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Research Article

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Quality assessment of seized Sildenafil-labelled tablets

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Abstract Phosphodiesterase type 5-inhibitor (PDE5-i) such as sildenafil and its analogues are among the most popular medicines bought through online retailers. Many reports however show that these products often are of poor quality or do not have a marketing authorization in the recipient country and thus may pose serious health threats to consumers. Qualitative and quantitative investigations were carried out on two quality attributes of sildenafil-labelled tablets, seized by the Luxembourgish customs from 2016 to 2020: sildenafil content and presence of undeclared active pharmaceutical ingredients (API). 649 samples, predominantly bought in India, were investigated. Results indicate an overall quality increase over the 5 years, but still 46.4% of the analysed samples were not conform to standard European quality regulations, with 18.2% of the products contaminated with at least one undeclared active pharmaceutical ingredients and 35.1% having a sildenafil content significantly different from claimed dosage. Despite an overall increase in quality over the last 5 years, many samples still contain adulterants with potentially serious health risks or non-conform API concentrations.

Keywords Sildenafil, Quality assessment, Contaminants, Substandard medicines

Introduction

According to the European Medicines Agency, falsified medicines are "fake medicines that pass themselves off as real, authorised medicines" [1]. In order to obtain marketing authorization in the European Union, medicines have to pass through a well-defined evaluation of quality, safety and efficacy, and can only be bought through licensed pharmacies and approved retailers [2].

While clear identification of substandard and falsified (SF) medicines is not always easy [3], they are found in all regions of the world and all types of medicines are affected [4]. The median prevalence has been estimated to be 25% in a study covering the period from 2013 to 2018 [5]. In Western Europe and North America, falsified medicines most often are "life-style" drugs, such as nootropics, steroids, anorectics and erectile dysfunction medications. SF medicines may fail to improve patients' health, may cause antimicrobial resistance, drug-resistant infections or unanticipated adverse effects, resulting in higher mortality and/or morbidity. A recent review reported more than 7000 causalities including at least 3600 deaths from 2006 to 2017 in developing and developed countries [4].

The increasing use of SF medicines is favoured by the proliferation of online pharmacies [6]. In addition, in case of drug shortages, patients may seek alternative supply routes, as experienced in the recent Covid-19 pandemic [7]. A survey conducted in the United States highlighted that many online pharmacies did not comply with practice standards or applicable laws [8], however, low prices, ease of access and relative anonymity make them highly attractive. The number of people purchasing medicines from internet pharmacies has increased in recent years [9] and the trend suggests a continuous increase for the future [10], despite the well-documented risks [11].

Not surprisingly, PDE5-i, prescribed for treatment of erectile dysfunction, are among the most popular medicines sold online [12]. Severe side effects have been reported in the literature. In Singapore [13] and in the United States [14], PDE5-i tablets had been contaminated with glibenclamide, an antidiabetic prescribed to treat diabetes mellitus type 2,

resulting in several deaths. A case of hepatotoxicity induced by adulterated sildenafil has been described [15]. A recent report from Japan revealed that only 40% of vardenafil tablets purchased on the internet were genuine [16].
In this article, the results of an investigation on the quality of sildenafil-labelled tablets seized by the Luxembourgish customs from 2016 to 2020 are presented. The goal was to determine their quality in terms of API content, presence of contaminants and undeclared APIs. The stated API and screening for contaminants were assessed using gas chromatography coupled to a mass spectrometer (GC/MS). Sildenafil was quantified using high performance liquid chromatography coupled to a UV detector (HPLC/UV).

Material and Methods

Sample collection

Packages suspected to contain unapproved medicinal products were seized by Luxembourgish customs. After a 6-month storage period, during which the buyer may object the seizure and claim his product, seizures are generally destroyed. Samples confiscated from January 2016 to December 2020 were anonymized and transferred to our laboratory for investigation.

Sample preparation and analysis

If available, 3 tablets from each load of sildenafil tablets were analysed. Each tablet was weighed and then divided into two parts, one was used for qualitative GC/MS analysis, and the other was weighed again, crushed, extracted, diluted, filtered and used for quantitative HPLC/UV analysis.
Qualitative GC/MS analysis was carried out on a 7890A GC coupled to a 7693 series autosampler and a 5975C mass selective detector (Agilent, Waldbronn, Germany) according to a previously published method [17]. Compounds were identified using commercial MPW, Wiley NIST and SWGDrug libraries. HPLC/UV assays were carried out on a 1290 series HPLC (Agilent, Waldbronn, Germany) equipped with a Waters Symmetry C18 column (150mm x 3.9mm x 5µm) using published isocratic chromatographic conditions [18].

Results

Sample collection

In 5 years, 5570 medicinal products were seized. 3038 products (54.5%) were labelled as PDE5-inhibitors including 991 labelled as containing sildenafil.
Among the 991 sildenafil samples, 882 (89.0%) were originating from India; other countries of origin were China, Russia, Malaysia, Philippines, Germany, Hungary, Singapore, Switzerland, and the US. Tablets and jellies were by far the most prominent dosage forms, with 649 (65.5%) and 211 (21.3%) samples, respectively against 131 (13.2%) samples with other formulation types (*i.e.*, liquid, capsules and chewable tablets). Therefore, Sildenafil labelled tablets represent 11.7% of all seized samples by Luxembourgish customs.

Screening of tablets for contaminants

118 samples (18.2%) were contaminated with at least one undeclared API. Single, double and triple/quadruple contaminations occurred 97 (14.9%), 14 (2.2%) and 7 times (1.1%), respectively. In total, 16 different contaminants were detected. Ibuprofen (anti-inflammatory) was the most frequently encountered product (38 samples), followed by dapoxetine (30 samples) and metronidazole (antibiotic, 20 samples). Undeclared PDE5-i Tadalafil and Vardenafil were present in one sample each. A summary of samples contaminations is presented in Figure 1.

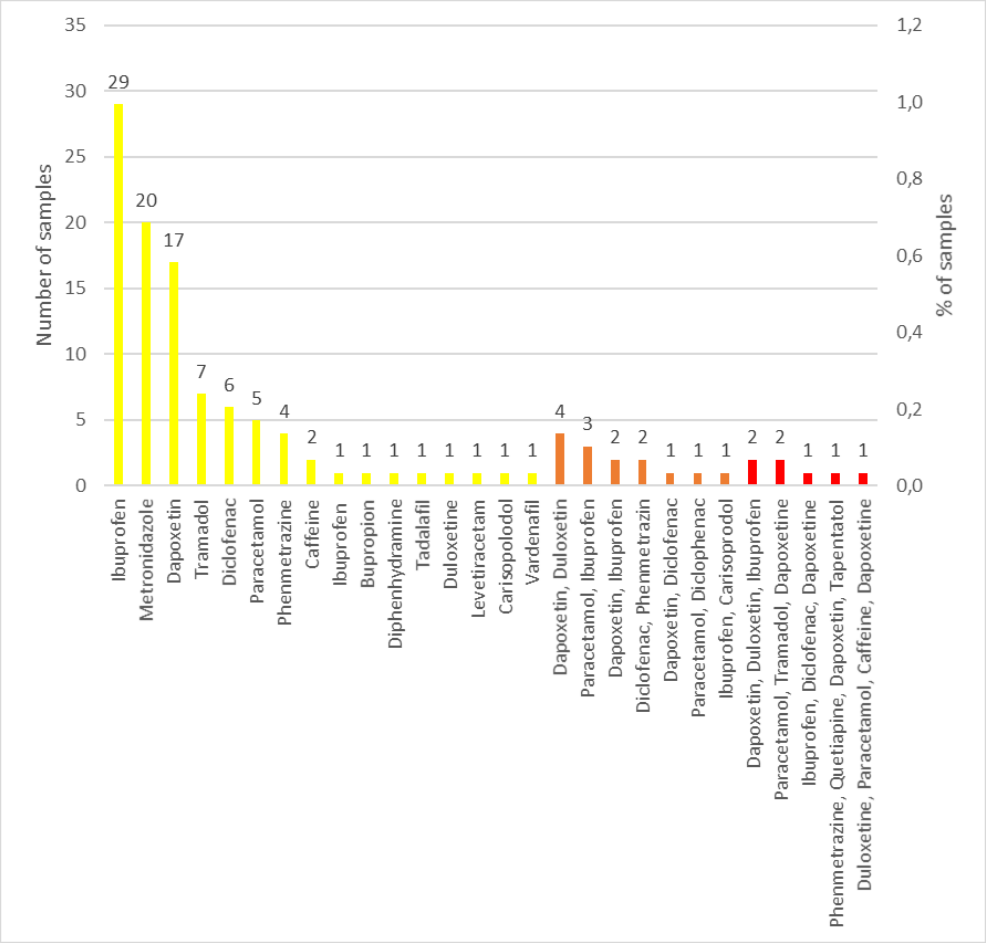


Figure 1: Undeclared contaminants in sildenafil samples (Single, double and multiple contamination separated).

The presence of contaminated samples varies from a maximum of 36.5% in 2016 to a minimum of only 1.1% in 2017. No clear trend in contamination occurrence was observed over the last 5 years (Figure 2).

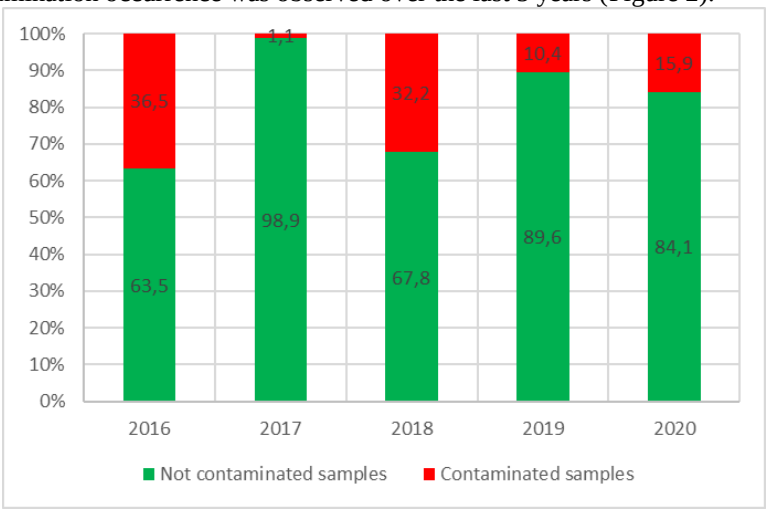
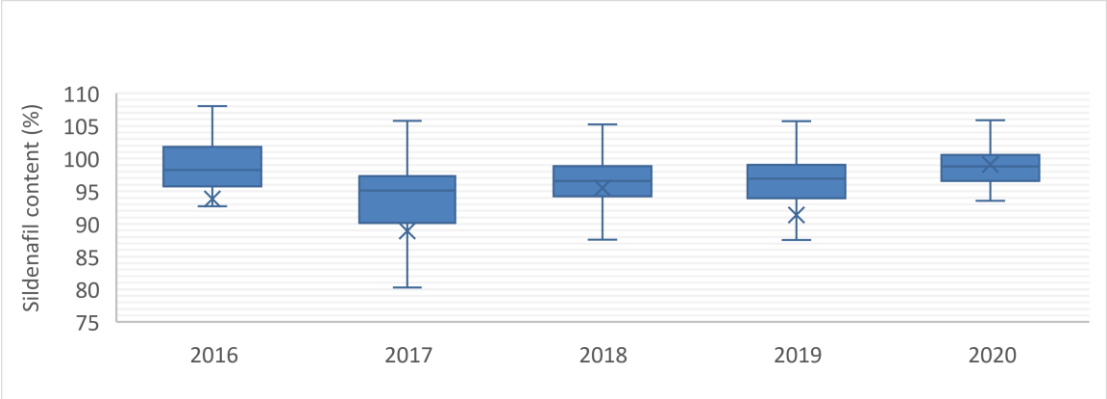


Figure 2: Percentage of contaminated samples from 2016 to 2020.

Sildenafil content in tablets. Package labelling indicated sildenafil content of 25, 50, 75, 100, 120, 130, 150 and 200 mg. Sildenafil was detected in all samples. The content of sildenafil, expressed in percentage normalized to the expected amount in the sample, was calculated using the following formula:

Sildenafil content (%) = c * V * Dilution factor * m_{sample} / $m_{½\ sample}$ * Potency factor / $cont_{exp\ sild}$ * 100
with c: measured concentration of sildenafil in the sample solution ($mg.L^{-1}$); V: Volume (0.1 L); Dilution factor: 25/5; m_{sample}: mass of the whole sample (mg); $m_{½\ sample}$: mass of half of the sample (mg); Potency factor: purity of the reference standard as indicated by the manufacturer and used for calibration; $cont_{exp\ sild}$: content per unit of sildenafil labelled on the packaging information of the sample (mg).

Variations in the sildenafil content were observed from 2016 to 2020 (Figure 3).



Mean	*93.8*	*88.9*	*95.5*	*91.3*	*99.1*
Median	98.2	95.1	96.5	96.9	98.8

Figure 3: Box plot of Sildenafil concentration distribution (x = mean).

From 2016 to 2020, global mean sildenafil concentration measured was 93.7% of labelled content; the median concentration was 97.0%, the standard deviation was 19.3% and the distribution negatively skewed. Minimum content was as low as 18.8% detected in 2017 and maximum as high as 202.8% detected in 2019. Mean and median content were always below 100% and lowest in 2017.

Sildenafil content was considered conform to label specification if the results were within 95-105% [19] of the expected content. Results <95% or >105% were considered outside specifications. In this study, 30.0% of samples were <95% of the labelled sildenafil content, 64.9% were within the 95 - 105% range and 5.1% were >105%. Several trends were observed (Figure 4):

- From 2016 to 2017: A decrease in samples with conform sildenafil content and a decrease in samples with overdose, a significant increase in samples with underdose sildenafil content,
- From 2017 to 2020: An increase of samples within the conformity acceptance criteria from 46.7% in 2017 to 78.8% in 2020 and a decrease in samples with underdose (48.9 to 12.4%).

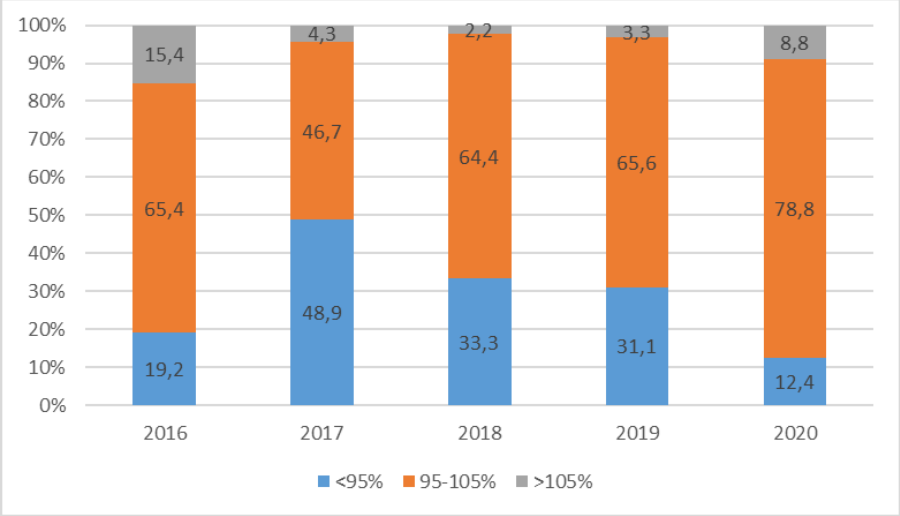


Figure 4: Percentage of conform samples related to the sildenafil content

Combined quality assessment. Combined quality was assessed by considering dosage quality and purity. Each of the two quality attributes, when considered individually, showed inconsistent trends over the years, with presence of contaminants being lowest in 2017, but expected sildenafil content conformity almost doubling from 2017 to 2020. The two quality attributes were considered together in the definition of "quality", which lead to four groups (Table 1**Error! Reference source not found.**)

Table 1: Combined quality distribution of Sildenafil tablets.

		Sildenafil content	
		95% - 105%	**<95 or >105%**
Absence of contaminant(s)	Yes	53.6% (conform)	28.2%
	No	11.2%	6.9%

Regardless the year of seizure, 53.6% of the samples were considered as conform, defined as those with sildenafil in the 95% - 105% range and absence of contaminants. Results over time are summarized in Figure 5.

A consistent and continuous improvement trend of sildenafil tablets was observed, with a highly significant increase in the frequency of conforming samples between 38.5% in 2016 and 67.3% in 2020 ($p<10^{-3}$). However, in 2020 still about one third of all samples did not reach the acceptance criteria in terms of active ingredient content and/or purity standards.

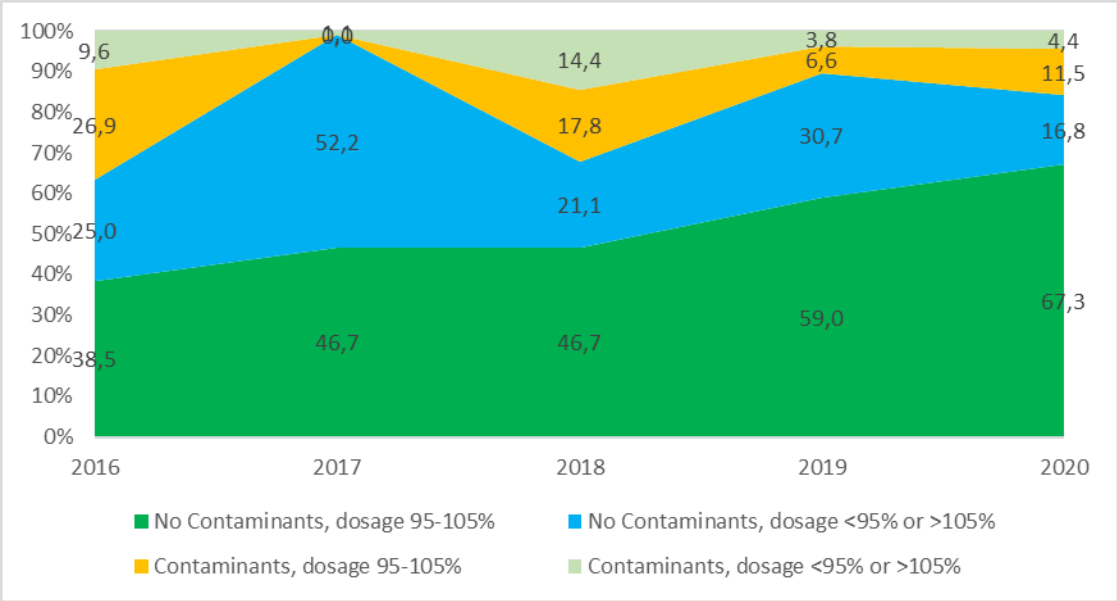


Figure 5: Summary of sildenafil sample quality

Discussion

Sildenafil was found in all samples but about 18.2% of them contained undeclared APIs. The presence of these APIs may be explained by non-standardized production chains, manufacturing errors and the absence of strict quality control, in overall the lack of GMP (Good manufacturing practices). If this is probably true in most cases, doubts remain for the presence of dapoxetine used for treatment of premature ejaculation, and pain medicines such as diclofenac, ibuprofen and paracetamol. An intentional contamination with pain medicines can also not be excluded. In the absence of declaration, the user may perceive the products as of higher quality because of diminished side effects (bladder, bone, chest, eye, muscle pain and/or headache). Beside of being illegal, such practices present a severe medical and ethical misbehaviour.

About 5.1% of the samples had a sildenafil content >105%, increasing the risk of severe side effects (cardiovascular system, hypotension), while 30% of the samples were underdosed. Sildenafil content below the expected level has been found in previous studies [12] and may induce diminished therapeutic effects.

46.4% of the samples were non-conforming with the labelled specifications. However, the frequency of samples with sildenafil content conforming to the specifications increased significantly from 2017 to 2020, reaching 67.3% in 2020. This quality increase contrasts to previous results from the past reported in the United Kingdom regarding samples seized from 2005 to 2009 (10% of samples were within 10% of the claimed tablet content) [20] or from 2000 to 2004

in the Netherlands (only about 3% of the samples were conforming) [21]. However, the non-conforming dosage and combination of drugs have not be studied which can potentially represent serious health risks.

Conclusion

Percentage of sildenafil labelled medications lacking marketing authorization in Europe and hereby seized by national custom authorities increased from 38.5% in 2016 to 67.3% in 2020. Despite this increase, serious risks remain as samples are still contaminated with undeclared pharmacologically active compounds, which may pose health risks to consumers. Combatting SF medicines remains an important topic in public health, that has to be addressed by scientists, testing the quality of products, by law makers and pharmaceutical companies defining quality standards and security measures, and last but not least by physicians, informing and educating their patients.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgments

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