Saudi Journal of Engineering and Technology (SJEAT)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-6272 (Print)
ISSN 2415-6264 (Online)

Evaluation of the Physic-Chemical and Microbiological Quality of Dexamethasone Sodium Phosphate Injection in the City of Douala

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Original Research Article

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Article History
Received: 25.08.2018
Accepted: 04.09.2018
Published: 30.09.2018

DOI:
10.21276/sjeat.2018.3.9.2

Abstract: The main objective of this work was to evaluate the physico-chemical and microbiological qualities of dexamethasone sodium phosphate injection in the city of Douala. The experimental type was conducted from December 2016 to June 2017. It consisted of carrying out tests of physic-chemical and microbiological parameters on 14 batches of injectable DSP, conditioning, pH, TLC identification, dosage of active ingredient, the clarity, nominal volume and sterility of the drug were analyzed. The technics used were a visual inspection, thin layer chromatography, UV/visible spectrophotometry and microbiological technics, were that of direct seeding. The results were compared in each sector (formal and informal) and in both. Thus, it was found that the injectable DSP, which came from the formal sector had a non-compliance rate of 12.7%, while that from the informal sector a non-compliance rate of 39.7%. In both sectors, the injectable DSP had a non-compliance rate of around 26.2%. Both formal and informal circuits were all affected by non-compliance, although there is a predominance in the informal circuit.

Keywords: Quality, physicochemical, microbiological, compliance, non-conformity.

INTRODUCTION

Since its inception in 1948, the World Health Organization (WHO) has been concerned with health for all and the quality of pharmaceuticals [1]. In addition, one of the key elements of a pharmaceutical policy is the guarantee of the safety, efficacy and quality of the medicines available in the country.

Throughout the last decades, quality control procedures have been applied to many drugs, which have resulted in a global explosion of counterfeit medicines, which could destroy the progress made so far in the fight against disease [2]. In some countries in Africa and South-East Asia, this rate would reach 30% or even 70%. Recently the American Journal of Tropical Medicine and Hygiene (AJTMH) in 2015 estimated that nearly 41% of the 17,000 samples of drugs used against tropical diseases tested did not meet the required quality standards [2]. In Africa, different pharmacological families are found in street drugs, analgesics represent in volume the best-selling family with the rate of 81%, followed by anti-inflammatories 8%, antimalarials 7%, antibiotics 3%, and others, families 1%.

Corticosteroids are drugs whose discovery has upset the treatment of certain diseases such as systemic lupus erythematosus, psoriasis and Addison's disease [3]. Despite the age of corticosteroid therapy, it remains a highly used therapeutic, generally and locally in clinical specialties. Because of its effectiveness and rapidity of action, it is often the first-line treatment for most of these diseases [4]. It is estimated that in France 0.2 to 0.5% of the population receive systematic corticosteroid treatment prolonged beyond more than three months [4]. In England around 1% of the adult population and up to 2.5% of the population over 70 years of age takes long-term corticosteroid therapy. In the United States, a new corticosteroid therapy is instituted in ten million people a year. The anti-inflammatory and immunomodulatory effect of corticosteroids is particularly sought after in therapy. Corticosteroids, however, are providers of multiple side effects, responsible for their bad reputation. The majority of adverse reactions, which may occur during systemic corticosteroid therapy, are predictable because they are related to the pharmacological effect. In a prospective french study of 80 patients, 71% of patients reported at least one side effect [4]. In Cameroon, despite the presence of structures that ensure the quality control of drugs, such as the Directorate of Pharmacy and Medicine (DPM), the General Inspection of

Pharmaceutical Services (IGSP), and the National Laboratory of Quality Control of Drugs and Expertise (LANACOME), the quality of the drug is still a matter of great concern [5]. De nombreux travaux ont été réalisés sur le contrôle qualité des corticoïdes dans le pays, mais aucun n'a été publié sur la dexaméthasone sodium phosphate (DSP). L'objectif principal de cette étude a été d'évaluer la qualité physico-chimique, bactériologique et fongique de la DSP injectable disponible dans les secteurs formel et informel de la ville de Douala. Les objectifs spécifiques ont été de : contrôler la qualité physicochimique de la DSP injectable dans les secteurs formel et informel, effectuer les tests microbiologiques sur la DSP injectable dans les secteurs formel et informel et comparer les données obtenues dans chaque secteur et dans les deux secteurs à la fois. Much work has been done on the quality control of corticosteroids in the country, but none has been published on dexamethasone sodium phosphate (DSP). The main objective of this study was to evaluate the physico-chemical, bacteriological and fungal qualities of the injectable DSP available in the formal and informal sectors of the city of Douala. The specific objectives were: to monitor the physic-chemical quality of the injectable DSP in the formal and informal sectors, to perform microbiological tests on the injectable DSP in the formal and informal sectors and to compare the data obtained in each sector and in both sectors that time.

MATERIALS AND METHODS

Type, location and period of study

The experimental study was carried out on the one hand at the Multidisciplinary Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala and on the other hand at the Multidisciplinary Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. The study ran from December 2016 to June 2017. The collection of samples was made in the various districts of the city of Douala. Wholesalers, in general hospitals, central hospitals, district hospitals, CMA, health centers for the formal sector and various street trading points for the informal sector. The collection of the reference substance was made by the purchase in a pharmacy of Douala of a pharmaceutical specialty DEXAZEM-I of the laboratory ZMC.

MATERIALS

Glassware and other laboratory equipment

The following material was used: blocked glass tube (50 mL) used for storage of small volume solutions; Graduated cylinders (10mL, 50mL) made of pyrex that measure and for the measurement precise volumes of solutions; pyrex beaker (50 mL) used for storing solutions; volumetric flask (50 mL, 100 mL) for the measurement of precise volumes; separating funnel (100 ml) to separate the various constituents of a solution; syringes (5 mL, 1 mL) to collect small amounts accurately; desk lamp for visibility during the mirage test; silica gel TLC plates for the deposits of the substances to be analyzed; micropipettes; chromatography tank for the migration of the compounds tested; petri dishes for microbial culture.

Reactive

For pH measurement, distilled water (H2O) and DSP (C22H28FNa2O8P), for reference and samples. For the clarity test, the water freed of CO2 and DSP (C22H28FNa2O8P), for reference and samples. For nominal volume measurement, the DSP (C22H28FNa2O8P), for reference and samples. For the mirage test, the DSP (C22H28FNa2O8P), for reference and samples. For TLC identification, DSP (C22H28FNa2O8P), for reference and samples, alkaline phosphatase solution, chloroform (CHCL3), acetone (C3H6O) dichloromethane (CH2CL2), sulfuric acid heated to 105° (H2SO4).

For the spectrophotometric assay, DSP (C22H28FNa2O8P) for reference and samples, dichloromethane (CH2CL2), phenylhydazine (C6H8N2) and sulfuric acid (H2SO4) [8]. For the microbiological control test, a thioglycolate broth, for the cultivation of anaerobic bacteria and a trypticase-soy broth, for the cultivation of aerobic bacteria and molds as well as DSP (C22H28FNa2O8P), for reference and samples [6].

Equipment

As equipment we had have: Incubateur BIOBASE type SCSJ-3; Autoclave LS-BB75L; balance de précision10- 3modèle RS232Ctype BK-JA5003B; pHmètre HANNA.

METHODOLOGY

Physicochemical tests

Control packaging

Secondary packaging

The inspection of the secondary packaging was carried out as follows. It was a question of checking among others: the name of the drug, the dosage; the qualitative and quantitative composition of active substances per unit of intake; the pharmaceutical form and the contents by weight, by volume or by dosage units; the list of excipients; the mode of administration [7].

Primary packaging

In the case of primary packaging, the space dedicated to the affixing of information was very often small. This was why the list of information that must appear on this element was restricted. This list includes: the name of the drug, the dosage, the dosage form; the name of the marketing authorization holder of the medicinal product; the manufacturing lot number; the date of manufacture; the expiry date; the mode of administration; the content by weight, by volume or by units [7].

Mirage test

The process involves inspecting ready-to-use solutions and reconstituted solutions to ensure they are clear and free of any visible particles. The test was performed manually, that is to say without a mirage table. Each DSP bulb was placed on a white background and lit with a desk lamp [8].

Clarity test

As the clarity test is only recommended for solutions, it essentially consists in avoiding the distribution and use of solutions containing particles. Clarity was determined by mixing a solution of 0.10 g of DSP in 10 mL of carbon dioxide-free water. Subsequently the observation of the prepared solutions was done visually in the light of the day [8].

Measurement of the pH

PH is a characteristic parameter of an aqueous solution. It represents by convention its acidity and its alkalinity. It makes it possible to maintain the stability of the active ingredient in a solution. The process consists in diluting 1mL of DSP in 5mL of CO2-free water and then measuring the pH value, which norm is between 7.5-9.5 [8].

Measurement of nominal volume

The nominal volume is the volume of the drug substance indicated on the drug label by the manufacturer. In order to meet the nominal volume requirement, the containers are filled with a volume slightly higher than the nominal volume. The realization was done by selecting 5 ampoules from which the contents were removed individually using 5 mL syringes, making sure that there were no air bubbles and then unloading the contents of each syringe in a graduated cylinder [6].

Identification by thin layer chromatography
Principle

Thin Layer Chromatography (TLC) is a simple and fast method for monitoring the evolution of a reaction or testing the purity of organic compounds. It is based on a capillary migration of the compounds, resulting from the driving force by the solvent (solubility) and the retention strength by the silica gel plate (absorption) [9].

Operating mode

Five microliters of each the prepared sample and reference solutions are deposited with a micropipette on the deposition zones. The solutions are prepared as follows: Dilute a quantity of the injection equivalent to about 5 mg of DSP with 25 mL of water and extract with two quantities, each of 25 mL of dichloromethane. Discard the dichloromethane each time and transfer the aqueous phase to a 50 mL volumetric flask, dilute to volume with water and mix. Pipette 5 mL into a sealed 50 mL glass tube and incubate at 37 ° for 45 min with 5 mL of alkaline phosphatase solution. Extract with 25 mL of dichloromethane. Evaporate 15 mL of the dichloromethane extract to dryness and dissolve the residue in 1 mL of dichloromethane [9]. A solvent system CHCL3/C3H6O/H2O was introduced into the tank and then allowed to saturate for 5 min. The plate was then placed in a tank saturated with elution solvent vapor. One edge of this plate dipped in a solvent bottom, taking care to avoid any contact between the point deposition of the samples and the solvent. The latter then migrated by capillarity towards the top of the plate. The solution of 50% w/v sulfuric acid sprayed on the plate heated to 105 °C was used for revealing the stains [9]. After being revealed, the tasks were marked in pencil so that they can remember their position even after they had faded.

Determination of the DSP by UV/VIS spectrophotometry
Principle

The assay was based on measuring the absorbance of the DSP samples at the $A = \varepsilon .L.C$, with A: absorbance; Ɛ: the molar absorption coefficient in L/moL/Cm; L: the tank width in Cm; C: the concentration of the solution in moL/L.

Dosage

Dissolve 10 mg of DSP in 5 mL of distilled water and make up to 100 mL with ethanol, take 2 mL of this solution and add it to a sealed glass tube and add 10 mL of a solution of sulfuric acid-phenylhydrazine, finally mix. After heating in a water bath at 60 °C for 20 min, then cool immediately. Measure the absorbance at a wavelength of 419 nm after having previously measured the blank. The determined absorbance values make it possible to draw the calibration curve showing the equation of the regression line which made it possible to calculate the exact concentration of DSP in the respective samples [8].

Microbiological test
Principle

The test applies to substances, preparations and products which according to the International Pharmacopoeia must be sterile. It consists in incubating samples inoculated in media for 14 days at temperatures that favor the growth of microorganisms. The sterility test must be carried out under strict conditions intended precisely to avoid contamination of the substance to be evaluated in order to avoid the risk of sepsis. Care must be taken to ensure that the test does not affect the microorganisms it has revealed. The sterility test is performed under aseptic conditions, for example under a laminar flow hood located in an insulator.

Sterility test

Several culture mediums are proposed both for the detection of aerobic bacteria and for anaerobic bacteria, the choice of the medium is made according to whether its ability to ensure the growth of a wide range

of microorganisms has been demonstrated and that it satisfies the effectiveness test of the medium in the presence of the preparation to be examined. The following culture mediums are suitable for carrying out the sterility test : the thioglycolate liquid medium, mainly intended for the detection of anaerobic bacteria: *Staphylococcus aureus* and *Pseudomonas aeruginosa*, also allows the detection of aerobic bacteria: *Clostridium sporogenes*; the casein and soy hydrolyzate agar medium, which is mainly used for the detection of aerobic bacteria: *Bacillus subtiles*, also allows the detection of yeasts and molds: *Aspergillus niger* and *Candida albicans* [6].

The method used was that of direct inoculation: First by incorporation, a volume of 1 mL of each sample in Tryptic agar agar from undivided Petri dishes. The manipulation was carried out close to a flame. The dishes were incubated at a temperature of 35 °C for 14 days. Then by inoculating a total volume of each sample into a sterile thioglycolate broth contained in 15 mL eppendorf tubes. The cultures were observed every day until the 14th day for a possible appearance of a microbial growth.

Statistical analysis of data

The database Social Science (SPSS) version 18. was built and analyzed. Microsoft Office (Excel) was used for formatting tables from SPSS and the production of graphics. The data was presented in tables and graphs.

RESULTS

Paramètres physico-chimical parameters

Secondary packaging

Six batches representing 85.7% of DSP showed compliance with secondary packaging in the formal sector and 2 batches representing 28.6% of DSP showed conformity to secondary packaging in the informal sector. And in both sectors, there were 8 batches representing 57.1% compliant and 6 batches representing 42.9% non-compliant.

Primary packaging

Seven batches or 100% from the formal sector all had a conformity of their primary packaging and 4 batches or 57.1% showed a conformity of their primary packaging in the informal sector. And in both sectors, there were 11 batches or 78.6% compliant and 3 batches or 21.4% non compliant.

Mirage test

All 14 lots of DSPs were mirage test compliant.

Clarity of solution

Five batches or 71% were in compliance with the clarity of solution in the formal sector and 2 batches or 29.0%, conform to the clarity of solution in the informal sector. And in both sectors, there were 7 batches or 50.0% compliant and 7 batches or 50% non-compliant.

pH test

The 14 batches of DSP all had pH test compliance. They all had a pH between 7.5 and 9.5.

Nominal volume

Two batches or 28.6% showed non-compliance with the nominal volume in the formal sector, and 4 bathes or 57.1% were non-compliant with the nominal volume in the informal sector. In both sectors, there are 8 batches (57.1%) compliant and 6 batches (42.9%) non-compliant.

Identification by CCM

Six batches representing 86% had formal TLC identification test compliance and 1batch representing 14.0%, showed compliance with the TLC identification test in the informal sector. And in both sectors, there were 7 batches representing 50% compliant and 7 batches representing 50% non-compliant.

Determination of active ingredient by spectrophotometry

Five batches or 71% comply with the determination of active ingredient in the formal sector and 3 batches or 43% comply with the determination of active ingredient in the informal sector. And in both sectors, there were 8 batches or 57% comply and 6 batches or 43% non-comply.

Microbiological test

The results on the sterility test showed that no batch was positive for this test, i.e no growth was obtained after 14 days in all 14 lots.

Frequencies of non conformities and conformities in the formal and informal sectors

Using a multiple response analysis, the total compliance and non-compliance frequencies were determined for the formal and informal sectors. The injectable DSP that came from the formal sector represented a compliance rate of 87.3% and that from the informal sector represented a compliance rate of 60.3%. And in both sectors, the injectable DSP had a compliance rate of around 73.8% and a non-compliance rate of around 26.2%.

DISCUSSION

Of the 14 batches analyzed in our study, 26.2% were non-compliant. This percentage was higher than that of Sacko Fatoumata [10] in Bamako, which had found a percentage of non-compliance of 5.76% and whose work concerned the quality control of injectable dosage forms in the National Laboratory of the Health. And lower than that of Ndong [11] in Mali, which had found a percentage of non-compliance of 41.86% and whose work concerned the quality control of veterinary oxytetracycline for veterinary use available in Mali.

The following results were found in our study non conformity of the secondary packaging, package insert, primary packaging, TLC identification, nominal volume, clarity and dosage of active ingredient. On the contrary, all batches were in accordance with the mirage, pH and microbiological tests.

Secondary conditioning had a formal non-compliance rate of 14.3% and 71.4% in the informal sector. Primary packaging had a non-compliance rate of 0% in the formal sector and 42.9% in the informal sector. These results are superior to those obtained by Penbengueri [12] in Yaoundé, whose study focused on the pharmaceutical evaluation of injectable ceftiaxone marketed in the legal and illegal markets of Yaoundé, and in which, it showed that 2 batches of 12% from the informal sector were non-compliant. And Menyeng [13] in Douala, whose study focused on the quality of injectable gentamicin available in the formal and informal sector of the city of Yaoundé, instead inspected the primary packaging and showed that there were 25% non-compliant in the formal sector and 37.5% non-compliant in the informal sector. A defect in the packaging testified to the non-compliance with good manufacturing Practices and this can lead to poor compliance, intoxication, deterioration of the dosage form, as well as an absence of traceability of the products. The TLC identification of the DSP, had a rate of non-compliance in the formal sector of 14% and 86% in the informal sector. These results did not agree with those of Ndong [11] in Mali, Penbengueri [12] in Yaoundé and Menyeng [13] in Douala, all of which had 100% compliance with active ingredient by TLC identification. These results present alarming levels in the sense that the active principle (P.A) is the substance responsible for the pharmacological action; a drug devoid of P.A then loses the drug sense.

Nominal volume had a formal compliance rate of 28.6% and 57.1% in the informal sector. This result was higher than that obtained by Menyeng [13], who obtained 0% of non-compliance in the formal sector and 18.8% of non-compliance in the informal sector. This highlights the non-compliance with good manufacturing practices.

The clarity of the solution, obtained a rate of non-compliance in the formal sector of 29% and 71% in the informal sector. The mirage of the solution, had 100% compliance in both sectors. The pH had 100% compliance in both sectors. These results are not consistent with those of Ndong [11], who found 16.65% of non-compliance with pH, and with those of Menyeng [13], who found 25% of non-compliance with pH in the formal sector and 18.8% of non-compliance with pH in the informal sector. The pH of a product plays an important role when its tolerance by the organism and its conservation. Unadjusted pH values can therefore have a major effect on the stability of the drug and influence its deterioration, causing disturbances in the body after or at the time of injection. The active ingredient had a rate of non-compliance in the formal sector of 29% and 57% in the informal sector. The underdetermination of the active ingredient decreases the expected therapeutic effect of the drug, thus leading to an inefficiency of the treatment and in the long term the appearance of bacterial resistance to the active ingredient. The microbiological test gave 100% sterility compliance in both sectors. This result was identical to that obtained by Menyeng [13].

CONCLUSION

The main objective of this study was to evaluate the physico-chemical, bacteriological and fungal qualities of the injectable DSP available in the formal and informal sectors of the city of Douala. Of the 14 batches, 26.2% were non-compliant following physico-chemical and sterility tests. The secondary packaging, the package leaflet, the primary packaging, the TLC identification, the nominal volume, the clarity of the solution and the dosage of active ingredient are the physico-chemical parameters found to be non-compliant. The mirage, the pH and the sterility test are the physic-chemical parameters found to be in conformity. The formal and informal sectors had respectively 12.7% and 39.7% of non-compliance with all tests. The various analyzes carried out showed that 26.2% of the injectable DSP was non-compliant in the city of Douala.

ACKNOWLEGMENTS

We thank Dr ETAME LOE Gisèle (Email: giseloetame@yahoo.fr ; P.O. Box 15515 Douala Cameroon; Tel: 237 699 833 818), General Manager of Industrial Laboratory, GENEMARK for their financial support.

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