Journal of Pharmaceutical and Biomedical Analysis 131 (2016) 133–139

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

The quality of sildenafil active substance of illegal source

Peter H.J. Keizers[a,*], Andrea Wiegard[b], Bastiaan J. Venhuis[a]

[a] National Institute for Public Health and the Environment, P. O. Box 1, 3721 MA, Bilthoven, The Netherlands
[b] Landeszentrum Gesundheit Nordrhein-Westfalen, Fachgruppe Arzneimitteluntersuchung, Von-Stauffenberg-Straße 36, 48151 Münster, Germany

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 8 August 2016
Accepted 27 August 2016
Available online 28 August 2016

Keywords:
Active substance
Sildenafil
Illegal medicines
Quality

ABSTRACT

There must be a large market for active pharmaceutical ingredients of illegal source to support the huge and lucrative business of trade in illegal medicines. The active substances found in illegal pharmaceuticals may differ from their legal counterparts concerning purity and associated risks for the health of the user. In this study we show two examples in which the active substance sildenafil, used in erectile dysfunction products, was not of European Pharmacopeia quality. In one case milligram-scale amounts of a 2-mercaptobenzothiazole contamination were found, in another case the mesylate salt rather than the monograph based citrate was used. For the user of products containing these active substances, the risks of side effects increase through the inherent properties of the impurity and the chance of overdosing. The fact that the users are most likely not aware of the poor quality of the products adds up to the health risk of using prescription medication without consulting medical professionals.

1. Introduction

Trading falsified and other illegal medicines is lucrative as there is a considerable demand to economically priced medicines that can be obtained without visiting a physician to obtain a prescription [1]. There is a substantial use of illegal medicines; it was conservatively estimated in 2014 that more than 60% of the erectile dysfunction drug sildenafil used in the Netherlands came from sources other than the legal pharmacies [2]. This sildenafil is consumed through falsified erectile dysfunction products, adulterated food supplements and other not registered medicines [3,4]. Clearly, there must be a trade in active substance underlying this use of illegal medicines, the scale of which – in the case of sildenafil – must be similar to that of its legal counterpart.

Because of the uncontrolled conditions in which illegal active substances are produced, concerns may arise about the quality of these products related to their purity and the identity of the impurities [5]. For example, testing of the drug substance gentamicin sulfate revealed that the quality of active substances may vary; 30% of the tested batches of gentamicin appeared to contain large quantities of impurities [6]. Falsification of batches and/or the mixing of batches could be the causes of the large variation found. Another example is the accidental mix of sildenafil and the antidiabetic drug glibenclamide. The two actives were found in strongly varying ratios in illegal erectile dysfunction medicines and supplements causing an outbreak of hypoglycaemia in South East Asia [7,8]. Reports of harm due to this incident continue up to present day [9].

The production process, production location and starting materials used in the manufacturing of an active substance in a medicine should comply with the approved medicines registration dossier. An active ingredient produced differently should not be used in a pharmaceutical formulation. The health risk of falsified active substances particularly lies in the unexpected presence of (genotoxic) impurities. A single batch of active substance may be used for the production of up to 200.000 dose units, making the impact of falsification a large one. Yet, an active substance falsification will probably only be noticed if leading to acute intoxications in a country with a well-functioning health care system [10]. At the moment there is little scientific or regulatory research dedicated to active substance falsifications.

In this study we have received a sample dubbed sildenafil active substance from the Dutch Health Care Inspectorate. This sample was part of three one kilogram packages send by regular mail. The white crystalline powder was packed in aluminium foil bags, wrapped by cardboard from washing powder packaging. The sample was analysed for identity and quality to assess possible health risks from an active substance produced outside of the regular medicines chain. A second sample was 100 mg Kamagra tablets seized by the German police. Kamagra is a sildenafil containing erectile dysfunction product, not registered in the EU.

* Corresponding author.
E-mail address: peter.keizers@rivm.nl (P.H.J. Keizers).

http://dx.doi.org/10.1016/j.jpba.2016.08.027
0731-7085/

2. Experimental

2.1. Materials

Sildenafil citrate was obtained from Pfizer (Capelle aan den IJssel, the Netherlands) and Fagron (Barsbüttel, Germany). MBT was obtained from Sigma-Aldrich (Seelze, Germany). All other chemicals were of analytical grade or higher and obtained from default suppliers. Relevant structures and atom numbering are shown in Fig. 1.

2.2. Methods

2.2.1. FTIR spectroscopy

Spectra were acquired using both a handheld Thermo TruDefender FTX (HazmatLINK, Lauriston Park, UK) and a benchtop Bruker Vertex 70 Fourier Transform infrared spectrometer (Bruker Nederland BV, Leiderdorp, the Netherlands). With both spectrometers spectra were acquired using the Attenuated Total Reflection (ATR) setup.

2.2.2. UPLC-QTOF-MS/MS

The sildenafil active substance was dissolved in methanol and diluted 1000-fold in volumetric flasks in eluens containing 87% component A (5 mM ammonium formate, adjusted to pH 3.0 using formic acid) and 13% component B (0.1% formic acid in acetonitrile), before injection in the LC–MS. Briefly, the chromatographic separation was performed using a Waters Acquity™ ultra-performance liquid chromatography (UPLC) system fitted with a HSS C_{18} column (150 mm × 2.1 mm i.d., 1.8 μm; Waters Chromatography B.V., Etten-Leur, NL). Detection of the analytes was carried out using a Waters Synapt™ G2 quadrupole time of flight (QTOF) mass spectrometer (Waters Chromatography B.V., Etten-Leur, NL) with a Z-spray electrospray ionization (ESI) source operating in the positive ion mode. MS^E data were acquired in the resolution mode (≥ 20.000 FWHM). Chromatographic and mass data were acquired and analysed using Waters MassLynx v4.1 software. The presence of an analyte was confirmed by retention time, MS and MS/MS using a reference standard.

2.2.3. UHPLC-Orbitrap-MS/MS

52 mg of the grinded tablet was dissolved in 25.0 ml of a mixture of 50% acetonitrile and 50 % water. The chromatographic separation was performed using a Thermo Scientific Accela ultra-high performance liquid chromatography UHPLC system (Thermo Scientific, Dreieich, Germany) coupled with an hybrid-ion trap-Orbitrap mass spectrometer (Thermo Scientific LTQ-Orbitrap XL). The chromatographic separation was carried out on a Waters Aquitiy UPLC BEH C8 column (100 × 2.1 mm, 1.7 μm, Waters GmbH, Eschborn, Germany) using a gradient (mobile phase A: 0.1% formic acid, mobile phase B acetonitrile with 0.1% formic acid). Detection of the analytes was carried out using an electrospray ionization (ESI) source operating in the positive ion mode. Data was collected with the mass resolution of 60.000 FWHM. Chromatographic and mass data were acquired and analysed using XCalibur 2.1.0 software (Thermo Scientific, Dreieich, Germany). The presence of MBT and sildenafil was confirmed by retention time, MS and MS/MS using reference standards. MBT eluted at 4.21 min, with an MH^+ of 167.99393 Da, fragmenting to *m/z* of 136.02174 and 109.01049.

2.2.4. Raman microscopy

A DXR Raman microscope (Thermo Scientific, Madison USA) was employed to record Raman spectra of the products. Measurements were carried out using a 10× objective, a 780 nm laser with a laser power of 14 mW, a collection time of 10 s for a spectrum, a slit width of 50 μm, at a range of 50- 3420 cm^{-1}.

2.2.5. Melting point

The melting point of the sildenafil active substance and the sildenafil citrate reference standard were determined using a Büchi M-565 apparatus. The melting point of the reference standard was found to be 182.6 °C and the melting point of the product was found to be 263.4 °C.

2.2.6. NMR spectroscopy

The sildenafil active substance and sildenafil citrate reference standard were prepared in duplicate and dissolved in d_6DMSO (8 mg in 0.5 ml). A drop of methanesulfonic acid was added to one of the duplicate samples. ^{1}H spectra were acquired on a Bruker AV 500 MHz nuclear magnetic resonance (NMR) spectrometer (Bruker, Wormer, the Netherlands) equipped with a 5 mm Triple TXI-Z-Gradient probe operating at 298 K. All samples were automatically tuned, matched and shimmed. Spectra were calibrated to the TMS peak at 0.0000 ppm and processed and analysed using Topspin 3.0 software (Bruker, Wormer, the Netherlands).

2.2.7. HPLC-DAD

The sildenafil active substance was analysed for purity using the HPLC-DAD method as described in the sildenafil monograph (European Pharmacopeia 8.0).

MBT and sildenafil in the Kamagra sample were quantified by dissolving 100 mg (accurately weighed) of the grinded tablet in 5 ml methanol in a 100.0 ml volumetric flask, diluted with 70 ml methanol/water (1:1), sonicated and filled up to 100.0 ml. The solution was filtered through a 0.45 μm membrane filter. The chromatography was performed with a Merck Hitachi LaChrom Elite HPLC (VWR-Hitachi, Darmstadt, Germany) fitted with Prodigy ODS 3 coloumn (150 × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany). Analysis was carried out in isocratic mode with 50% of solvent A (0.05 M potassium dihydrogen phosphate buffer, adjusted to pH 2.1 using phosphoric acid) and 50% of solvent B (methanol). Reference solutions were prepared by dissolving each standard in 5 ml methanol, sonicate and diluted with methanol/water (1:1) to the final concentration of 95 μg/ml sildenafil citrate and 3.5 μg/ml MBT.

3. Results and discussion

From a visual inspection the sildenafil active substance seemed to contain pure white powdered crystals, a first indication of a professional, well performed synthetic procedure. No positive identification could be obtained however with the handheld FTIR spectrometer carrying a database containing a spectrum of sildenafil citrate. The product was identified as containing sildenafil using LC–MS (Fig. 2). In an attempt to explain the false negative result of the handheld FTIR spectrometer, subsequently a benchtop FTIR spectrum and a Raman spectrum were taken from the sample. Clearly, the spectra were different from reference sildenafil citrate, indicating the presence of a different polymorphic form or salt (Fig. 3). The melting point of the product was determined to be 263 °C, significantly higher than that of the reference standard at 183 °C. The higher melting point ruled out polymorphism and pointed towards the presence of a sulfonate salt. In general, it is known that sulfonate salts generate higher melting points than other salts [11]. No sulfonates could be identified in the LC–MS data so NMR spectroscopy was employed to determine the presence of organic salts in the product. The product clearly lacked the citrate peak at 2.6676 ppm and the piperazine ethylene peaks at 2.9619 and 2.5562 ppm are absent (See Table 1 and Fig. 4). Instead, the product displayed some other peaks at 3.8148, 3.4893,