	1.2 (± 0.05)	1.1 (± 0.03)	1.85 (± 0.07)	3.46 (± 0.01)
Chloride (< 0.2ppm)	0.72 (± 0.01)	0.63 (± 0.01)	0.82 (± 0.01)	Not detected
Sulphate (< 0.2ppm)	0.09 (±0.05)	0.15(±0.0054)	0.25 (± 0.05)	Not detected
Nitrate (< 0.2 ppm)	0.15 (± 0.04)	0.16 (± 0.05)	0.31(± 0.04)	1.71(± 0.06)
Total dissolved solids (10 ppm max)	3.53 (± 0.01)	2.30 (± 0.05)	2.38 (± 0.05)	6.2 (± 0.05)
pH (5-7 max)	7.02 (± 0.01)	7.65 (± 0.01)	7.53 (± 0.04)	4.40 (± 0.05)
Endotoxin (0.25 units/mL)	Not detected	Not detected	Not detected	Not detected
Bacteria-count (cfu/100 mL)	No growth	No growth	No growth	No growth

(**S4** is an expired sample), (n =5).

DISCUSSION

Evaluation of physical characteristics:

The physical QC testing of the studied brands (Table 2) shows that all sample solutions were clear and transparent with no sign of turbidity or bad odor. This can be attributed to efficient filtration system through all the steps of purification, however, based on the evaluation of water quality in the finished product at the point of release it can not be enough to ensure its stability and safety, thus the evaluation of other parameters during transport, handling and storage through the shelf life can be more efficient. an expired sample S_4 where then included in the study to see the possible different changes after expired validity.

Evaluation of chemical quality:

i) Conductivity testing

The test of conductivity performed to investigate the presence of ions that cause the water for injection more conducting to current. All samples passed the conductivity test, except S3, this indicate that the sample has high concentration of chemical impurities in source of water which was not easily removed during pre-treatment stage.[26] The higher conductivity level could be resulted from the presence of ions and traces of heavy metals coming from the container substance or the change of temperature during bad storage condition. Results can be compared to the higher conductivity of S4, the expired sample.

ii) The test of pH

According to USP (1231), Normal pH range set at pH (5-7), reasons for setting a high limit are that high pH can promote hardness scale precipitation and accordingly can increase the calcium and other cationic ions. Low level of pH has an antibacterial effect against Pseudomonas and other common water bacteria but low pH water can increase the corrosion rate of pipping and tubing. In the tables 4.2 above, all samples were above the limit of pH by small variations, which can be attributed to the presence of alkaline effect either due to the contamination of the source of water or the leaching of substances from the container material. Also during the purification and pretreatment system thereby preventing total removal. Low pH of the expired sample S4, this could be formation acidic impurities lowered the media more acidic, this confirmed by the absence of total alkalinity in the sample that neutralize the acidity.

iii) Evaluation of the chemical quality

The chloride ions concentration was higher in S1 and S2, this could be attributed to the lack of an efficient ion exchange system used to trappe the ions during the purification procedures or pretreatment of feed water or as result of residual chloride ions from the chlorine used as disinfectant in the water supply.

The presence of nitrate can indicate the presence of organic matter traces, either from contamination of the feed water or traces of nitrogen containing impurities coming from biological wastes in the final stages of stabilization or run-off from heavily fertilized fields. A remarkable high level of nitrate was found in S3, or higher rate of decretive of container substance due to temperature and storage conditions which mainly made from Bisphenol (BPA).[27]

Sulphate was detected in higher concentration in sample S3. Solutions in contact with the plastic container may leach out certain chemical components from the plastic in very small amounts; however, biological testing was supportive of the safety of the plastic container materials. Exposure to temperatures above 25°C/77°F during transport and storage will lead to minor losses in moisture content. Higher temperatures lead to greater losses.

Evaluation of microbiological quality

The microbial evaluation was tested as endotoxin coliform, the studied brands have no any microbial contamination nor growth was developed during the period of testing. The USP restricted the level of endotoxin to be not more than 0.25 USP endotoxin units per ml to comply with these specifications, to minimize microbial contamination WFI.

CONCLUSION

As the objective of this work was to assess the chemical and microbiological quality of WFI of different brands in the local market, this study revealed some deviations which should be closely followed. Generally, the efficient control of preparation steps during the production of water for injection is a determining steps, no other main errors than failed to certain application of GMP procedures since the used package individually was not exceeding the amount of 10 ml vials for parental use, substances of the container, therefore, can permeate from the container into the over wrap but it is insufficient to affect the solution significantly. However, harsh condition of higher temperature and humidity can permit the leach of the content of the container into the WFI, leach can be due to also certain solutions those in contact with the flexible container along the expiration period.

The pharmacopeail specifications (USP, EU. Ph and BP) are considering the free of foreign substances either of microbial origin or chemical entity is essential.

Water for Injection should be sterile and cause no harm to the patient. However, the amount of water is small enough to produce high danger effects and toxicity, but during prolonged treatment the accumulation of certain potential substances can be seriously effective other organs.

It is clear that the QC assessment in postmarket evaluation is very essential to discover the substandard and counterfeit products early before it spreads into the market. The follow up of good procedures for transport, storage and handling can decrease the harsh effects of temperature, humidity and pressure and safe WFI products can be marketed under close supervision.

REFRENCES

1. Hultavist, A. (2007) Practical Guidelines for Qualification of Purified Water Systems, Pharm,Technol. Europe 19 (12).

2. USP 1231 (2017) Water for Pharmaceutical Purposes, USP29-NF24 Page 3056.

3. WHO (2003) Expert Committee on Specifications for Pharmaceutical Preparations, Thirty Seven Report Geneva No 908 Anenex 4.

4. Ismail H. Suryadiansya H. (2002) Thermoplastic elastomers based on polypropylene/natural rubber and polypropylene/ recycle rubber blends, *Polymer testing* 21(4), 389-395.

5. PDA J M (2001) One-way Distribution System for Water for Injection. Process Microbiological Quality Control and Meeting Regulatory Requirements, *Journal of Pharmaceutical Science and Technology* 55(1), 3-9.

6. EMA (2020) Guideline on the quality if water for pharmaceutical use. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-water-pharmaceutical

7. Japanese Pharmacopoeia (2016) Water for injection specification, 17 Edn, p. 17151, https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0019.html

8. Poore L J, Zauber. E and Oser N (1970) Detection of endotoxin in the blood of patients with sepsis due to Gram-negative bacteria, *The New England Journal of Medicine* 283, 1313-1316.

9. Gupta I Chakraporty Roy J Firinas S, and Mitra S (2021) Separation and Purification Technololgy Nanocarbon immobilized membranes for generating bacteria and endotoxin free water via membrane distillation, 259, 118133.

10. Pharmacopeial Forum (2010) Chapter Water for Injection. Design and Operation of Purified Water and Water for Injection Systems, 31(3), 803.

11. European Pharmacopoeia (2020), Guideline on the quality of water for pharmaceutical use, 10th Edition, Monograph 0169 p. 524.

12. ISO 3696 (1987) Water for analytical laboratory use -*Specification and test methods* p 23.

13. FDA (2001) Guide to Water for Pharmaceutical Injection, Dept. Of health, Education and wellfare, N 46

14. United States Pharmacopeia (2023) 3rd Edition, National Formulary 38th Edition, United States Pharmacopeia Convention, Inc., Rockville, MD. p.672

15. US Environmental Protection Agency (2009) Maximum Contaminant Levels (MCLs) 123.

16. FDA Guidance for Industry (2004) Sterile Drug Products Produced by Aseptic Current Good Manufacturing Practice 9.

17. Ph. Eur. (2016) General particulate contamination: sub-visible particles, in: The European Pharmacopoeia, 8th edn., (Chapter 2.9.19)

18. USP (2023) General Chapter Particulate Matter in Injection, 44- nf 39, p 59

19. Tran T, Kupiec TK, Trissel LA. (2006) Quality-control analytical methods: particulate matter in injections, Int J Pharm Compd 10 (3), 202-204.

20. Hach company US 2021, Enterococci (Coliforms) Membrane Filtration, Proposed Method 1600

21. Hach company US 2021, Total Aerobic Bacteria, Yeasts and Molds-Paddle Testers DOC 316.53.01223

22. Hach company US 2021, Hardness, Calcium-Titration Method using EDTA Method 8204.

23. Hach company US 2021, Acidity, Phenolphthalein-Sodium Hydroxide with a Buret Method 8010

24. Hach company US 2021 Sulfate SulfaVer 4 Method 8051.

25. Hach company US 2021, Chloride Mercuric Thiocyanate Method 8113.

26. PDA J. M (2001) One-way Distribution System for Water for Injection. Process Microbiological Quality Control and Meeting Regulatory Requirements, *Journal of Pharmaceutical Science and Technology* 55(1), 3-9.

27. Latif Abdul a b 1, Xiongyuan Si 1, Kai Sun a, Youbin Si a (2021) Degradation of bisphenol A in aqueous environment using peroxy mono sulfate activated with carbonate: Performance, possible pathway and mechanism, *Journal of Environmental Chemical Engineering* 9(4), 105419.