

Substandard/Falsification Antibacterial Agents: A Systematic Review of Liquid Chromatographic and Spectrophotometric Methods for Their Detection

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Abstract

Falsified antibacterial agents continue to pose serious public health problems around the world. They are notably responsible for resistance emergence in the treatment of infectious diseases that can lead to death. The aim of this study was to summarize the literature on the methods developed and validated to detect poor quality antibacterial agents in order to assess the impact of these available methods in the fighting against Counterfeit/substandard medicines. Thus, studies published in the time from January 2000 to July 2017, were accessible via Google Scholar and Pubmed and allowed to analyze 41 papers. The majority (30) of developed and validated methods concerned LC methods, 9 regarded UV/V is spectrophotometry and two studies simultaneously developed both methods. Antibacterial agents belonging to beta-lactams group were the most concerned by the methods developed and validated (39%), followed by quinolones (18%) and macrolides (12%). Regarding active ingredients, amoxicillin (12%) was the most concerned by the development and validation methods. In spite of several available methods to detect substandard drugs, antibacterial agents for which methods were developed and validated were the most concerned by worldwide detected non-conformities. The fight against this scourge should not be only limited by their detection, but it also requires an effective involvement of different actors notably: health authorities, international organizations, pharmaceutical industries etc.

Keywords

Counterfeiting/Falsification/Substandard Antibacterial, Liquid Chromatography/Spectrophotometry UV/Vis

1. Introduction

Counterfeit/substandard drugs are becoming an increasing source of risks for public health [1]. They are responsible for several cases of morbidity and mortality in the world [2]. These drugs notably increase the risks of treatment failure in various diseases but can also be at the origin of drug poisoning [3]. Moreover, suboptimal dosages of antibacterial agents undoubtedly contribute to the emergence of bacterial resistance [4] [5].

Despite a series of studies on counterfeit/substandard medicines and efforts provided by the health authorities in various countries to fight them, the rate of dubious quality drugs continues to rise [3] [6] [7]. It is reported that more than 10.5% of drugs in circulation worldwide are of low quality (counterfeit, substandard) [8].

In addition, several studies indicate that because of the lack of pharmaceutical regulation and/or controls, the circulation of counterfeit/substandard drugs mainly affects developing countries, through an expanding illicit market [9] [10] [11] [12]. In these countries, the falsification of antibacterial agent and other antiparasitic agents is particularly prevalent, with alarming and life-threatening consequences for the most prevalent diseases, such as malaria, tuberculosis and other opportunistic HIV infection-related diseases [4] [13]. Moreover, a major cause of the increasing failures rate in the management of infectious diseases in low-income countries resides in the use of low-quality antibacterial agent. In this context, it is opportune to combat the circulation of counterfeit/substandard antibacterial agent [13]. This increasing rate in the management of infectious diseases due to counterfeit/substandard antibacterial agent, represents the main problem of public health. It is established that an effective fight against counterfeit and substandard drugs relies on their detection, notably through a careful visual inspection of the product, the packaging and the galenic form [11]. Other elements can be also checked, such as the accuracy of bar codes and holograms eventually indicated on the packaging. However, the increased sophistication of counterfeiters often allows them to correctly reproduce the packaging and appearance of galenic forms, so that the visual examination alone cannot differentiate the authentic from the counterfeit [11] [14].

The investigation of drugs authenticity can be also conducted to detect falsified drugs. It consists essentially to contact the manufacturers and regulatory authorities of marketing and manufacturing countries; the manufacturer can formally attest drug authenticity which he has produced [12] [13]. Indeed, Drug regulatory authorities can provide essential information on legality of products

which are in circulation in the country. But, It is not obvious to obtain always reliable information from the manufacturer if himself decided to falsify his own product for beneficial reasons, this can be achieved either by reducing the cost of presentation of packaging or by using less expensive excipients than those used in the manufacturing of original product, either by producing the same drug in another country where labor is not at expensive or even reducing the exact dose of active ingredient that is eventually expensive. In addition, it is not always easy to achieve this investigation successfully because of the incertitude that exists to have a frank collaboration with the manufacturers and the health authorities of some countries.

The considerations mentioned above indicate that these methods are limited to ensure an optimal detection of falsified drugs. Thus, analytical methods offer an interesting alternative to detect counterfeit/substandard drugs. So, there are several analytical methods including: gas chromatography, high performance liquid chromatography (HPLC), mass spectroscopy, UV/VIS spectrophotometry, near infrared spectroscopy (NIR), capillary electrophoresis, thin layer chromatography, ... [15] [16].

Several studies focused on development and validation methods analytical to determine the content of active ingredient. Some of them are complex and expensive; others are simple, inexpensive and rapid [17] [18]. It has been reported that among the analytical methods used to analyze drugs, HPLC was the most commonly used method to control drugs quality [19]. Indeed, HPLC is a separation technique widely applied in quantitative and qualitative analysis [16]. Elsewhere, most of laboratories in low-income countries do not always have the capacity to get sophisticated equipment such as Liquid Chromatography, mass spectroscopy, capillary electrophoresis, to apply analytical methods. They use simple and less costly methods, among which UV/VIS spectrophotometry has an important role in the detection of poor quality medicines [17]. UV/VIS spectrophotometry is a non-separative technique which is related to the interaction of light with matter [15].

Some reviews focused on the usual methods of detecting counterfeit/substandard drugs [14] [16]. Kovacs *et al.* (2014) identified methods for detecting counterfeit/substandard drugs, they classified them according to their cost, the need of sample preparation, the need of reagents, etc. [20]. A systematic review was also conducted on analytical methods to detect counterfeit/substandard drugs; the study covered 2010-2016 [21]. The authors focused specifically on digital technologies that exist to ensure the integrity of the supply chain to combat fake medicines. The above studies didn't sufficiently study a certain number of parameters such as: the active ingredient concerned by the validated and developed methods, the information on the diluent, mobile phase and the wavelength in which the methods were developed and validated. This present study takes into account those parameters. Thus, the aim of this study is to summarize the literature on the methods developed and validated to detect poor quality antibacterial agents in order to assess the impact of these available methods in the fight

against counterfeit medicines.

2. Method

The literature choice was made using online databases: Google Scholar and Pubmed. The combination of terms such as: “detection counterfeit/substandard drugs”, “method detection counterfeit drugs”. In order to be more specific, the combination of the following words was also performed: “spectrophotometric/LC/development/validation antibacterial agent”, method determination antibacterial agent substandard/counterfeit. The articles concerned by this study are those published in 2000 and July 2017. The review was performed in accordance with the PRISMA statement [6] [22].

We considered only articles published in English and French. We took into account studies which concerned exclusively LC and UV/visible spectrophotometry methods developed and validated to determine the content of active ingredient in pharmaceutical formulations. In addition, drugs involved were those having antibacterial action.

Elsewhere, studies focused only on 1) quality control of drugs, 2) development and validation of methods of drugs not having an antibacterial effect, 3) active ingredient didn't clearly mentioned for the developed method 3) methods developed and validated but whose determination of the content of active ingredient were carried out in biological fluids (serum, plasma, urine, blood, bronchial secretions) were not taken into account.

First, the examination of articles titles were done in order to judge the conformity of articles according to our inclusion criteria. Thus, some articles were excluded from the study because the title didn't meet our inclusion criteria. About articles having titles that raise up doubts or were ambiguous, the analysis of abstracts were necessary to be applied. Full-text of all titles that appeared to fit the purpose of the present study were obtained and analyzed to determine if they met inclusion criteria defined of the study.

The articles selected were evaluated in accordance to 14 parameters that were established in this study, these parameters were taken from each selected article: the active ingredient concerned, the type of method concerned, the apparatus(s) used, the mobile phase used, the diluent(s) used, the wavelength, the linearity, the specificity, the precision, the accuracy, the robustness, the correlation coefficient, the limit of detection and quantification. The data entry was done on the Microsoft Excel (version 2013) and data analyze were performed on Epi-info software (version7.0).

3. Results

3.1. General Aspect

We identified 853 (**Figure 1**) articles in the database selected. 742 articles were excluded from the study for several reasons 1) they were not published between 2000 and July 2017; 2) it concerned systematic reviews article; 3) only quality

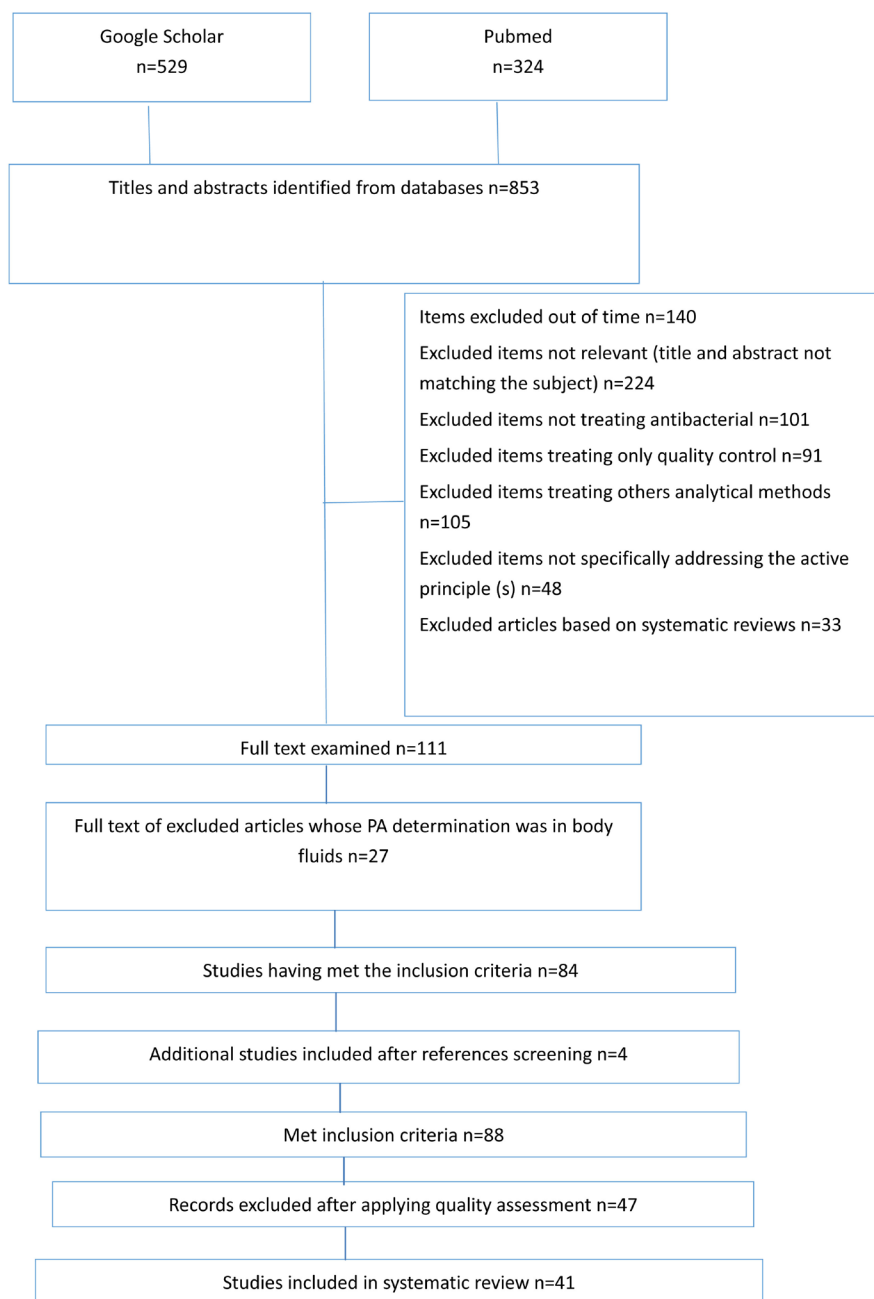


Figure 1. PRISMA flowchart.

control of drugs were concerned; 4) they treated others methods than UV/vis spectrophotometric and LC; 5) the active ingredient concerned in the study had not an antibacterial action; 6) they treated other subjects which did not correspond to the aim of our objective of study. Thus, we retained first a total of 111 items.

Of these 111 items, 27 were excluded mainly because UV/vis spectrophotometric and HPLC methods developed for the antibacterial agents were performed in biological fluids (serum, bronchial secretions, plasma, and urine). It is important to note that 4 additional studies were included after references screening.

All 88 articles did not meet 14 evaluation criteria established, 27 met 13 criteria, 11 met 12, and respectively five, three and one studies met nine, 11 and 10 criteria. A total of 41 studies met therefore all 14 criteria established in the study.

Of these 41 studies, five studies were published in 2014, five in 2012, five in 2011, four in 2015, three studies were published in 2008 and in 2013, while other years did not exceed two publications. Any publication was not identified during 2000, 2001, 2005 and 2010.

3.2. Characteristics of Methods and Active Ingredients

As described above, LC is considered as the gold standard analytical in drug analysis. 30 studies used LC to develop and validate methods in order to determine the content of active ingredient of antibacterial agents. However, nine studies used UV/vis spectrophotometry and two studies simultaneously developed both methods. Studies based on HPLC used an average wavelength of $260.8 \text{ nm} \pm 40.92$. The range was of 205 to 360 nm. On the other hand, in UV/vis spectrophotometry, the average wavelength was 418.3 ± 101.72 and the range was of 210 to 540 nm.

In 7 studies [23]-[29], UV/vis spectrophotometry methods developed of active ingredients consisted on the formation of a colored compound, which shown a maximum absorption, measurable on a spectrophotometer at a specific wavelength, whereas 4 studies [30] [31] [32] [33] did not proceed on the formation of a colored compound. Elsewhere, studies carried out on HPLC were based on reversed phase chromatography coupled to UV detector.

33 antibacterial agents (Table 1) were concerned in the development and validation methods. Beta-lactams were widely represented with 13 antibacterial agents (39%), quinolones were concerned with six active ingredients (18%), and they were followed by macrolides (12%) for four antibacterial agents, three for cyclins (9%) and two actives substances belonging to lincosamides (6%) and two others to nitro-imidazoles (6%) Others groups: aminoglycoside, beta-lactamase inhibitor and antituberculous drugs provided only one active ingredient.

Development and validation of methods to determine the content of amoxicillin were carried out in five studies (12%), three were determined by LC method and two by UV/vis spectrophotometry method. The detection system of amoxicillin was performed at 215, 254 and 283 nm by LC [34] [35] [36]. In UV/vis spectrophotometry the content of amoxicillin were achieved at 397 nm and the method was based on the selective oxidation of amoxicillin with cerium (IV) or iron to give an intense yellow coloring product [29]. Another study developed UV/vis spectrophotometry method by using 4 different wavelengths: 390 nm, 520 nm, 435 nm, and 415 nm (Salem, 2004). The study used 4 procedures: 1) nitration and subsequent complexation with a nucleophilic reagent; 2) nitrosation and subsequent metal chelation; 3) coupling with diazo reagent; and 4) reaction with copper and extraction of the resulting chelate into chloroform [28].

Doxicyclin were studied in 4 studies (10%), all of them were developed by using LC methods at 360 nm, 277 nm, 310 nm and 410 nm, respectively [37] [38]

Table 1. Characteristics of methods based on HPLC and UV spectrophotometry.

Antibacterial agent	Number of times used	Method type	Diluent	Mobile phase	Detection system (nm)	Reference
Clavulanic acid	2	HPLC	Water	methanol:KH ₂ PO ₄ (95:5)	215, 0	[35]
		HPLC	Acetonitrile:water (1:1)	acetonitrile:KH ₂ PO ₄ (70:30)	228	[50]
		HPLC	Water	methanol:KH ₂ PO ₄ (95:5)	215, 0	[35]
		Spectro	methanol:water (2:98)		397, 0	[29]
Amoxicilin	5	HPLC	KHPO ₄	Methanol:KHPO ₄ :(5:95)	254	[36]
		Spectro	HNO ₃ :HSO ₄ :water (2:2:96)		390, 520, 435, 415	[28]
		HPLC	KH ₂ PO ₄ et methanol (95:5)	methanol:KH ₂ PO ₄ (5:95)	283	[34]
		HPLC	Methanol	méthanol:H ₃ PO ₄ (80:20)	210, 0	[46]
Azythromycin	3	Spectro	Methanol		540, 0	[27]
		HPLC	acetonitrile:water (40:60)	acetonitrile:KH ₂ PO ₄ (50:50)	215, 0	[47]
		Spectro	methanol:water (2:98)		397, 0	[29]
Cefadroxyl	2	Spectro	HNO ₃ :HSO ₄ :water (2:2:96)		390, 520, 435, 415	[28]
Cefdinir	1	Spectro	Methanol		210, 0	[25]
		HPLC	Water	KHPO ₄ :acetonitrile (80:20)	230, 0	[43]
Cefoperazone	2	Spectro	HNO ₃ :HSO ₄ :water (2:2:96)		390, 520, 435, 415	[28]
Cefozopran	1	HPLC	Water	Ammonium acetate:acetonitrile (92:8)	260	[54]
Cefpirome	1	HPLC	Water	Ammonium acetate:acetonitrile (90:10)	270	[55]
		HPLC	acetonitrile:water (1:1)	acetonitrile:KH ₂ PO ₄ (70:30)	228	[50]
Cefpodoxime	3	HPLC	acetonitrile:methanol:water (20:50:30)	acetonitrile:methanol:acetate trifluore (30:50:20)	235	[45]
		HPLC	Methanol	acetonitrile:KH ₂ PO ₄ (70:30)	248	[56]
Cefprozyl	1	Spectro	methanol:water (2:98)		397, 0	[29]
Ceftiofur	1	Spectro	Water		292, 0	[31]
Ceftrizoxime	1	HPLC	methanol:water (20:80)	methanol:water (20:80)	290, 0	[57]
		HPLC	Methanol	Mobile phase A: methanol:KH ₂ PO ₄ :triethylamine (30:69.7:0.3); mobile phase B: acetonitrile	277	[37]
Claritromicin	2	HPLC	Methanol	Mobile phase A:KH ₂ PO ₄ :octanesulfonic acid (5:1) phase B: acetonitrile (Phase B)	210	[39]
		HPLC	acetonitrile:HCl 0.1 N (50:50)	Phase A: buffer carbonate:acetonitrile (90:10) Phase B: buffer carbonate:acetonitrile (20:80)	214	[58]
Demeclocycline	1	HPLC	HCl 0.01 M	Phase A: acetonitrile:sodiul edelate:phosphate tetrapropylammonium hydrogene:water (2:35:35:28) Phase B: (30:35:35:0)	280	[48]
Dicloxacillin	1	HPLC	acetonitrile:methanol:water (20:50:30)	acetonitrile:methanol:acetate trifluore (30:50:20)	235	[45]
Doxicyclin	4	HPLC	HCl 0.001 M	water-acetonitrile (60:40)	360	[40]

Continued

				Mobile phase A:		
		HPLC	Methanol	methanol:KH ₂ PO ₄ :triethylamine (30:69.7:0.3); mobile phase B: acetonitrile	277	[37]
		HPLC	Methanol	Mobile phase A: KH ₂ PO ₄ :octanesulfonic acide (5:1) phase B: acetonitrile (Phase B)	310	[39]
		HPLC	KH ₂ PO ₄ :methanol (2:8)	KH ₂ PO ₄ :methanol (3:7)	400	[38]
Enrofloxacin	1	HPLC	KH ₂ PO ₄ :Acetonitrile (75:25)	acetonitrile:KH ₂ PO ₄ (75:25)	267	[59]
		HPLC	Buffer citrate:acetonitrile (52:48)	buffer citrate:acetonitrile (52:48)	292	[53]
Gatifloxacin	2	Spectro	Water		412, 415, 417, 414	[23]
Gemifloxacin	1	Spectro	Methanol		270	[30]
		HPLC	methanol:water (50:50)	methanol:orthophosphoric acid (60:40)	225	[26]
Imipenem	1	Spectro	Water		525	[26]
		HPLC	acetonitrile:water (3:7)	acetonitrile:buffer phosphate:hydrogeno sulfate tetrabutylammonium:water (21:5:3:71)	232	[44]
Josamycin	1	HPLC	acetonitrile:water (1:1)	water:acetonitrile (6:5)	260, 265, 270, 275, 280	[60]
Levofloxacin	1	HPLC	Ethanol	acetonitrile:buffer phosphate (89:11)	220	[61]
		HPLC	Water	KH ₂ PO ₄ :acetonitrile (9:1)	298,0	[32]
Meropenem	1	Spectro	Water		298,0	[32]
		HPLC	KHPO ₄	KHPO ₄ :methanol (95:5)	254	[36]
Metronidazole	2	HPLC	acetonitrile:methanol (80:20)	NaHPO ₄ :acetonitrile (65:35)	319	[51]
		HPLC	Methanol	methanol:KH ₂ PO ₄ (62:38)	254	[42]
		Spectro	Water		623, 660	[24]
Moxifloxacin	4	HPLC	acide phosphorique 0.1%	water (triethylamine2%):acetonitrile (90:10)	290	[41]
		Spectro	1) HCl 0.1 N 2) tampon phosphate		296, 289	[33]
Norfloxacin	1	HPLC	Methanol	Mobile phase A: KH ₂ PO ₄ :octanesulfonic acid (5:1) phase B: acetonitrile (Phase B)	310	[39]
		HPLC	HCl 0.01 M	Phase A: 0.05% trifluoroacetic acid in water; phase B: acetonitrile:methanol:tetrahydrofuran (80:15:5)	254	[49]
Oxytetracyclin	2	HPLC	Methanol	methanol:acetonitrile:buffer phosphate (12.5:12.5:75)	253	[52]
Rifampicine	1	HPLC	acetonitrile:KH ₂ PO ₄ (50:50)	acetonitrile:KH ₂ PO ₄ (50:50)	238	[62]
Roxithromycin	1	HPLC	Ethanol	KH ₂ PO ₄ :acetonitrile (50:50)	205	[63]
Spectinomycin	1	HPLC	acetonitrile:tampon phosphate (89:11)	acetonitrile:buffer phosphate (89:11)	220	[61]
Tinidazole	1	HPLC	Methanol	Mobile phase A: KH ₂ PO ₄ :acide octanesulfonic acid (5:1) phase B: acetonitrile (Phase B)	310	[39]

[39] [40]. On the other hand, UV/vis spectrophotometry method permitted to develop and validate methods to quantify the amount of moxifloxacin [24] [33].

In addition, LC method were also used to determine the content of moxifloxacin, the wavelength were performed at 254 nm and 290 nm [41] [42]. One of the method used to develop the LC method of moxifloxacin consists to separate and determine impurities and degradation products of moxifloxacin in its pharmaceutical forms [41].

The methods developed of cefpodoxime were all based on LC method [43] [44] [45]. Azithromycin has been also studied in 3 studies (7%). HPLC method were useful for 2 of them whereas 1 used UV spectrophotometry which were based on the reaction of azithromycin with p-chloranil in order to obtain a compound colored in red and measurable at the spectrophotometer at 540 nm [27]. The detection system of azythromycin used in HPLC was performed at 210 nm and 215 nm [46] [47].

Potassium clavulanate, clarithromycin, metronidazole and oxytetracyclin were all performed by HPLC [35] [36] [37] [39] [48] [49] [50] [51] [52]. Two wavelengths (215 and 228 nm) permitted to detect potassium clavulanate. The detection system was performed at 319 nm for metronidazole, 253 nm for oxytetracyclin, 210 and 277 nm for clarithromycin.

Cefadroxyl were concerned in two studies (5%), all of them used UV/vis spectrophotometry method. Indeed, one study used four different wavelengths: 390 nm, 520 nm, 435 nm, and 415 nm as mentioned above with the case of amoxicillin [28]. In another study, the wavelength were performed at 397 nm and the method was based on the selective oxidation of these drugs with either Ce (IV) or Fe (III) in acid medium to give an intense yellow colored product [29].

Gatifloxacin were concerned in 2 studies (5%), which one used UV/vis spectrophotometry method based on the formation of yellow ion pair complexes between the basic nitrogen of the drug and three sulfonphthalein acid dyes and the wavelength were performed at 412 nm, 415 nm, 417 nm and 414 nm [23]. The wavelength performed in HPLC for gatifloxacin was at 292 nm [53]. Cefoperazone were also concerned in 2 studies, one used UV/vis spectrophotometry method as it described above about Cefadroxyl [28]. The system detection in HPLC method of cefoperazone was at 292 nm [43].

Others antibacterial agents (cefzopran, cefpirome, ceftroxime, clindamycin, demeclocycline, dicloxacillin, enrofloxacin, imipenem, levofloxacin, lincomycin, meropenem, norfloxacin, rifampicin, roxithromycin, spectinomycin and tinidazole) were concerned in one study by using HPLC method as shown in **Table 1**. On the other hand, cefdinir, cefprozyl, ceftiofur and gemifloxacin were concerned also in one study, but UV/vis spectrophotometry method was used. It fit to note that two different studies used the same wavelength in the development and validation of a method of metronidazole and oxytetracyclin by LC. It fit to note that, three studies used the same wavelength (254 nm) to describe the LC method of amoxicillin, metronidazole, moxifloxacin and oxytetracyclin. The LC method of amoxicillin and azythromycin has also been performed at the same wavelength (215 nm) in two different studies [36] [42] [49].

3.3. Diluents, Mobile Phase and Others Validation Parameters

The use of diluents is essential, whatever UV/vis spectrophotometry or LC method. The water were the preferred diluent (9 studies) for preparing antibacterial agents [23] [24] [26] [31] [32] [35] [39] [54] [55]. Indeed, of these 9 studies, water was used as diluent in 4 studies which concerned LC methods. Four others concerned UV/vis spectrophotometry methods. In one study water were used as diluent to develop LC and UV spectrophotometry method [32].

Methanol was used in 8 studies: 5 for LC and 3 for UV/vis spectrophotometry method [25] [27] [30] [37] [39] [46] [52] [56] whereas the mixture acetonitrile:water was used in 4 studies. The mixture acetonitrile:phosphate buffer in 3 studies [59] [61] [62].

The proportion of solvent mixture was different in studies mentioned above, except for two studies, where the same mixture solvent and proportion were used [50] [60]. Moreover, the diluent mixtures were often used for LC methods except two studies which used a solvent mixture (methanol:water) to develop a UV/vis spectrophotometry method [28] [29]. Other diluents were used in one or two studies as detailed in **Table 1**.

Regarding the mobile phase, mixture acetonitrile:buffer were used in 10 studies for the development and validation methods whereas the mixture methanol:buffer were used in 5 studies as mentioned in **Table 1**. It fit to note that 1) two studies used the same mobile phase (acetonitrile:buffer phosphate) at the same proportion (70:30) for the same antibacterial agent (cefepodoxime), except the wavelength (248 nm and 228 nm) and the diluent (methanol and acetonitrile:water) [50] [56].

The parameters validation: specificity/selectivity, precision, accuracy, robustness, linearity, correlation coefficient and limit of detection and quantification, were determined in all 41 studies concerned.

4. Discussion

Poor quality antibacterial agents are real threats for the public health, mainly because of the phenomena of resistance that they can cause [4] [5]. Several cases of resistance has been reported notably, the case of the management of urinary tract infections which were successfully treated with colistin. Indeed, the emergence of the mechanism resistance of colistin via an MCR-plasmid was reported [64]. However, the fight against poor quality antibacterial agents can contribute to prevent the emergence of antimicrobial resistance [5]. In other words, an effective fighting against poor quality antibacterial agents, is to detect them. This shows that, the methods to detect falsified antibacterial agents are therefore a way which can guarantee also a better management of infectious diseases. This fighting requires the application of prior factors that are mainly less or not applied in many developing countries: 1) registration and authorization of drugs by the health authorities before their marketing; 2) promoting the use of WHO prequalified drugs [9].

Elsewhere, the purpose of this review were to summarize LC and UV/visible spectrophotometry methods developed and validated to detect antibacterial agent drugs of poor quality in terms of active ingredients. It should be noted that, the quality of a drug don't always depend on a correct amount of active ingredient, other methods can be also taken into account such as dissolution test and disintegration test which can have an effect on therapeutic response.

In this study, several studies (31) used LC to develop and validate methods. The choice of LC methods may be justified by the fact that the results provided by the LC are generally reliable because of the high accuracy, sensitivity and selectivity obtained by this method comparing to UV/vis spectrophotometry. But we can note some of its disadvantages notably the high cost as reported by Kovacs *et al.* (2014), LC device costs about \$50.000 and its use requires a highly qualified technician, it requires electricity at all times but also the consumption of reagents should be taken into account [20].

Antibacterial agents for which methods were developed and validated belonged to beta-lactam group (39%), followed by quinolones (18%), lincosamides (18%), macrolides (18%) and cyclins (9%). Besides, amoxicillin were the most concerned by the development and validation of methods. Also, it was the most falsified antibacterial agent for which non-conformities were reported in 29 countries [13]. Moreover, Delepierre *et al.* (2012) indicated that antibacterial agents belonging to beta-lactam group were the most counterfeited in the world (50%), followed by quinolones 12%, 11% for macrolides and lincosamides and 9% for cyclins [4]. These observations, show that there is a correlation between antibacterial agents for which methods are developed and substandard antibacterial agents detect in the world. However, the availability of these methods does not allow the eradication of falsified medicines. In other words, the fight against this scourge should not be only limited by their detection, but it also requires effective involvement of different actors notably: health authorities, policies, international organizations, pharmaceutical industries, and pharmacists...

It has been noted that, LC method used the lower wavelength values compared to those used in UV/vis spectrophotometry: The minimum wavelength value to develop LC methods were 205 nm and 360 nm for the maximum value whereas in UV spectrophotometry the minimum value was 210 nm and 540 nm for the maximum value. This can be explained by the fact that the UV detector used in LC is more sensitive and permit a rapid detection which doesn't require great wavelength values. In their study, Kogawa *et al.* (2012), listed LC methods developed to quantify doxycycline in biologicals liquids [40]. This list indicated that the minimum wavelength value were 230 nm and 363 nm for the maximum value. It is clear that there is no significant difference between the values obtained in this study even if they studied on one molecule whereas several antibacterial agents were concerned in this study [40].

Knowledge of solubility is important in the pharmaceutical field. It allows scientists to choose the best diluent which can dissolve an active ingredient or a

combination of two or three active ingredients in a sample [65]. This choice depends on the physico-chemical characteristics of the molecules under study. We reported in this study that water was the most used diluent, followed by methanol. This confirms that water is not only the main diluent but also the most used in the pharmaceutical field [65] [66]. In addition, the use of water has many advantages notably: ease to use, not dangerous for the manipulator and the environment and improvement of reactivity and selectivity. It is also less expensive compared to several other diluents [65] [66]. This is interesting for developing countries where acquisition solvent is a great problem to analyze drugs in laboratories. However, medications are often slightly soluble in water and their solubility should be increased. The study conducted by Jouyban (2008) indicated the methods used to improve the aqueous solubility of drugs, among these methods there is co-solvability, hydrotropism, complexation, ionization and the use of surfactants [67]. In this study, the active ingredients diluted in water were clavulanic acid, amoxicillin, cefoperazone, ceftiofur and cefpirome for HPLC and ceftiofur, gatifloxacin, imipenem and moxifloxacin for UV/vis spectrophotometry. For meropenem, Mendez *et al.* (2003) used water as a diluent to develop both methods [32].

By analyzing antibacterial agents for which water has been used as diluent, 6 of them belonged to beta-lactam group and 2 to quinolones. The beta-lactams are constituted of penicillins and cephalosporins are weak acids characterized by high polarity and form the soluble salts in water [68]. On the other hand, quinolones are also weak acids but their aqueous solubility is low [69].

The choice of mobile phase depends primarily on the nature of the compounds to be separated. The mixture acetonitrile:phosphate buffer were the most used (11 studies) for methods development, followed by methanol: buffer (5 studies). The predominance of the use of acetonitrile can be justified by its physical properties which are exceptionally well adapted in LC. In addition mobile phases which containing other solvents often provide chromatograms wide and asymmetry peaks [70]. Yet, acetonitrile has some disadvantages including its toxicity and its high cost compared to other solvents such as acetone and methanol.

5. Conclusion

The aim of this study was to summarize liquid chromatography and UV/vis spectrophotometry methods developed and validated to detect antibacterial agents of poor quality in terms of active ingredient content in order to assess the impact of these available methods in the fighting against counterfeit/substandard medicines. It has been reported in this study that the antibacterial agents for which methods were developed and validated were the most concerned by worldwide detected non-conformities. Indeed, antibacterial agents for which methods were developed and validated belonged to beta-lactam group (39%), followed by quinolones (18%). In addition, amoxicillin (12%) was the active ingredient of the most concerned by the development and validation methods. This present study

showed that it is obvious that the fight against substandard and falsification agent antibacterial should not be only limited by their detection, but it also requires an effective involvement of different actors notably: health authorities, international organizations, pharmaceutical industries etc. Moreover, the tracking of poor quality drugs cannot be therefore interrupted as far as the counterfeiters do not admit to being defeated. Despite the development of several methods to detect falsified drugs, counterfeiters continue to develop strategies to escape to the detection of falsified drugs that they are producing. Thus, there is a need to diversify and update methods detection. In addition, considering that falsified drugs are mainly consumed in poor countries, it is desirable to propose methods which are financially and technically feasible and require a minimum of infrastructure.

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Conflicts of Interest

The authors declare no conflict of interest.

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