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Leone Peter Andrade

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Detection of Impurities in Anti-infective Generic Drugs in Brazil by Liquid Chromatography-Mass Spectrometry

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Impurities found in generic medicinesmay contribute to loss of efficacy and adverse side effects when administered to patients suffering from various diseases. Methods of analysis of the quality of drug products are well advanced. Herein, we used Liquid Chromatography with Diode Array Detector coupled to Mass Spectrometry to detect the presence of organic impurities and determine the quantity of the Active Pharmaceutical Ingredient (API) present in representative Antibiotics (2) and Antifungals (2). Possible impurities were detected in some of the generic drugs in both classes of anti-infective drugs. No impurities were detected in the amoxicillin. The compounds 3'-N,N-Di(demethyl) azithromycin (azithromycin impurity E) and 3'-De(dimethylamino)-3'-keto azithromycin (azithromycin impurity N) were detected in generic azithromycin. For itraconazole, the compounds cis-[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-4-yl-methyl)-1,3-dioxolan-4-yl]methylmethanesulfonate and trans-[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl]methylmethanesulfonate, besides a third substance identified as 2-(2-Butyl) -4-{4-[4-(4-methyloxy-phenyl)-piperazin-1-yl]-phenyl}-2,4-dihydro-[1,2,4]-triazol-3-one were detected as possible impurities. Interestingly, an additional peak was noted in the chromatogram for the generic fluconazole, in addition to the peak of the API; however, none of known impurities of fluconazole were identified. We conclude that tests in addition to bioequivalence measurements may be required to assess post-market generic quality. Such surveillance of generic quality should be performed routinely.
Keywords: Generic Drugs. Mass Spectrometry. Drug Impurities.

Impurities may be present in medicinal products generated from poorly controlled manufacturing conditions,poor-quality substrates and/or API synthesized by a process different from than the certified synthesis pathway [1,2]. The impact of the use of low-quality drugs is related to the adverse effects caused by the presence of undesirable substances, such as impurities and worse, it may contain theincorrect Active Pharmaceutical Ingredient (API) [3].

The adulteration of drugs has been grouped into five categories: (i) copies of the authentic medicine, butwith changes in the quantityof API; (ii) products with incorrect API, which may be of inferiorquality; (iii) preparations with an absence of API; (iv) medicines with very high or very low API content; (v) contamination with known and/or unknown impurities [4,5].

Due to lower costs, the use of generic drugs has been promotedin many countries [6]. Policies to reduce costs and increase the availability of medicines have contributed to the prevention of drug shortages, in addition to improving accessibility to drugs invarious therapeutic classes, especially in developing countries [7-9]. Analytical methods for rapid and accurate drug testingare necessary to combat the increasing number of adulterated drugs as well assophisticated counterfeits [10-15]. The deployment of such analytical technology will have an important role indetecting drug tampering. The safety of medicines depends on the absenceof impurities and accurate API levels. Effective and reproduciblemethods fordetecting drug impurities and API levels may provide insightsinto the quality of generic drugs available in the global marketplace [16,17].

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High-performance liquid chromatography (HPLC) has been a common technique for the determination of impurities in pharmaceutical products [11]. For basic identification and quantification of the components of the sample, UV/Vis spectrophotometry data is gathered using a diode array detector (DAD). Additionally, this analytical technique can be coupled to mass spectrometry (MS) for the structural identification of components of interest such as impurities resulting from improper synthesis [18,19]. Thus, chromatographic techniques have been powerful tools in the analysis and characterization of adulterated medicines because they assess the composition of the analyzed sample [10].

Herein we analyzed the generic antibiotics amoxicillin trihydrate, azithromycin dihydrate, and the generics antifungals fluconazole and itraconazole by high-performance liquid chromatography to detect the presence of impurities in the products.

Generic drugs were provided by the Minister of Health of Brazil (MH). The generic medicines provided were selected from batches stored at the MH that were ready to be distributed to the population. Each generic and branded drug tablet or capsule was prepared for HPLC-DAD-MS analysis by extracting API in methanol, with the exception of amoxicillin trihydrate (in water). The analyses were tested in collaboration with other laboratories at a referral laboratory in Boston, MA - USA (personal communication from Elucida Research). Certified standards of the API for the antibiotics (amoxicillin trihydrate and azithromycin dihydrate), were purchased from Toronto Research Chemicals (Toronto, Canada) for comparative analysis.

API Impurity and Quantification Analysis were performed as follows: Stock solutions were prepared at 1-5 mg mL-1 in an aqueous or organic solvent to match the corresponding sample preparation for each compound. Analytical curves for each compound were constructed at the following concentrations: 0.05, 0.1, 0.2, 0.25 and 0.5 mg mL-1.

Tablets were crushed using an Agate mortar and pestle and solubilized in an aqueous or organic solvent at 1 – 5 mg mL-1, depending on the nature of the API. For products delivered in gelatin capsule form, the two halves of the capsule were separated and the contents were solubilized in aqueous or organic solvent. Solutions were stirred at least 4 hours to ensure uniform distribution of material. The active pharmaceutical ingredient (API) was then separated from insoluble excipient by centrifugation at room temperature for 30 minutes. The supernatant (containing the API) was then removed and stored at 4°C before HPLC-DAD-MS analysis.

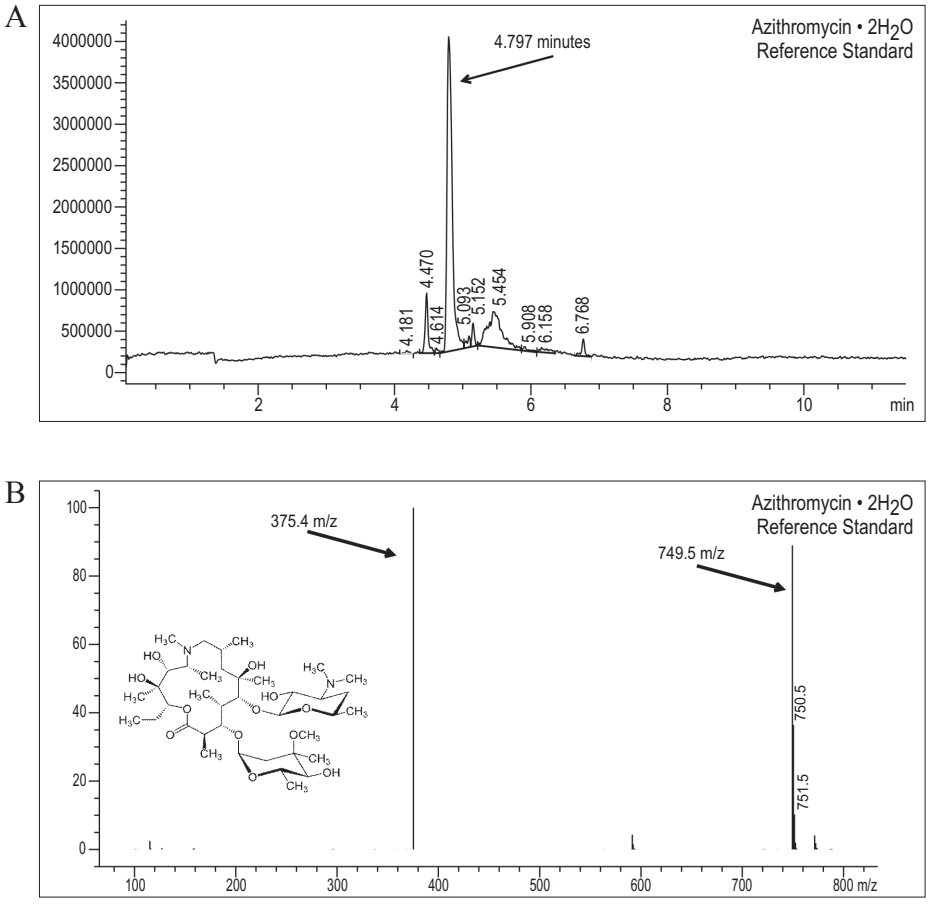
Each sample was diluted down to a nominal concentration of 0.2 or 0.5 mg mL-1, and then the actual concentration was determined based on the analytical curve. Results were reported as a percentage of expected concentration compared to standard solutions as well as compared to branded formulations when applicable. For impurity analysis, samples were run at 0.5 mg mL-1.

Samples and standard solutions were run on an Agilent 1260 Series LC, equipped with a diode array detector (DAD) and an Agilent 6120B MSD for mass spectrometry analysis. All samples were run on a Poroshell 120 EC-C18 4.6 X 100 mm 2.7 μM column. For the evaluation of impurities, the drug samples were analyzed using the mass spectrometric detector, described above, in SCAN mode (m/z 100 to 800). For the quantification of the APIs, the diode array detector (DAD) was operated at the wave lengths according to the optimal absorbance of electromagnetic radiation for each analyte.

Figure 1A shows the chromatograms and mass spectrum for the pure azithromycin dihydrate standard. In Figure 1A, the peak at 4.797 min corresponds to azithromycin, as evidenced by the characteristic mass spectrum and associated molecular weights (Figure 1B). The molecular weight of azithromycin is 749 g mol-1.

Figure 2A shows the LC-MS analysis, where the peak at 4.832 min corresponds to generic azithromycin while the 6.052 min peak corresponds to a potential impurity not seen in the standard formulation. In Figure 2B, we show the mass spectrum analysis of this peak, which

Figure 1. Chromatogram obtained by LC-MS (SCAN mode) for pure azithromycin dihydrate (A) and mass spectrum of pure azithromycin dihydrate (B).



revealed the characteristic of the mass-to-charge ratio associated with azithromycin, which also matches the pattern of the standard azithromycin. The molecular weight of azithromycin is 749 g mol-1, as shown in Figure 2B. Also, in Figure 2A, we noted several smaller peaks, which were also seen in the standard formulation.

The peak seen at 6.052 min (Figure 2A) did not have a corresponding peak in the standard formulation. Analysis of the mass spectrum of this peak (Figure 3) revealed a chemical entity with a molecular weight around to 720 g mol-1. According to Chang et al. (2015) (20), there are two possible impurities in the azithromycin with similar molecular weight. One of the molecules, azithromycin EP impurity E (3'-N,N-Di(demethyl) azithromycin), has a molecular weight of 720.9 g mol-1 (Figure 4A). Another molecule, azithromycin EP impurity N (3'-De(dimethylamino)-3'-keto azithromycin), has a molecule weight of 719.9 g mol-1 (Figure 4B).

The reference and generic formulations of amoxicillin hydrated were analyzed for impurities using LC-MS as previously described. The amoxicillin trihydrate standard (2.992 min) and both the reference (2.985 min) and generic (2.982 min) amoxicillin trihydrate show a similar peak as shown in Figure 5. The mass spectrum of the standard, the reference and the generic confirmed that the only peak observed in both chromatograms correspond to amoxicillin hydrated. Nevertheless, no other peaks were noted. Thus, it confirms that no impurities were encountered in both reference and generic amoxicillin.

Figure 2. Chromatogram obtained by LC-MS (SCAN mode) for generic azithromycin dihydrate (A) and the mass spectrum of 4.832 min peak for generic azithromycin dihydrate (B)

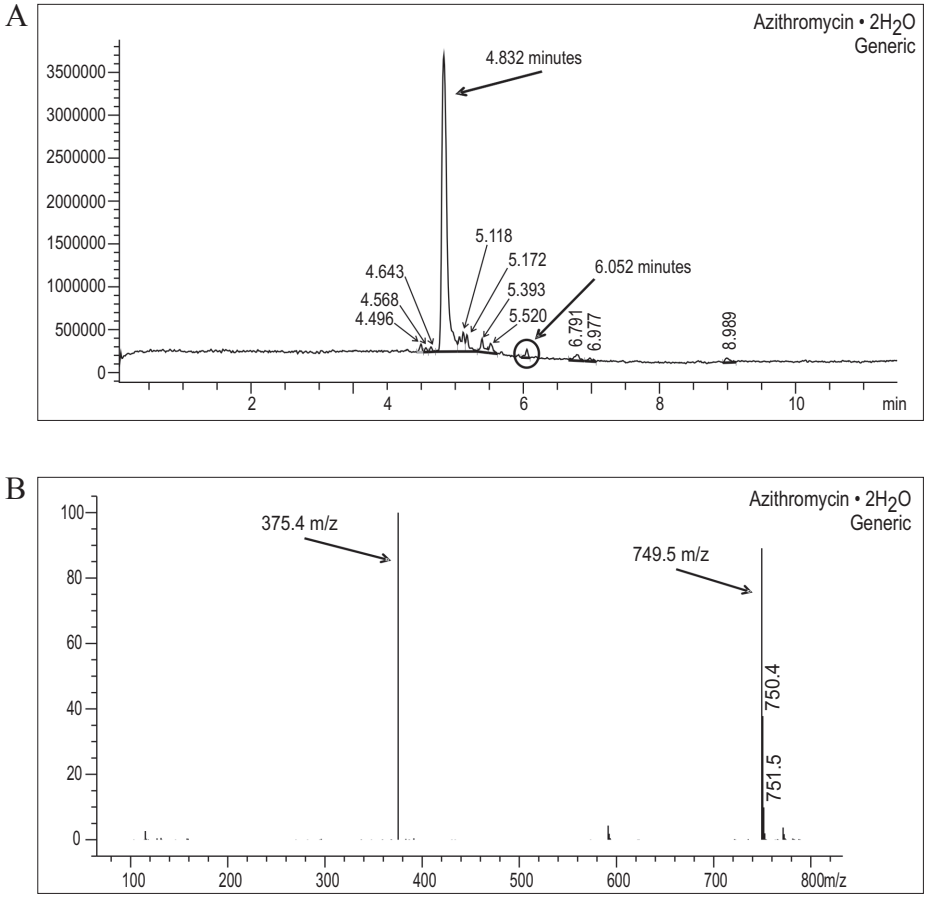


Figure 3. Mass spectrum of 6.052 min peak for generic azithromycin dihydrate. The pattern of this mass spectrum indicates a chemical entity with a molecular weight around 720 g mol-1 that was not seen in the standard formulation.

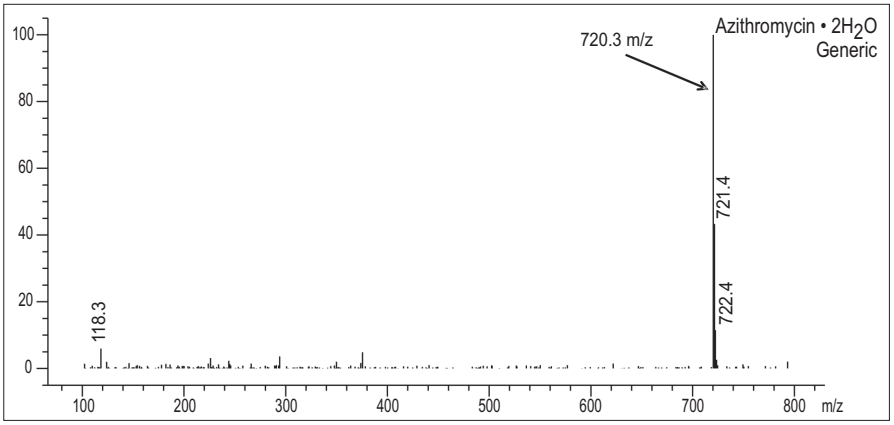


Figure 4. Chemical structure of possible impurities found in the generic formulation of azithromycin dihydrate. (A) 3'-N,N-Di(desmethyl) azithromycin (CAS#: 612069-27-9, azithromycin EP impurity E), which has molecular weight of 420.9 g mol-1 and (B) 3'-Des(dimethylamino)-3'-keto azithromycin (CAS#: 612069-25-7, azithromycin EP impurity N), which has molecular weight of 419.9 g mol-1.

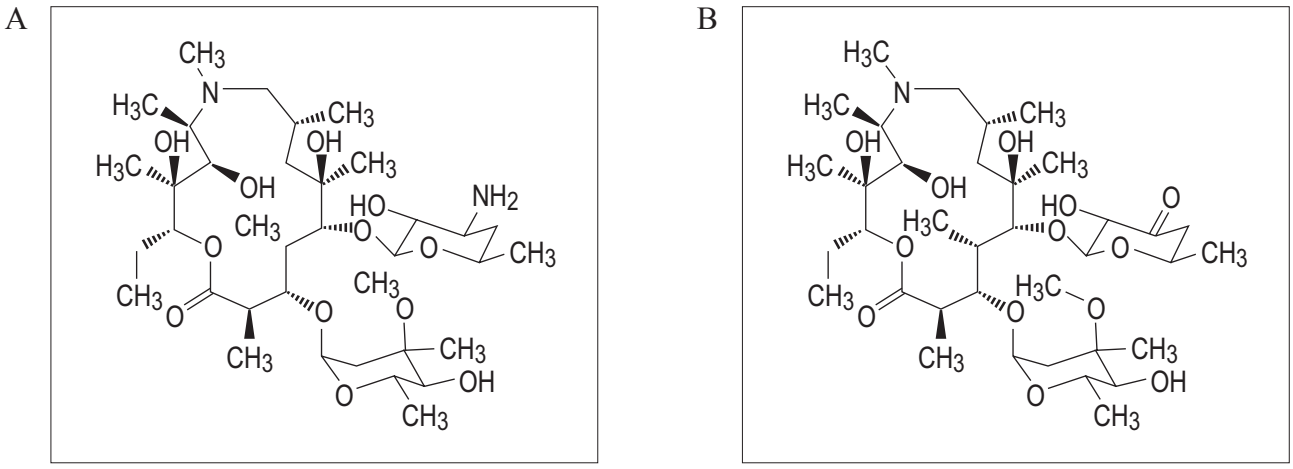
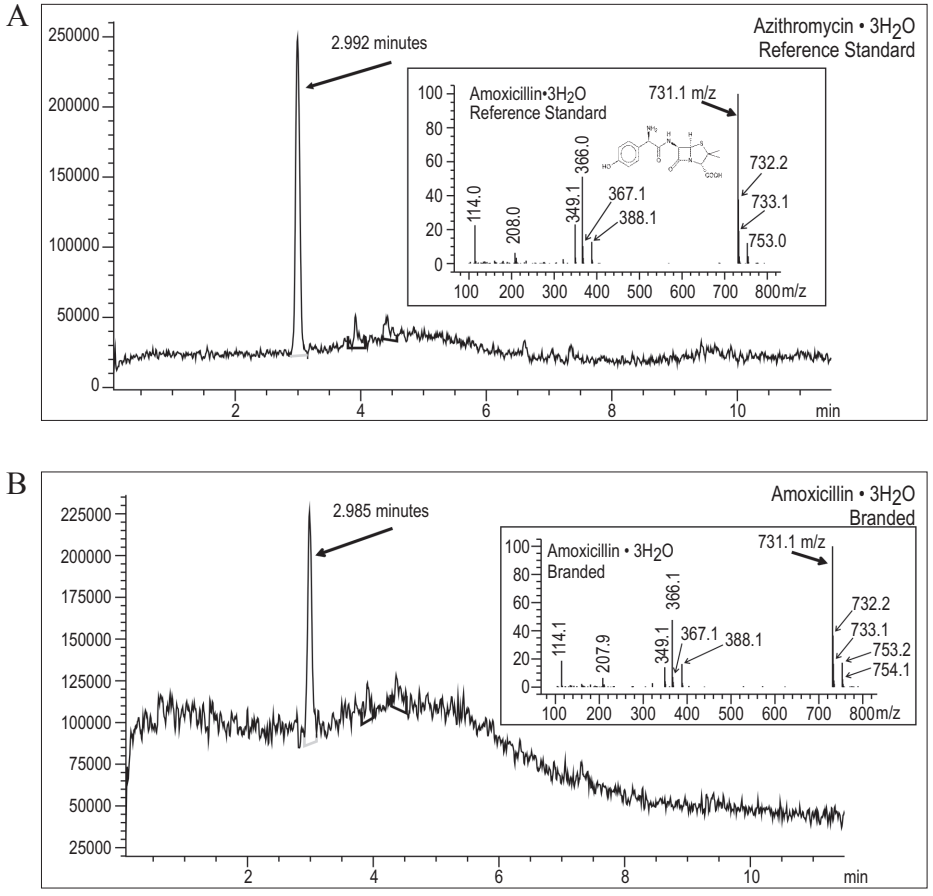


Figure 5. Chromatograms and mass spectrums obtained for (A) standard amoxicillin trihydrate, (B) reference amoxicillin trihydrate and (C) generic amoxicillin trihydrate.



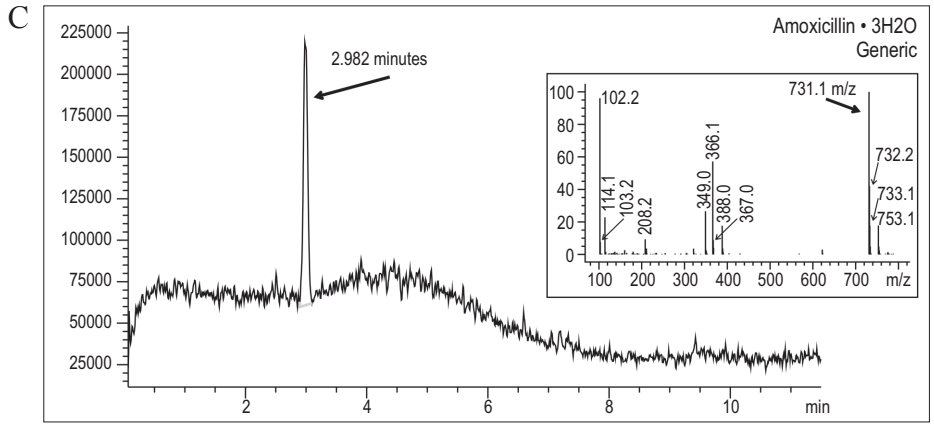
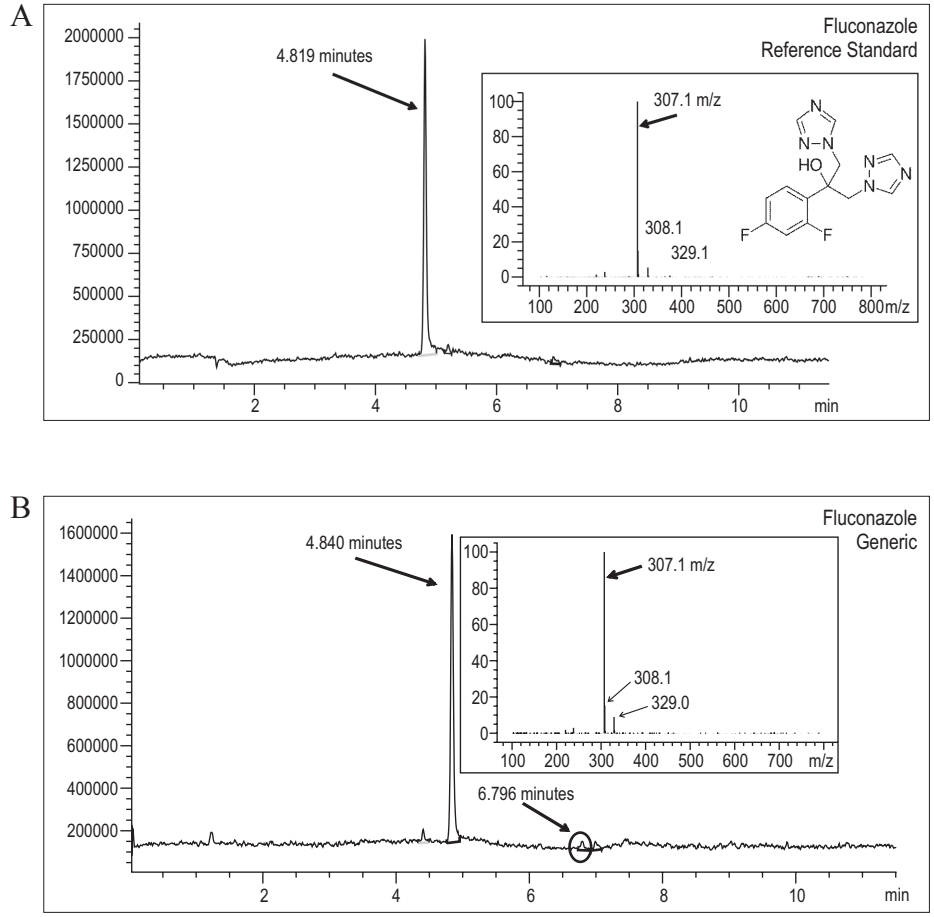


Figure 6 shows the chromatogram (LC-MS) and mass spectra associated with the standard and generic fluconazole.

Figure 6. Chromatogram obtained by LC-MS (SCAN mode) and mass spectra for standard fluconazole (A) and generic fluconazole, which the main peak corresponds to fluconazole (4.840 min) (B).



The peak at 4.840 min corresponds to fluconazole, as evidenced by the characteristic mass-to-charge ratio associated with fluconazole seen in the mass spectrum of this peak (Figure 6), in addition to the comparison with the pure standard of this substance. Additionally, there is a small peak at 6.796 min, which did not have a matching peak in the pure standard. Further analysis of the mass spectrum of this peak (Figure 7) shows a fragment with a molecular weight of 227 g mol-1. A search of known impurities of fluconazole did not reveal any molecules with the same molecular weight, but it should be noted that this does not mean that any of the known impurities are not present.

Figure 7. Mass spectrum of 6.796 min peak for generic fluconazole.

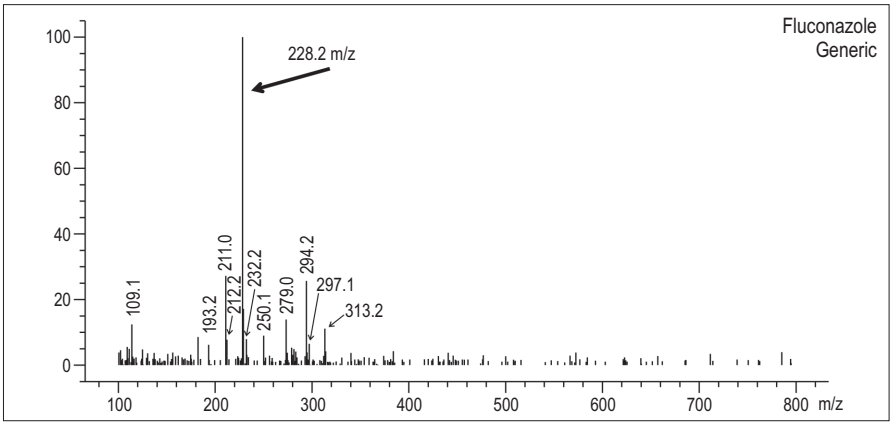
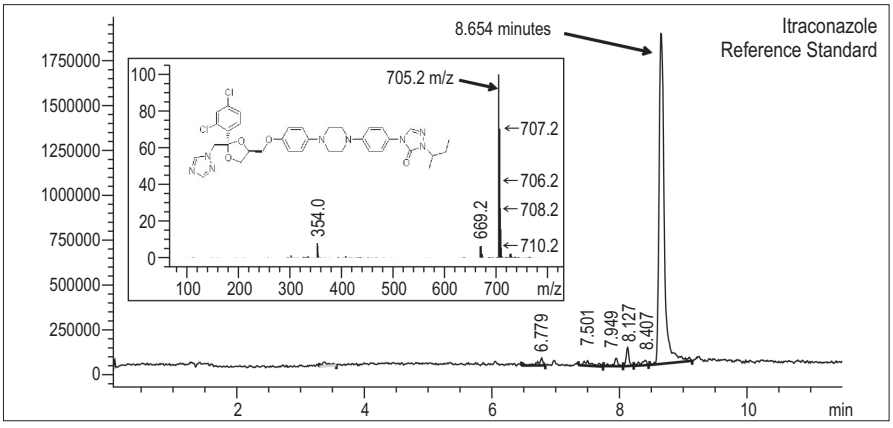


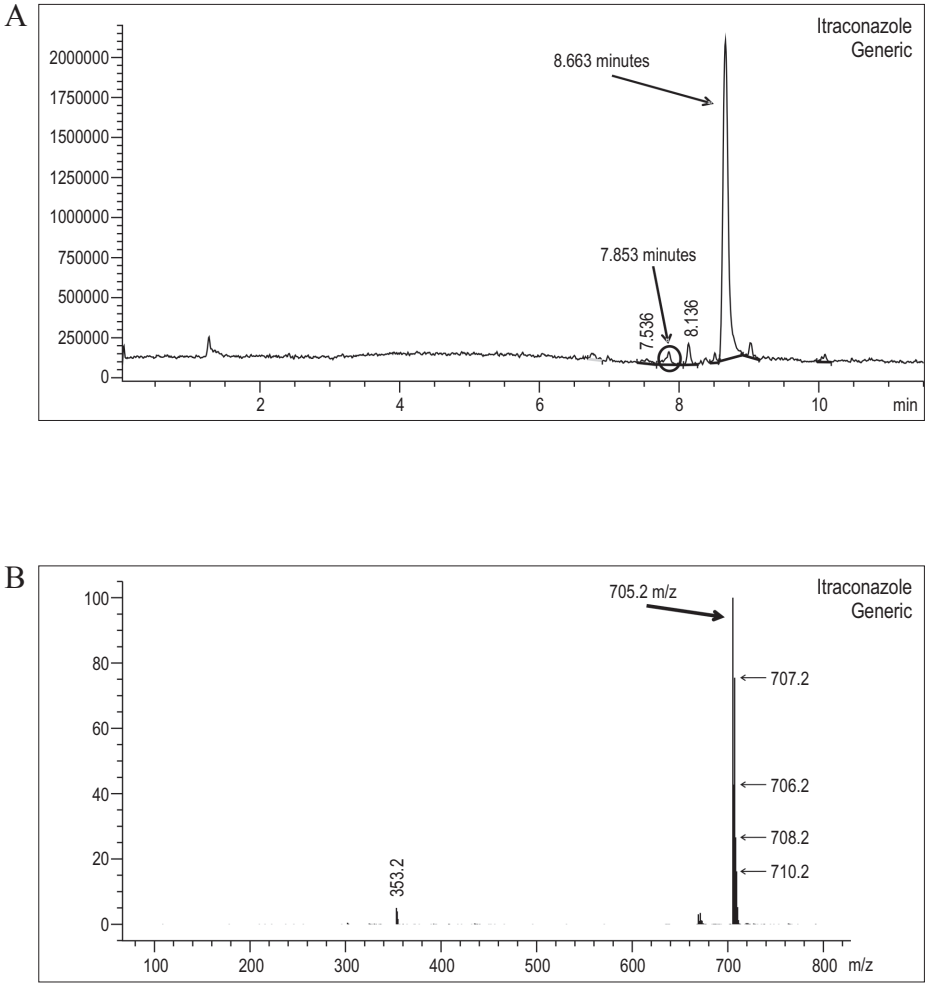
Figure 8 shows the mass spectrum for the pure itraconazole standard, which corresponds to the peak at 8.654 min from LC-MS chromatogram. The molecular weight of itraconazole is 705.65 g mol-1. There are also several peaks that differ only by one mass unit: 706.2, 707.2, 708.2, and 710.2 m/z, which indicate that itraconazole can ionize in several different ways, only differing by the mass of a few hydrogen atoms. It is essential to know when identifying possible impurities – the molecular weight indicated by a mass spectrum is not guaranteed to be the actual molecular weight of that particular chemical entity.

Figure 8. Chromatogram obtained by LC-MS (SCAN mode) and mass spectra for standard itraconazole.



For the generic itraconazole, the peak at 8.663 min corresponds to itraconazole, as evidenced by the characteristic mass-to-charge ratios and pattern associated with itraconazole seen in the mass spectrum of this peak (Figure 9). There are several other small peaks (with retention times shown) that indicate chemical entities that have conjugated moieties. In particular, there is a small peak at 7.853 min on the LC-MS scan, which does not have a matching peak in the standard. Analysis of the mass spectrum of this peak (Figure 9C) reveals the presence of a chemical entity with a primary fragment with a mass-to-charge ratio of 408.3 m/z. As mentioned earlier, it is unclear whether this corresponds to a molecular weight of 407 g mol-1 or 408 g mol-1 based on how itraconazole ionizes with ESI.

Figure 9. Chromatogram obtained by LC-MS (SCAN mode) for generic itraconazole (A), Mass spectrum of 8.663 min peak for generic fluconazole (B) and Mass spectrum of 7.853 min peak for generic fluconazole (C).



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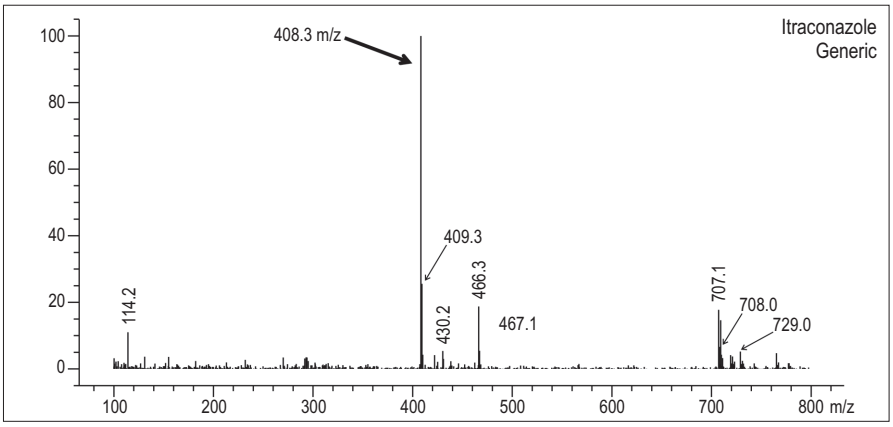
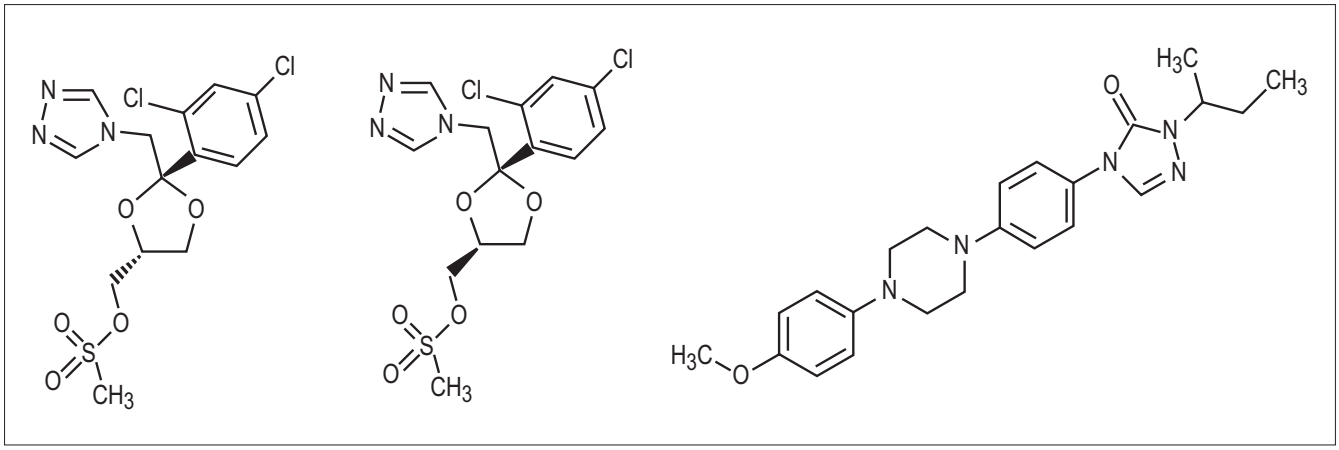


Wharton et al. in 2014 found different molecules with the mentioned molecular weights may be known impurities of itraconazole (21). Two molecules (Figure 10 A and B) are cis-trans isomers: cis-[2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-4-yl-methyl)-1,3-dioxolan-4-yl]methylmethanesulfonate, and trans-[2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl] methylmethanesulfonate, respectively. Both of these molecules have a molecular weight of 408.3 g mol-1. A third molecule (Figure 10C) is a known impurity according to the European Pharmacopoeia (itraconazole EP impurity A). This molecule is 2- (2-Butyl) -4-{4-[4-(4-methyloxy-phenyl)-piperazin-1-yl]-phenyl}-2,4-dihydro-[1,2,4]-triazol-3-one (CAS#: 252964-68-4) and has a molecular weight of 407.5 g mol-1.

Figure 10. Structure of (A) cis-[2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-4-yl-methyl)-1,3-dioxolan-4-yl]methylmethanesulfonate (CAS#: 67914-86-7); (B) trans-[2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl]methyl methanesulfonate (CAS#: 854372-78-4) and (C) 2-(2-Butyl)-4-{4-[4-(4-methyloxy-phenyl)-piperazin-1-yl]-phenyl}-2,4-dihydro-[1,2,4]-triazol-3-one (CAS#: 252964-68-4), known impurities of itraconazole.



These data indicate that the generic itraconazole appears to contain an impurity not found in a standard formulation of itraconazole, which and has a prominent mass fragment of 408.3 m/z.

Given the results discussed above, Table 1 shows a summary of the presence of organic impurities in generic and reference Brazilian medicines, as well as the evaluation of the API content present in each of the generic drugs analyzed.

Conclusion

We conclude that impurities which can interfere with the safety and efficacy of drugs are not routinely analyzed prior to registration for the treatment of human diseases. It is critical to explore the influence of such generic impurities with respect to their efficacy in addition to a bioequivalence test.

Table 1. Summary of the results of identification of impurities and quantification of the API of generic medicines compared to reference medicines.

Therapeutic Class	Compound Description	Sample Impurity*	Possible	% API vs Branded*
Antibiotic	Amoxicillin trihydrate	Reference	ND	n/a
		Generic 1	ND	97.7
	Azithromycin dihydrate	Generic 1	Azithromycin EP impurity E, N	n/a
Antifungal	Fluconazole	Generic 1	Unknown (mass ~ 227 g mol-1)	n/a
Itraconazole	Generic 1		cis-trans isomers of known impurity	n/a

*ND = not detected; n/a = not applied.

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Lethal Ovitrap CIMATEC: A New Trap for Arbovirus Transmitting Mosquitoes

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A lot of countries have difficulty in combating *Ae. aegypti*, due to the high adaptability of this mosquito to the urban area. The National Program for Dengue Control (PNCD) is responsible for allocating the most activities to combat *Ae. aegypti* in districts of Brazil (basic sanitation, education, chemical control). Despite the technological advances in the 21th century, there was little social commitment with the conception of a formal device in the fight against *Ae. aegypti*. Senai-Cimatec developed a trap (Lethal Ovitrap Cimatec™) based on new technologies to control *Aedes aegypti*. So, the aim of this study was to compare the traditional traps on the market with the LOC™ trap, created by Cimatec. We performed two experiments to evaluate the performance of the traps against the mosquitoes. The tests occurred in a monitored and closed environment at the Central Laboratory of Bahia (Entomology Laboratory) (LACEN/BA). The larvae, water, and substrate for the experiment in the three containers had the same origin; three cages were kept in the entomology sector with the same conditions of temperature and humidity. The results showed that LOC™ has several advantages when compared to the traditional traps, such as: capacity to eliminate the immature forms of the vector in a few hours; entrapment and extermination of larvae and/or adults forms by asphyxiation; high lethality for mosquitoes and ability to reduce the *Aedes* population in an area by removing from the environment future generations of mosquitoes; installation; it is attractive to mosquitoes; no needs of weekly monitoring; no power consumption; simple manipulation and low-cost; water as a protection factor and not as a risk factor; it does not use insecticide or any toxic agent harmful to human health. So, the use of LOC™ has advantages over traditional traps, directing the biotechnology market to new solutions with low-cost, easy to handle and non-toxic to man and the environment in the fight agains *Ae. aegypti*.
Keywords: Traditional Ovitrap. LOC™. Arboviruses. *Ae. aegypti.*

The impacts of the current epidemiological scenario of arboviruses in Brazil, characterized by Dengue, the dissemination of ZIKV and CHIKV virus, and the re-emergence of FMV in the Extra-Amazon region were enough to establish a public health emergency by the Ministry of Health and the World Health Organization [1].

Brazil recorded 113,381 suspected cases of Dengue Fever, 43,010 of Chikungunya, 7,911 of ZIKV Fever and 3,140 cases of Yellow Fever according to the Ministry of Health (2017) [2]. In that period, 17 deaths from Dengue, 9 deaths from CHIKV, and 240 deaths from Yellow Wild Fever were confirmed.

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The entomological situation reveals that *Ae. aegypti* is found dispersed in most Brazilian cities [3]. The Ministry of Health has tried to implement control actions for this vector; however, the results have not been satisfactory, since infestation by remains high [4]. Only four Brazilian states (Acre, Amapá, Piauí and Sergipe) have not yet registered the presence of *Ae. Albopictus* [5]. However, most Brazilian states have sufficient *Ae. aegypti* to initiate and maintain the transmission of DENV and other arboviruses.

A lot of countries have difficulty in combating *Ae. aegypti*, due to the high adaptability of this mosquito to the urban area [6]. In the last two decades, the intensification of the vector combat has been carried out mainly through chemical control, which has led to a resistance of *Ae. aegypti* to many groups of insecticides [7]. Therefore, additional effort from the health sector is required, with an estimated cost of R$ 1 million per day [8].

The National Program for Dengue Control (PNCD) is responsible for allocating the most activities to combat *Ae. aegypti* in the districts of Brazil, such as investment in basic sanitation and education [3], chemical control, and other essential performances [6].

The areas of water accumulation (focal treatment) applied chemical control with larvicidal action. The control of adult insects uses two chemical control modalities: the spraying of insecticides with residual effect in the walls of breeding places, which are susceptible to the proliferation of immature forms (larvae and pupae), denominated perifocal treatment; and the spatial treatment of insecticide by ultra-low volume (UVB), indicated for outbreak and epidemic situations [1].

New strategies to combat vectors have been adopted to reduce the population of mosquitoes to minimize the impact of chemical larvicides on human health and in the environment, such as biological control based on the use of natural enemies or biological toxins. Different groups of organisms (bacteria, protozoa, fungi, and viruses) with potential use against *Ae. aegypti* have been evaluated. Among them, *Bacillus thuringiens isisraelensis* (B.t.i) and *Bacillus sphaericus* (B.s.) have been used to control mosquitoes. B.t.i has been used in the control of *Ae. aegypti* because it is effortless to prepare the formulation and because of the properties that allow its growth on a wide variety of substrates [10].

Environmental management should be considered an equally relevant strategy for mosquitos' controls. Among the ecological management actions, there are the elimination and removal of breeding sites in the home environment, the storage, collection and final disposal of solid waste, control of the border vegetation of the breeding sites, drainage and earth moving services, as well as investment in the primary sanitation system, and the participation and education of the community [11].

More recently, full integration of control alternatives has been devised, combining physical and biological management control: the use of specific adulticides and the application of alternative (natural) larvicides with low impact on human health and in the environment. However, this kind of strategies cannot be dissociated from education of the community to achieve success in combating vector insects [12].

Despite the technological advances in the 21th century, with the use of new machines, materials, and production processes, there was little social commitment with the conception of a formal device in the fight against *Ae. aegypti*. Little effort was made to create tools, such as traditional traps, to combat the mosquitoes.

Senai-Cimatec developed a trap (Lethal Ovitrap Cimatec™) based on new technologies to control *Aedes aegypti*.

The aim of this study was to compare the traditional traps on the market with the LOC™ trap, created by Cimatec, for the ability to visualize the presence of the eggs and eliminate larval forms of *Ae. aegypti*.

Traditional Traps

The traditional oviposition trap consists of a black-plastic container with a large hole (Figure 1). The volume capacity is 300 mL, but only 200 mL of water is added, equivalent to the size of the LOC™ trap. The Eucatex palette - 6.0cm by 2.5cm plywood - is placed inside the trap, similar to that used in LOC™.

Lethal Ovitrap Cimatec™ (LOC™)

The LOC™ trap is made up of a transparent cylindrical plastic container, containing two pieces: a cap with a recess and a funnel in the center. The cover has three openings, through which the female mosquitoes enter to perform oviposition. Besides being the access door to the vector, the cover protects the trap from objects or other materials that may fall in it and clog it. The funnel is tilted, and the outer wall is roughened to increase egg adhesion because *Aedes aegypti*

Figure 1. Traditional Ovitrap.

Figure 2. LOC trap.

females prefer to lay eggs on rough walls (Figure 2).

The LOC™ does not have sensors, light, nor a mechanical barrier nor a physical barrier. There is no card with glue, insecticide, nor a fan and attractive chemicals. It is a simple trap, easy to handle, low-cost and uses no energy in its operation.

The trap is excellent, with a quality certification guaranteed by the responsible institutions, which ensure its long-term durability in combating vectors.

LOC™ Operation

The *Ae. aegypti* female deposits the eggs on the wall of the funnel. In contact with the water, the eggs will hatch, and the larvae will emerge. The newborn mosquito larvae descend to the bottom of the container, looking for food passing through the rod. However, the larvae need to return to the surface to breathe, but they cannot reach the top of the rod, which has a 45º degree inclination and is usually submerged, and get trapped and die by asphyxiation.

When the level of the container is not full, the larvae tend to survive to adult form, but the mosquitoes also cannot get out because they even cannot reach the top of the rod, and die inside the container. The cover must be unscrewed to release the water with the dead larvae or insects to remove the dead larvae or adults.

LOC™ uses the color black and the presence of water to attract the *Culicidae*. The females of the *Ae. aegypti* are invited to the trap and lay the eggs in the funnel. The rounded form and the dark color are considered visual stimuli since they simulate a quiet place to rest for the adult mosquitoes to lay their eggs.

In this trap, any egg that hatches is unable to release the adult mosquito into the external environment due to its internal design. Thus, this trap can reduce the population of *Ae. aegypti* in urban areas by collecting eggs, trapping larvae and pupae, hampering the emergence of adult mosquitoes.

Installation of LOC™

The trap must be installed at ground level or the height of 1.20 to 1.50 cm from the floor and can be hung by the handle or under a flat surface, with natural water. The trap should be placed preferably indoors or in the perimeter, in shaded places, protected from direct rain, and with little movement of people or animals.

LOC™ requires that the owner maintain the trap full of water. If the trap is full, it will kill the larvae within a few hours later. On the other hand, if it is partially complete, the larvae will survive, but the adults will die from drowning after exhaustion.

The Experiments

We performed two experiments to evaluate the performance of the traps against the mosquitoes. The tests occurred in a monitored and closed environment at the Central Laboratory of Bahia (Entomology Laboratory) (LACEN/BA). The larvae, water, and substrate for the experiment in the three containers had the same origin; keeping three cages in the entomology sector with the same conditions of temperature and humidity.

We collected a total of 90 larvae of *Culicidae* from a single artificial local at LACEN/BA. These larvae were introduced into three (03) traps in separate cages:

Trap 1: Black Hole Trap was filled with water, i.e., no oxygen was available inside for larval breathing. Thirty larvae at different stages (2nd, 3rd, and 4th stages) were introduced. It was observed that the larvae descended immediately through the funnel to the bottom of the container. The trap was placed inside cage 1 to ensure the containment of future adults in case of larvae survival. Larval return to the surface by the funnel was not observed.

Trap 2: Black Hole trap was partially filled with water. Thirty larvae at different stages (2nd, 3rd, and 4th stages) were introduced. The larvae were introduced into the funnel with the help of a pipette, where it was observed that they immediately entered the container. The trap was put into cage 2. The return of the larvae to the surface via the funnel was also not observed.

Trap 3: An open cylindrical pot was partially filled with water, without a funnel. Thirty larvae in 2nd, 3rd, and 4th stages were introduced. The container was put into cage 3.

Three cages were used in the laboratory environment. Ten females and two males of *Ae. aegypti* were put into each cage. The females were inserted engorged on the exposure date (01/07/2016). Two oviposition traps were placed within each cage: the traditional ovitrap and the LOC™ trap. In both traps, a Eucatex palette was inserted.

Reading for the presence and number of eggs in the palette was performed daily from 07/04 to 07/08 (five days) for one week from the start date of the experiment (01/07/2016). The time recommended to monitoring the ovitraps in the field. A magnifying glass counted the eggs of the mosquitoes.

In cage one (1), the LOC™ had more eggs (48 eggs) in the palette than the traditional ovitrap palette (45 eggs) (Table 1). In cage two (2), the traditional ovitrap had more eggs (122) than the LOC™ trap (57 eggs) (Table 2). In cage three (3), the LOC™ trap had more eggs in the palette (147) than the traditional lovitrap (25 eggs) (Table 3). In terms of the total number of eggs collected, the LOC™ trap had more eggs (252) compared to the traditional trap (192) (Table 4).

Water was added to the traps to the limit of the container on the last day of reading (07/08/16) for the presence and quantification of eggs (07/08) to cause the contact of the water with the eggs in the palettes to hatch and release the larvae.

Larvae counting began on 07/04/16. In the three cages of the experiment it was observed that the LOC™ trap did not present any live *Ae. aegypti* larvae: we counted twenty-two dead *Ae. aegypti* larvae in cage 1; fifteen dead *Ae. aegypti* in 1st stage larvae in cage 2; and fifty-two dead *Ae. aegypti* in 1st stage larvae in cage 3 (Table 5).

Cage three (3) presented adult forms of *Culicidae*, no winged forms of the vector were observed in cages 1 and 2. No *Culicidae* survived in any of the three cages, indicating that the LOC™ worked both full and partially filled with water.

This experiment was repeated in the laboratory to confirm results and then applied in the field, to validate the project and establish final adjustments, completing the design stage.

Table 1. Number of *Aedes aegypti* eggs by traps in cage 1 (traditional ovitrap x LOC™).

Read Date (Eggs)	Traditional Ovitrap	LOC™
07/04/16	0	0
07/05/16	0	0
07/06/16	19	16
07/07/16	0	0
07/08/16	26	32
Total	**45**	**48**

Table 2. Number of *Aedes aegypti* eggs by traps in cage 2 (traditional ovitrap x LOC™).

Read Date (Eggs)	Traditional Ovitrap	LOC™
07/04/16	0	0
07/05/16	0	0
07/06/16	17	15
07/07/16	0	0
07/08/16	105	42
Total	**122**	**57**

Table 3. Number of *Aedes aegypti* eggs by traps in cage 3 (traditional ovitrampa x LOC™).

Read Date (Eggs)	Traditional Ovitrap	LOC™
07/04/16	0	0
07/05/16	25	147
07/06/16	0	0
07/07/16	0	0
07/08/16	0	0
Total	**25**	**147**

Table 4. Total of *Aedes aegypti* eggs by traps (traditional ovitap x LOC™

Cages	Traditional Ovitrap	LOC™
1	45	48
2	122	57
3	25	147
Total	**192**	**252**

Table 5. Total of *Aedes aegypti* larvae in LOC™ traps.

Cages	LOC™	
	Live larvae	Dead larvae
1	0	22
2	0	15
3	0	52
Total	**0**	**89**

Comparison Between LOC™ and Traditional Ovitraps

The LOC™ project was carried out in stages dependent on each other, involving literature review, laboratory and field tests, to consolidate a marketable, efficient and compatible product for the needs of the community in the combat of *Ae. aegypti*.

The initial phase of the design process included the definition of the problem, a result of the consumer's need. This stage is crucial and decisive for the development of the method. It addressed a current issue in daily life, the incidence of dengue, which persists despite government efforts and campaigns. First of all, a trap to capture dengue vectors was chosen to be redesigned.

The second phase consisted of the analysis of traps existing in the market, i.e., the collection of data on already existing products. The study of all the data collected on commercial traps provided suggestions of what should not be done and what needed to be improved.

Formal studies helped identify alternatives to solve the problem in the phase of alternative models and sketches. Thus, the pre-selected options were analyzed to establish the best set of improvements for the final prototype. We did a study of economic feasibility and sale price after selecting the best alternative that fully met consumer needs and project specifications, and in parallel, the drawings for the construction of the prototype.

In the fight against *Ae. aegypti* and *Aedes albopictus*, no trap is recommended by the National Program for Dengue Control. There is also no commercially available trap sufficiently capable of decreasing the vector population and thus reducing dengue transmission. The trap created by our group from Cimatec (Lethal Ovitramp Cimatec™) is better than traditional traps on the market.

The LOC™ is a trap that can also be used in monitoring and early detection of Dengue and Yellow Fever vectors, such as larval traps (larvae trap) and ovitraps (egg traps). LOC™ is a lethal trap with a function to reduce the *Aedes* population in the environment and future generations of mosquitoes.

If compared to the traditional ovitrap, as our study showed, it contains functional and aesthetic improvements. One of the main differentials of this trap is the angle in the nozzle of the funnel, which is the main item responsible for the dispensing with the sieve. As a result, the constant handling of the trap is not required, reducing the risk of mishandling and malfunctioning.

Existing traps in the market were essential in aiding the design of LOC™. For example, we mention the funnel, which is located above the trap in the LOC™, so the volume of water in the trap causes the death of the captured larvae in only a few hours. LOC™ can, therefore, reduce *Aedes* vectors, which can have a huge impact on public health.

LOC™ has several advantages when compared to the traditional traps, such as: capacity to eliminate the immature forms of the vector in a few hours; entrapment and extermination of larvae and/or adults forms by asphyxiation; high lethality for mosquitoes and ability to reduce the *Aedes* population in an area by removing from the environment future generations of mosquitoes; installation, since it can be installed in different contexts and at different heights; it is attractive to mosquitoes – dark color of cover and funnel, and presence of water; no needs to be monitored weekly; no power consumption; simple manipulation and low-cost; water as a protection factor and not as a risk factor; it does not use insecticide or any toxic agent harmful to human health.

So, the use of LOC™ has advantages over traditional traps, directing the biotechnology market to new solutions with low-cost, easy to handle and non-toxic to man and the environment in the fight agains *Ae. aegypti*.

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Identification of Sex Using Linear Skull Measures: The Importance of Imaging in Biotechnology

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There are several methods used in the identification process of human remains. The most of them are based on comparing of *antemortem* and *postmortem* data available. Although the technique of fingerprinting is considered more accurate in many cases, it cannot be used when the bodies are mutilated, decomposed, burned, or fragmented. This article aims to compare the metric values obtained by Galvão (1994) and Saliba (2001) to differentiate male and female through dry skulls, using the measurement of the Radiocef Studio 2 Program. It was used 16 teleradiographs (11 females and 5 males). The linear measurements used in this article were: 1. The bodies stature of the mandible; and 2. Distance Nasium-Front Nasal Spine. Several radiological techniques are used to aid the human identification process for determining sex, ethnic group, and age. The analyses of X-rays and Computer Tomography (CT) scans, *antemortem* and *postmortem*, have been an important tool for human identification in forensic dentistry, especially with the refinement of techniques acquired with the advancement of radiology and CT scans. We concluded that the knowledge of the best method by forensic dentists with a careful application of the technique and report's interpretation is essential to fulfilling the necessary characteristics for a successful identification of sex using skull measures.

Keywords: Radiology. Forensic Dentistry. Sex. Cranial Measures.

The human identity is the set of own and exclusive characters of a person: physical, functional, psychic, born or acquired, such as name, age, state, profession, sex, physical defects, fingerprints. Thus, the identity could be defined as the collective aspect of a set of characteristics by which something is recognizable or known [1].

Identification is the process by which one determines the identity of a person or a thing [2]. Personal identification is essential in Forensic Medicine.

Establishing *postmortem* human identity is one of the significant areas of study and research in dentistry and legal medicine. These forensic sciences study the human body in various stages of *postmortem*: sprung, torn, charred, macerated, decomposed, and skeletonization.

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Some methods used for identification include fingerprints, anthropological and radiological examinations, genetic analysis (DNA), and dental analysis (especially in carbonization cases).

The process of human identification by radiographic comparison is a technique little used in Legal Medicine, despite presents a satisfactory and unquestionable results.

The participation of dentistry in the processes of *postmortem* human identification is present since the initial procedures (general identification: estimates of sex and age, ethnic group determinations, stature, diagnosis of spots or liquids in the oral cavity, definition of cause and time of death) until the irrefutable possibility of the individual identification. The skull and teeth assume relevant identification in situations of substantial destruction of the human body (spoils, fragments, bony or carbonized mortal remains) because of the higher resistance when compared to the other structures. The skull and teeth are often the only elements from which identity can be derived: race, age, stature, and sex.

The human skeleton of children and pre-teenager has qualitative characteristics little pronounced, providing few or no subsidy to make possible the identification of sex. In these cases, it is required other methods to determine sex, such as DNA, sexual chromatin, or teeth. [3].

The human skeleton develops after puberty under the influence of hormones, environment, and musculature, presenting differential characteristics, such as prominences, roughness, crests, apophyses, protrusions, and structures that characterize the sexual dimorphisms.

These characteristics are more prominent and evident in men than in women, in which these characteristics are more delicate and less prominent.

Ramirez (1990) [4] defines the male skull as larger than the female, which is more rounded, childlike. The frontal bone is more upright, and the parietal is smaller; the supraorbital ridges are sharp; the superciliary arches are less protruding; the front nasal joint is more curved; the styloid apophyses are short and thin; there are occipital protuberance and unmarked necklines; the mastoid processes are smaller; the occipital condyles are broad, short, and the jawless are robust. In man, the cranial thickness is greater; the forehead tilted back; the parietal bones are larger; the glabella is prominent; the superciliary arcs are protruding; the ridges above the blunt orbital; the frontal-nasal joint is angular; the styloid apophyses are long and thick; the mastoid processes are protruding and separated (sole shoe shape); the prominent occipital protuberance and regions of muscle insertions, and the more robust jaw. The palate has a broad and shallow form in males, different from females in which it is narrower and more profound, having thinner dental arches.

The bones that form the basin and the skull have reliable characters for determining sex. However, the degree of certainty of the diagnosis varies according to the number and nature of the parts examined. For example, the ethnic group should be known to identify the sex, due to the characteristics of each group.

Nowadays, the sexual difference has been studied under metrical parameters, transforming physical and qualitative characteristics into numerical values that, submitted to statistical analysis, derive metrical relations or discriminant functions. This analysis is endowed with single or multiple variables from the same measurement bone or from various structures, depending on the population that the data were collected [5-7].

In specific populations, the use of computers and mathematical models provide more reliable and accurate results in sexual determination [8,9].

Abe (2000) [6] *apud* Inoue *et al*. (1992) agree that these models enable a less experienced examiner to discriminate sex as precisely as an experienced observer. However, the statistical analysis does not replace the experts, since their competence is essential to avoid errors.

Historically, the application of radiology in the forensic sciences was introduced in 1896, just one year after X-rays were discovered by Roentgen to demonstrate the presence of lead bullets in a victim's head. Schüller proposed the possibility of using radiological images of the facial sinuses for identification purposes in 1921.

Galvão and Vitória [11] studied skulls of the Brazilian population, belonging to individuals of known sex and age group over 20 years in 1994. They concluded that the dimensions of the foramen magnum are larger in men than in women.

Saliba [12], in 2001, carried out a study of sex determination through the area formed by the triangle of the upper face, using 168 dry skulls of adult humans, over 20 years. After analysis, the Nasium-Anterior Nasal Spine (N-Ena) measure was 57.15 to 60.06 for female and 61.25 to 63.68 for male.

The present work chooses for a more precise and rational mathematical method for sex determination and aims to compare the measures obtained by the study models of Galvão (1994) and Saliba (2001), with the criteria acquired, using the measurement performed in lateral teleradiography by the following linear measurements of the cranium: 1. Height of the mandible body and 2.

Nasium-Anterior Nasal Spine by the program Radiocef Studio 02. Therefore, we proposed:

1. To verify the existence of sexual dimorphism using two linear measurements, performed by the program Radiocef Studio 2, in lateral teleradiography, belonging to individuals of known sex;
2. To check the degree of correctness of the values found in dry skulls, in the studies surveyed, with the radiographic values obtained.

The sample consisted of lateral cephalometric radiographs of 16 individuals (11 female and 5 male), without previous orthodontic treatment and without presenting moderate to advanced bone loss. The IOBA Clinic provided the samples' data. All the individuals showed balanced facial profiles and absence of retrognathia or prognathia of the bone bases and typical vertical pattern. All radiographs were performed on the same Orthophos Panoramic X-ray apparatus from Siemens, with the same distance and intensity. The procedure was carried out by a single examiner to avoid discrepancies between the measures obtained. The values analysed were obtained by marking the points Nasium (N), Mental (Me), Infradental (Id) and Anterior Nasal Spine (Ena) by the program Radiocef Studio 02 and the measurement of the following linear measures: height of the mandible body (Id-Me) and distance Nasium-Anterior Nasal Spine (N-Ena) (Figure 1).

These values were compared with those found in the researched literature. For the height of the mandible body, the values used as parameters were those found by Galvão in 1994 [11] (Table1). The study of Saliba (2001) [12] was used for the previous Nasium -Anterior Nasal Spine measure, considering this study presented statistically significant differences for the confirmation of sexual dimorphism, in agreement with earlier studies - Peixoto (1931) [13] and Ávila (1958) [14] (Table 2).

We compared the authors, and it showed that the mandible body height (Id - Me) for the measurement in millimeters, when compared both sex, had a compatibility of 27.27%, with the values found by Galvão [11] in 1994 for females; and 100% for males (Table 3). There was an agreement with 81.81% to female and 80% for the male when compared the distance Nasium-Anterior Nasal Spine (N-Ena) in millimeter, with the study of Saliba (2001) [12] (Table 3). According to the proposed comparison and applied methodology, we conclude that:

1. Linear Nose-Spine (N-Ena) measurements, considered as acquired in human skull belonging to known sex, showed compatibility with Saliba (2001) [12] for sexual differentiation;
2. For the measurement Height of the mandible body (Id-Me), in the same study, a concordance of 27.27% was found with the values found by Galvão (1994) [11] for the female sex, and 100% of compatibility for males;
3. Among the two measures analysed, the Nasium-Anterior Nasal Spine (N-Ena) measurement demonstrated more excellent reliability in the obtained results.

Due to the variety of methods available, the legal dentistry professional can choose the method that best fills the characteristics necessary for the success in the sex identification, taking care in the correct application of the technique and the accurate interpretation of the results.

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We would like to thank CIMATEC, Federal University of Bahia (UFBA) (School of Odontology, Department of Radiology) and clinic IOBA for all support in the development of this study.

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Figure 1. Cephalometric points and linear measurements of the skull.

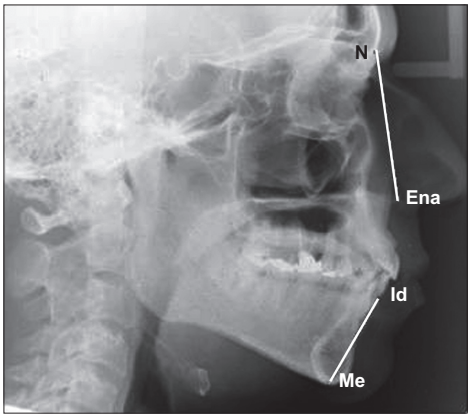

Table 1. Parameter height of the mandible body (Id-Me) were found by Galvão (1994) [11].

	Male			Female	
Parameter	**Confidence**	**Probable**	**Undetermined**	**Confidence**	**Probable**
Height of the mandible body (Id-Me) (mm)	>41.00	33.50-41.00	31.00-55.50	<27.00	27.00-31.00

Table 2. Nasium-anterior nasal spine measure by Saliba (2001) [12].

	Minimum	**Normal**	**Maximum**
Female	57.15mm	58.61mm	60.06mm
Male	61.25mm	62.56mm	63.68mm

Table 3. Data result.

Sample	**Sex (Id-Me)**	**Body height of the jaw (N-Ena)**	**Distance nasium-anterior nasal spine**	**City**
1	F	32.81mm	53.22mm	Salvador
2	F	29.12mm	50.55mm	Salvador
3	F	28.69mm	57.40mm	Salvador
4	F	38.70mm	57.19mm	Salvador
5	F	30.89mm	44.97mm	Salvador
6	F	39.94mm	57.93mm	Salvador
7	F	39.36mm	60.41mm	Salvador
8	F	35.01mm	57.45mm	Salvador
9	F	39.44mm	59.95mm	Salvador
10	F	36.04mm	59.40mm	Salvador
11	F	32.62mm	59,92mm	Salvador
12	M	40.82mm	61.61mm	Salvador
13	M	36.55mm	63.37mm	Salvador
14	M	37.99mm	56.18mm	Salvador
15	M	34.04mm	61.31mm	Salvador
16	M	41.00mm	63.39mm	Salvador

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Liposome Drug Delivery in Cancer Chemotherapy: Review and Multifactorial Analysis

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We reviewed the use of liposomes for cancer therapy using computational biostatistics. We used virtual libraries, such as PubMed, LILACS, National Health Institute (NHI) and the Food Drug Administration (FDA) to conduct the review. Cluster analysis and correlation were developed using the Tanimoto coefficient (TC) of 0.7 using the modeling tool ChemMine Tools from databases (PubChem, DailyMed, DrugBank, Drug @ FDA). The results pointed fifteen molecules in the pharmaceutical form of liposome for the oncological clinic. Of these, 13 are classified by size into small-molecules and were analyzed by computational statistical modeling. Of these, only 4 were approved for use by the FDA, and 9 are in phases of research by the pharmaceutical industry as liposomal formulations. Essential differences in physical-chemical properties and molecules structure were observed, indicating original proposal in the development of liposomal as an anticancer drugs.

Keywords: Bioinformatics. Cancer. Drug delivery. Liposomal.

The cancers have in common the disordered and malignant growth of several cell lines with the capacity to invade other tissues far from the place of origin, forming secondary tumors or metastases [1]. The disease is currently one of the world's public severe health problems. According to Globocan 2018, a project by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), there were 14.7 million new cases of cancer and a total of 9.8 million cancer deaths worldwide in 2018, and a projection of almost 21.4 million new cases until 2030 with 13.2 million cancer deaths [2].

So, new antitumoral drugs are in tests as well as new methods to deliver these drugs. Due to the limitations and toxic events of conventional chemotherapies, several nanocarrier delivery systems have been developed and extensively used for drug delivery to cancer cells [3-5]. A large number of nanocarriers such as liposomes, polymeric nanoparticles, micelles, nanotubes, are already in the market, or under research and evaluation for cancer treatment [6]. Reports reveal an estimated global growth of liposome drug delivery (LDD) around $ 3.6 billion by 2020 in the global economic market and pharma industry [7].

The structural diversity and encapsulated drugs, as well as the mechanics of action liposomes, created a new technological perspective. However, there are challenges in methods for the preparation of liposomes, methods for efficient drug delivery, and procedures for the entrapment the drugs in the liposome. So, the innovations in liposome technology target researching that make it possible to predict new formulations and drugs for antitumoral treatments.

This review summarizes the types of methods used for the preparation of liposomes, mechanism of drug loading and potential therapeutic applications in cancer therapy, and provide current information on the liposomal products as a potent anticancer delivery drugs, either in clinical use or in clinical trials.

Liposomes

Liposomes have been widely used for several therapeutic applications, primarily as delivery drugs to reduce the toxic effects in the standard cell. They have also been used to optimize the bioavailability

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of drugs in areas such as pharmacology, oncology, cell biology, immunology, genetic engineering, and therapeutic and preventive medicine.

Liposomes have a larger surface area as compared to bigger particles, which can be easily modified to encapsulate a high amount of drug, increasing the blood circulation time, and enhancing the accumulation of drugs in solid tumors by permeability and retention (EPR) effect as well as selective targeting of tumor cells [8-10]. They also improve the solubility, bioavailability and pharmacokinetic properties of chemotherapeutics [9,11,12].

Liposomes have an unusual sphere structure, globular lipid bilayers of 50-1,000 nm. They are versatile, notably in size, surface charge and have phospholipid bilayer membranes similar to plasmatic membranes, which favored the permeability of the molecules to get into the cell [13]. Therefore, these structures can encapsulate and deliver both hydrophilic and hydrophobic substances.

Drug Delivery Systems (DDS)

DDS are methods of administering drugs to reduce side effects and increase therapeutic efficacy, such as reduction of toxicity for anticancer agents [7,13]. DDS represents an advance in pharmaceutical technology compound to achieve a therapeutic effect in drug delivery for several treatments [13,14].

Liposomes are a potential DDS [15] (Figure 1). For example, niosome are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or their lipids [16] by sub-micron (colloidal) of types MLVs (size ≥0.05 μm), LUVs (size ≥0.10 μm), SUVs (size =0.025-0.05 μm) [17], and consist of advanced DDS liposome (Figure 2) [18].

Gregoriadis and colleagues [19] consider two aspects that justify the promising future of liposomes drug delivery (LDD): reducing toxic side effects, and incoming of the drug in tumor cells. The studies by liposomes have been showing that the absorption of the drug by tumor cells is superior to drugs used in chemotherapies [20].

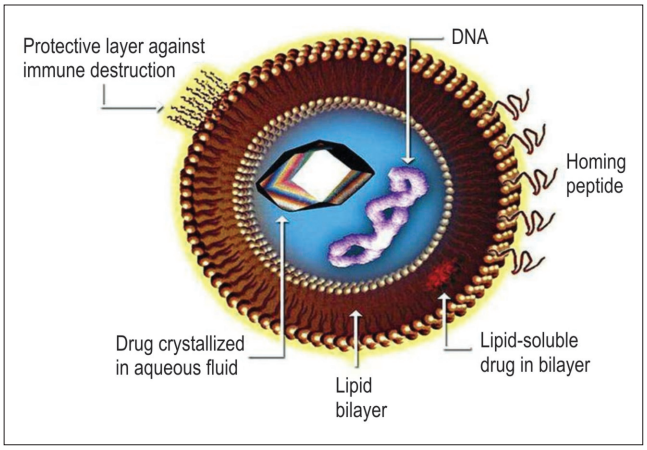
Designing Liposomes Drug Delivery (LDD)

The physicochemical properties of the liposomes are generally useful for characterizing a drug formulation, which must be more stable to avoid changes in product quality, including leakage of the drug. So, chemical, manufacturing and control (CMC); pharmacokinetics and bioavailability; and labeling documentation for liposome products submitted to new drug applications (NDAs) and abbreviated to new drugs (ANDAs) have been reviewed by the Center for Drug Evaluation and Research (CDER) [21] and recommended by the FDA for Industry.

The production of liposomes involves the use of an active agent and a phase lipid/lipid drug. So, the variety of natural or synthetic lipids, the different sizes, and molar rays, lead to a diversity of liposomes. In this aspect, the inherent properties of the carriers' structures and the nature of the drug to be encapsulated could affect biological half-life and modify drug absorption due to the relation between such structures and physicochemical characteristics of the drug substance (Table 1) [22].

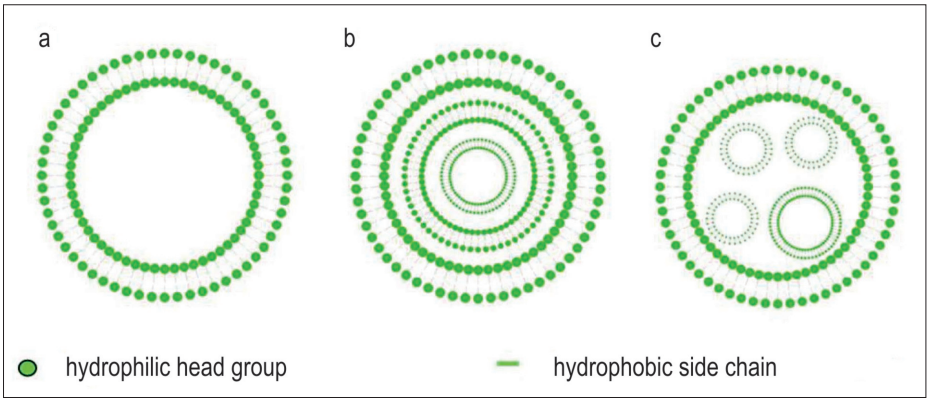
At present, the designing stage of the liposome is still in the beginning. So, the choice of the

Figure 1. Illustration of liposome for drug delivery.



Source: Çağdaş M. et al. (2010) [15].

Figure 2. A schematic structures of non-ionic surfactant vesicle.



(a) unilamellar vesicle, (b,c). multi-lamellar vesicle [18].

best liposome design and reagent quality may be decisive for the testing phases for drug approval.

Liposomes Drug Delivery in Cancer

FDA approvals 16 news therapies for cancer with new pharmaceuticals, including new molecular entities (NMEs) and new Biologic License Applications (BLAs) between 2013-2016. The pipeline of 836 drugs for cancer using liposomes in 2016, 123 were for lung cancer, 106 for leukemia, 92 for lymphoma (including non-Hodgkin lymphoma), 82 for breast cancer, 58 for brain tumors, 53 for skin cancer (including melanoma) [23].

According to Institute for Healthcare Informatics (IMS) on Global Use of Drugs [24], many potential applications of liposomes in immunotherapies and targeted therapies will be a promising field in oncology and drug delivery in 2020 [25].

Regarding clinically used liposome-based products in cancer chemotherapy active agent indicated for the treatment of different types of cancer are Doxorubicin (DOX) (Doxil®/ Myocet®), Daunorubicin (DAU) (DaunoXome®), Cytarabine/Ara-C (Depocyt®), Mifamurtide (Mepact®), Vincristine (Marqibo®), Irinotecan (Onivyde™). Also, the pharmaceutical industry is testing for some new active substance (NAS) Tecemotide, T4 endonuclease V, Cisplatin, Lurtotecan, Paclitaxel, all-trans retinoic acid for treating different types of cancer [26]. For example, Idarubicin (IDA), a drug exclusively used for the treatment of leukemia through thermosensitive liposomes (TSL) in combination with hyperthermia (HT) demonstrated to be more prominent tumor growth inhibition in leukemia than in the conventional form [27].

Some of these drugs have already in Phase II in Japan, as Stimuvax® (Tecemotide/L-BPL

Table 1. Effect of the nature of the drug on the formation of niosomes.

Nature of drug	Leakage from the vesicle	Stability	Oher properties
Hydrophobic drug	Decreased	Increased	Improved transdermal delivery
Hydrophilic drug	Increased	Decreased	-
Amphiphilic drug	Decreased	-	Increased encapsulation, altered electrophoretic mobility
Macromolecule	Decreased	Increased	-

Modified from KM Kazi et al. (2010) [22].

25) [28]: a liposome vaccine against the cancer cells that overexpress Mucin 1 (MUC-1) (Figure 3), a glycoprotein antigen expressed in cancers such as multiple myeloma and colorectal, breast cancer, prostate and ovarian cancers. However, the results of Stimuvax® were disappointing to the investigational therapeutic vaccine to enter Phase III (PhIII) studies in Non-Small Cell Lung Cancer (NSCLC), mainly due to flops in Phase III lung cancer trial and breast tumors [28-30]. Even so, the pharmaceutical company, Merck, will be responsible for the clinical development, commercial producing and marketing, estimated the sales to reach about €350m in 2019 [28].

Pfizer developed and produced the drug Xalkori® (Crizotinib) [31] for the treatment for Non-Small Cell Lung Cancer (NSCLC) and metastatic anaplastic lymphoma kinase (ALK) approved by FDA in August 2011 using liposomes [32]. Crizotinib is a receptor tyrosine kinase inhibitor that inhibits ALK and c-MET, and it is also been using in a liposome as a target-antitumoral drug [33,34].

Darolutamide by Bayer and Orion [33] have reported positive results in Phase III clinical trial (ARAMIS) in patients with non-metastatic castration-resistant prostate cancer using liposomes [35].

Avapritinib, an inhibitor of KIT and PDGFRα D842V mutant kinases, is under clinical development in China at present (2019) for GI Stromal tumors [33,36].

Analysis of Liposome Drug Delivery in Anticancer Therapy

We performed a database of chemical molecules, substances, compounds, bioassay, drugs and drugs for therapeutic or clinical medicine in liposomes using (PubChem) (National Institutes of Health - NIH), DailyMed [37], DrugBank [38,39], Drug@FDA [40], Pubmed (Medline), Cochrane Foundation, Isi Web of Science. Our focus was to find molecular similarities and correspondence for both liposome drug and drug delivery molecule for anticancer therapies, both approved and already in use, as well as those that are released by the FDA for in phases 3,2,1 or investigative research.

Fifteen molecules were found in the pharmaceutical form of liposome for oncological clinic. Of these, 13 are classified by size into small-molecules and were analyzed by computational statistical modeling. Of these only four were approved for use by the FDA, and nine are in phases of research by the pharmaceutical industry as liposomal formulations (Table 2). Tecemotide (Stimuvax®), Vincristine (Marquibo®), and

Figure 3. Proposed mode of action of L-BPL 25.

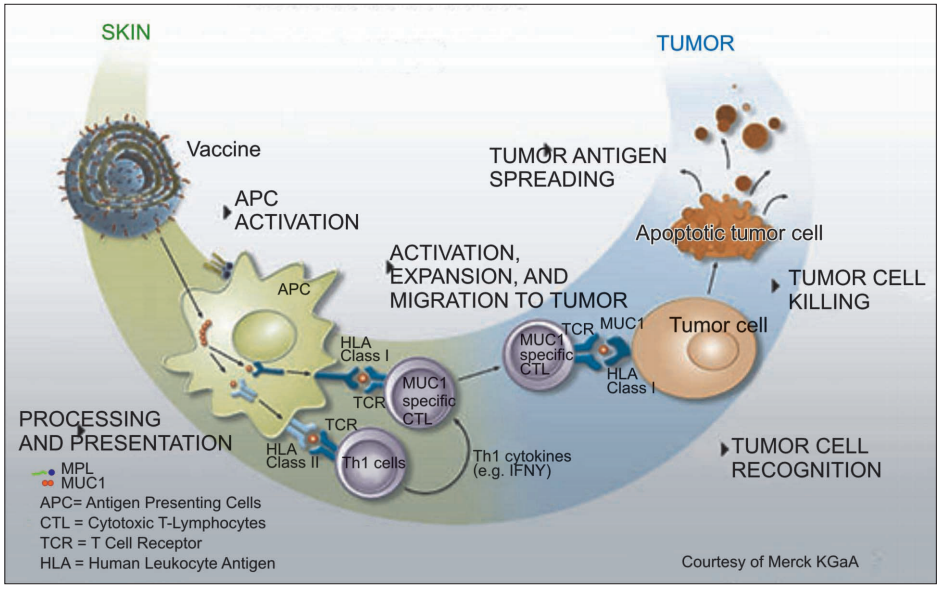
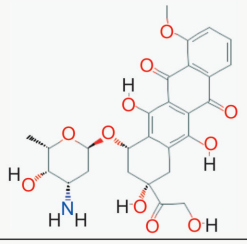
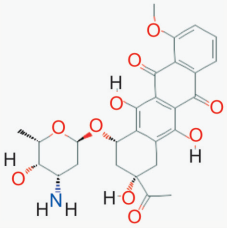
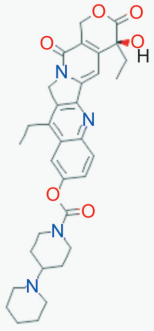
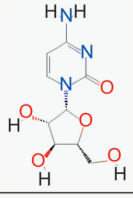
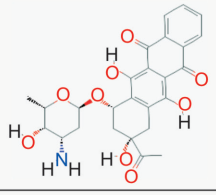
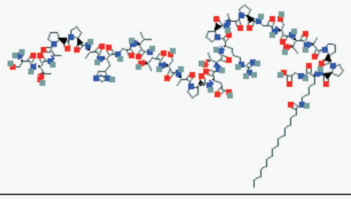
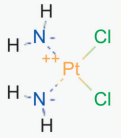
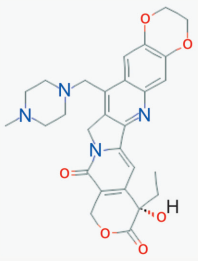
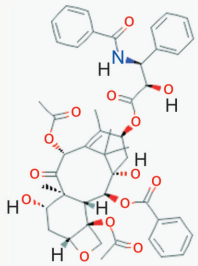
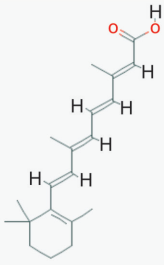
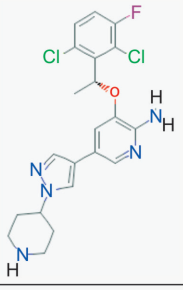
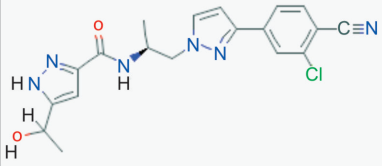
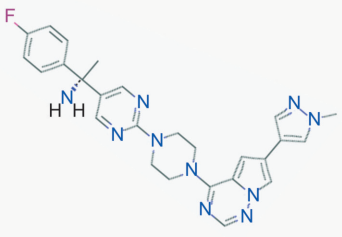


Table 2. Therapy and anticancer LDD approved by FDA and in investigative research.

Active Agent	Formula	Molecular Weight g/mol	Structure Molecular	Treatment	FDA
Doxorubicin 31703	$C_{27}H_{29}NO_{11}$	543.520		Several solid tumors	Prescription
Daunorubicin 30323	$C_{27}H_{29}NO_{10}$	527.520		Leukemia	Discontinued
Irinotecan 60838	$C_{33}H_{38}N_4O_6$	586.678		Colorectal cancer	Prescription
Cytarabine 6253	$C_9H_{13}N_3O_5$	243.2166		Acute non-lymphocytic leukemia, acute lymphocytic leukemia and blast phase of chronic myelocytic leukemia	Discontinued
Idarubicin 42890	$C_{26}H_{27}NO_9$	497.5		Acute myeloid leukemia (AML) in adults	Investigation
Tecemotide 16208013	$C_{124}H_{203}N_{33}O_{38}$	2764.181		Prostate câncer, rectal carcinoma	Investigation
Cisplatin 57021978	$Cl_2H_4N_2Pt$	298.035		Treat various types of cancers, including sarcomas, e.g. small cell lung cancer, and ovarian cancer, lymphomas and germ cell tumors Phase 01	Investigation

The pink's lines is active agent approved FDA and used liposome-based products for treatment various types of cancer.

(continued Table 2)

Active Agent	Formula	Molecular Weight g/mol	Structure Molecular	Treatment	FDA
Lurtotecan 60956	$C_{28}H_{30}N_{406}$	518.57		Antineoplastic activity	Investigation / BioAssay
Paclitaxel 36314	$C_{47}H_{51}NO_{14}$	853.918		Carcinoma of the ovary, and other various cancers including breast cancer	Investigation for liposome form
All-trans Acid Retinoic 444795	$C_{20}H_{28}O_2$	300.442		Acute promyelocytic leukemia (APL)	Investigation
Crizotinib 11626560	$C_{21}H_{22}Cl_2FN_{50}$	450.339		Non-small cell lung cancer	Prescribed
Darolutamide 67171867	$C_{19}H_{19}ClN_6O_2$	398.851		Prostate câncer	Descontinued
Avapritinib 118023034	$C_{26}H_{27}FN_{10}$	498.57			Investigation

Mifamurtide (Mepact®) were not evaluated in this analysis.

We use ChemMine® for small molecules data analysis relevant in chemical biology, chemical genomics, and drug discovery [41]. ChemMine® proved to be an excellent software to study similarity, clustering, prediction of a small molecule, integrating the physicochemical and bioactivity properties through PubMed [42].

Few similarities are found between the liposome-based products in Cancer Chemotherapy approved (Graphic 1). So, the diversity physicochemical characteristics of a drug molecule in terms of therapeutic class and chemical structures, involving different functional groups are factors considered essential to meet FDA recommendations about liposomes as a tool with anticancer drugs.

The graphics multidimensional scaling (MDS) is an array of item distances. The coordinates are assigned to each item in a low-dimensional space to represent the distances graphically in a scatter plot.

The distance matrices required for the clustering of MDS were calculated by comparisons of all against all compounds using measures of similarity of atom pairs, the similarity of substructures and transforming similarity scores generated in distance values. According to the model proposed by ChemMine®, basic descriptor type that is defined by the shortest paths among the non-hydrogen atoms in a molecule are atom pair. The TC calculated the similarity and the distance values were calculated by subtraction (1 - Tc). Then, the coordinates are assigned to each item in a low-dimensional space to plot the distances in a scatter plot. We determined Tc = 0.7. The coefficient varies between 0-1, the higher, the greater, and the greater the similarity. The axes V1 and V2 represent the chemical space in two directions. A higher correlation between the structures contained in the pink band on Graphic 2.

HC results were developed based on the molecular weight and physicochemical properties heat map Joe Lib descriptors. The JOELib tool computes 38 descriptors for each compound. The result showed the most similar of all molecular, almost identical is cluster hierarchical formed by DOX/ DAU and IDA. IDA is closer in physicochemical properties of DAU than dox. DAU has been discontinued for economic issues unrelated to the product's quality, safety or efficacy.

Boron (B), Bromine (B), Iodine (I), Phosphorus (P), Sulfur (S), SO2, -SO2, -OSO, -NO2, and hydrophilic groups are not present in all active agents.

The molecular weight, an import characteristic for liposome development varies in green range by almost all molecules, except Cytarabine, All-trans- Acid retinoic, Darulutamide and Paclitaxel.

Several halogen atoms are down (blue for green), but more in the cluster formed by Crizotinib, Daurulutamide, and Avapritinib.

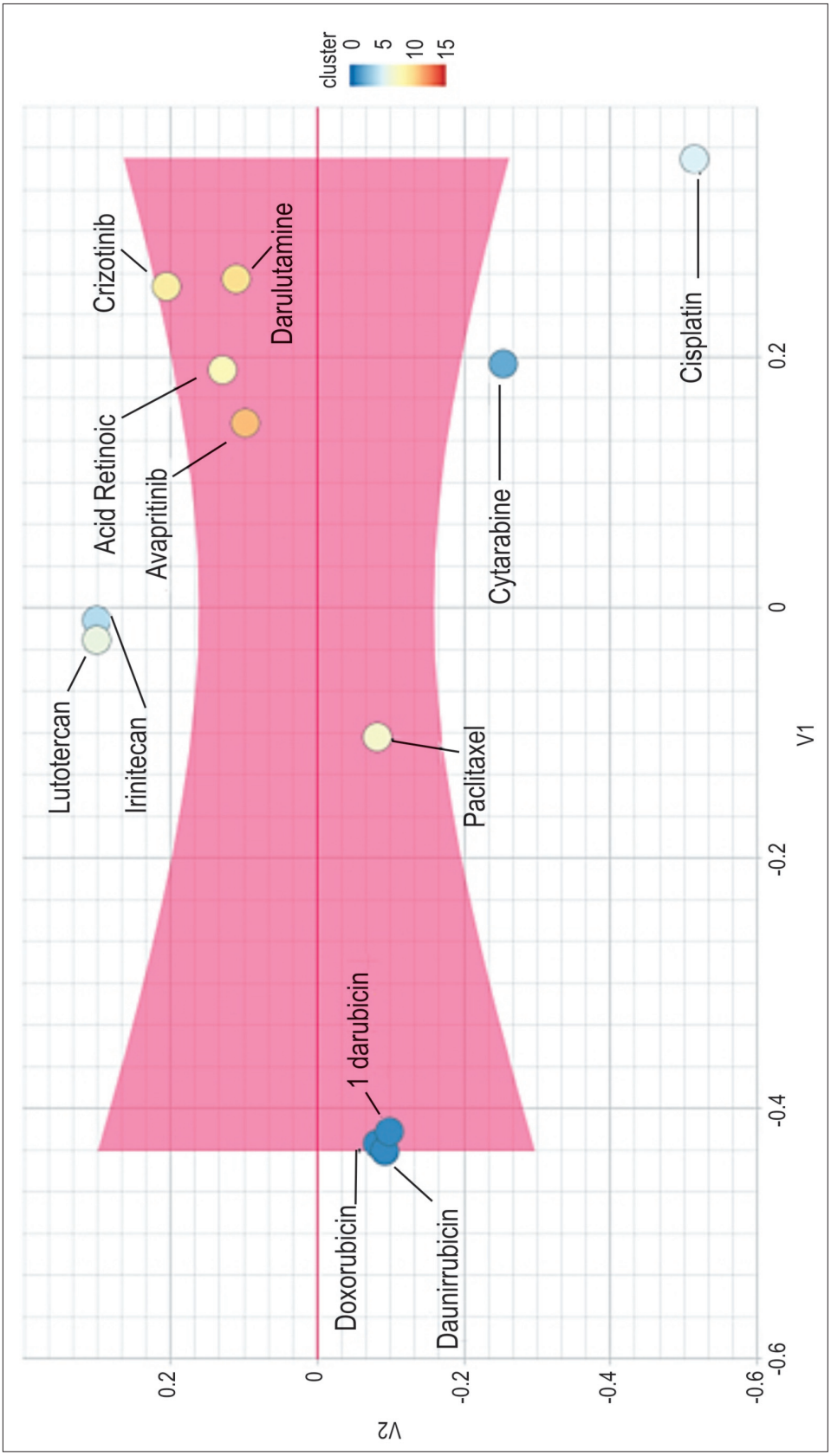
The number of primary groups and acid groups between the active agents, Lurtotecan showed the most significant amount of basics groups. However, with the predominance of acid groups, eight of the fifteen compounds analyzed contained more acid groups, highlighting all trans-acid-retinoic.

Lurtotecan and Irinotecan were outside the correlation range due to out of the correlation range due to the presence of a primary group in the first and greatest geometrical diameter in second. On the other hand, Cytarabinestands out from all other compounds by having lower: molecular weight, P-hydroxybenzaldehyde, heavy bonds, number of atoms, number of bonds, geometrical diameter, and other descriptors.

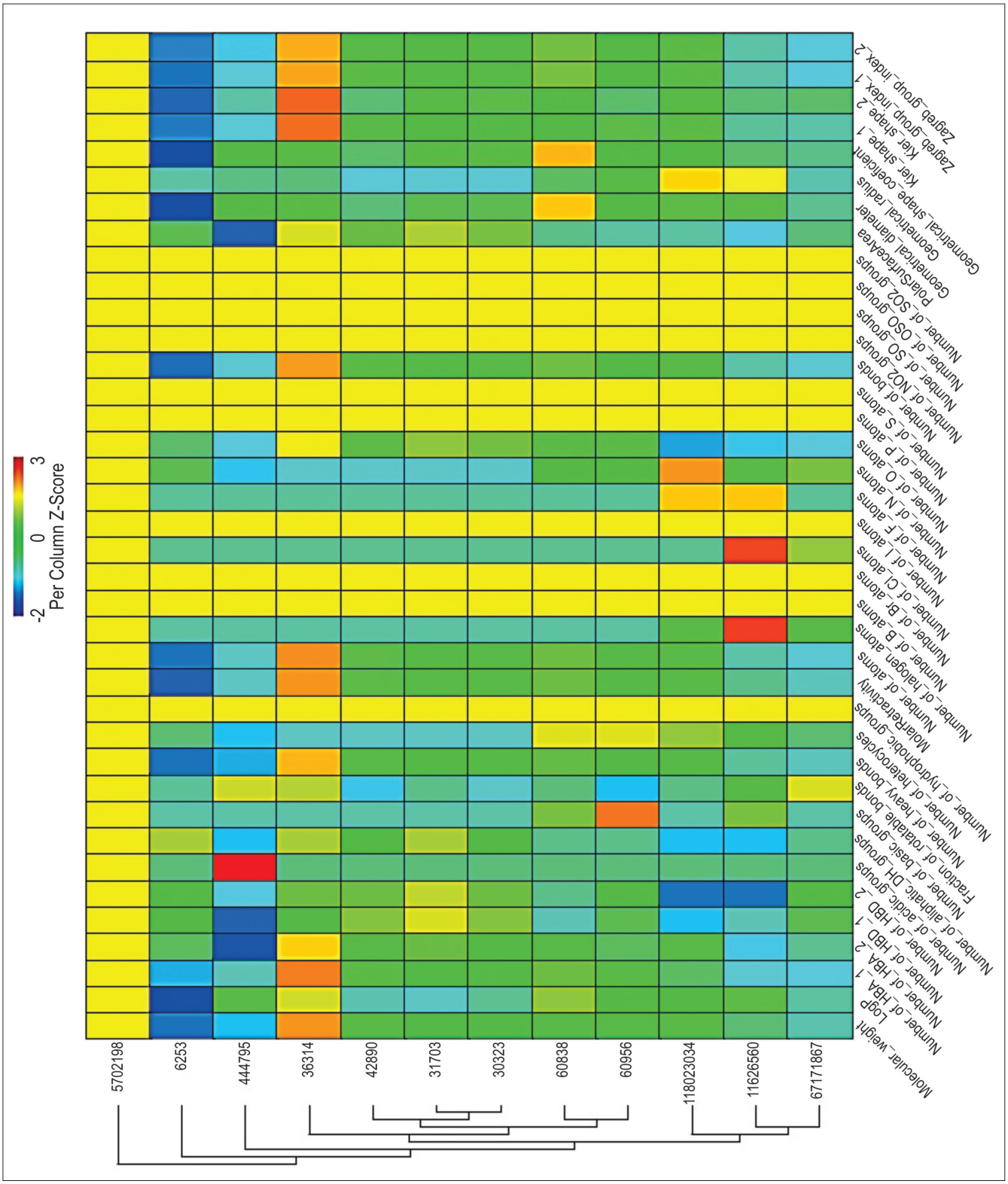
Conclusion

The use of liposome as anticancer therapy is restricted. However, liposomes are an essential research niche for NAS prospecting

Graphic 1. Multidimensional Scaling Clustering (MDS) of the NAS liposome in clinical oncology.



Graphic 2. Hierarchical Clustering Results of the NAS liposome in clinical oncology.



in which the evaluation of the physical and chemical characteristics of the pharmaceutical by computational methods can provide valuable information for the development of LDD in antineoplastic therapy. Nevertheless, it is essential to make a point of time-based understanding of physicochemical and bioactivity properties may be useful in finding methods of predicting more assertive experimental bed paths.

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The Management of Electro-Medical Equipment in Intensive Care Units: Assurance of Traceability and Metrological Reliability

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Physiological measurements in the health sector have been supported by the rapid evolution of medical equipment technologies. The health sector increasingly requires the development of mechanisms and applications that assure the metrological reliability of the results obtained using the equipment for the diagnosis, treatment, and monitoring of the clinical evolution of patients. This study demonstrates the weaknesses in the control of metrological parameters related to electro-medical equipment (EMS) in Brazil, specifically those used in Intensive Care Units (ICU), where reliability is critical in terms of survival, sequelae or death. We discuss essential determinants to ensure physiological measurements, such as the limitations of legislation/standards, laboratory infrastructure, voluntary accreditations, including a brief history and indicators of patient safety in Brazil and the United States of America, as well as data from research at Hospital Units (HUs) located in Salvador, Bahia, Brazil.

Keywords: Metrology. Health. Electro-Medical Equipment. Intensive Care Unit.

The basic principles of metrology are essential to several branches of technology because it is the science of measurement and its applications [1].

Recent advances in this science inside industrial environments have brought countless benefits related to measurement and operation reliability, traceability, quality, and safety. These are characteristics which are neither well-established nor present inside the Brazilian hospital environment.

The development of instruments has contributed to the manufacture of increasingly modern electro-medical equipment with the advances in science and technology since the XX century. This development has enabled biomedicine to measure several physiological parameters in isolated or parallel ways.

Essential signals, such as body temperature, heart or pulse rates, blood pressure, breathing frequency, and oxygen saturation are measured uninterruptedly and the results are necessary to diagnose disease, evaluate the patient's general clinical status, to observe response to therapy and can be used to help in rapid decision-making by professionals in Intensive Care Unit (ICU) or Emergence Room (ER). It is because these signals and other physiological measurements determine the basis of clinical problem solving [2]. Patients are more susceptible to hospital infections and adverse events (AE) in an ER.

An AE is a situation that occurs during the treatment that is not defined by the patient's clinical conditions. An AE is not an error, neglect or poor quality. It means an unwanted care result related to therapy or diagnosis. An AE attached to a mistake is an avoidable adverse event [3].

Research performed at Harvard Medical School concluded more than 20% of the ER patients suffered an avoidable AE [4]. Avoidable AEs are the ones of the most important consequences of the lack or inadequacy of metrological control of EEM. Therefore, there is a need for precise measurements and with the Unit International System (UIS) traceability. There is also need a law to control the metrological management of the EEM, laboratory infrastructure with accreditations to competent organizations and users capable of understanding the bad functioning or measurement error in equipment.

The risk of using equipment without accurate metrological evaluation increased emphasis after 1990 as a result of the Harvard Medical Practice

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Study publication, in which standards for the quantification of health services were established. They showed that from a total of 30,195 hospitalizations in the United States of America (USA), 1,133 AEs registered were preventable [5]. From there, the community began to devote greater attention to AEs. In 2000, the publication of the report "to err is human" by the Institute of Medicine (IoM) estimated that about 44,000 to 98,000 annual deaths in the United States of America (USA) were due to the failure of medical assistance. Approximately 1 million patients admitted to US hospitals a year were victims of AE assistance, more than half of them from mistakes that could have been prevented [6].

In 2002, the first world debate on the subject took place, sponsored by the World Health Organization (WHO) at the 55th World Health Assembly: "Quality of Care: Patient Safety"; then, in 2004, the "World Alliance for Patient Safety" was launched by WHO, of which Brazil was a signatory. In 2013, the National Patient Safety Program (NPSP, by Ordinance GM No. 529/MS) was created in Brazil and the resolution of the Collegiate Board (RDC No. 36) of the National Health Surveillance Agency (ANVISA-Brazil) introduced the obligatory core creation of NPSP in health establishments [7] with the goal of passing the record and treat the AEs. The chronology of the reference points regarding AEs in the world and Brazil is described in Figure 1.

The competent agencies, such as WHO, IoM and ANVISA have since been debating and developing legislation for patient safety and health care related AEs. WHO estimates that one in every 10 patients suffers damage when receiving medical care in developed countries, and in developing countries, the number of events is higher [7].

In Brazil, despite the recording of AEs being compulsory since 2014, the number of records in the NPSP corresponds to only 1.1% of the medical institutions in the country. In the period of 2014-2018, the country registered 205,290 occurrences of AEs, and the ERs in the HUs sector recorded the second highest number [8].

The metrological control of EEM used in ERs, with calibrations and tests traced to IS ensures not only the safety, reliability, and accuracy required by a sector of high complexity for the care of patients at risk or severe, but also a reduction in the occurrence of avoidable AEs.

Aspects of Certification and Metrological Control of EEM in Brazil

No product of interest to health, national or imported, can be industrialized, offered for sale or handed over to the Brazilian market before being registered in the Ministry of Health in Brazil (Law nº 6,360/1976). Law nº 9,782/1999 gives ANVISA the responsibility of regulation, control, and inspection of the products and services that involve risk to public health. This includes the granting of product registration (Law nº 9,782/1999) [9]. Resolution nº 444/August 1999 from ANVISA established the model adopted to ensure the safety of medical electrical equipment (model), adopting the technical standards of NBR

Figure 1. Chronology of reference frames for AEs.

- 1990 – Harvard Medical Practice Study – Adverse Events;
- 1999 – Institute of Medicinen (IOM) - to err is human;
- 2002 – OMS: 55ª World Health Assembly - resolution 55.18 wha: quality of care, patient safety;
- 2004 – OMS: 57ª World Health Assembly - global alliance for patient safety;
- 2013 – ANVISA: National Patient Safety Program (PNSP), Administrative Rule No. 529;
- 2015 – ANVISA: Integrated Plan for Health Management of Patients in Health Services - Monitoring and Investigation of Adverse Events and Evaluation of Patient Safety Practices

IEC 60,601 - 1 series: Electro-Medical Equipment. Part I - General Requirements for Safety; and Private Professional Standards -2. The compliance with the requirements established through the "good manufacturing practices for medical products" was determined for all suppliers of medical products by RDC 59/2000. The registry requirement from ANVISA grants certification of conformity to specific technical standards, issued by Certification Organizations for Products (COP) accredited by the National Institute of Metrology, Standardization and Industrial Quality (INMETRO-Brazil). The compliance results in the protection of the physical integrity of the users as well as the implementation of a good cycle between the regulator and productive systems [10].

The International Organization of Legal Metrology (IOLM) elaborates recommendations in which metrology is based on Technical Regulations for Measurement (TRM) prepared by INMETRO in Brazil. TRM puts several categories of measuring instruments, metrological and technical requirements for use and confirmation under central government control [11]. After delivery of any medical equipment to the customer, except for only clinical glass mercury thermometers, non-invasive mechanical aneroid sphygmomanometers and EEM used in the field of ionizing radiation, there is no law to regulate nor make compulsory metrological control tracing their performance over their life span. Currently, the only tool used in the direction of metrological reliability of EME in use is the voluntary accreditation of health establishments, which is conducted through national or international entities, including the International Standardization Organization (ISO), the National Accreditation Organization (NAO), the Joint Commission for Accreditation of Health Organizations (JCAHO), the American Association of Blood Banks (AABB). These entities are established agencies accredited to ensure the reliability of EEM, tracking calibrations as well as preventive and corrective maintenance [10].

According to the National Supplementary Health Agency (NSHA), by 2016, in Brazil, 133 hospitals had met important quality criteria as to the standard of care provided to the population. The data showed the performance of establishments according to three indicators: voluntary accreditation, hospital readmission, and patient safety. It was the first time that the NSHA offered information about the attributes of providers, helping consumers to monitor and evaluate the services received [12].

About Survey

The survey consisted of a literature review of theses, dissertations, and articles published in scientific meetings, journals, specialized journals, electronic consultation, analysis from ANVISA's reports and other relevant documents. There were also visits and questionnaires applied from May to July 2018 in UHs, of which two belong to SUS, and one is private, all located in Salvador, Bahia, Brazil. We searched for works that focus on the importance of metrology management of medical equipment of health, medical technology, metrology in health, adverse events in the hospital environment specifically in ERs, and legislation or specific standards.

Results and Discussion

There are 421 million hospitalizations annually and about 42.7 million AEs in the world. In the USA, these events are the third cause of death, reaching 400,000 deaths per year [13]. In 2002, a study in the United Kingdom showed that 28% of mercury sphygmomanometers and 42% of aneroides had errors of over 4mmHg. The study also revealed that only one in every 54 physicians had taken the care to maintain and calibrate their sphygmomanometers [14]. INMETRO conducted a study of calibration verification of sphygmomanometers used in hospitals in Juiz de Fora, São Paulo, and Rio de Janeiro, in 1997. The results indicated that 61% of the checked sphygmomanometers had an error more significant than the maximum allowed, which is 4mm Hg [15]. In 2018, in Brazil, there were 321,321 health care establishments of different sizes [16] and only 3,401 SPs, which registered 205,290 AEs and 1,157 deaths/type of incidents from March/2014

to May/2018. Bahia had 4,256 records. Of the total, 192,937 cases occurred in Hospital Unit (HUs), and 56,602 episodes occurred in UTIs [8] object of this study. A comparative study of three UHs was carried out in the city of Salvador, Bahia, one a member of a private network and two of the Unified Health System (SUS), all of them had ICUs, with 42.8 and 120 beds, respectively. None of them had a dedicated team for the metrological management of EEM, and only one had a member of staff with additional training in metrology. Two of them had regular maintenance and metrology management of EEM and intermediate checks in at least one hospital sector. Two had voluntary accreditation (ONA and QMENTUM) and only one registers AEs.

Brazil has 321,321 registered medical institutions and despite the compulsory creation of the NPSP, only 3,401 (1.1%) take part in NPSP (1.1%). This is taking into consideration that the NPSP provides unification in cases of several basic units in the same region. At best, with the unification of the number of establishments reduced to 50%, the country should have approximately 161,000 NPSPs, which means that the real number of EAs in the country is far higher than what is known today. Despite the lack of reporting AEs, the ERs were the second type of service to register the highest rates over the last four years, accounting for 29.3% of incidents attributed to hospitals. If we assume that only 10% of these incidents are caused by failures or absence of metrological control of EEM, this would already indicate 5,660 incidents which could have been avoided. The survey also shows that no law or regulation makes the metrological control of EEM compulsory after commercialization in Brazil, nor any network of qualified laboratories and calibration service providers and tests that could meet the demand [10]. Furthermore, a set of actions that promote the further training in Metrology for users/ managers is necessary, EEM in the dissemination of metrology culture, HUs health metrology, in-depth studies for the determination of uncertainty of measurement and other metrological parameters to promote error prevention in any sector.

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Osteoma: A Case Report Based on Image Technology

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Osteomas are benign mesenchymal tumors, characterized by proliferation of compact or modularly bone. They are small, slow-growing lesions, usually asymptomatic and detected in young adults. This tumors can affect the paranasal sinuses and are often diagnosed with incidental findings through imaging tests. Osteomas are typically restricted to the craniofacial skeleton and rarely found in other bones. Osteoma of the gnathic bones may be peripheral or endosteal. The osteomas' etiology is controversial and still unknown. It is more frequent in the frontal sinuses, corresponding to 57% of the paranasal sinuses osteomas, followed by the ethmoidal and maxillary sinuses. Computed Tomography (CT) is the gold standard to assess the location, extent, and aspects of the injury. The objective of this paper is to present a case report of osteoma diagnosed by computed tomography (CT) scan, indicating the importance of the technology of imaging in the medicine diagnostic.
Keywords: Osteoma. Paranasal Sinus Osteomas. Frontal Sinus Osteoma. Computed Tomography.

Osteomas of the frontal sinus is uncommon but not rare [1-8]. Recent surveys confirm that the frontal sinus is the most common location of this benign neoplasms [7]. Osteomas are the most common benign tumors that arise in the paranasal sinuses and the nose, with a slow-growing rate, well-circumscribed, indolent lesions that develop predominantly into the frontal sinus (80% of the sinus localization) [6-8]. In the beginning, small osteomas are usually asymptomatic [3,4,6-8]. The clinical symptoms depend on the location and the size of the tumor [6]. The most common symptom is a frontal headache or facial pain. Osteomas are usually identified accidentally by X-Ray or CT scan images [6]. The cause of frontal sinus osteoma is unknown and speculative. Many theories have been proposed, but it is uncertain [3-8].

From the histological point of view, there are three types of sinus osteomas [2-8]:

1. Eburnated (ivory, compact type) – very dense, with no evidence of Haversian canals;
2. Mature, spongious osteomas – osseous trabecules associated with fibrous tissue and collagen fibers;
3. Mixed types – with both (1 and 2) histological types.

Two main protocols are used in these tumors: 1. Conservatory: if the osteoma is small and asymptomatic, the better perform is to wait and check the progression of the neoplasm; and 2. Surgical treatment: if the osteoma has rapid-growth, presence of infections, severe pain, or orbital complications because of the tumor extension, the surgery is the best procedure [6].

A 37-year-old right-handed female was attended in the clinic of diagnostic imaging at Camaçari city (Bahia, Brazil) to evaluate the maxillary sinuses. During the analysis of the sinus X-ray of the face, a clear radiopaque image was found, located near the upper middle contour of the right orbit, ethmoidal sinus and inferior contour of the frontal sinus (Figure 1). The computed tomography (CT) confirmed the osteoma in the region (Figure 2). Based on the patient clinical history, radiographic images, and histopathology of the lesion, the treatment chosen was conservative.

Osteoma can be observed in any age group, being more prevalent in the third and fourth

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Figure 1. The sinus X-ray of the face.

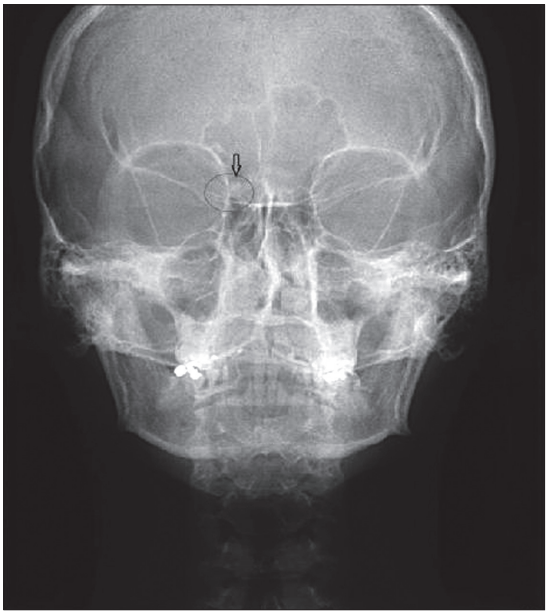
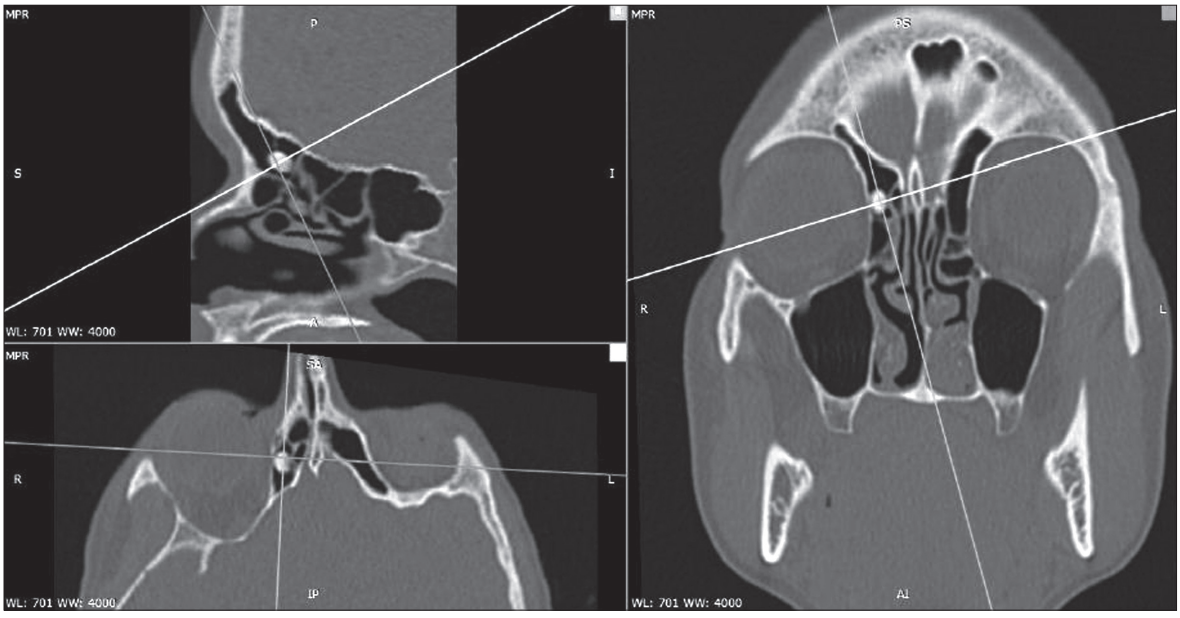


Figure 2. Osteoma visualized on computed tomography (CT).



decades of life. The etiology of the osteomas is unknown, in which many theories could be admitted, such as trauma, embryology, infectious diseases, and genetic. Osteomas are benign, indolent, slow-growing tumors of the skull and facial bones, commonly arising around the paranasal sinuses. In about 80% of cases, they are found solely within the frontal sinus, however the ethmoidal, maxillary, and rarely sphenoid sinuses might be affected as well. The osteoma cannot be considered as the causative agent of the headache, but could be regarded as the complaint that led to

complementary radiological examination, such as CT.

Diagnosis of osteomas is frequently made incidentally in X-rays, but more especifically with the technology of computed tomography scans. Therefore, the careful evaluation of the radiologist is essential to identify osteomas due to their small size, because they may go unnoticed.

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