Science and Justice 57 (2017) 283–295

Contents lists available at ScienceDirect

Science and Justice

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

ATR-FTIR characterization of generic brand-named and counterfeit *sildenafil*- and *tadalafil-based* tablets found on the Brazilian market

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ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form 3 April 2017
Accepted 20 April 2017

Keywords:
Viagra
Cialis
Excipients
Generic medicine
Manufacturer identification
Spectral deconvolution

ABSTRACT

Viagra and Cialis are among the most counterfeited medicines in many parts of the world, including Brazil. Despite the many studies that have been made regarding discrimination between genuine and counterfeit samples, most published works do not contemplate generic and similar versions of these medicines and also do not explore excipients/adjuvants contributions when characterizing genuine and suspected samples. In this study, we present our findings in exploring ATR-FTIR spectral profiles for characterizing both genuine and questioned samples of several generic and brand-name sildenafil- and tadalafil-based tablets available on the Brazilian market, including Viagra and Cialis. Multi-component spectral matching (deconvolution), objective visual comparison and correlation tests were used during analysis. Besides from allowing simple and quick identification of counterfeits, results obtained evidenced the strong spectral similarities between generic and brand-named tablets employing the same active ingredient and the indistinguishability between samples produced by the same manufacturer, generic or not. For all sildenafil-based and some tadalafil-based tablets tested, differentiation between samples from different manufacturers, attributed to slight variations in excipients/adjuvants proportions, was achieved, thus allowing the possibility of tracing an unknown/unidentified tablet back to a specific manufacturer.

1. Introduction

It is nearly 20 years now since Viagra, the first phosphodiesterase 5 (PDE5) inhibitor, reached the market and transformed erectile dysfunction (ED) treatment [1,2]. Aside from its initial development as a potential treatment for angina and its enormous success as an on-demand oral treatment for ED, today's therapeutic potential uses of sildenafil and other PDE5 inhibitors, like tadalafil, include treatment of pulmonary hypertension, heart disease, diabetes, cancer and benign prostatic hyperplasia, among other possible indications [1,3,4]. Even if new uses for PDE5 inhibitors were to be disregarded, the estimated worldwide increase of ED cases, possibly afflicting more than 322 million men around 2025 [5], coupled with recreational use and misuse, in spite of the potential physical and psychological dangers [6–8], should be more than enough to keep the ongoing demand (prescribed or not) for this drug class.

As a side-effect of its own success, PDE5 inhibitors are among the most counterfeited medicines around the world, specially in Brazil, where about two thirds of counterfeit medicines seized by local authorities belong to this class [9,10].

From the forensic point of view, analysis of questioned PDE5 samples and identification of counterfeits has been achieved based on multiple approaches, including physical profiling (PP) [11,12], Ultra Performance Liquid Chromatography with Electro Spray Ionization and Mass Spectrometry(HPLC-ESI-MS) [13], Near Infrared (NIR) and Raman Spectrometry [14,15] and Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) [16].

The great majority of published works, however, is focused on Viagra and Cialis alone and only considers the infrared spectra of sildenafil and tadalafil, mostly ignoring that each tablet is a solid mixture composed by the active ingredient (AI) and several other excipients/adjuvants. In fact, for Viagra (50 mg) and Cialis (20 mg), excipients/adjuvants mass amount to 77.7% and 94.5% of each tablet, respectively. Therefore, their presence and contributions to the

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http://dx.doi.org/10.1016/j.scijus.2017.04.009
1355-0306/

spectral profile of the tablets must be taken into account during analysis.

Also, the ongoing demand for PDE5 inhibitors, coupled with patent expirations and the expansion of generic drugs market in many countries, including Brazil, prompted many generic and brand-named alternative versions of Viagra and Cialis to be available at drugstores. Many of these new sildenafil- and tadalafil-based tablets are manufactured by companies other than Pfizer (maker of Viagra) and Eli Lilly (maker of Cialis), and, therefore, may present different spectral characteristics, regardless of bioequivalency. As counterfeit versions of these new tablets may soon be reaching the streets, new studies concerning their characteristics, specially when compared to Viagra and Cialis, are needed.

Here, we report our findings in characterizing some of the sildenafil- and tadalafil-based tablets legally available on the Brazilian market. Samples studied encompass not only Viagra and Cialis, but several other tablets, generic and brand-named, manufactured and marketed by different pharmaceutical companies. Analysis of ATR-FTIR spectral profiles was used to identify not only the AI of each sample, but also the main excipients/adjuvants present. Multi-component spectral analysis (deconvolution), objective visual comparison and correlation allowed genuine samples from different manufacturers to be distinguished from each other. The same procedures were successfully used to identify unknown and counterfeit seized samples. All analyses were made without explicitly recurring to chemometrics, the technique usually employed on most previous published works, thus making the proposed approach more easily accessible to forensic analysts working at laboratories equipped with modern ATR-FTIR spectrometers.

2. Materials and methods

2.1. Samples

Genuine tablets of sildenafil 50 mg and tadalafil 20 mg were purchased from local and regional trusted drugstores. Sildenafil samples consisted of 26 generic tablets from 7 pharmaceutical companies and 19 branded tablets from 6 pharmaceutical companies. Tadalafil samples consisted of 13 generic tablets from 5 pharmaceutical companies and 10 branded tablets from 4 pharmaceutical companies. Viagra and Cialis samples were included as part of the brand-named tablets. The brand name, pharmaceutical company, manufacturer and composition of each type of tablet were obtained from the packages and accompanying leaflets. The mass, shape and color of each individual tablet were also registered.

Questioned tablets, seized by local law enforcement forces, consisted of 24 tablets packaged as generic sildenafil from 2 pharmaceutical companies, 13 tablets packaged as Viagra, 1 tablet packaged as Dejavú and 12 tablets packaged as Cialis. Other 15 unpackaged/unbranded tablets with unrecognizable or no identification marks, but suspected to be PDE5 inhibitors, were treated as questioned tablets as well.

After external physical characteristics (mass, shape and color) were registered, the external layer of each tablet was carefully removed. The remaining cores were then crushed and homogenized.

The total number of tablets studied was 133 (45 genuine sildenafil-based, 23 genuine tadalafil-based, 38 questioned/seized sildenafil-based, 12 questioned/seized tadalafil-based and 15 questioned/seized totally unknown tablets).

2.2. Instrumentation

ATR-FTIR spectra of the crushed tablet cores were taken using a Nicolet™ *iZ*10 spectrometer equipped with EverGlo IR source, DLaTGS room temperature IR detector and single-bounce Smart Orbit™ accessory module with diamond ATR crystal. All hardware from Thermo Fischer Scientific Inc. (USA).

Each spectrum was averaged over 16 scans, taken at 4 cm^{-1} resolution, maximum detector window aperture and minimum interferometer mirror speed, in the range of 4000–400 cm^{-1}. Background signal was averaged over 8 scans prior to each measurement.

2.3. Data analysis

Analysis of collected spectra was carried out using the software suite accompanying the spectrometer, which included the OMNIC™ 9.1.27, used for data acquisition and single spectrum comparisons (including correlation), and the OMNIC™ Specta™ 2.0, used for multi-component database comparison (deconvolution). The Specta software allows for multi-component spectral matching of mixtures containing up to 4 unknown components or 1 known component (spectral bulk) and 4 unknown contaminants. All software from Thermo Fischer Scientific Inc. (USA).

A spectral IR library, containing profiles of pharmaceutical-grade raw materials purchased from local compounding pharmacies, was previously build and used as reference database for multi-component/deconvolution spectral analysis, as well as for the objective visual comparison between tested tablets. The library included reference spectra for both AI studied (sildenafil and tadalafil) and most excipients/adjuvants listed by manufacturers in the leaflets accompanying genuine tablets (microcrystalline cellulose, lactose, magnesium stearate, calcium phosphate dibasic and sodium croscarmellose), along with more than 100 other ingredients employed by pharmaceutical companies (AIs and excipients/adjuvants), most of them found on tablet formulations.

For the characterization and comparison between genuine samples, the spectral profiles of all individual tablets sharing the same identification, regardless of lot/batch numbers, were averaged altogether, thus producing a single spectral profile for each type of genuine tablet studied. The averaged profiles were normalized and used as a reference standard for the corresponding type of tablet. Each standard thus generated was then submitted to multi-component spectral matching (deconvolution), in order to assess which constituents could be automatically identified, allowing immediate confrontation with leaflet data. Correct identification of the tablets core composition was enhanced through the use of the spectral IR library generated from the same experimental setup and containing reference spectra for all target compounds. Objective visual comparison between reference profiles for each type of tablet was performed by overlaying all profiles sharing the same AI and manually checking for similarities and differences, taking into account specific spectral bands from each component previously identified, specially the excipients/adjuvants. In the final stage of the characterization/comparison, the correlation between all reference profiles sharing the same AI was measured.

Analysis of questioned tablets was conducted much the same way as the characterization and comparison between genuine tablets, whenever possible, with minor modifications. Seized tablets packaged together had their mass and color evaluated prior to the removal of the external layer and tablet core crushing and homogenization. A single average profile was produced from all tablets within the same package. The averaged profile from each questioned package was then compared to the standard corresponding to the package identification, the same occurring for tablet mass and color. For unpackaged (unmarked/unidentified) questioned tablets, some of which were received broken or even partially crushed, evaluation of mass and color could not be properly made and were thus disregarded. The spectral profile of each individual unidentified sample was normalized and compared to each of the genuine standards available for composition, spectral profile and correlation, in order to verify if a reasonable match could be found.