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Analysis by LC-Ms/Ms of Drugs Sold in the Markets of Kara and Lome

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Abstract: The falsification of medicines is a worldwide scourge, but one that particularly affects developing countries, causing a great deal of damage, including deaths. Fake medicines are seized by customs every year. However, very few studies have looked at the qualitative and quantitative analysis of medicines found in Africa, both in pharmacies and on the markets. This study aims to analyze several drugs purchased in Togo in the cities of Kara and Lomé to ensure that they actually have the active ingredients indicated inside, which is one of the guarantees of their quality. The samples were analyzed by HPLC coupled with MS/MS which is a method of choice for the identification of molecules of interest. Analysis of the spectra obtained showed that the samples purchased on the markets (analgesics, anti-inflammatory drugs and antibiotics) contain all the active ingredients indicated on the package. This result is reassuring because 100% of the samples analyzed are of good quality. However, it will be necessary to make additional studies by adding other classes of drugs such as anti-malarials that are most affected by falsification on the African continent. A quantitative study should also be done to determine the amount of each active ingredient in the tablets studied. It will be interesting to study the excipients contained in drugs because, although they are not active principles, they have an influence on the metabolism of active molecules.

Keywords: Drugs, LC-MS/MS, Kara, Lomé

1. Introduction

A drug is a substance or composition with curative or preventive properties with respect to human or animal diseases [1]. Drugs are therefore used to prevent diseases or symptoms, alleviate pain, slow down the disease process, cure or treat diseases. The health of individuals is a major concern and that is why the third Sustainable Development Goal (SDG3) is to empower individuals to live healthy lives. In this context, access to essential medicines or drugs is included in point 3.8 of the SDGs [2]. Although in developed countries access to medicines has improved considerably in recent years [3], drugs of dubious, inferior or counterfeit quality are extremely abundant on the market which alarms worldwide [4]. Fake medicines represent a major danger to public health and cause economic losses throughout the world [5-7]. They are more prevalent in developing countries and especially in Africa [8]. Indeed, 42% of trafficking cases reported to the World Health Organization's global surveillance system come from sub-Saharan Africa [9]. Fake medicines can lead to excess morbidity and mortality [10]. Although it is impossible to make an accurate inventory of the number of deaths attributable to this practice, the WHO nevertheless estimates that it is the cause of 800 thousand to one million deaths per year worldwide [11]. The report of the United Nations Office on Drugs and Crime (UNODC) of 31 January 2023 reveals that 270,000 deaths per year in sub-Saharan Africa are due to the consumption of falsified antimalarial drugs and that more than 160,000 deaths of children in the same region are directly attributable to the consumption of false antibiotics [12]. An estimated 122350 deaths of children under the age of five each year in 39 countries in sub-Saharan Africa are linked to the use of low-quality antimalarial drugs [13]. Togo is resolutely committed to the fight against fake medicines and it is also around this issue that was held, on January 17 and 18, 2020 in Lomé a summit called «Lomé Initiative on fake medicines», bringing together seven African governments (Togo, Congo-Brazaville, Uganda, Niger, Senegal, Ghana and The Gambia)

committed to fighting and criminalizing this activity, which poses a threat to public health, the security of countries and their economies [14]. All therapeutic categories are affected by counterfeiting, whether «vital» drugs, «comfort» drugs or high value-added drugs [15]. Most published studies on substandard and falsified drugs have focused on antimalarials [16, 17], and antivirals [18], but data on the most consumed drugs such as analgesics and antibiotics are scarce. Among analgesics, paracetamol or N-(4-hydroxyphenyl)-acetamide, is one of the most popular and widely used drugs for the treatment of pain and fever. It is also the best-selling drug in the informal circuit [19]. It is in this context that this study was conduct by analyzing by HPLC and GCMS several samples of paracetamol and antibiotics obtained in the cities of Kara and Lomé in Togo to verify the compliance of active ingredient composition by report to data reported on packaging.

2. Materials and Methods

2.1. Sampling

The medicines were purchased in the cities of Kara and Lomé from re-sellers located at the Kara market and the Assiyéyé market in Lomé. The drugs chosen were analgesics and antibiotics which are among the best-selling and easily accessible drugs. Indeed, for other more specific drugs, the distrustful ladies told us not to sell them. In total, 5 different drugs namely paracetamol, GMOL 500, SOCOMOL, IBUCAP and LETAP were purchased from 6 different vendors or 30 samples in total.

2.2. Tools

The UHPLC system included a Thermo Scientific/Dionex HPLC UltiMate 3000 liquid chromatograph (Germany) consisting of a quaternary gradient pump LPG-3400A high pressure variable flow rate, a cooled autosampler WPS-3000TSL Analytical with 100 µL sample loop, TCC-3000SD column furnace and diode array detector with range from 190 to 800nm VWD-3400RS (230V, 50/60Hz, 750W). The column used was a C18 Hypurity Advance column (5 µm, 150 4,6mmi.d., Thermo-Hypersil Keystone, Bellefonte, PA). The flow rate was 1.0 mL/min and the injection volume was 20µL. The injection time was 20min with a gradient of 10% ultra pure water 90% acetonitrile. The detector was set to λ = 200 nm; λ = 254 nm and λ = 350 nm. All analyses were performed at room temperature.

Chromeleon™ Chromatography Data System (CDS) Software (ThermoFischer) was used for data processing and peak integration.

Mass spectra were performed by the CESAMO (ISM, Bordeaux, France) on a QExactive™ benchtop Orbitrap mass spectrometer coupled to a Vanquish UHPLC system (Thermo Scientific, San Jose, USA). The instrument is equipped with an ESI source and spectra were recorded in the negative / positive mode. Source parameters were as follows: spray voltage 3,2 kV, capillary temperature 320°C, S-Lens RF level 55,0, auxiliary gas temperature 300°C, sheat gas 25, auxiliary gas flow 10.

Full MS scans were acquired over the range 50-1300 m/z with a mass resolution of 140000. The target value (AGC) was 1E6, and the maximum allowed accumulation time (IT) was 50 ms.

Analysis were processed by direct infusion (FIA). Samples were introduced by injection through a 20 µL sample loop into a 300 µL/min flow of methanol from the LC pump.

XCalibur software version 4,1 was used for data acquisition and FreeStyle software version 1,5 for processing (Themo Scientific, San Jose, USA).

2.3. Drugs and Reagents

HPLC-grade solvents including methanol and acetonitrile (Fisher Scientific, Fair Lawn, NJ), and high purity distilled water were used in the study. The tablets of paracetamol GMOL 500, paracetamol, ibuprofen-paracetamol and oxytetracycline were purchased from several vendors in the cities of Lomé and Kara (Togo).

2.4. General Procedures

Sample preparation

Each tablet was weighed and finely ground into a powder. The capsules were weighed and opened and the powder was recovered. Methanol (1mL) was added to 1mg tablets and the mixture was stirred until the powder dissolved completely. The samples were then processed according to HPLC-MS procedures.

2.5. HPLC

The mobile phase at t = 0 consisted of a mixture of water/acetonitrile in the proportions 10:90 (v/v). Then a gradient was applied to arrive at t = 20min at a mobile phase composed of a water/acetonitrile mixture in the proportions 90:10 (v/v). Injection of each sample (20µL) was performed and chromatographed under the LC conditions described above.

2.6. GCMS

Drugs stock solutions (1000 µg/mL) were prepared in methanol. Injections were made for each drugs and chromatographed under the previously described GC conditions.

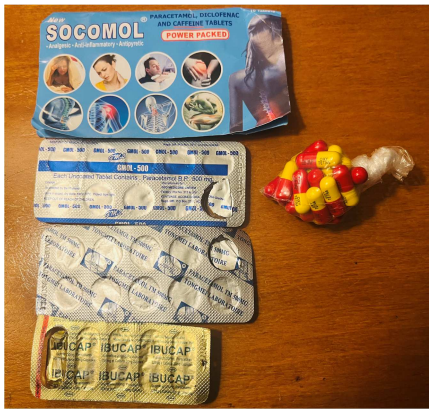

Figure 1. *Drugs purchased in Lomé and Kara (Togo).*

3. Results and Discussion

3.1. Aspect and Packaging of Drugs

All drugs, except drug M5, were sold in a package that looked compliant, with a package insert and expiration dates that did not reveal any errors or inconsistencies that could be found on falsified drugs [20]. On the other hand, the capsules of the drug M5 are sold are no packaging or leaflets, regardless of where it was purchased, but they are just attached in a plastic bag (Figure 1). This packaging is problematic because there is no package leaflet that accompanies the drug, expiry dates are not known, the lot from which these capsules come is also not known. The consumer cannot know the possible adverse effects of the drug and other necessary information to know. This is problematic, especially since it is not a health professional who sells these drugs by giving advice, but a market saleswoman.

3.2. Each Drugs Composition

The weight and composition of each tablet, as described on the drug box, is recorded in Table 1.

Table 1. *Composition of each drugs purchased.*

	Drug name	Form	Weight (mg)	Composition
M1	Paracetamol	Tablet	576,5	Paracetamol
M2	GMol-500	Tablet	586,7	Paracetamol
M3	Socomol	Tablet	646,7	Paracetamol/Diclofenac sodium/Caffeine
M4	Ibucap	Capsule	666,5	Ibuprofen/Paracetamol/Caffeine
M5	Letap	Capsule	418,6	Oxytetracycline

Reading the composition of the drugs on the boxes determined that four of the drugs sampled contain paracetamol which is the most consumed analgesic in the world, as the predominant active ingredient [21]. M4 is a drug that contains ibuprofen, which is both analgesic and non-steroidal anti-inflammatory [22]. The drug M3 contains diclofenac which is also a non-steroidal anti-inflammatory. For the drug M5 which does not contain packaging, an internet search was carried out to find out the composition of this drug and it appears that the only active ingredient contained in these capsules would be oxytetracycline, a broad-spectrum antibiotic. It is known that all categories of drugs are victims of falsifications, inducing a decrease in quality, including the absence of active ingredient in the tablet. It was decided to check that the active ingredients that were described on the tablet's box were actually present.

3.3. Analysis of Each Drug

HPLC-MS/MS was used to analyze all drugs and then interpret the mass spectra. Figure 2 shows the LCMS spectrum of drug M1.

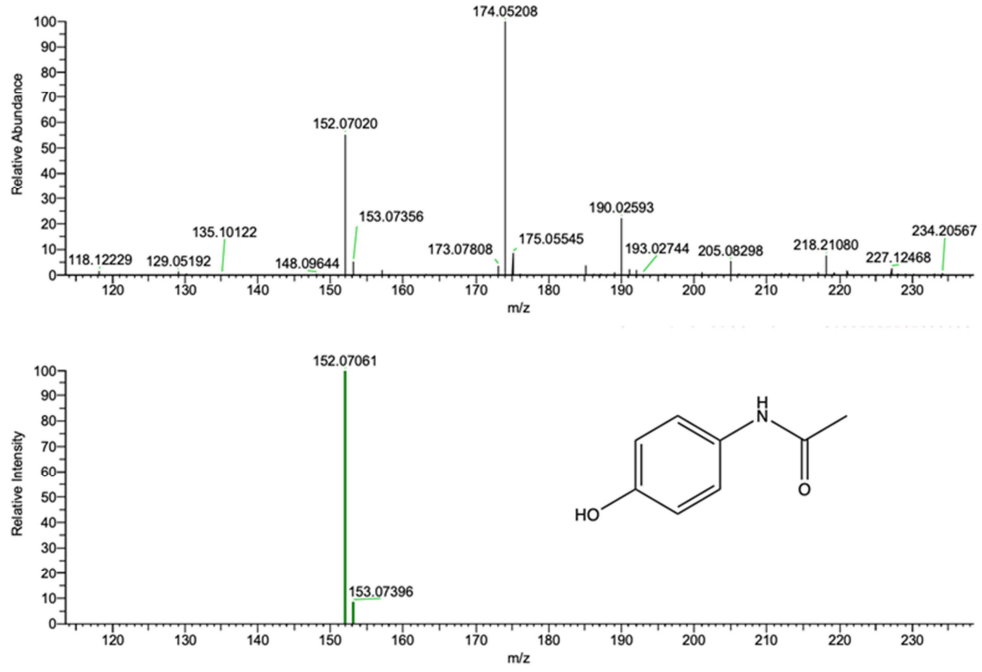

Figure 2. *Drug M1 LCMS Spectrum.*

In Figure 2, there is 3 predominant peaks in relative abundance that are m/z = 152; m/z = 174 and m/z = 190. These three peaks confirm the presence of paracetamol whose molar mass is 151 g/mol with the peak m/z = 152 corresponding to the peak of paracetamol with an additional proton, m/z = 174 corresponds to the mass of paracetamol

with a sodium atom (M_{Na} = 23g/mol) and peak m/z = 190 corresponds to paracetamol with addition of potassium (M_K = 39g/mol). In relative intensity, the only peak present is that of m/z = 152. With all these observations, it is possible to confirm that the drug does contain paracetamol as an active ingredient. All samples of this drug taken from different vendors have the same spectrum. Qualitatively, this first drug can be considered good.

Figure 3 shows The LCMS spectrum of drug M2.

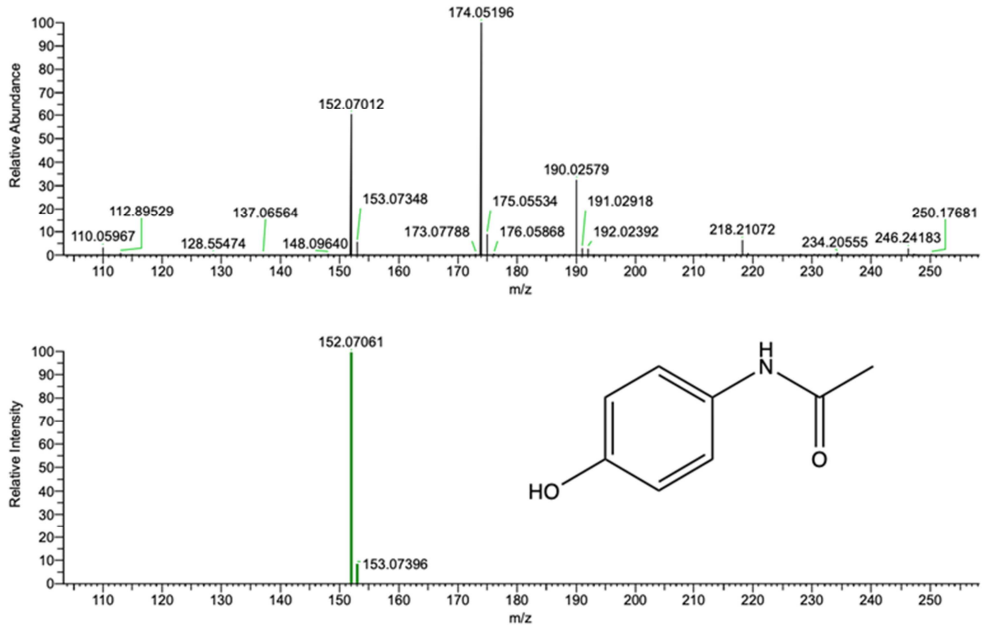

Figure 3. *Drug M2 LCMS Spectrum.*

Figure 3 has almost the same profile as Figure 2 with the same predominant peaks in terms of relative abundance namely m/z = 152; m/z = 174 and m/z = 190. The interpretation is therefore exactly the same as before and it is possible to say that the drug M2 contains the paracetamol which is the only active ingredient indicated as present in the tablet. This drug is therefore qualitatively correct since it actually contains the indicated active ingredient.

The LCMS spectrum of M3 is shown in Figures 4 and 5.

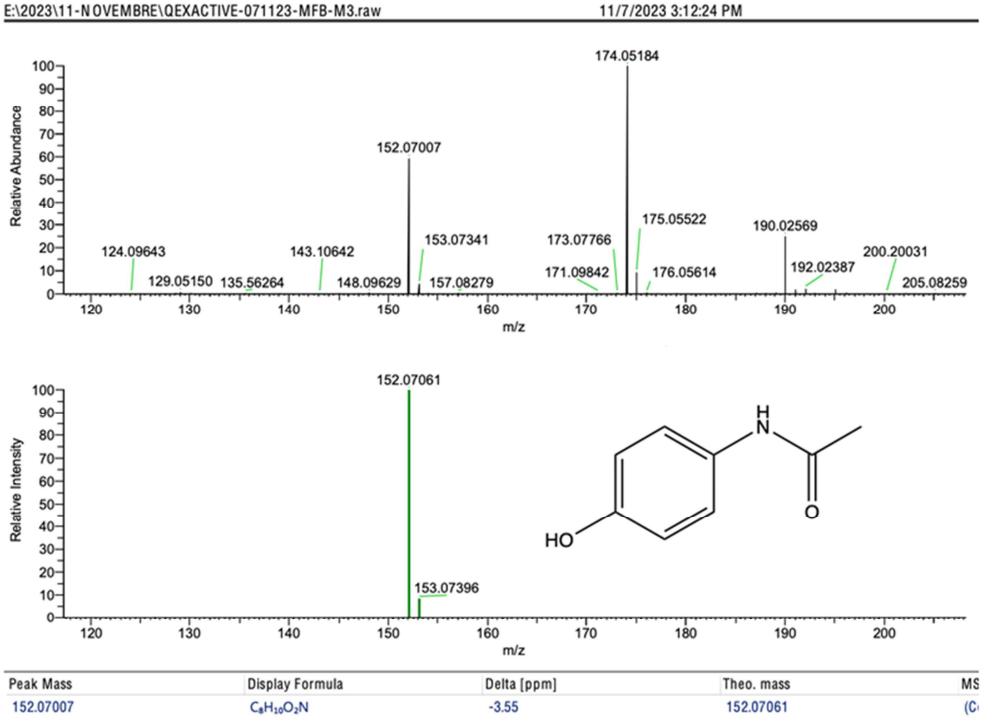

Figure 4. *Drug M3 LCMS Spectrum.*

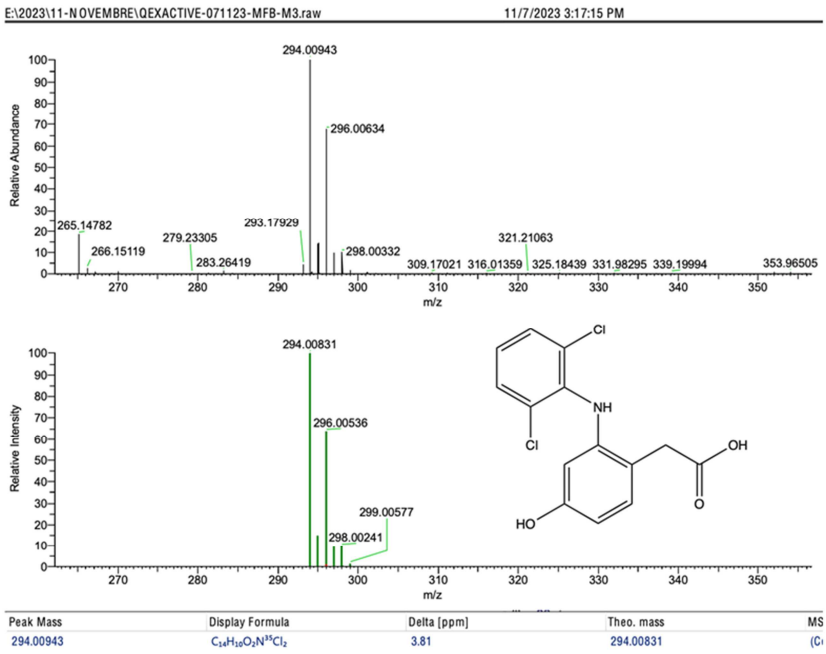

Figure 5. *Drug M3 LCMS Spectrum.*

Figure 4 has almost the same profile as Figures 2 and 3 with the same predominant peaks in terms of relative abundance namely m/z = 152; m/z = 174 and m/z = 190. It can therefore be said that drug 3 also contains paracetamol, in accordance with the data listed on the box of the drug. Figure 5 shows that drug 3 contains another molecule in addition to paracetamol. The predominant peak is that at m/z = 294 which corresponds to the gross formula $C_{14}H_{10}O_2NCl_2$. This peak confirms the presence of diclofenac of the crude formula $C_{14}H_9O_2NCl_2$ with an additional proton. The two main active ingredients are clearly identifiable in mass spectrometry. On the other hand, it is mentioned that the tablet contains caffeine but does not contain much less than the other two molecules, but it could not be clearly identified in mass spectrometry. This is probably due to its low concentration compared to the other two compounds. It is still possible to say that the drug 3 is of good quality since the active ingredients of therapeutic interest namely paracetamol which calms the pains and helps to lower the fever, and diclofenac, anti-nonsteroidal inflammatory whose role is to calm joint pain, are present in the tablet.

For drug M4, the molecules identified are found in Figures 6 and 7:

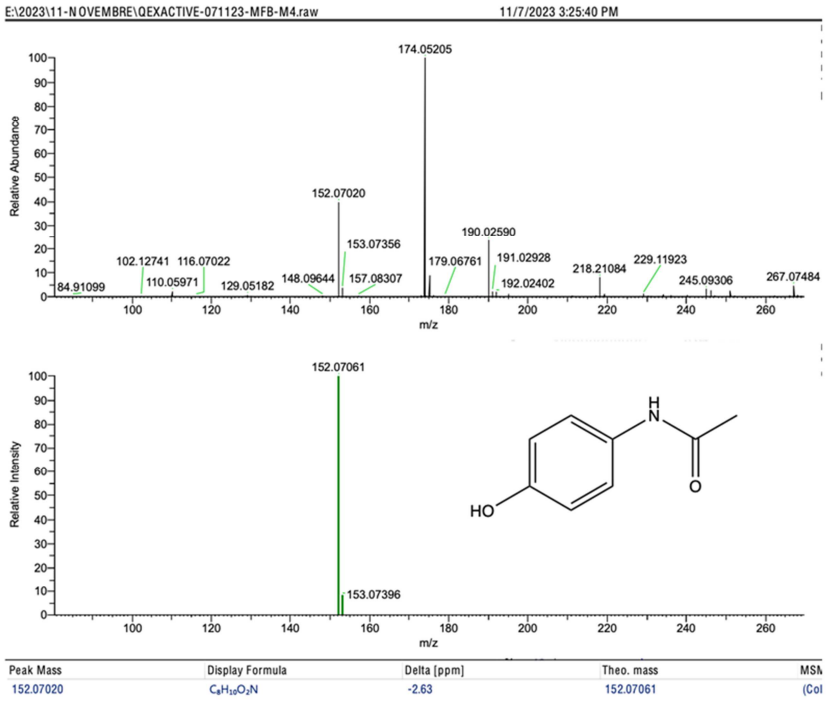

Figure 6. *LCMS spectrum of drug M4.*

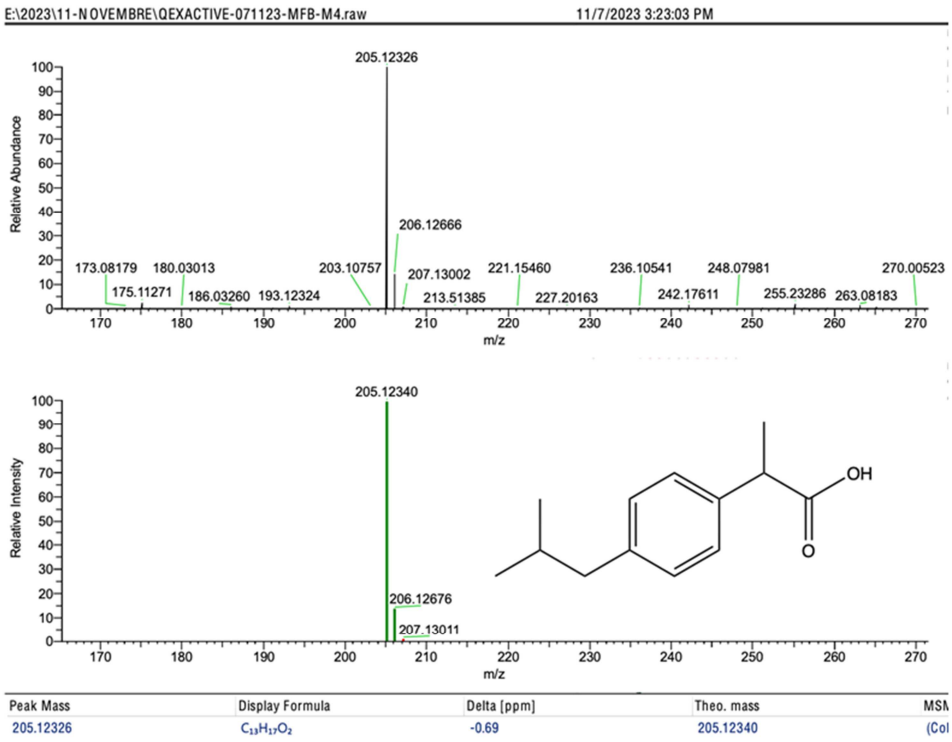

Figure 7. *LCMS spectrum of drug M4.*

In Figure 6, the characteristic peaks corresponding to the presence of paracetamol in this drug namely m/z = 152, m/z = 174 and m/z = 190 are found. In Figure 7, a parent peak at m/z = 205 and a peak at m/z = 206 are observed. The crude formula corresponding to this peak of greater relative abundance is $C_{13}H_{17}O_2$ which corresponds to the carboxylate ion of ibuprofen which lost a proton during electrospray. On the other hand, the caffeine that is indicated on the box of drug 4 does not appear on the mass spectrum, perhaps because of its low content that makes it difficult to identify.

The results of the analysis of drug M5 are shown in Figure 8:

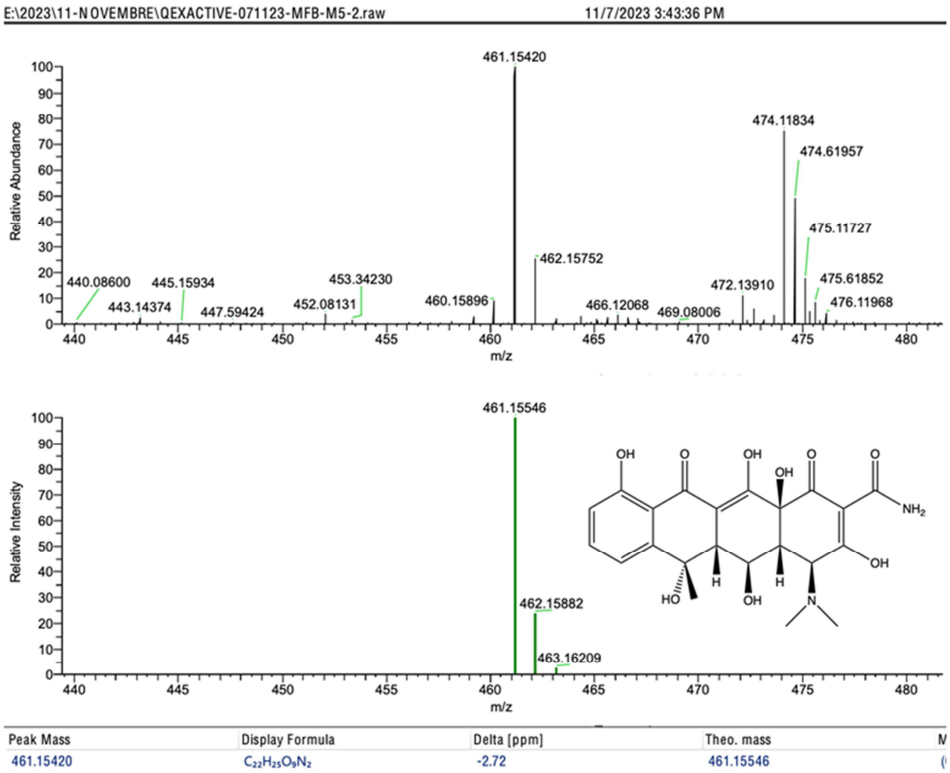

Figure 8. *M5 LCMS Spectrum.*

In Figure 7, it appears that the peaks with the highest relative abundance are those for which m/z = 461 and m/z = 474. These two peaks correspond to M+1 of the oxytetracycline of formula $C_{22}H_{25}O_9N_2$ and molar mass M = 460g/mol, the peak at m/z = 461 is the peak with an additional H^+ in the peak at m/z = 474 corresponding to the compound with Na^+. This reinforces the fact that the drug M5 contains the active ingredient that is described.

This work represents the first study of drugs quality in Togo. Thanks to its sensitivity and specificity, liquid chromatography mass spectrometry is a powerful technology and a method of choice for determining and analyzing the ingredients contained in drug formulations [23, 24]. In general, all drugs that have been analyzed contain the active ingredients that are indicated on the package or found on the internet. On the other hand, the caffeine that is supposed to be present in the M3 and M4 drugs has not been clearly identified, probably because of its too low content to give a visible peak with a significant relative abundance compared to the other molecules contained. The drugs analyzed, observing the packaging and with the identified molecules, therefore seem to be of good quality. So that's a positive thing. However, in developing countries, antimalarial drugs are the most victims of falsification [25]. The samples analyzed contained only analgesics, anti-inflammatories and antibiotics. It therefore seems logical that no clear evidence of falsification has been found. This does not mean, however, that this type of drug is not falsified in developing countries and in particular in Togo where this study was conducted, because the number of samples analyzed is limited. In fact, a similar study carried out in Niger on paracetamol-based medicines showed that the majority of the samples analysed were of poor quality, as they contained a lower concentration of active ingredient than the norm, and one was a counterfeit medicine [26]. Analysis of antibiotics purchased in the UK, Ghana and Nigeria showed that 60% of samples purchased in Ghana and Nigeria did not comply with standards [27]. This study is only the beginning, and should in the future contain a larger number of samples including antimalarial drugs to check if they are falsified or not. A quantitative study would also be interesting to determine the effective amount of active ingredient in each drug. This requires the purchase of each pure active ingredient in order to make a specific dosage via a calibration curve, which has not been possible due to a lack of resources but this is a perspective for the continuation of work on this theme. It is reassuring to know that the samples analyzed are among the most commonly consumed drugs, even in Africa, and despite not having been purchased in pharmacies, are good qualities.

4. Conclusion

This study which is one of the few conducted in Togo has shown that common drugs purchased and analyzed, including analgesics and anti- pain inflammatory, are good qualities since they do contain the active ingredients mentioned on the package leaflet, despite the fact that these drugs were not bought in pharmacies but in the market. Unfortunately, caffeine could not be identified in the drugs believed to contain it, probably because of its low concentration. These observations are encouraging but remain to be deepened, in particular with a quantitative study to specifically dose each active ingredient. It will also be necessary to increase the number of samples to be analyzed and to be interested in other drugs such as antimalarial drugs and drugs prescribed for non-communicable diseases such as high blood pressure or diabetes.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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