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Research Article

Analysis of the Physico-Chemical Conformity of Antibiotics: Case of Amoxicillin 500 mg Capsule at the National Health Laboratory Distributed in Health Structures in Mali

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Abstract

Introduction: Amoxicillin belongs to the family of β-lactams and is one of the mostly used in hospitals for the management of bacterial infections especially in Mali. It has a broad spectrum of activity from the group of aminopenicillins or aminobenzylpenicillins (group A penicillins). Microbiological and physico-chemical control has evolved with the development of biotechnology. All bacterial species or germs are affected by the phenomenon of resistance to antibacterials, which often poses real therapeutic problems. Based on previous studies in Mali, amoxicillin is still recommended in pharmacies and university hospitals to treat bacterial infections. It sometimes happens that patients are not satisfied with this amoxicillin-based prescription in our teaching hospitals. The objective of this study was to control the physico-chemical quality of amoxicillin 500 mg in capsule form.

Methodology: This is a physico-chemical study of amoxicillin capsules dosed at 500 mg received at the National Health Laboratory in Bamako. We analyzed 10 batches of five (5) boxes (10 platelets/box) of amoxicillin. We used either HPLC, or TLC or Dissolu-test to control the quality of amoxicillin capsules for the presence of the active principle and to ensure fake amoxicillin capsules were not distributed. We used as reference, the standards contained in the pharmacopoeias in use.

Results: The results of the physicochemical tests revealed that the samples analyzed complied with the standards of the Pharmacopoeias in use: The weight of our capsules was between 0.5g and 0.6g. All the tested samples were compliant with a disintegration time of 15 minutes at 37°C ±2°C. The peaks of standard amoxicillin and that of the tested samples appeared at substantially equal retention times, i.e. at 3.249 min and 3.248 min. respectively.

Conclusion: Our work aimed to assess the quality of antibiotics used in Mali with the analytical means available at the LNS. All the batches of amoxicillin analyzed have not presented any cases of non-compliance so they can be distributed in health structures. This type of study should be extended to the other pharmaceutical forms of amoxicillin in the form of powders to be reconstituted dispensed in health centers in Mali.

Keywords: amoxicillin500 mg capsule, compliance test, HPLC

INTRODUCTION

The antibiotic is a chemical substance produced by micro-organisms. More often, lower fungi have the power to oppose to the multiplication of microbial germs by exerting either a bacteriostatic or bactericidal effect [1]. Amoxicillin (Figure 1a) is an antibiotic, bactericidal antibacterial from the family of beta-lactams (Figure 1b), from the group of penicillins A or amino-penicillins. Beta-lactam antibiotics belonging to the penicillin family (Figure 1c) have played an important role in human medicine for decades due to their broad spectrum of antimicrobial activity, good absorption and their high tissue penetration [2]. This drug is used in the treatment of several infections due to germs defined as sensitive (*Streptococcus*, *Staphylococcus aureus*, *Enterococcusfecalis*, *Monocytogenes*, *Clostridiumsp*, *Actinomyces*, *etc.*) in particular in their manifestations [3].

Microbiological and physico-chemical controls have evolved with the development of biotechnology throughout the production chain and at the level of the finished product to be identified [4]. Since the introduction of antibiotics into the

therapeutic arsenal of infectious diseases, microorganisms have developed means of defense to become insensitive to antibacterials. All bacterial species or germs are affected by the phenomenon of resistance to antibacterials will sometimes pose real therapeutic problems [5]. This resistance may be due either to either the quality of the amoxicillin administered to the patients or to the patients' non-compliance with the treatment. Based on previous studies in Mali, amoxicillin continues to be recommended in pharmacies and university hospitals to treat bacterial infections. It sometimes happens that patients are not satisfied with this amoxicillin-based prescription in our teaching hospitals. According to a 2015 study in Chad which showed by CHPL a range of amoxicillin (Bactox^{X} , Amolin^{R}) had impurities such as Co-oligomers of amoxicillin, amoxicillin penicillinic acid, 6-amino penicillinic acid and N-penicillan-6-yl at retention and content times respectively equivalent to (0.68min, 0.18%), (0.78min, 0.18%), (0.87min, 0.13 %), (4.5min, 0.2%) [6] . Of all the 12,861 prescriptions recorded in the pharmacy department of the Gabriel Touré Hospital between January 1, 2004 and June 30, 2004, 6,000 prescriptions including 39.8% from the pediatric department were included in that study. Twenty-six antibiotic molecules had been prescribed with amoxicillin on the top of the list in 30.5%. The prescription of antibiotics has reached high levels in the Malian hospital setting. The authors have recommended measures to optimize the antibiotic therapy in hospital [7]. According to the world health organization (WHO), 25% of the drugs used in developing countries are either fake drugs or of inferior quality; five percent (5%) of the antibiotics sold in the world are counterfeit (among other counterfeit drugs). Many cases have shown harmful effects on human health. In extreme cases, we can observe the aggravation of the pathologies treated, so it is important to ensure the quality of these drugs [8]. Studies carried out on amoxicillin in Senegal gave 22% cases of non-compliance due to the absence of the expiry date without which it is impossible to estimate the lifespan of a drug and therefore to judge its stability. It is in this option that the WHO has recommended that manufacturers include on the label of products information relating to their period of use, the expiry date and a date of manufacturing [9]. We therefore initiated this study on amoxicillin in capsule form to verify its physico-chemical quality at the National Health Laboratory in Bamako, Mali.

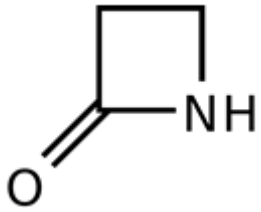


Figure1: noyau bêta-lactame[10]

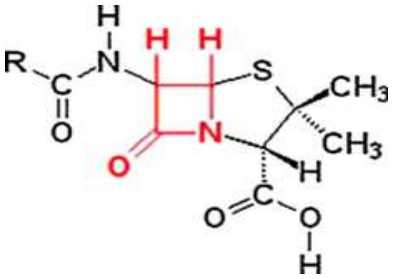


Figure 2: structure générale des pénicillines [11]

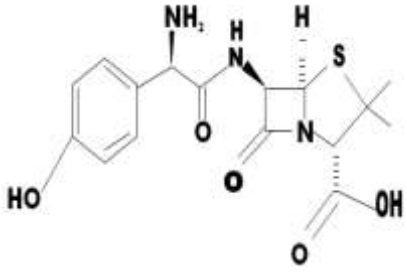


Figure 3 : structure chimique de l'amoxicilline [11]

MATERIAL AND METHODS

Study Site and Period

We conducted a 3-month analytical study from September to December 2020 based on the quality control of amoxicillin capsules at the drug quality control department of the National Health Laboratory (LNS) in Bamako, Mali. We chose to carry out this study at the LNS because it is a public scientific and technological structure, which has mandate and is well equipped for drug quality control.

Material

Apparatus and Solvents: All the devices and equipment used during the production:

HPLC, pH-meter, Precision electronic balance, Dissolute-test, Visible ultraviolet spectrophotometer, Disintegrator, Thermometer, Vial, Hood, Acetonitrile, Methanol for HPLC brand analysis, Monobasic potassium dichromate, Distilled water, Syringe (5/30 mL), Magnetic bars. The reference product was trihydrated amoxicillin (Sigma-Aldrich, Germany) with titer: 100.2% (858.7 µg/mg).

Sampling

We received from the *Pharmacie Populaire du Mali* (PPM) and analyzed at the LNS ten (10) batches of samples, each batch consisting of five boxes of ten (10) 500 mg amoxicillin capsule strips using assay techniques recognized by the American and European Pharmacopoeias

Methods

Visual examination

Labeling: On the packaging, we checked that the following information appeared on the packaging of the medicines: product name, name of the PAs, the quantity of active ingredients contained in each capsule, batch number assigned by the manufacturer, date of manufacture and expiration of the product, name and address of the manufacturer, the instructions on the conservation and storage of the product in a nutshell we have adopted by the same procedure as Diop A, *et al.,* 2009 [9].

Appearance Physical and organoleptic character: consisted in identifying the aspect, the color, the odor, the shape, the surface and the thickness of the capsules.

Mass Uniformity:

We individually weighed 20 capsule units using a RADWAG micro balance. Then we calculated the mean, the standard deviation and the Coefficient of Variation (CV).

$$\%M = \frac{(M1-M2)}{M1}\ x\ 100$$

$$CV = \frac{\text{Ecart-type}}{\text{Moyenne}} * 100$$

Legend:

%M : mass loss;
M1 : mass of the tablets before the test;
M2 : mass of the tablets after the test.

Dissolved-test:

We carried out the dissolution test of the different batches of amoxicillin using the palette under the conditions described by the United States Pharmacopoeia (USP) -2020 [12]:

- Test duration: was 60 min;
- Dissolution medium: distilled water;
- Volume of dissolution medium: 900 mL;
- Dissolution medium temperature: 37 ± 0.5 ºC;
- Blade rotation speed: 75 rotations per minute.
- A thermometer that allows you to take the temperature of each container
- Tolerance: not less than 80% of the labeled amount of amoxicillin dissolved in 60 minutes

Assay by UV Spectrophometer:

Preparation of the reference control solution

We weighed 0.025799 g of standard amoxicillin, which we introduced into a 100-mL flask with distilled water up to the line of the cheek. We then took 1 mL of this solution and introduced it into a 25-mL flask filled with distilled water. We had a concentration of 0.010316 mg/mL [12].

Sample preparation

A capsule of 500 mg of amoxicillin is introduced into each vessel containing 900 mL of distilled water and the test was carried out for a period of 60 minutes. We took 30 mL from this dissolution beaker. By taking this sample, we made sure that the syringe was halfway into the beaker containing our solution.

- At the end of the test, 1 mL of the solution was taken and filtered with the 0.45 µm filter then diluted in 50 mL of distilled water. We obtained a final concentration of 0.01111 mg/mL.
- Then we determined the amount of dissolved amoxicillin using the visible UV spectrophotometer at the maximum absorbance wavelength of about 272 nm in comparison with a standard solution having a known concentration in the same medium [12].

$$\textbf{Absorbance} = \frac{absorbance\ ech}{absorbance\ std} \times \frac{[std]}{[ech]} \times 100$$

Disintegration test:

Six (6) capsules were taken from the batch of each controlled specialty. The water bath of the disintegrator was heated. In each 1L beaker, we put 700 mL of distilled water. The beakers were placed in the water bath and the temperature was checked frequently using a thermometer until it reached 37°C ± 2. The drugs were then introduced into each of the six (6) tubes of the basket of disintegration which were placed on the arm of the apparatus. A stopwatch was triggered after starting the engine ensuring the vertical movement of the basket [12].

High Performance Liquid Chromatography (HPLC):

Instrumental and analytical conditions: The analysis was carried out with a CLHPAGILENT 1260 system (see Figure 4 under the following conditions:

✓ **Stationary phase: A C** $_{18}$ column (250×4.5 mm×5 µm) was used.

✓ **Buffer Preparation:** We weighed 6.8009g of monobasic potassium phosphate and dissolved it in a 1L flask then topped up with distilled water. We adjusted the pH to 5.0 ±0.01 using potassium hydroxide (NaOH).

✓ **Mobile phase:** 960 mL of buffer were taken, then 40 mL of acetonitrile. We filtered the mixture using a 0.45 µm filter then degassed for 45 minutes.

✓ **Pump flow rate:** The device operated with a flow rate of 1.5 mL/minute.

✓ **Detector:** UV detection was performed at 230 nm.

✓ **Injection:** The injection volume was 10 µL.

Preparation of the standard solution: 0.03002 g of amoxicillin standard was weighed accurately then introduced into a 25-mL volumetric flask and completed with the buffer. A concentration of 1.20008 mg/mL was obtained.

Sample preparation: A quantity of sample powder containing 500 mg was weighed and then introduced into a 100-mL volumetric flask filled with buffer. We adjusted for about five (5) minutes then took 1mL of this solution and put it in a 10-mL vial and completed with buffer. The solution was filtered through a 0.45µm filter. Then we moved on to reading [12]. The tolerance was 90.0 to 120.0% of the labeled amount of amoxicillin.

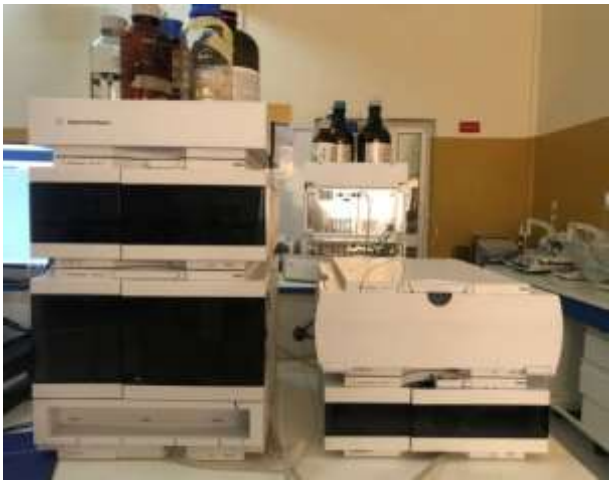


Figure 4: High Performance Liquid Chromatography

RESULTS AND DISCUSSION

The results of the visual examination of the packaging of the different batches of capsules are recorded in Table I

Table I: Visual examination results

Samples	Colors	form	Odor	Batch manufacturer name and expiration date	Primary condition
Amoxicillin 500 mg	Dirty white and orange	capsule	Scentless	Yes	Printed Aluminum blister Amoxicillin 500 mg

We observed that the visual examination was up to the standard. Our results were similar to those obtained by Bokola Tinni SONHON in 2012 in the RCI [13] all the amoxicillin samples collected were compliant on the visual inspection plan. On the other hand, our results were from the study carried out in Dakar showed that the physical and visual inspection revealed on 34 samples, five cases of non-compliance (23.5%): 80% of the non-compliant samples came from the illicit sector and 20% from the private sector. No non-compliance was observed for samples from the public sector [9].

Uniformity of Mass and Dimensions

The results relating to the mass uniformity tests and the disintegration time are in Table II.

Table II: Results of mass uniformity and disaggregation test.

Analysis number	Average weight (g)	Coefficient of Variation(%°	Disintegration time	Disaggregation standard
20-001	0.632275	1.591586563%	07'59''	≤30''
20-002	0.631695	1.83097641	15'07''	
20-003	0.557915	3.97559076	11'20''	
20-004	0.55903	4.77500522	11'50''	
20-005	0.58339	2.13857241	12'10''	
20-006	0.59091	2.10733484	08'57''	
20-007	0.61677	2.7736994374	13'13''	
20-008	0.612655	1.79398038	09'30''	
20-009	0.61664	2.679329434	09'08''	
20-010	0.5842375	1.87410262	09'39''	

': minutes and **'':** Second

The weight of our capsules was between 0.5g and 0.6g. According to the European Pharmacopoeia, when the Average Weight is greater than 300 mg (0.3g), be included within the limit ± 7.5% of their average mass, the Coefficient of Variation (CV) must not exceed 5%. However, all the samples tested are compliant with a disintegration time of 15 minutes at 37°C ± 2. Our results are different from those obtained by Diop A, *et al* , 2009 [9] in Dakar that a sample of amoxicillin (amox 4) and a sample of ampicillin (ampi 3), from the illicit sector, had a disintegration time greater than 30 minutes.

Dissolution test

Table III shows us the dissolution time of the different batches of our capsules analyzed.

Table III: Results of the dissolution of the different batches

Analysis number	Vessel 1%	Vessel 2%	Vessel 3%	Vessel 4%	Vessel 5%	Vessel 6%
20-001	91.2	92.2	93.3	92.3	93.4	92.3
20-002	91.1	90.1	92.1	92.1	92.4	92.1
20-003	94.4	95.4	95.2	95.4	94.3	94.2
20-004	93.3	93.1	93.2	93.1	92.9	90.4
20-005	90.2	90.1	90.2	89.4	90.1	89.1
20-006	91.2	91.4	89.1	89.4	89.4	89.00
20-007	91.4	91.2	92.1	92.2	92.4	92.4
20-008	91.4	92.2	95.2	94.1	90.4	90.4
20-009	90.6	88.2	92.1	92.2	91.3	90.2
20-010	89.1	91.2	90.4	90.4	90.3	89.3

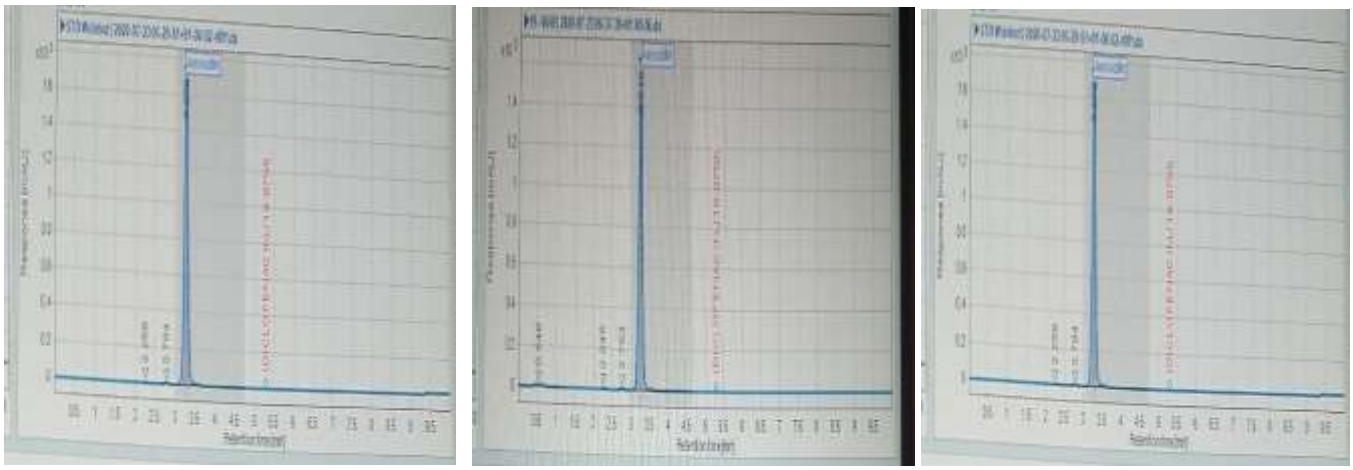
From the results shown in the dissolution of capsules, USP standards require that the dissolution rate should not be less than 80%. The capsules comply with the USP.S standard in terms of the dissolution test our results are similar to those obtained by Bokola Tinni SONHON in 2012 (RCI) [13] where the results were compliant for the six (6) batches analyzed. Four (4) out of six (6) products, or approximately 66%, therefore do not meet the quality standards prescribed by the American Pharmacopoeia USP 32 NF 27. The same trend is observed in a study carried out in Niger by Idrissa H. *et al* . . in 2005 a study in which approximately 62% of the anti-infectives tested did not meet the standards among forty-five (45) products analyzed [14].

Identification and dosage of the active ingredient by HPLC

Table IV represents the average of the retention times of the standard and the sample (figure 5).

Table IV: Results of PA content retention time

Analysis No.	Concentration in %	Concentration in mg	Review time (min)	
			Standard	Scale
20-001	105.3259714	526.629856905	3,249	3.25
20-002	106.5430359	532.715179665	3,249	3,248
20-003	109.3042732	546.52136589	3,249	3,248
20-004	102.3616548	511.808273875	3,249	3,251
20-005	100.9322935	504.661467635	3,249	3,248
20-006	107.698439	538.492195345	3,249	3.25
20-007	111.4955392	557.477769618	3,249	3,247
20-008	110.9887533	554.943766595	3,249	3,251
20-009	109.1688758	545.84437875	3,249	3,251
20-010	90.095329	450.47664525	3,249	3,248



(a) : Standard (b) : Echantillon 20-002 (c) : Echantillon 20-009

Figure5: Image of the chromatograms of two samples (b,c) and a standard (a) by HPLC.

The observation of the chromatograms (Figure 3) showed that the peaks of the samples were similar to that of the standard. We then deduced that our samples were compliant according to the monograph. The standard and sample amoxicillin peaks appeared at approximately equal times at 3.249 min and 3.248 min respectively. The peaks of the samples are similar to those of the standard. It was deduced that the samples contain the active ingredients of amoxicillin.

The results of the identification and dosage tests of the active ingredients, obtained by thin layer chromatography (TLC), prove the existence of placebo drugs, under dosed or with impurities in all drug sales sectors in Senegal [9] (Diop A, *et al* , 2009).

The results obtained in our study were different from those of the WHO carried out in Cameroon, Madagascar and Chad [15] and in which 30% (47/157) of the antibiotic samples tested were non-compliant; 57% (27/47) of non-compliances were due to an under-dosage of active ingredient and 26% (12/47) to the absence of active ingredient. The study carried out by Pennaforte [16] who has reported a similar result in Cameroon with 28% (27/96) of non-conformities: 52% (14/27) of them by under-dosage of active ingredient, and 41% (11/27) by the presence of a bad active ingredient.

A study was interested in various anti-infective drugs among the most widely used (Amoxicillin, Benzylpenicillin, Chloramphenicol, Sulfamethoxazole / Trimethoprim,

Tetracycline, Mebendazole, Metronidazole and Quinine) and sold on the street in Cambodia. A total of 144 samples were analyzed, of which 108 were found to be compliant and 36 non-compliant [17]. The problem of poor drug quality does not only affect Africa.

CONCLUSION

The purpose of this work was to determine impurities in amoxicillin samples taken at the PPM to know their compliance with international standards. The results of physicochemical tests such as PA content, dissolution, breaking strength revealed that the samples analyzed complied with the standards of the Pharmacopoeias used. The PA content was compliant for the samples of amoxicillin belonging to the family of the WHO essential drugs.

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