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Short Communication

IDENTIFICATION OF COUNTERFEIT MEDICINES FOR ERECTILE DYSFUNCTION FROM AN ILLEGAL SUPPLY CHAIN

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The appearance of counterfeit medicines in supply chains is a global public health problem that may seriously affect patients. Counterfeit drugs do not meet quality standards and do not declare their real composition and/or source for the purposes of fraud. They may be generic or innovative, they may contain genuine constituents in a fake packaging, or wrong ingredients, or inactive ingredients, or an incorrect quantity of the active substance. In Croatia, no cases of counterfeit medicines have been detected so far, but the Agency for Medicinal Products and Medical Devices has received 34 samples of medicines and other products for testing from Zagreb City Police. The samples included medicines for erectile dysfunction: sildenafil, tadalafil, and vardenafil. Twenty-three samples of tablets without marketing authorisation in Croatia were tested with high-performance liquid chromatography (HPLC) for the declared sildenafil and tadalafil content. Samples labelled 1 (batch T/33), 3 (batch T/33), 5 (batch 4), 6 (batch M0016J), 10 (batch T-070235), 12 (batch T-070544), 15 (batch 314833201), 16 (batch 832718474), and 17 (batch 504830028) containing sildenafil and samples labelled 20 (batch 070356), 21 (batch 05668), and 22 (batch T 378 5) containing tadalafil did not contain the active substance within the acceptable 95 % to 105 % margin of deviation from the declared content. While most samples cannot be described as fake with a reasonable amount of certainty, there is still a suspicion of counterfeit. A correct conclusion can be drawn only with the assistance of the manufacturers and by conducting additional laboratory tests.

KEY WORDS: *HPLC, pharmaceutical crime, quality control, sildenafil, tadalafil, vardenafil*

According to the World Health Organization (WHO) definition, counterfeit medicines form part of a broader category of pharmaceutical products which do not meet quality standards in force, with the difference that they are deliberately and fraudulently mislabelled with respect to their real composition and/or source. Counterfeit medicines may be both branded and generic and may contain genuine constituents in a fake packaging, or wrong ingredients, or inactive ingredients, or an incorrect quantity of the active substance (1, 2). The use of such medicines poses a health risk in that the intended effect of a medicinal product is not achieved, which may result in unexpected adverse effects, anaphylaxis, resistance to medicinal product, or other health problems (3-5). Most industrialised countries with an efficient system and market surveillance regulation have a low percentage of such products, mostly below 1 %, whereas in many countries of Africa and parts of Asia and Latin America the market share of such products exceeds 30 % (6-10). Counterfeit medicines in the European Union are mostly the "lifestyle" drugs, including medicinal products for erectile dysfunction and obesity (11). Apart from these, there are fake medicines for oncological, cardiac, psychiatric, and infectious diseases (12-14). This trend

in pharmaceutical crime may further rise, because the driving force behind it are high gains and high sales accompanied by utter disregard for the health of patients, which makes it a global problem (15-18).

In Croatia, no cases of counterfeit medicines have been detected so far in licensed pharmacies or in wholesale chains. But on the illegal drug market 34 samples have been seized. The products were classified in three groups, the first of which included products authorised for marketing in Croatia, the second comprised products without marketing authorisation, and the third products which are not medicines but rather medical devices or dietary supplements (Figure 1). The first and the second group consisted of 26 samples indicated for the treatment of erectile dysfunction, declared to contain active substances sildenafil, tadalafil, or vardenafil (Figure 2).

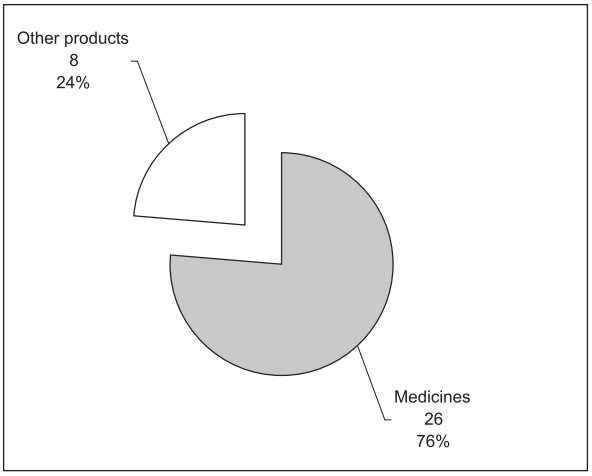

Figure 1 *Medicines and other products from illegal supply*

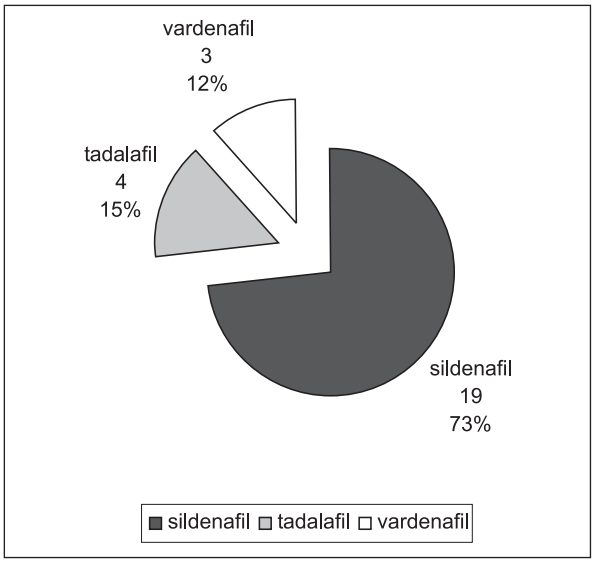

Figure 2 *Medicinal products from illegal supply according to their active pharmaceutical ingredients*

The purpose of this study was to determine the safety of samples identified as sildenafil and taldalafil in view of possible counterfeiting.

MATERIALS AND METHODS

The samples were submitted to the Agency as the regulatory authority by Zagreb Police Department. High performance liquid chromatography (HPLC) was used to separate sildenafil and tadalafil from their potential degradation products, process-related impurities, and formulation constituents according to manufacturer instructions. Sildenafil and tadalafil were quantified by comparing chromatographic peak areas of sample solutions with reference standard solutions. The following reference substances were used to prepare reference solutions: sildenafil citrate; Pfizer (USA), lot 0015-QSC-23 and tadalafil; Lilly (USA); lot 991085.

To determine active substances in tablets of the illegal products, we used a liquid chromatograph equipped with a column oven, a variable wavelength ultraviolet absorption detector, and a sample injection system. Sildenafil and tadalafil content in test samples was calculated as percentage of declared content of sildenafil and tadalafil per tablet.

Chromatographic conditions for sildenafil and tadalafil identification are given in Table 1.

RESULTS AND DISCUSSION

If a finished medicinal product is licensed for marketing in Croatia and the manufacturer is based outside Croatia, the distributor or the importer shall apply to the Agency for quality verification for every imported batch or for Agency's consent if its quality has been tested in the European Union. A comparison with samples licensed for marketing in Croatia (sildenafil 50 mg tablets, sildenafil 100 mg tablets, tadalafil 20 mg tablets, vardenafil 10 mg tablets, vardenafil 20 mg tablets) showed that samples submitted by the police were not authorised for marketing in Croatia, and were most likely intended for distribution in countries other than Croatia or for an illegal drug market.

Medicinal products lacking marketing authorisation for Croatia may be imported only with a special import license issued by the Agency. Since its foundation, the

Table 1 *Chromatographic conditions for sildenafil and tadalafil identification*

	Sildenafil	Tadalafil
Column	Symmetry Shield RP 18, 150 mm x 3.9 mm; 5 µm	Symmetry Shield RP 18, 150 mm x 3.9 mm; 5 µm
Detector	UV 290 nm	UV 285 nm
Column temperature	30 °C	30 °C
Injection volume	20 µL	10 µL
Buffer	0.05 mol L^{-1} triethylamine solution modified to pH 3.0±0.05 with 1:10 diluted phosphate acid or 0.01 mol L^{-1} NaOH	
Mobile phase	buffer : acetonitrile : methanol (58 :17 : 25);	acetonitrile : water with 0.1 % (v/v) trifluoroacetic acid (35 : 65)
Flow rate	1.0 mL min^{-1}	1.0 mL min^{-1}

Agency has never issued a special import licence for any of the reference medicinal products (C/Finegra 100 mg tablets[1], Sildofil 100 mg tablets[2], Cobra 100 mg tablets[3], Kamagra™ 100 mg tablets[4], Novagra forte 100 mg tablets[5], Vega 100 mg tablets[6], Venegra 100 mg tablets[7], Virecta 100 mg tablets[8], Apcalis plus 20 mg tablets[9]).

Other submitted samples not identified as medicines or medical devices are not within Agency's scope of responsibility (Figure 1). One can not discard the possibility that some of these products are classified as medicines in other countries. This group of products includes Femi-X[10] tablets, manufactured by Danish Pharmaceutical Industries Ltd., that have been authorised for marketing in Croatia as a dietary supplement intended to increase libido in women. Other products, Wollust Tropfen, Erotic Fluid, and Original Inverma Yohimbinum[11] were manufactured by Inverma Chemie, Germany. According to the declaration, these products are intended to increase potency and contain ginseng extract and yohimbine at very low concentrations otherwise typical of homeopathic products.

During authorisation of authentic medicines, the Agency for Medicinal Products and Medical Devices approved the manufacturer's specifications which were used in the Agency for testing the first group of drugs. The tests included verification and quantification of the active substance against reference substance using high-performance liquid chromatography (HPLC).

The Agency does not possess any manufacturers' documentation or analytical instructions for medicines lacking marketing authorisation in Croatia. As, according to the declaration, the medicines from the second group contained the same active substance as the authorised medicinal products, we used the same methods as for licensed finished medicinal products to verify and quantify them**.** As not all submitted batches had enough samples to meet manufacturer's instructions for analysis, we tested only two samples per batch, and the results were not statistically analysed.

Sildenafil samples were prepared in accordance with the procedure for Viagra® tablets manufactured by Pfizer. Two samples containing one tablet each were tested from each batch (Table 2, Figure 3).

Tadalafil samples, again two per batch, were prepared by combining three individual tablets in a 250 mL volumetric flask and processing them in accordance with the procedure for Cialis tablets manufactured by Lilly (Table 2, Figure 4).

The presence of the active substance has been confirmed in all tested samples, so in qualitative terms all samples conformed to declarations. Table 2 and Figure 5 show the quantitative composition in the tested samples of the first and second group of medicines. Samples labelled 1 (batch T/33), 3 (batchT/33), 5 (batch 4), 6 (batch M0016J), 10 (batch T-070235), 12 (batch T-070544), 15 (batch 314833201), 16 (batch 832718474), 17 (batch 504830028) 20 (batch 070356), 21 (batch 05668), and 22 (batch T 378 5), contained the active substance outside the acceptable 95 % to 105 % margin of deviation from the declared value. These deviations confirmed drug defects and the suspicion of counterfeit (Figure 6).

[1] Manufacturer is not specified
[2] Future Pharmaceuticals
[3] MB C
[4] Aajanta Pharma Limited
[5] Brown & Burk (UK) Ltd.
[6] Manufacturer is not specified
[7] Anvaxx Laboratory, USA
[8] EVA Pharma
9 MS Pharma
[10] Femi-X A/S
[11] Inverma Arzneimittel

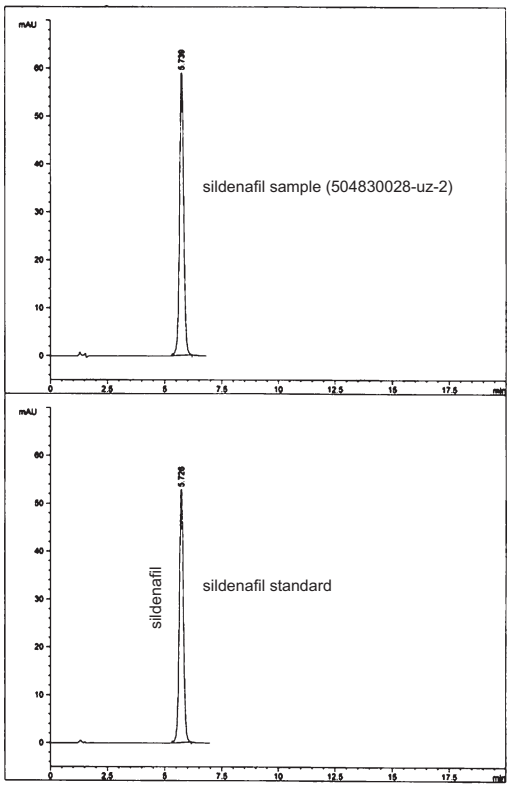

Figure 3 *A typical chromatogram of sildenafil sample and reference standard*

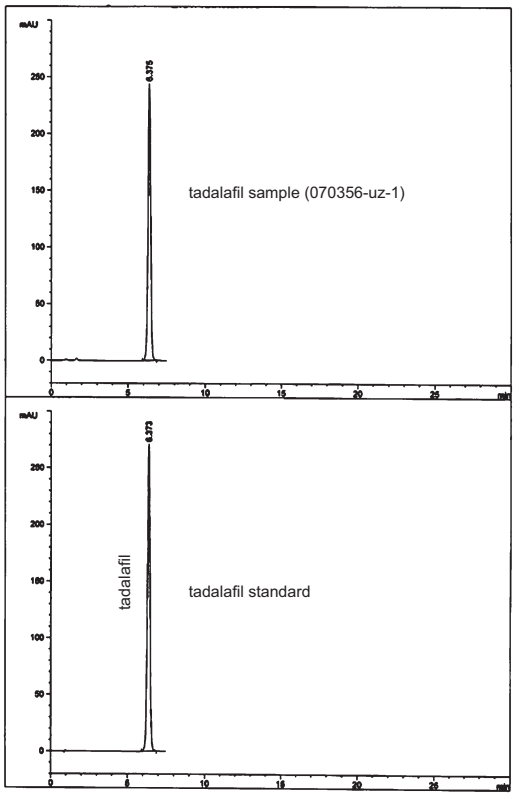

Figure 4 *A typical chromatogram of tadalafil sample and reference standard*

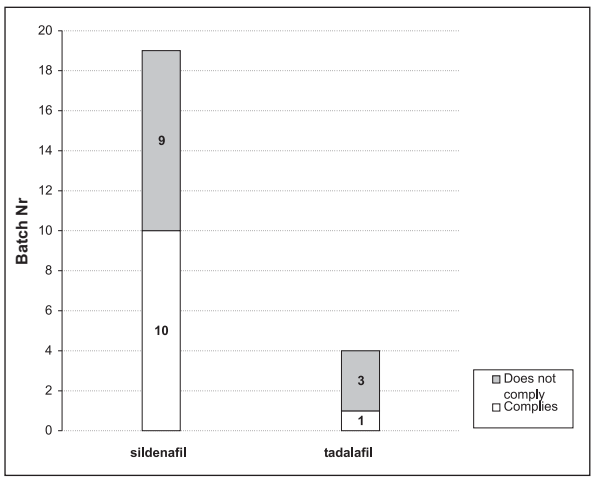

Figure 5 *Test results of sildenafil and tadalafil tablets*

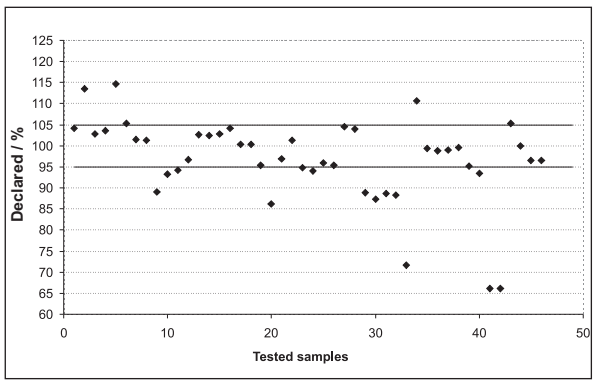

Figure 6 *Deviation from active substance content in tablet samples*

CONCLUSION

Even if test results fall within acceptable margins, one can safely verify the authenticity of a medicine only after receiving manufacturer's opinion based on complete batch data. Manufacturers alone possess complete data and in case of suspicion can run a series of additional target tests in their laboratories, including additional tests for purity profiles of active substances, excipients used in the finished drug product, type and material of primary and secondary packaging, or the quality and credibility of printing. These additional tests can make it easier and quicker to see whether a drug is authentic or fake; indeed, they are sometimes the only means to do this. Sufficiently discriminating analytical techniques are also needed,

Table 2 *Test results of sildenafil and tadalafil containing tablets*

Sample no.	Proprietary name	Manufacturer	Active substance / dose	Package	Batch	Content	
						mg per tablet	Deviation / %
1	Sildofil 100	Future Pharmaceuticals	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	T/33	104.1 113.6	104.1 113.6
2	Sildofil 100	Future Pharmaceuticals	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	T/20	102.9 103.6	102.9 103.6
3	Sildofil 100	Future Pharmaceuticals	sildenafil / 100 mg	4 tablets in a blister	T/33	114.7 105.3	114.7 105.3
4	C/Finegra	not specified	sildenafil / 100 mg	4 tablets in a blister	2036	101.4 101.2	101.4 101.2
5	Cobra 100 mg	MB C	sildenafil / 100 mg	4 tablets in a blister	4	89.1 93.3	89.1 93.3
6	Kamagra*Trade Mark 100 mg	Aajanta Pharma Limited	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	M0016J	94.2 96.7	94.2 96.7
7	Novagra-Forte	Brown & Burk (UK) Ltd.	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	NVFH 0028	102.6 102.4	102.6 102.4
8	Novagra-Forte	Brown & Burk (UK) Ltd.	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	NVFH 0030	102.9 104.2	102.9 104.2
9	Novagra-Forte	Brown & Burk (UK) Ltd.	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	NVFH 0031	100.3 100.4	100.3 100.4
10	Vega 100	not specified	sildenafil / 100 mg	4 tablets in a blister	T-070235	95.3 86.1	95.3 86.1
11	Vega 100	not specified	sildenafil / 100 mg	4 tablets in a blister	T-070237	96.9 101.2	96.9 101.2
12	Vega 100	not specified	sildenafil / 100 mg	4 tablets in a blister	T-070544	94.8 94.1	94.8 94.1
13	Vega 100 Asia	not specified	sildenafil / 100 mg	4 tablets in a blister	T-2106	96.0 95.4	96.0 95.4
14	Venegra	Anvaxx Laboratory, USA	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	PO226C	104.6 103.9	104.6 103.9
15	VIAGRA 100 mg	Pfizer Inc.	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	314833201	88.8 87.3	88.8 87.3
16	VIAGRA® 100 mg	Pfizer USA	sildenafil / 100 mg	4 tablets in a blister packaged in a carton	832718474	88.6 88.3	88.6 88.3
17	VIAGRA 50 mg	Pfizer Inc.	sildenafil / 50 mg	4 tablets in a blister packaged in a carton	504830028	35.8 55.4	71.7 110.7
18	Virecta	EVA Pharma	sildenafil / 100 mg	3 tablets in a blister packaged in a carton	703291	99.4 98.9	99.4 98.9
19	Virecta	EVA Pharma	sildenafil / 100 mg	3 tablets in a blister packaged in a carton	704391	99.0 99.5	99.0 99.5
20	Apcalis Plus 20	MS Pharma	tadalafil / 20 mg	2x2 tablets in a blister packaged in a carton	070356	19.0 18.7	95.2 93.5
21	Cialis® 20 mg	Lilly ICOS	tadalafil / 20 mg	2x2 tablets in a blister packaged in a carton	05668	13.2 13.2	66.1 66.1
22	Cyvel 20 mg	Barakat Pharmaceutical Industries	tadalafil / 20 mg	2 tablets in a blister	T 378 5	21.1 20.0	105.4 100.0
23	Tadlis 20	Orient Pharma	tadalafil / 20 mg	2x2 tablets in a blister packaged in a carton	702099	19.3 19.3	96.6 96.6

such as the near infrared spectroscopy (NIR), which is a mandatory method for fingerprint identification of medicinal products by manufacturers (19-21). Such tests are designed to identify the manufacturing site and deviations from manufacturer's standards for each manufactured batch.

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Sažetak

IDENTIFIKACIJA KRIVOTVORENIH LIJEKOVA ZA EREKTILNU DISFUNKCIJU IZ ILEGALNOG LANCA OPSKRBE

Pojava krivotvorenih lijekova u lancima opskrbe globalni je javnozdravstveni problem koji može imati ozbiljnih posljedica za pacijenta. Krivotvoreni lijekovi ne zadovoljavaju propisane standarde kakvoće te su u svrhu prijevare drugačije deklarirani u odnosu na njihov stvarni sastav i/ili podrijetlo. Mogu biti generički, ili inovativni, mogu sadržavati ispravne sastojke, ali pogrešno pakiranje, pogrešne sastojke, ne sadržavati djelatnu tvar ili ne sadržavati dovoljnu količinu djelatne tvari. U Hrvatskoj do sada nisu zabilježeni slučajevi krivotvorenih lijekova, ali je Agencija za lijekove i medicinske proizvode od policijske uprave Zagrebačke zaprimila 34 uzorka lijeka i ostalih proizvoda u svrhu ispitivanja. Od lijekova, radilo se o lijekovima za erektilnu disfunkciju sildenafilu, tadalafilu i vardenafilu. 23 uzorka tableta koji nemaju odobrenje za stavljanje lijeka u promet u Hrvatskoj ispitana su metodom tekućinske kromatografije visokog učinka (HPLC) na deklarirani sadržaj sildenafila i tadalafila. Ustanovljeno je da uzorci pod oznakama 1 (serija T/33), 3 (serijaT/33), 5 (serija 4), 6 (serija M0016J), 10 (serija T-070235), 12 (serija T-070544), 15 (serija 314833201), 16 (serija 832718474), 17 (serija 504830028) koji su sadržavali sildenafil te uzorci 20 (serija 070356), 21 (serija 05668), 22 (serija T 378 5) i 23 (702099) koji su sadržavali tadalafil, ne sadržavaju djelatnu tvar unutar prihvatljivih granica odstupanja od 95 % do 105 % od deklarirane vrijednosti. Iako se ne može sa sigurnosti za većinu uzoraka reći da se radi o krivotvorini, ipak se može govoriti o sumnji na krivotvorinu, a cjeloviti zaključak može se izvesti jedino uz suradnju i mišljenje proizvođača te provedbom dodatnih laboratorijskih ispitivanja.

KLJUČNE RIJEČI: *farmaceutski kriminal, HPLC, provjera kakvoće, sildenafil, tadalafil, vardenafil*

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