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Development of a hydrophilic cream based on metronidazole, furadantin and chloramine to treat the Buruli ulcer: formulation and stability study

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Abstract
Buruli ulcer, also called "mbasu", is the third mycobacteriosis affecting humans, after leprosy and tuberculosis, which it even exceeds in terms of prevalence in certain areas of DR Congo. The World Health Organisation ranks it among emerging diseases. The treatment of this wound is complex.
The objective of this study is firstly to prepare a hydrophilic cream based on metronidazole, Furadantine and chloramine, secondly to develop an HPLC-UV method for the quality control of this cream and finally, to study the stability of this product during its use.
The results obtained in this study show that the cream used has good pharmaco-technical properties including good spreadability, ease of sampling and no lumps. The developed HPLC-UV method makes it possible to simultaneously analyze the three active ingredients in a relatively short time. Then, the product has good stability during its time of use.
This hydrophilic cream is an important advance in the treatment of this disease.

Keywords: Cream, Buruli ulcer and HPLC

1. Introduction
The wound is a lesion produced by a mechanical agent whose wounding action exceeds the resistance of the affected organ; as far as the skin is concerned, it represents an interruption of the cutaneous surface and therefore a potential entry point for an infectious agent [1, 2].
Disruption of skin integrity can occur in a number of contexts: surgeries, burns, radiation, cuts, tears, scratches, abrasions, rubbing, pinching and squeezing [1, 2]. There are also certain endogenous factors (extent and depth of the wound, deficiency in certain coagulation factors, inflammation, oxidative stress ...), exogenous (bacterial and / or fungal infection, staining, etc.) and iatrogenic (use of dressings). unsuitable, poor care technique ...) present in a wound that can lead to the development of infections and the appearance of oxidative, inflammatory, and antiangiogenic phenomena likely to make the healing process difficult, and the wound becomes incurable [3]. This is particularly the case of Buruli ulcer commonly referred to as MBASU in Kinshasa [4-6].
Wounds are often conditions neglected by emergency and first aid services, this is probably due to the fact that they are not life-threatening although they are at the origin of major socio-professional handicaps [2, 7]. Open wounds pose a risk of serious bacterial infection, including gas gangrene and tetanus, which can lead to long-term disability, chronic wound or bone infection, and even death [2, 7].
Currently, we use several solutions to overcome these problems, we have among others, wound dressings, the use of antiseptics, antibiotics, corticosteroids, anti-inflammatory nonsteroidal and antifungal. But this does not take into account all associated phenomena delaying healing [3, 6]
Thus, an antibiotic-based treatment was set up at Kinshasa University Clinics under the name of Kibadi® solution. The latter is prepared extemporaneously by mixing the tablets of metronidazole, chloramine and Furadantine.
However, the therapeutic success of this product can be compromised by its various disadvantages, in particular the fact that two of these active ingredients are not soluble in the water used as a solvent, therefore there will be a serious problem of stability and then the quality is not not determined that there is no official method of quality control of this product.
This work involves first the development of a simple pharmaceutical form including a cream based on these three active ingredients and the development of an analytical method capable of simultaneously detecting the three active ingredients for the purpose of consider a physico-chemical stability study during the use of this product. Second, we plan to verify the effectiveness of the cream produced on a few isolated cases.

2. Experimentation
2.1 Matériel chimique
HPLC grade methanol was supplied by the Merck laboratory (Darmstadt, Germany). HPLC quality water was provided by kim Pharma (Kinshasa, DR Congo) using a Milli-Q Plus 185 (Massachusetts-MA, USA).
The chemical substances Metronidazole (100.0%), Furandatin (100.0%) and Chloramine (100.0%) were provided by the Arauphar laboratory (Kinshasa, DR Congo). Excipients for reconstitution of the cream were also provided by the same laboratory.

2.2 Formulation of the cream

In a beaker the variable amounts of cetyl alcohol and peanut oil were heated slightly to 40 ° C. On the other side, the lauryl sulphate was dissolved in water under slight agitation. Then, the lipid phase was incorporated in the aqueous phase. While stirring vigorously in a mortar and using a pestle, until obtaining a homogeneous mixture finally to avoid any lumps. The mixture is put to rest, the formulation of the cream was observed. Then it was packaged in 40g pomps.

2.3 Appareils

To carry out our experiments, we used as a chromatographic column a XBridge C18, 250 x 4.6 mm ID, (5 µm dp) preceded by a guard column XBridge guard C18, 20 mm x 4.6 mm, (5 µm dp) both of Waters (Milford, Massachusetts - MA, USA).

The experiments were carried out with vwr Hitachi brand liquid chromatography equipment coupled to a UV-DAD 5430 detector. The entire chromatography system is controlled by a DellTM computer (Hangzhou, China) using software. Chromaster. The mobile phases were charged at 1 mL / min. All the chromatograms were recorded in the range of 210 nm to 400 nm with a resolution of 1.2 nm and an acquisition frequency of 2 Hz. The integration of the peaks was carried out at 220 nm and the volume of injection was 10 µL.

The pH values of the different buffer solutions were adjusted using a Villeurbanne Cedex ION check 10 - Radiometer Analytical Analyzer (Lyon, France) coupled with Kyosha Industries Kyosha label printer (Bezons, France). We used the Mettler-Toledo AX 204 analytical balance with a Mettler-Toledo RS-P42 label printer from Mettler-Toledo GmbH (Zurich, Switzerland).

The ultrasonic bath used to facilitate the dissolution of the few compounds was of the Branson 2210 E-DTH type from Branson Ultrasonic BV (Utrecht, The Netherlands).

2.4 Preparation of solutions

2.4.1 Preparation of metronidazole

In a 10.0 mL graduated flask, enter exactly weighed amounts of 10 mg metronidazole. Add 5ml of methanol, dissolve and bring to the line with the same solvent. Take 1 ml of the solution obtained place in a 10.0 ml graduated flask and bring to volume with the mixture of water HPLC and methanol 50/50 (ie 10 ml of water and 10 ml of methanol). The final solution is dosed at 100 µg /ml.

2.4.2 Preparation of furandatine

In a 10.0 mL graduated flask, enter exactly weighed amounts of 10 mg furandatin. Add 5ml of methanol, dissolve and bring to the line with the same solvent. Take 1 ml of the solution obtained place in a 10.0 ml graduated flask and bring to volume with the mixture of water HPLC and methanol 50/50 (ie 10 ml of water and 10 ml of methanol). The final solution is dosed at 100 µg / ml.

2.4.3. Preparation of chloramine

In a 10.0 mL graduated flask, enter exactly weighed amounts of 10 mg chloramine. Add 5ml of methanol, dissolve and bring to the line with the same solvent. Take 1 ml of the solution obtained place in a 10.0 ml graduated flask and bring to volume with the mixture of water HPLC and methanol 50/50 (ie 10 ml of water and 10 ml of methanol). The final solution is dosed at 100 µg / ml.

2.4.4. Preparation of the standard solution

In a 10.0 mL graduated flask, weigh exactly 10 mg of metronidazole, 10 mg of furandatin and 10 mg of chloramine. Add 5ml of methanol, dissolve and bring to the line with the same solvent. Take 1 ml of the solution obtained place in a 10.0 ml graduated flask and bring to volume with the mixture of water HPLC and methanol 50/50 (ie 10 ml of water and 10 ml of methanol). The final solution is dosed at 100 µg / ml.

2.4.5. Preparation of essay solution

In a 10.0 ml graduated flask, introduce an exactly measured volume of 10 ml of the cream®. Dissolve with methanol and bring to the line with the same solvent. Then introduce into a graduated 10.0 ml flask, a volume 1 ml of the solution obtained and bring to the line with the water-methanol mixture (50/50 v / v is 10 ml of water and 10 ml of methanol). The final solution is dosed at 100µg / ml.

3. Results and discussion

3.1. Formulation of the cream

The first step in this study is the formulation is a cream through the mixture of different raw materials, leading to the production of a homogeneous, stable with well-defined properties. Table I represents the different formulas tried to find in the end the best

Table 1: Cream formulation test

Composition	Formule 1	Formule 2	Formule 3	Formule 4	Formule 5
Cetyl alcohol	17.24g	18.17g	20,00g	12.76g	35,00g
Lauryl sulfate Na	3.45g	1.875g	4,00g	4.08g	5,00g
Water	52,00g	45.45g	50,00g	51.02g	40g
Chloramine T	1.041g	0.20 g	0.25g	0.30g	1.50g
Nitrofurantoine	1.041g	0.20 g	0.25g	0.30g	1.50g
Metronidazole	1.041g	0.20 g	0.25g	0.30g	1.50g
Parfum Silky	3.50g	2.50g	1.50g	1.80g	2.00g
Nipagin	0.50g	0.50g	0.50g	0.80g	0.10g

The formulas were evaluated pharmaco-technically and the results obtained are shown in Table II.

Table 2: Pharmaco-technical characteristics of prepared creams

Characteristic	Formule 1	Formule 2	Formule 3	Formule 4	Formule 5
Lumps	Absence	Absence	Absence	Absence	Absence

Caking time	10min	14min	6min	8min	5min
Easy sampling	Poor	Good	Poor	Poor	Poor
Color	white	white	white	white	white
pH	6	5	5	5	6
Spreadability	Poor	Good	Poor	Poor	Poor
Separation of phases	Absent	Absent	Absent	Absent	Absent
Viscosity	Very viscous	Very viscous	Good viscous	Poor viscous	Poor viscous

The results obtained show that the formula 2 is the best because it has a sampling facility that the other 4 but also good spreadability, no lumps, no phase separation. This formula will be used for the rest of our work.

3.2 Analyse of the Chromatographie Liquide à Haute Performance

An HPLC-UV method has been developed to analyze this combination of metronidazole, furandatin and chloramine. Being in the presence of the three active ingredients, the gradient mode was desirable to separate in a reasonable time these molecules (Table III).

Table 3: Elution gradient of the mobile phase

Time (min)	Water (%)	methanol (%)
0.0	95.0	5.0
9.0	5.0	95.0
12.0	5.0	95.0
13.0	95.0	5.0
20.0	95.0	5.0

The chromatogram obtained in this chromatographic condition is shown in figure 1.

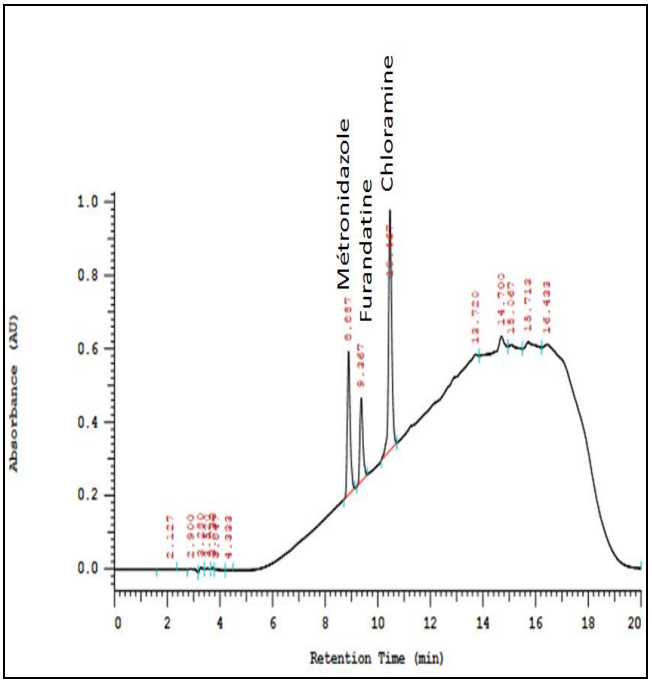

Fig 1: Chromatogram obtained at 220 nm

This method has the advantage of separating the three active ingredients to less than 10 minutes. The chromatographic udders were recognized thanks to the individual injections of control solution and to the UV spectra of each molecule. These spectra are shown in Figures 2,3 and 4.

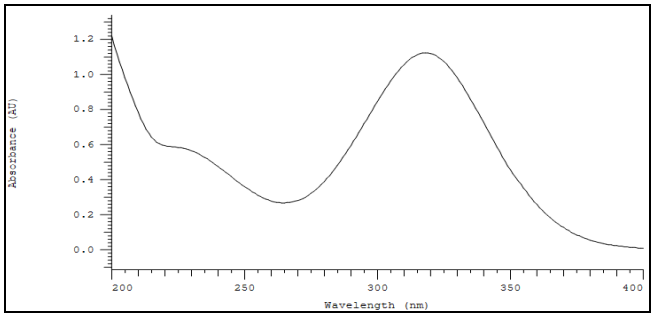

Fig 2: Uv Spectra of métronidazole

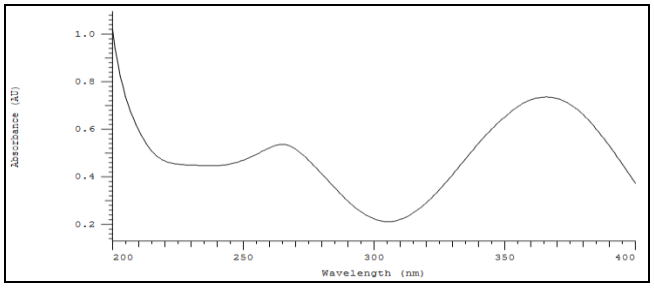

Fig 3: Uv Spectra of furandatine

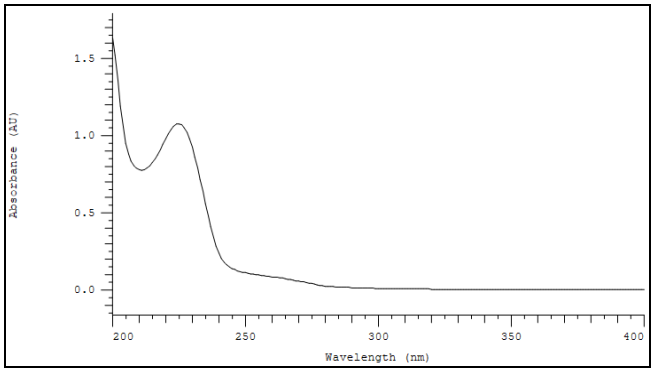

Fig 4: Uv Spectra of chloramine

3.3 Study of the stability of the cream formulated

The developed method allowed us to follow the stability of the cream formulated during its use.

The cream was stored for 7 days at room temperature, ie 30 ° C. with a relative humidity of 60% ± 5%. The results of analysis obtained are shown in Table IV.

Table 4: Stability study for 7 days of the cream formulated

Active subtances	Day 1	Day 4	Day 7
Metronidazole	99,4%	99,1%	99,4%
Furandatine	97,8%	96,7%	95,4%
Chloramine	102,4%	99,2%	90,7%

We found that the cream was stable under these conditions of study for 7 days of its storage during use.

Conclusion and perspectives

The main objective of this study was to propose a cream starting from the Kibadi solution which was used at Kinshasa University Clinic in the treatment of wounds commonly called MBASU. Then develop an HPLC analysis method to study the physicochemical stability of this formulation during its use.

The results obtained in this study show a cream of good quality because it does not have lumps, no phase separation with a pH of 5.00.

Then, an HPLC-UV method was developed to simultaneously analyze the three active ingredients. This method has been applied to demonstrate the stability of the cream during its duration of use ie 7 days. This is a major step forward in the treatment of Buruli ulcer, which is very common in Kinshasa.

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