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ORIGINAL ARTICLE

Comparative dissolution study on counterfeit medicines of PDE-5 inhibitors

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Abstract Counterfeit medicines are a growing problem in both developing and industrialised countries. In general the evaluation of these medicines is limited to the identification and the dosage of the active ingredients. In this study in vitro dissolution tests were conducted on two sets of counterfeit medicines containing PDE-5 inhibitors (sildenafil citrate and tadalafil). The dissolution profiles were statistically compared to the ones of the genuine products using the f_2-method and a comparison at each time point using the Cochran test.

The results showed low equivalences between counterfeit and genuine products as well as higher variations around the mean dissolution value at the different time points for the counterfeit products.

1. Introduction

Nowadays it is estimated that about 10–15% of the medicine market worldwide is covered by counterfeit products. However, the highest precedence of counterfeit medicines can be found in developing countries with a weak regulatory system, where it is estimated that about 30% of the medicines on sale are counterfeited. In some countries this percentage can even be as high as 60% of the medicines on sale. In industrialised countries the problem is less severe with less than 1% of the medicine market affected [1–3]. Between industrialised and developing countries, differences also exist in the type of counterfeit medicines encountered. In developing countries life-saving medicines, like antibiotics, anti-malaria products, HIV-inhibitors, etc. are often counterfeited [4–7], while in industrialised countries they are mainly life-style drugs like PDE-5 inhibitors and anorexics [8]. However, it should be mentioned that counterfeited antineoplastic and cardiovascular drugs were also detected in the western world [9].

The treatment of diseases with high untreated mortality rates such as malaria, pneumonia, meningitis, and AIDS with counterfeit medicines will lead to not only an increase of mortality and morbidity, but also an increasing risk of developing microbial resistance. The latter is due to the fact that counterfeit medicines

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often contain active ingredients in sub-therapeutic dosages. In this case, even genuine medicines can become inefficient [10]. For counterfeited life-style drugs, the risks are more situated in the presence of toxic impurities or components, unexpected active ingredients or unauthorized and untested analogues or designer molecules, a wrong dosage, exceeding the maximum daily intake and wrong, missing or inadequate information concerning the use of the drug [8].

WHO [11] defines the counterfeit drug as: "one which is deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without the active ingredients, with insufficient active ingredient or with fake packaging." Next to this definition WHO also defines the substandard medicine (also called out of specification (OOS) products) as "a genuine medicine produced by manufacturers authorized by the National Medical Regulatory Authority which do not meet the quality specifications set for them by national standards." By definition, the latter group of medicines should not be present in the market. If they are, a problem has occurred with the control of the legitimate supply chain or there have been unscrupulous activities and reselling of those medicines that should have been destroyed [10].

In literature numerous papers can be found in which several analytical techniques are used for detection and characterisation of counterfeit medicines [1]. These papers, however, limit themselves to the detection of counterfeits, the identification of the active ingredients, the dosage of these active components, and in some cases the presence of impurities. Though the effectiveness, the toxicity and the risks for public health can also be influenced by other parameters, it should be kept in mind that most of the counterfeit medicinal products on the market are manufactured without taking into account the principles of good manufacturing procedures and that they are sold without any kind of quality control. This may lead to the fact that even if products are found with the correct active ingredient and in the correct dose, the quality of the finished product can influence the effectiveness/secondary effects of the product. These influences can be due to the type or nature of the used excipients, which can differ from the ones of the genuine products, to the production process like the pressure used to compress the tablets, and to the storage and transport conditions of the finished products.

Only a limited number of papers have already addressed this issue by performing in vitro dissolution tests with genuine, generic and counterfeit medicines. Most of these studies were conducted for anti-malarial products on the market in developing countries [4,12–14]. Gaudiano et al. [4] performed a quality analysis of counterfeit and substandard anti-malarials on the informal market in Congo, Burundi and Angola. They found that about 50% of the samples showed sub-standard technological properties and very low dissolution profiles, which could affect bioavailability and bioequivalence in comparison with branded products [4].

In this paper a dissolution study for counterfeit and imitation samples, containing PDE-5 inhibitors, was performed by using in vitro dissolution testing. Genuine, counterfeit and imitation samples of Viagra® and Cialis® were analysed in order to obtain dissolution profiles. These profiles were statistically compared. To our knowledge it is the first time such a study is conducted on counterfeit products found on the European market. As a consequence the knowledge about the dissolution profiles of counterfeit products is important in the risk assessment of these products. This risk assessment can both be used to focus custom campaigns on the most dangerous products and otherwise to sensitise the public for the problem based on the scientific data, in order to lower the demand for these counterfeit products.

2. Materials and methods

2.1. Samples

All counterfeit and imitation samples were donated by the Federal Agency for Medicines and Healthcare Products (FAMHP) in Belgium. Genuine samples of Viagra® were kindly provided by Pfizer SA/NV (Puurs, Belgium). Eli Lilly SA/NV (Benelux) kindly provided genuine samples of Cialis®.

2.2. Chemicals and reagents

The reference standards for sildenafil citrate and tadalafil were kindly donated by Pfizer SA/NV (Puurs, Belgium) and Eli Lilly SA/NV (Benelux), respectively.

Acetonitrile HPLC grade was purchased from Biosolve (Valkenswaard, the Netherlands). Formic acid 99% and sodium dodecyl sulphate were purchased from VWR international (Leuven, Belgium). Ammonia solution 25% (m/m) and chloric acid 36% (m/m) were purchased from Merck (Darmstadt, Germany).

2.3. Instrumental conditions

2.3.1. Dissolution

The dissolution tests were performed using a Sotax AT6 and a Sotax AT7 Smart dissolution device (Sotax, Alschwil, Switzerland). The dissolutions were performed following test device 2 (paddle) as described by the European Pharmacopoeia [15]. The rotation speed was set at 50 rpm and the vessels temperature at 37.0 ± 0.5 °C. For the sildenafil-containing samples 1 L of 0.5% sodium dodecyl sulphate (SDS) was used as dissolution medium, while 900 mL of 0.01 M HCl was used for the tadalafil-containing samples.

Two millilitres of dissolution medium were sampled at 5, 10, 15, 30, 45, 60 and 120 min, respectively, after starting the test. The amount of dissolution medium sampled was not replaced. The loss of medium was corrected during calculation of the dissolution profile [16]. Due to the limited amount of sample available, all the samples were only tested in triplicate.

Tablets and gels were just added to the dissolution medium at the start of the dissolution tests. Capsules and soft caps were positioned in a metallic scaffold, especially designed for dissolution tests with apparatus 2, and added to the dissolution medium at the start of the tests.

2.3.2. HPLC

The quantifications in both samples and dissolution media were performed on a Waters 2695 Alliance® chromatographic system (Waters Corporation, Milford, USA). The system consisted of a quaternary pump, a temperature controlled autosampler and a column heater, coupled to a Waters® 2998 Diode Array Detector (DAD). The output signal was monitored and processed using the Waters Empower®2 software. The analysis was performed on a Waters XTerra RP18 (150 mm × 4.6 mm, 5 µm) chromatographic column using a gradient with a 10 mM ammonium formate buffer

pH 3.5 as aqueous phase and acetonitrile as organic modifier. The gradient started at 70% buffer. The gradient reached 65% buffer in 5 min and went further down to 55% in 3 min. Afterwards a plateau of 20% buffer reached in one minute and held for two minutes before returning to the initial conditions. The flow was 1 mL/min; the column temperature was set at 30 °C; the sample temperature was set at 15 °C and detection was performed at a wavelength of 254 nm. For the samples containing sildenafil citrate the injection volume was set at 5 µL, while for the tadalafil-containing samples it was set at 20 µL [17].

2.4. Preparation of standards and samples

Stock solutions of sildenafil citrate and tadalafil of 1.0 mg/mL and 0.2 mg/mL, respectively, were prepared using acetonitrile/water (50/50) as solvent.

Calibration standards of 25, 50, 100 and 150 µg/mL for sildenafil citrate were prepared by dilution of the stock solution with acetonitrile/water (50/50). Tadalafil calibration standards of 5, 10, 20 and 40 µg/mL were prepared using the same procedure.

Before dissolution the dosage of sildenafil citrate and tadalafil in each of the samples was assayed. This was done on one dosage unit, due to the limited amount of sample at our disposal. The dosage unit was brought into 100 mL of acetonitrile/water 50/50 and stirred for at least 30 min. The obtained solutions were then diluted 10 times with the same solvent and filtered through a 0.2 µm polytetrafluoroethylene syringe filter (∅ 13 mm, VWR international). Each sample solution was injected twice. Concentrations were back calculated using the calibration lines obtained with the prepared standards and calculated using least-squares regression.

2.5. Calculations

All calculations were performed in Excell® 2010 for Microsoft Windows®. The dissolution profiles were compared using the f_2-method as recommended by the United States Pharmacopoeia [18–20]. The percentages of dissolution at each time point were compared using the Cochran test, which is an adapted *t*-test that can be used in the case of heteroscedastic data [21].

3. Results and discussion

3.1. Quantification of the active ingredients

Eighteen counterfeit and imitation samples for both Viagra® and Cialis® were tested for their dissolution. For each sample the content of sildenafil and tadalafil was determined on one dosage unit. This was done due to the limited number of dosage units per sample available.

3.1.1. Sildenafil-containing samples

Table 1 shows the set of 18 samples containing sildenafil used for the dissolution testing. Based on the packaging, the marks on the blisters and/or the tablets, all the samples should contain 100 mg of sildenafil per dosage unit. However, the content determination on one dosage unit showed that the content varied between 51.57 and 114.33 mg per dosage unit and that 6 samples (samples 1, 3, 13, 14, 15 and 18) did not meet the quality criteria of 95–105% of the active ingredient as set for pharmaceutical specialities. These results should be treated with caution since the dosage was determined on only one dosage unit and following the European pharmacopoeia criteria for content uniformity, an individual dosage unit can deviate between 75% and 125% of the dosage claimed by the manufacturer [15]. This would mean that only two samples (samples 1, 13) do not meet the quality criterion for content.

3.1.2. Tadalafil-containing samples

Table 2 shows the 18 counterfeit samples containing tadalafil used for dissolution testing.

Based on the packaging, the marks on the blisters and/or the tablets, all samples should contain 20 mg of tadalafil per dosage unit. However, the content determination on one dosage unit showed that the content varied between 17.15 and 21.85 mg per dosage unit and that 9 samples (samples 3–6, 10, 12 and 15–17)

Table 1 Counterfeit samples of Viagra® with their galenic form and their content determined on 1 dosage unit.

Sample no.	Product name	Galenic form	Content (mg/unit)	% theoretical content
1	–	Tablets	51.57	51.57
2	–	Soft tablets	104.85	104.85
3	Viagra	Tablets	87.03	87.03
4	Suhagra	Filmcoated tablets	98.56	98.56
5	–	Tablets	103.79	103.79
6	Filagra	Tablets	99.55	99.55
7	Kamagra	Chewing tablets	102.81	102.81
8	–	Filmcoated tablets	101.63	101.63
9	–	Filmcoated tablets	96.13	96.13
10	–	Filmcoated tablets	96.73	96.73
11	–	Soft tablets	100.05	100.05
12	Lady-Era	Tablets	97.77	97.77
13	Sildigra	Soft tablets	59.16	59.16
14	Afrika black ant rouge	Capsules	114.33	114.33
15	Hercules capsules	Capsules	94.46	94.46
16	Vajra	Capsules	100.67	100.67
17	Hard Ten Days	Capsules	96.16	96.16
18	Kamagra	Oral gel	92.68	92.68

Table 2 Counterfeit samples of Cialis® with their galenic form and their content determined on 1 dosage unit.

Sample no.	Product name	Galenic form	Content (mg/unit)	% theoretical content
1	–	Filmcoated tablets	21.01	105.05
2	–	Tablets	19.12	95.60
3	–	Filmcoated tablets	18.90	94.50
4	Tadora	Filmcoated tablets	18.39	91.95
5	–	Soft tablets	18.03	90.15
6	–	Chewing tablets	17.15	85.75
7	–	Filmcoated tablets	19.60	98.00
8	–	Filmcoated tablets	19.01	95.05
9	–	Filmcoated tablets	20.54	102.70
10	Erectafil ST	Soft tablets	18.36	91.80
11	–	Chewing tablets	21.08	105.40
12	TADALA	Filmcoated tablets	18.25	91.25
13	–	Tablets	21.22	106.10
14	TAZALIS	Tablets	20.78	103.90
15	–	Filmcoated tablets	18.86	94.30
16	–	Filmcoated tablets	18.37	91.85
17	–	Filmcoated tablets	21.85	109.25
18	TDFIL	Tablets	19.24	96.201

did not meet the quality criteria of 95–105% of the active ingredient as set for pharmaceutical specialities. Again caution is advised with these results since the quantification was performed on a single dosage unit.

3.2. *Dissolution tests*

3.2.1. *Sildenafil-containing samples*

Each of the counterfeit/imitation samples as well as a genuine Viagra® sample containing 100 mg sildenafil was tested for dissolution. These tests were performed in triplicate (three dosage units per sample), due to the limited amounts of sample units available.

Fig. 1A shows the mean dissolution profiles (of three dosage units) of the 12 counterfeited tablets tested and the genuine product. Fig. 1B shows the ones obtained for the four capsules, the soft capsules, the oral gel and the genuine product. The standard errors at each time point are given in Table 3. The figures represent the cumulative percentages of sildenafil liberated from the formulations in function of time, where 100% dissolution is reached when the complete amount of sildenafil present in the formulation is liberated. From the profiles it can be concluded that for the genuine product 100% (±4.51%) dissolution is reached at 30 min. This is also the case for samples 1, 5, 6 and 7, but it can be observed that the release from these samples is slower during the first 15 min, compared to the genuine tablets.

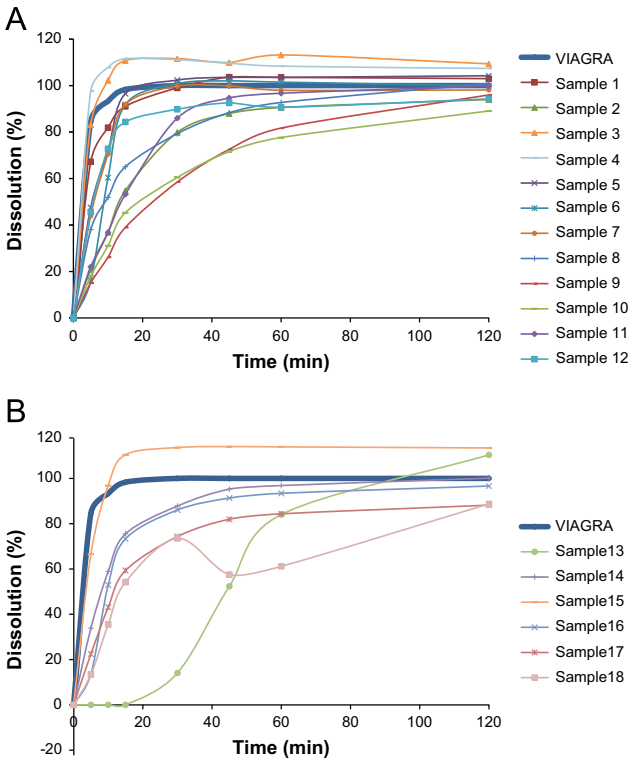

Fig. 1 (A) Mean dissolution profiles of Viagra® 100 mg and 12 counterfeited tablets; (B) mean dissolution profiles of Viagra® 100 mg and the samples of capsules (samples 14, 15, 16, 17), soft capsules (sample 13) and oral gel (sample 18).

For seven samples, from which four were tablets (samples 2, 9, 10 and 12), two capsules (samples 16 and 17) and the last oral gel (sample 18), 100% release was not reached within the duration of the test (120 min). The oral gel (sample 18) also showed a decreased dissolution between 30 and 45 min. This was confirmed when repeating the test with three new samples and is possibly due to the exhaustion of the solubilising effect of the gel compounds. For the tablets of sample 3 and the capsules of sample 15, 100% dissolution was yet achieved at 10 and 15 min, respectively, while for the tablets of samples 8 and 11 and the capsules of samples 13 and 14, the 100% release was attained at 120 min.

From the dissolution profiles it could be observed that for 14 of the 18 samples the release of sildenafil from the dosage units was slower compared to Viagra®.

For an objective comparison of the dissolution profiles the f_2-values as described by Freitag et al. [18] were calculated. It is considered that two dissolution profiles are equivalent when the f_2-value is between 50 and 100, which means that the profiles show a difference of maximum 10% at each time point.

Table 4 shows the f_2-values calculated for each of the 18 counterfeited samples toward the dissolution profile of genuine Viagra® 100 mg. From this table it can be seen that only sample 1 has an f_2-value above 50, meaning that the dissolution profile of this sample can be considered as equivalent to the one of the genuine product, though it should be kept in mind that this sample contains only 51% of the labelled dose (100 mg) and therefore cannot be considered as equivalent to the genuine product. All other samples have f_2-values situated in the range of 7–50, where samples 3 and 4 have values close to 50. These samples can therefore be considered as not equivalent to Viagra® 100 mg.

Table 3 Standard errors for each time point of the dissolution of Viagra® and the 18 counterfeited/imitated samples.

Sample no.	Time (min)							
	0	5	10	15	30	45	60	120
VIAGRA	0.00	13.22	9.29	6.34	4.51	3.29	3.32	2.59
1	0.00	6.46	4.53	1.81	1.79	1.03	0.58	0.38
2	0.00	16.08	17.31	19.89	12.66	7.38	5.37	2.20
3	0.00	10.17	1.83	1.46	2.00	1.69	7.69	2.34
4	0.00	5.05	3.68	1.59	1.72	1.57	1.54	1.05
5	0.00	13.45	15.70	5.10	2.12	0.61	0.81	0.88
6	0.00	150.96	17.14	7.36	2.81	1.24	0.93	1.29
7	0.00	16.04	12.60	8.23	1.87	1.95	1.37	0.53
8	0.00	17.50	19.59	18.06	10.45	5.68	3.29	1.03
9	0.00	7.67	3.56	3.78	9.38	9.46	8.77	5.31
10	0.00	38.11	30.63	31.84	21.65	17.74	15.19	9.27
11	0.00	9.64	8.78	11.61	8.14	5.35	4.55	3.15
12	0.00	10.02	13.07	11.29	6.84	4.67	0.58	3.60
13	0.00	0.00	0.00	173.21	57.32	15.80	6.51	4.67
14	0.00	3.00	4.19	4.42	4.17	3.34	3.35	2.05
15	0.00	29.41	14.38	15.73	13.06	11.07	9.79	8.98
16	0.00	84.65	20.46	15.98	11.87	9.22	8.40	7.09
17	0.00	75.49	28.56	15.80	13.39	15.84	14.33	14.91
18	0.00	84.13	11.22	7.02	18.22	83.74	83.47	3.62

Table 4 f_2-Values calculated for the 18 counterfeited samples toward the dissolution profile of Viagra® 100 mg.

Sample no.	f_2-Value	Sample no.	f_2-Value	Sample no.	f_2-Value
1	**53.07**	7	37.41	13	7.36
2	21.31	8	27.38	14	29.90
3	*49.44*	9	16.23	15	43.73
4	*47.24*	10	17.38	16	24.08
5	39.18	11	21.80	17	21.94
6	26.95	12	36.51	18	39.05

In order to obtain a more thorough comparison of the dissolution profiles, the dissolution at each time point was compared statistically. Since the *F*-tests at the different time points showed that the data were heteroscedastic, an adapted *t*-test, called the Cochran test [21], was performed in which the mean dissolution at each time point of the counterfeited samples was compared to the value obtained for the genuine product. Table 5 shows the *t*-values as calculated for each time point and each counterfeit sample toward the dissolution of the genuine product. From the results it can be concluded that only for two samples (samples 1 and 15) the *t*-value is smaller than the critical value (2.920) and this at each time point. This means that based on the Cochran test the dissolution profiles of these two samples are equivalent to the one of the genuine product, though again sample 1 contains only 51% of the labelled dose and can therefore not be considered as equivalent with the genuine product. For all other samples significant differences are observed for at least one time point.

For sample 1 both the f_2-value and the Cochran test indicate an equivalence with the dissolution of the genuine product, since the f_2-value is higher than 50 and the Cochran test does not show a statistically significant difference for the different time points in the profile. For sample 15, however, the f_2-value was lower than 50 (43.7) while the Cochran test could not detect any difference between the dissolution values at each time point of the sample and the genuine product. When examining the dissolution profiles (Fig. 1B) it can be seen that the dissolution of sample 15 is indeed less equivalent to that of Viagra® compared to sample 1. The standard deviations for sample 15 are much higher than for sample 1 and the genuine product. To conclude it can be stated that none of the samples can be considered as equivalent to the genuine product, since sample 1 contains only 50% of the labelled dose and the f_2-value for sample 15 is lower than 50.

3.2.2. *Tadalafil-containing samples*

As for the samples containing sildenafil, each of the selected counterfeit/imitation samples as well as a genuine Cialis® sample, containing 20 mg of tadalafil, was tested for dissolution. Again the dissolutions were only performed in triplicate.

Fig. 2 shows the mean dissolution profiles obtained for the 18 counterfeited/imitation tablets and the genuine product. The standard errors at each time point are given in Table 6. The figures represent the cumulative percentages of tadalafil liberated from the formulations in function of time, where 100% dissolution

Table 5 *t*-Statistics calculated for the Cochran test at each time point for each counterfeited samples of Viagra® 100 mg (absolute values lower than the critical *t* value (2.920) are indicated in bold).

Sample no.	Time (min)						
	5	10	15	30	45	60	120
1	**2.510**	**2.092**	**1.947**	**0.358**	**−1.819**	**−1.817**	**−1.986**
2	9.456	9.082	6.007	3.155	**2.871**	**2.776**	3.147
3	**0.172**	**−1.746**	−3.348	−3.990	−4.550	**−2.448**	−4.452
4	**−1.846**	**−2.634**	−3.535	−3.945	−4.474	−3.890	−4.540
5	4.994	**2.605**	**0.394**	**−0.818**	**−1.834**	**−1.778**	**−2.665**
6	4.463	4.222	**1.216**	**−0.267**	**−0.989**	**−0.749**	**0.050**
7	5.331	3.179	**1.132**	**0.096**	**0.073**	**0.981**	**1.244**
8	6.206	5.362	4.337	3.797	3.376	**2.802**	**−0.609**
9	10.711	13.337	16.059	10.120	6.244	4.008	**1.217**
10	8.816	8.363	5.840	4.948	3.751	3.161	**2.189**
11	9.531	10.576	8.909	**2.918**	**1.531**	**1.036**	**0.083**
12	5.592	**2.760**	**2.123**	**2.319**	**2.383**	4.807	**2.321**
13	13.097	18.643	27.252	16.110	9.256	4.318	−3.119
14	7.820	6.599	5.577	3.637	**1.793**	**1.170**	**−0.058**
15	**1.384**	**−0.372**	**−1.138**	**−1.520**	**−1.862**	**−2.072**	**−2.214**
16	7.637	5.031	3.262	**2.165**	**1.667**	**1.340**	**0.799**
17	5.299	5.772	5.995	4.070	**2.334**	**2.172**	**1.528**
18	7.791	10.492	10.459	3.247	**1.522**	**1.314**	4.734

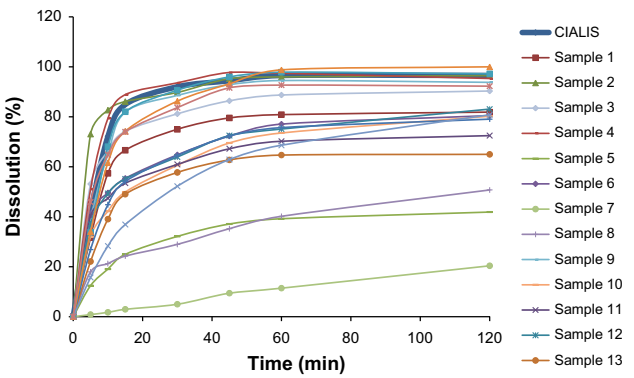

Fig. 2 Mean dissolution profiles of Cialis® 20 mg and 18 counterfeited tablets.

is reached when the complete amount of tadalafil present in the formulation is liberated. From the profiles it can be concluded that for the genuine product a maximum of 96.54% (±1.53%) dissolution is obtained after 1 h. Only one sample (sample 15) attains an equivalent percentage dissolution after 1 h. Two other samples (samples 2 and 4) reach a similar plateau, with the difference that the plateau is already attained at 45 min. Four samples (samples 1, 9, 13 and 18) reach a plateau at 60 min, but lower percentages of dissolution are attained. One sample (sample 16) reaches a dissolution of 100% after 2 h, while for all other samples no plateau is obtained. For these samples the amount of tadalafil released is still increasing after 2 h and the percentage dissolution for these samples at 2 h varies between 20% and 90%.

Visual inspection of the profiles also shows that for 11 samples the release of tadalafil from the formulation is slower than for the genuine product. For three samples (samples 2, 3 and 4) the release is faster, while for the remaining samples (samples 9, 10, 15 and 16) a comparable release rate can be observed.

Again for each of the counterfeited/imitated samples the f_2-values were calculated toward the dissolution profile of Cialis® 20 mg (Table 7). From these values it can be concluded that for six samples (samples 3, 4, 9, 15, 16 and 18) an f_2-value between 50 and 100 was obtained, showing that they have an equivalent dissolution profile with the genuine products. For the other twelve samples, the f_2-values are situated in the range between 5 and 45.

As for the sildenafil-containing samples, the Cochran test was performed for each of the counterfeited/imitated samples toward the dissolution profile of Cialis® 20 mg. The *t*-values for each of the 18 samples are given in Table 8. From the results it can be concluded that when all time points are compared individually, only for three samples (samples 4, 9 and 15) no statistically significant differences could be observed for all the time points compared to Cialis® 20 mg. For all other samples significant differences could be observed for at least one time point.

Three of the six samples (samples 4, 9 and 15) considered equivalent to Cialis® 20 mg based on the f_2-values are also considered as equivalent based on the Cochran test. For samples 3, 16 and 18 this is not the case. Samples 16 and 18 show significant differences for the amounts of tadalafil released at 30 and 120 min, respectively. It should also be mentioned that for these three samples standard deviations of the dissolution values were much higher than the ones obtained for samples 4, 9 and 15. For sample 3 the dissolution is significantly different from the one obtained for Cialis® 20 mg from 30 min on. Sample 3 can be considered as a borderline sample. It shows an f_2-value of 52.25, which is near the limit of 50 and the results of the two tests performed are not consistent. For this sample it would be advised to repeat the dissolution testing with more units, possibly resulting in less variation on the results and a lower f_2-value. Due to a lack of samples, this extra test could not be performed.

Generally it could be concluded that based on the two statistical tests performed and taken into account that the analysis was only performed in triplicate, samples 4, 9 and 15 can be considered as equivalent to Cialis® 20 mg, since the f_2-values were higher than 50 and at no time point a significant difference was detected with the Cochran test. For sample 3 no conclusion could be formulated.

Table 6 Standard errors for each time point of the dissolution of Cialis® and the 18 counterfeited/imitated samples.

Sample no.	Time (min)							
	0	5	10	15	30	45	60	120
CIALIS	0.00	33.37	24.20	12.77	2.30	2.33	1.53	1.54
1	0.00	40.94	29.35	18.16	11.23	7.79	5.84	5.08
2	0.00	2.48	1.99	0.97	1.69	3.10	1.14	0.71
3	0.00	13.60	7.92	5.18	2.71	0.77	1.80	0.70
4	0.00	25.45	8.22	5.92	1.55	1.34	1.22	0.88
5	0.00	6.27	12.67	9.03	2.52	1.92	1.03	1.09
6	0.00	3.42	3.36	4.59	2.46	1.76	1.83	2.57
7	0.00	20.31	15.48	23.20	26.43	23.88	26.94	19.72
8	0.00	39.74	43.78	42.05	38.73	31.09	28.86	20.77
9	0.00	11.24	6.50	0.92	0.77	2.32	1.79	1.85
10	0.00	5.97	6.76	3.62	2.92	2.62	1.21	0.75
11	0.00	10.63	7.34	6.83	5.03	4.68	3.33	4.41
12	0.00	15.67	0.20	1.78	3.65	5.19	6.47	6.19
13	0.00	29.56	26.13	19.89	18.76	16.52	15.88	13.43
14	0.00	9.51	9.20	9.83	7.47	5.84	5.51	3.73
15	0.00	23.42	7.17	2.99	1.91	1.94	1.43	1.72
16	0.00	11.61	3.67	2.39	1.43	2.52	1.87	1.59
17	0.00	7.40	3.51	7.77	1.43	3.12	3.07	1.97
18	0.00	6.66	5.04	4.88	3.71	3.68	3.80	2.21

Table 7 f_2-Values calculated for the 18 counterfeited samples toward the dissolution profile of Cialis® 20 mg.

Sample no.	f_2-Value	Sample no.	f_2-Value	Sample no.	f_2-Value
1	40.81	7	5.75	13	25.50
2	44.99	8	13.82	14	31.32
3	**52.25**	9	**71.13**	15	**71.06**
4	**64.99**	10	29.50	16	**57.74**
5	13.37	11	29.58	17	22.87
6	33.28	12	33.00	18	**58.89**

4. Conclusions

In general it can be concluded that for the counterfeit samples high variations of the dissolution values (Tables 3 and 6) were observed and this both between the dosage units of one sample as between the different counterfeited samples. Moreover, it was observed that for the majority of the samples (sildenafil and tadalafil), higher standard deviations were found at time points of 5, 10 and 15 min compared to the deviations found for the genuine products. The other time points showed lower standard deviations, which is characteristic for dissolution profiles reaching a plateau. The higher variations at the beginning of the dissolution profiles might be due to a difference in disintegration between the samples, but also between the dosage units of one sample. This can inherently be related to the quality of the counterfeit/imitation products. Possible factors responsible for the lower quality of the tablets are the variable particle size of the active ingredients, the use of less qualitative primary substances, the use of different excipients or the correct excipients in different amounts, improper transport and storage conditions, and differences in production process as to high compression for tablet production or low moisture content.

The results also showed that for the sildenafil-containing samples no sample and for the tadalafil-containing samples only three samples (16.7%) could be considered as having an equivalent in vitro dissolution profile with the genuine product. Taking into account that in vitro dissolution is an indicator for bioequivalence, this indicates that even when in a counterfeit product the correct active ingredient and dosage is found, the product can still be considered as a risk for public health. If the trend observed for PDE-5 inhibitors could be extended to life-saving medicines like antibiotics, anti-viral and anti-parasitic products, this can lead to ineffective treatment, development of resistance, and a loss of faith in health care, regulating authorities and the pharmaceutical industry.

Finally, it can also be stated that for the comparison of dissolution profiles, especially when not enough dosage units are available, the combination of both the f_2-methods as the comparison of the dissolution at each time point can be very valuable, leading to objective conclusions in line with visual observations.

Table 8 *t*-Statistics calculated for the Cochran test at each time point for each counterfeited samples of Cialis® 20 mg (absolute values lower than the critical *t* value (2.920) are indicated in bold).

Sample no.	Time (min)						
	5	10	15	30	45	60	120
1	**0.911**	**1.054**	**1.953**	3.395	3.916	5.484	5.764
2	−3.958	**−1.176**	**−0.314**	**−0.379**	**−0.384**	**0.599**	**−0.163**
3	**−1.263**	**0.555**	**1.619**	6.151	6.040	6.235	6.696
4	**−0.844**	**−0.671**	**−0.562**	**−1.044**	**−2.233**	**−0.799**	**1.249**
5	3.637	5.217	9.380	45.816	42.865	64.939	60.965
6	**0.060**	**2.230**	4.601	17.829	15.057	16.525	10.964
7	5.085	6.984	13.067	60.511	46.769	43.217	30.811
8	**2.633**	4.456	7.062	9.579	9.178	8.356	7.466
9	**0.331**	**0.681**	**0.486**	**2.692**	**0.876**	**1.551**	**2.093**
10	**1.185**	2.931	5.520	19.715	15.072	23.129	17.615
11	**0.162**	**2.406**	4.770	14.461	12.274	16.497	11.830
12	**0.525**	**2.266**	4.740	15.475	8.703	7.313	4.359
13	**2.209**	**2.834**	4.266	5.382	5.178	5.315	6.181
14	**1.815**	**2.638**	4.423	9.054	8.037	8.170	9.180
15	**0.852**	**0.389**	**0.455**	**0.872**	**−0.930**	**−1.131**	**−0.499**
16	**0.917**	**1.034**	**1.693**	4.076	**0.661**	**−1.655**	**−2.748**
17	3.224	4.344	7.420	30.752	18.489	18.824	12.545
18	**−0.509**	**0.712**	**1.659**	3.854	**1.213**	**1.746**	2.950

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2014.03.002.

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