Talanta 123 (2014) 78–88

Contents lists available at ScienceDirect

Talanta

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

Headspace–gas chromatographic fingerprints to discriminate and classify counterfeit medicines

D. Custers [a,b], M. Canfyn [a], P. Courselle [a], J.O. De Beer [a], S. Apers [b], E. Deconinck [a,*]

[a] Division of Food, Medicines and Consumer Safety, Section Medicinal Products, Scientific Institute of Public Health (WIV-ISP), J. Wytsmanstraat 14, B-1050 Brussels, Belgium

[b] Laboratory of Pharmacognosy and Pharmaceutical Analysis, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form
15 January 2014
Accepted 20 January 2014
Available online 31 January 2014

Keywords:
Counterfeit medicines
Residual solvents
Chemometrics
Fingerprints

ABSTRACT

Counterfeit medicines are a global threat to public health. These pharmaceuticals are not subjected to quality control and therefore their safety, quality and efficacy cannot be guaranteed. Today, the safety evaluation of counterfeit medicines is mainly based on the identification and quantification of the active substances present. However, the analysis of potential toxic secondary components, like residual solvents, becomes more important. Assessment of residual solvent content and chemometric analysis of fingerprints might be useful in the discrimination between genuine and counterfeit pharmaceuticals. Moreover, the fingerprint approach might also contribute in the evaluation of the health risks different types of counterfeit medicines pose. In this study a number of genuine and counterfeit Viagra® and Cialis® samples were analyzed for residual solvent content using headspace–GC–MS. The obtained chromatograms were used as fingerprints and analyzed using different chemometric techniques: Principal Component Analysis, Projection Pursuit, Classification and Regression Trees and Soft Independent Modelling of Class Analogy. It was tested whether these techniques can distinguish genuine pharmaceuticals from counterfeit ones and if distinct types of counterfeits could be differentiated based on health risks. This chemometric analysis showed that for both data sets PCA clearly discriminated between genuine and counterfeit drugs, and SIMCA generated the best predictive models. This technique not only resulted in a 100% correct classification rate for the discrimination between genuine and counterfeit medicines, the classification of the counterfeit samples was also superior compared to CART. This study shows that chemometric analysis of headspace–GC impurity fingerprints allows to distinguish between genuine and counterfeit medicines and to differentiate between groups of counterfeit products based on the public health risks they pose.

1. Introduction

Counterfeit medicines pose a huge threat to public health worldwide [1]. Not only developing countries are threatened, also industrialized countries are exposed to pharmaceutical counterfeiting. A counterfeit medicine is defined by the World Health Organization (WHO) as "one which is deliberately and fraudulently mislabeled 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 active ingredients, with insufficient active ingredients or with fake packaging" [2].

These forged medicines are mostly manufactured by uncontrolled or street laboratories without respecting Good Manufacturing Practices (GMP) [3]. They are not subjected to any form of quality control [4] and therefore their safety, efficacy and quality cannot be guaranteed [2]. Health risks, caused by counterfeit medicines, might be due to the presence of incorrect active ingredients, the absence of active ingredients, an incorrect dosage, the presence of high concentrations of potential toxic secondary components and fake packaging or documentation [5].

Assessing the actual extent of pharmaceutical counterfeiting is very difficult due to its illicit and clandestine character [5]. Moreover the size of the problem differs from region to region. It is estimated that about 1% of the total medicines market of industrialized countries, such as the United States, European countries, Japan, etc., consists of counterfeit medicines. In countries of the former Soviet Union about 20% of the medicines market is covered by counterfeit pharmaceuticals. This number reaches even more than 30% in African countries and parts of Asia and Latin-America. Furthermore, it is also estimated that approximately 50% of all medicines, bought online from websites which cover up their

* Corresponding author. Tel.: +32 2 642 51 36; fax: +32 2 642 53 27.
E-mail address: Eric.Deconinck@wiv-isp.be (E. Deconinck).

0039-9140/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2014.01.020

physical address, are fake [1]. In fact, the extension of the Internet is one of the main reasons for the increasing threat posed by counterfeit drugs, especially in industrialized countries [6]. The types of medicines which are most sold as counterfeit in industrialized countries are commonly referred to as 'life style drugs' and comprise phosphodiesterase type 5 (PDE-5) inhibitors, slimming products (containing anorexics) and anabolic hormones [6,7].

In most literature, the characterization of counterfeit medicines is based on the identification and quantification of the active substances present. Indeed, potential toxic secondary components, such as impurities, residual solvents, etc., are often not taken into account. As a result, a product can be regarded as relatively save for it might contain the right active substances in the correct dosage, while in actual fact high concentrations of potential toxic secondary components could be present. Since counterfeiters probably use inferior primary substances and manufacture these medicines without respecting any quality norm, the analysis of these secondary components becomes more important. The evaluation of residual solvents is fundamental for quality control of genuine medicines, especially for medicines intended for chronic use. Consequently, residual solvents are of great interest for the characterization of counterfeit medicines [7].

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) defines residual solvents as "organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products" [8]. Many of these solvents are known to be harmful to humans or the environment [9]. Furthermore, these chemicals have no therapeutic benefit and they may facilitate decomposition of pharmaceuticals [10]. Since it is not possible to completely remove residual solvents from drug substances and excipients, it is important that these impurities are eliminated to the extent possible in order to meet quality norms. ICH issued guidelines which not only recommend the use of less toxic solvents; they also recommend acceptable amounts for residual solvents in order to ensure the patient's safety [8]. These guidelines have been adopted by the European Pharmacopoeia, the United States Pharmacopoeia and the Japanese Pharmacopoeia [6].

ICH defines 4 classes of residual solvents. Class I solvents (e.g., benzene, 1,2-dichloroethane, etc.) are to be avoided because of their high toxicity or harmful environmental effect. Concentration limits vary between 2 and 8 ppm. 1,1,1-trichloroethane is classified as class I solvent because of its environmental hazard. Its concentration limit is set at 1500 ppm. Class II (e.g., acetonitril, methanol, toluene, etc.) consists of solvents which should be limited due to low toxicity [8]. Their limits range between 50 and 5000 ppm [7]. Class III solvents (e.g., ethanol, acetic acid, acetone, etc.) are considered to have low toxic potential and they are limited to 5000 ppm. Class IV (e.g., isopropyl ether, trifluoroacetic acid, etc.) is composed of solvents for which no adequate toxicological data are available [8].

The increasing interest in residual solvent assessment has led to the development of a large number of analytical techniques intended for the determination of these chemicals [9]. In general, most of these techniques are based on gas chromatography (GC) [7]. The European Pharmacopoeia mentions two gas chromatographic methods using static headspace injection and a flame ionisation detector. A mass spectrometer or, if needed, an electron-capture detector for the determination of chlorinated residual solvents may also be used. These two methods allow: (1) the identification of class I, II and III solvents; (2) to carry out a limit test for class I and II solvents and (3) to quantify class II solvents, if the limits are higher than 1000 ppm, and class III solvents [11]. Besides these two techniques, several other GC methods are described in literature using different injection techniques, such as split/splitless injection, headspace and solid-phase micoextraction [9,12–15]. Other techniques for residual solvent determination, used as alternatives to gas chromatography, are loss on drying, thermogravimetric analysis, differential scanning calorimetry, IR spectroscopy and NMR spectrometry. Many of these techniques have the disadvantage of being non-specific or they are characterized by high detection limits, making them often less suitable for residual solvents assessment [16]. Both groups of techniques, gas chromatography and alternatives, are reviewed by B'Hymer [16] and Grodowska et al. [17]. Even though many different analytical methods are available, gas chromatography remains the most powerful technique for residual solvent analysis [17]. The combination of headspace injection with GC–MS has also the advantage of a limited sample preparation effort, allowing fast analysis. Our group developed and validated its own GC technique for the identification and quantification of residual solvents [7]. This technique has the advantages of being fast and suitable for routine analysis of pharmaceuticals.

Despite the fact that GC is the most suited technique for residual solvent analysis, the use of GC impurity fingerprints is a fairly new concept in literature. The fingerprint approach is already extensively used in the field of Pharmacognosy for the identification and quality control of plants. This approach might be interesting for the identification of potential toxic secondary components in counterfeit medicines. A fingerprint is a characteristic profile which visualizes the composition of a sample. It can be obtained by usage of chromatographic, spectroscopic or electrophoretic techniques. However, chromatographic fingerprints are the most interesting fingerprints. By spreading information about the composition of a sample over time, they provide information about individual compounds [18].

In this paper the chromatograms, obtained by the headspace–GC–MS analysis of a set of genuine and counterfeit Viagra® and Cialis® samples, were used as fingerprints. These fingerprints were analyzed using different chemometric techniques. The purpose of this data-analysis was to test whether these techniques allow for the distinction between genuine and counterfeit medicines, based on the obtained fingerprints. Furthermore, it was tested if these methods can also discriminate between different counterfeit medicines based on the public health risk they pose.

2. Methods

2.1. Samples

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

2.2. Chemicals and reagents

2-Propanol, dichloromethane, acetone, ethanol absolute, acetonitril (all HPLC grade) and ethylacetate (pesti-S) were purchased from Biosolve (Valkenswaard, The Netherlands). Chloroform (for gas chromatography), benzene, tetrachloromethane (CCl_4) (for spectroscopy) and ethylbenzene (for gas chromatography) were purchased from Merck (Darmstadt, Germany). Toluene and cyclohexane were purchased from VWR prolabo (Fontenay-Sous-Bois, France). These solvents were used as reference standards. Dimethyl sulfoxide, which was used as solvent for the samples, was purchased from Merck.