Journal of Pharmaceutical and Biomedical Analysis 100 (2014) 215–229

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A general analytical platform and strategy in search for illegal drugs

Monika Johansson[a,b], Dick Fransson[a], Torgny Rundlöf[a], Ngoc-Hang Huynh[a,*], Torbjörn Arvidsson[a,b]

[a] Medical Products Agency, P.O. Box 26, SE-751 03 Uppsala, Sweden
[b] Division of Analytical Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala University, Biomedical Centre, Box 574, SE-751 23 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 11 May 2014
Received in revised form 22 July 2014
Accepted 24 July 2014
Available online 4 August 2014

Keywords:
LC–QTOF-MS
Quantitative NMR (qNMR)
Accurate mass determination
Suspected illegal medicines
Screening

ABSTRACT

An effective screening procedure to identify and quantify active pharmaceutical substances in suspected illegal medicinal products is described. The analytical platform, consisting of accurate mass determination with liquid chromatography time-of-flight mass spectrometry (LC–QTOF-MS) in combination with nuclear magnetic resonance (NMR) spectroscopy provides an excellent analytical tool to screen for unknowns in medicinal products, food supplements and herbal formulations. This analytical approach has been successfully applied to analyze thousands of samples. The general screening method usually starts with a methanol extraction of tablets/capsules followed by liquid chromatographic separation on a Halo Phenyl-Hexyl column (2.7 μm; 100 mm × 2.1 mm) using an acetonitrile/0.1% formic acid gradient as eluent. The accurate mass of peaks of interest was recorded and a search made against an in-house database containing approximately 4200 substances, mostly pharmaceutical compounds. The search could be general or tailored against different classes of compounds. Hits were confirmed by analyzing a reference substance and/or by NMR. Quantification was normally performed with quantitative NMR (qNMR) spectroscopy. Applications for weight-loss substances like sibutramine and orlistat, sexual potency enhancement (PDE-5 inhibitors), and analgesic drugs are presented in this study. We have also identified prostaglandin analogues in eyelash growth serum, exemplified by isopropyl cloprostenate and bimatoprost. For creams and ointments, matrix solid-phase dispersion (MSPD) was found to give a clean extracts with high recovery prior to LC–MS analyses. The structural elucidation of cetilistat, a new weight-loss substance recently found in illegal medicines purchased over the Internet, is also presented.

1. Introduction

All kinds of medicinal products, both branded and generic are subject to counterfeiting as reported by the World Health Organization (WHO). To define such products, WHO has introduced the terms 'spurious/falsely-labelled/falsified/counterfeit (SFFC) medicines, which are medicines that are deliberately and fraudulently mislabelled with respect to identity and/or source' [1]. Some of the counterfeit products have, after analysis, been found to lack active pharmaceutical ingredients (APIs), to contain too low or too high amounts or even to contain a different active substance. These SFFC products may pose a major threat to public health; in several cases, they have even led to deaths. To combat illegal medicines, operation Pangea (coordinated by Interpol) was organized. This operation brings together health regulators, national police, customs and the private sector. Its main goal is to increase public awareness of the dangers of buying medicines on line. It also aims to target the organized crime network, to disturb illegal trading and to identify individuals behind the websites. In May 2014, over 100 countries around the world participated; 9.4 million fake and illicit medicines with an estimated value of 36 million USD were seized, 239 arrests were made and more than 10,600 websites were shut down [2]. Since October 2011, the European initiative from the Council of Europe, the Medicrime Convention, has also been open for signature [3].

Falsified medicines are not controlled. It is therefore not known what these products contain. Neither the quality nor the efficacy of these products can be assured. To support the Swedish Medical Products Agency (MPA) in its effort to remove these products from the market and to better communicate their risk to public health, the MPA laboratory has developed screening procedures to identify and quantify unknown APIs in different types of products. Methods and instrumentation are different from those often used in ordinary

* Corresponding author at: Medical Products Agency, P.O. Box 26, SE-751 03 Uppsala, Sweden. Tel.: +46 18 174996; fax: +46 18548566.
E-mail addresses: imjo@telia.com (M. Johansson), Ngoc-Hang.Huynh@mpa.se (N.-H. Huynh).

http://dx.doi.org/10.1016/j.jpba.2014.07.026
0731-7085/

quality control work in compliance testing of medicinal products. This control of illegal medicines is also made in collaboration with other medical authorities in Europe, and the work is reported to the network of Official Medicines Control Laboratories (OMCL) coordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM) [4].

Analytical techniques used for identifying counterfeit drugs have been reviewed by Martino et al. [5]. The techniques discussed were colorimetry, thin-layer chromatography, gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS) and different vibrational spectroscopic methods. Also ^{1}H NMR has been proven very useful for detecting and quantifying illegal compounds. [6]. Chemical analysis can reveal if the product contains the right substance and the correct declared amount. However, whether the product is genuine or an imitation is a more difficult question to answer. Chemometrics and chromatographic fingerprint methods based on HPLC-UV data have successfully been used to investigate this issue based on analytical data for falsified Viagra® and Cialis®, two approved medicines to improve erectile dysfunction [7].

The substance sibutramine was detected and quantified in 11 out of 32 tested commercial weight-loss formulations by a screening procedure based on ultra-high-pressure liquid chromatography (UHPLC) with diode array detector [8]. In another study, the content of phosphodiesteras-5 (PDE-5) inhibitors and 10 analogues were detected and quantified in herbal dietary supplements using HPLC with charged aerosol detection [9]. Venhuis et al. [10] counted up to 46 different analogues of the PDE-5 inhibitors sildenafil, tadalafil and vardenafil identified in herbal food supplements in their summary report. In recent studies Wiest et al. [11] identified and quantified various API in herbal preparations using NMR and Desmedt et al. [12] found illegal skin whiteners in cosmetics using LC–QTOF-MS.

In this paper, we describe a general analytical platform used to identify and quantify unknown APIs in medicinal products, food supplements and herbal formulations. The platform consists of a combination of liquid chromatography coupled to mass spectrometry (LC–QTOF-MS) and NMR spectroscopy. The API is identified using LC–QTOF-MS and determination is made using quantitative NMR (qNMR) [13–15]. Most products consisting of tablets and capsules can be analyzed with LC–QTOF-MS after simple generic extraction with methanol. Sometimes, however, specific analytical methods need to be developed when the expected concentrations are very low, e.g., prostaglandin analogues in cosmetic products, or for difficult matrixes like creams and ointments.

The platform presented in this paper has been used for testing more than 1000 samples during the last 5–10 years. Some examples of the identification and quantification of falsified medicines are given. Results from the chemical analyses have been posted on the website of the MPA and warnings have been issued to the general public [16].

2. Materials and methods

2.1. Chemicals, reagents and samples

Sildenafil was supplied by Pfizer (Sollentuna, Sweden). Potency substances: acetildenafil, aildenafil, aminotadalafil, benzamidenafil, carbodenafil, homosildenafil, hydroxyhomosildenafil, nor-acetildenafil, norneosildenafil, piperiacetildenafil, pseudovardenafil, tadalafil, thioaildenafil, thiohomosildenafil, thiosildenafil, udenafil and vardenafil were obtained from TLC PharmaChem Inc. (Vaughan, Ontario, Canada, https://www.tlcpharmachem.com). Isopropyl cloprostenate, Item 10010016, was obtained from Cayman chemicals (Michigan, USA, https://www.caymanchem.com). Betamethasone-21-acetate was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Dexamethasone-21-acetate, triamcinolone acetonide, ephedrine and forskolin were purchased from Sigma–Aldrich (Stockholm, Sweden). Other reference substances referred to in this paper were obtained from the quality control unit within the Swedish Medical Products Agency.

Slimming products were obtained from the Swedish Customs. Fortodol was obtained from Swedish and Norwegian patients who had reported adverse reactions. The product was also purchased in different health shops in Sweden. Curcumine was purchased as the spice 'Curcuma longa' in a Swedish supermarket. The product for sexual potency enhancement was bought in a 'Love Store' in Stockholm, Sweden. Eyelash growth serums were provided by the manufacturers or purchased from Internet web shops. The cream found to contain corticosteroid was also purchased in a shop in Stockholm, Sweden.

Methanol, CHROMASOLV® gradient grade for HPLC, ≥99.9%, used for sample preparation, Acetonitrile, CHROMASOLV® gradient grade for HPLC, ≥99.9%, used for preparation of eluents, and sodium sulfate (Na_2SO_4) were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Formic acid, EMSURE® for analysis 98–100%, ammonium acetate, EMSURE® analytical grade and acetic acid EMSURE®, 100%, were obtained from Merck KGaA (Darmstadt, Germany). Florisil®, mesh 60/100 was from Supelco (Bellafonte, PA, USA).

NMR solvents dimethylsulfoxide-d_6 (DMSO-d_6, 99.9%), methanol-d_4 (CD_3OD, 99.8%) and chloroform-d ($CDCl_3$, 99.8%) with the chemical formulae and degrees of deuteration in parentheses as well as DCl (20% in D_2O), all manufactured by Armar Chemicals (Döttingen, Switzerland), were purchased from Glaser Lab-Kemikalier (Göteborg, Sweden).

NMR internal standard 3-sulfolene (98+%) was purchased from Sigma–Aldrich (Stockholm, Sweden), and 3,4,5-trichloropyridine (99.9+%) from Fisher Scientific (Stockholm, Sweden).

2.2. Experimental

2.2.1. Sample preparation

Prior to analysis, sample package and labelling were checked and the appearance of the tablet or capsule to be analyzed was recorded. For sample preparation, a number of units were then taken depending on sample type. At least three but preferably 10 tablets/capsules (depending on the number available) were used for analytical work. The tablets were weighed together on an analytical balance to obtain a mean weight and thereafter ground and mixed. The pulverized material was used for sample preparation prior to analysis with LC–QTOF-MS and/or NMR.

The analytical balance was a Genius ME 215P from Sartorius AG (Goettingen, Germany) and the micro-balance was an UMX2 from Mettler-Toledo AB (Stockholm, Sweden).

2.2.1.1. Sample preparation for LC–MS analyses. The general sample preparation for LC–MS was methanol extraction. A 20 mg sample of the pulverized material, accurately weighed on a micro-balance, was added to a test tube. Analytes were extracted by mixing the sample with 2.0 mL methanol for 20 s on a WortexGenie2 mixer (Scientific Industries, New York, USA) followed by 30 min extraction in a gentler Boule 440 mixer (Boule Medical AB, Sweden). Prior to LC–MS analysis, the extract was filtered through a GHB Acrodisc® 13 mm syringe filter with a 0.45 μm GHP membrane from Pall Corporation Life Sciences. The sample solution was further diluted with methanol if the response in the MS was too strong in the first analysis. Capsules were treated in the same way. The capsule content and the shell were normally analyzed separately.

For prostaglandin analogues, 100 mg of the eyelash growth serum was mixed with 150 μL of methanol and added directly into the auto-sampler vial. The vial was centrifuged at 3000 × g for 5 min