

Determination of sildenafil mixed into herbal honey mixture by ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry

Neira Mustabasic^{1*} and Selin Isik²

¹Ankara University, Institute of Forensic Science, Department of Forensic Chemistry and Forensic Toxicology, Cebeci Research and Application Hospital, 06590 Dikimevi, Ankara, Turkey

²Near East University, Faculty of Pharmacy, Department of Analytical Chemistry, 99138 Nicosia, Turkish Republic of Northern Cyprus

original scientific paper

DOI: 10.17508/CJFST.2017.9.2.13

Summary

There has been a number of reports of natural products contaminated with illegal adulterants that threaten consumer health because of their adverse pharmacological effects worldwide. In this study, a multi-residual ultra-performance liquid chromatography method with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) was applied for the identification of sildenafil added into a herbal honey mixture used as an immune system booster. Electrospray ionization (ESI) source was applied and operated in the positive ion mode. The mobile phase consisted of 0.1% formic acid aqueous solution/acetonitrile (70:30, v/v) using the isocratic gradient elution system at a detection wavelength of 290 nm. The compound of sildenafil added into traditional herbal mixed honey was identified according to the spectrum, chromatographic behavior, and mass spectral data were identified by comparison with the reference substance. The method is selective, sensitive and can be used to detect the sildenafil illegally added into traditional herbal medicinal preparations.

Keywords: ultra-performance liquid chromatography, quadrupole time-of-flight mass spectrometry, honey, sildenafil, herbal products

Introduction

Sildenafil, 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl) phenyl-sulfonyl]-4-methyl piperazine (Fig. 1), is a potent and selective inhibitor of a phosphodiesterase type 5 enzyme (Alkharfy, 2009). In 1998, sildenafil (*Viagra*[®]) was approved by the United States Food and Drug Administration (FDA) for the treatment of erectile dysfunction (Ren et al., 2012).

Unlike other approved medicines for erectile dysfunction, sildenafil does not directly cause penile erection, but induces reaction to sexual incitement. The medication acts by influencing enzymes in the nitric oxide pathway, causing a cascade of enzymatic reactions that enhance nitric oxide, causing more blood flow and better erections. When sexual stimulation causes local release of nitric oxide, sildenafil enhances the effect of nitric oxide on the corpus cavernosum by increasing the levels of cGMP in this tissue (Umrani et al., 1999; Rendell et al., 1999).

Sildenafil is rapidly absorbed following oral administration, has an onset of action within 25 to 60 minutes after dosing and has a short plasma half-life of approximately 4 hours (Rendell et al., 1999). The absolute average bioavailability after oral administration is about 40%. Sildenafil is subject to a

predominantly hepatic metabolism and converted to an active metabolite in reaction mediated by the low-affinity, high-capacity P450 (CYP) 3A4 isoenzyme (major route) and the high-affinity, low-capacity CYP2C9 isoenzyme (minor route). After either oral (PO) or intravenous (IV) dosing, sildenafil is rapidly cleared from the body as metabolites mostly in the feces and a minor amount in the urine (Umrani et al., 1999). Redness in the face and neck, indigestion, photophobia, blurred vision, and in rare conditions, ventricular arrhythmias, myocardial infarction and sudden hearing loss are side effects of constant sildenafil use (Maryam, 2016).

Male erectile dysfunction, the persistent inability to achieve or maintain an erection for satisfactory sexual performance, is a common medical problem. According to a random study, over half of all men between 40 and 70 years of age have experienced erectile dysfunction. It is also estimated that the number of men who have erectile dysfunction will double in the next 25 years, ultimately affecting more than 330 million men worldwide (Al-Tahami, 2014).

The use of herbal remedies and dietary supplements has greatly increased. There are reports that some herbal products advertised as “all natural” had been found to be adulterated with synthetic PDE-5 inhibitors such as sildenafil (Damiano et al., 2014).

*Corresponding author: mustabasic@ankara.edu.tr

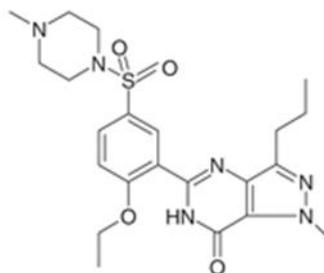


Fig. 1. Chemical structure of sildenafil

Illegitimate manufacturers produce hand-made counterfeit herbal supplements without any quality and purity standards under the authority of regulatory systems. There is a great common concern that herbal drugs are safe without any side effects. As a result of this belief, many patients trust herbal supplement manufactures and rely on what they say about their products (Maryam, 2016).

Recently, world's largest pharmaceutical companies have agreed with Interpol to provide the funding and other support to Interpol's battle against counterfeit drugs. The major pharmaceutical companies are involved in the problem with large investment plans (Damiano et al., 2014).

In this study, herbal mixed honey used as an immune system booster found in Ankara, Turkey was analyzed for the presence of sildenafil, the approved synthetic PDE-5 inhibitor. The UPLC/Q-ToF method was adopted for this study to investigate sildenafil presence simultaneously. In the present study we investigated the effect of different extraction media on the sildenafil detection by ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry illegally mixed into herbal honey mixture in Turkey. It is an automated detection and data filtering was performed using the Waters UNIFI software.

Materials and methods

This research was carried out at the Novagenix Bioanalytical R&D Centre, Herbal Product Analysis Laboratory, Ankara, Turkey.

Chemicals and materials

HPLC grade methanol, formic acid and acetonitrile (Merck, Germany, purity >99.9%) were purchased and used with no further purification. Millipore filtered water was obtained by passing the distilled water through a Milli-Q[®] system (Millipore Corp., Milford, MA). Sildenafil reference standard was obtained from

Sigma (St. Louis, MO) and dissolved in HPLC grade methanol.

Sample

The herbal honey mixture found in Ankara, Turkey, was used as an immune system booster. A premarketing regulatory review was sought for this honey and claimed suspicion on the presence of sildenafil or other PDE-5 inhibitors. Many products labelled as "herbal" or "all natural" (herbal/natural) escape a premarketing regulatory review through marketing as over-the-counter (OTC) dietary supplements.

Sample preparation

Three different extraction methods were applied to the sample. For the first extraction, 5 g honey mixture was dissolved in 15 mL methanol, for the second extraction 5 g honey mixture was dissolved in 15 mL acetonitrile, and for the third extraction 5 g honey mixture was dissolved in 15 mL acetonitrile/water (50:50, v/v). The samples were subjected to ultrasonic treatment for 15 minutes, and then cooled to room temperature. After the sonication, samples were centrifuged at 5500 rpm. Approximately 2 mL of each supernatant solution after microfiltration was added in three vials and placed in the UPLC autosampler. The samples were analyzed by UPLC/Q-ToF.

Standard preparation

Sildenafil stock standard solution was prepared by accurately weighing an amount equivalent to 5 mg of the drug, which was dissolved in 20 mL methanol to produce a concentration of 0.25 mg/mL.

Preparation of mobile phase

HPLC-grade acetonitrile and formic acid (Merck, Germany, purity >99.9%) were purchased and used with no further purification. The mobile phase was

0.1% formic acid aqueous solution/acetonitrile (70:30, v/v) filtered through a cellulose acetate membrane of 0.2 μm (Waters, USA) and degassed in ultrasonic bath for 5 minutes before being used. Milli-Q[®] ultrapure water was used for mobile phase preparation.

UPLC- MS Analysis

The samples were analyzed on a quadrupole time-of-flight mass spectrometer (Xevo G2-XS Qtof-MS, Waters Micromass, UK) with an electrospray ionization probe in positive ion acquisition mode (ESI(+)), coupled to an ultraefficient liquid chromatography Acquity[®] I-Class UPLC-TUV consisting of a binary pump, autosampler, column oven and a tunable UV-detector (Waters, USA). Scientific Information System from Waters (USA). Separation was performed in a column Acquity[®] UPLC BEH C18 (2.1 μm x 50 mm x 1.7 μm). Mobile phase flow rate was 0.7 mL/min. Column was maintained at 60 °C during the analysis. Chromatograms were monitored at 290 nm. Chromatographic separation lasted for 2 min. For LC-ESI (+) QTOF-MS system, ESI (+) source conditions were as follows; source temperature of 120 °C, capillary voltage of 3 kV, cone voltage of 40 V and collision energy 6 eV. The spectra were scanned in the range of m/z from 50 to 2000. The UPLC-Q-TOF-MS data of samples were acquired and analyzed by Waters UNIFI software.

Results and discussion

The chromatographic method for the simultaneous determination of sildenafil used in this study was developed and validated previously for the different analytical matrices (Ortiz et al., 2013). The standard and examined analytes were separated under the given chromatographic conditions.

The herbal honey mixture sample was extracted with different solvents, and different extraction media responded very similarly. Chromatogram of Sildenafil standard is shown in Fig. 2 with the retention time of 0.45 s. Representative chromatograms of sildenafil ($m/z = 474.5$) in different extract honey samples are shown in Fig. 3. Sildenafil was eluted within 2 min of the chromatographic run. The retention times for sildenafil in different extractants were 0.47 in methanol, 0.45 in acetonitrile, and 0.47 min in acetonitrile/water (50:50, v:v), respectively. The peaks were well-resolved with no overlapping. When compared to the standard, the herbal honey mixture sample contained sildenafil having similar retention time to the standard. UPLC/Q-ToF is used in pharmaceutical analytical laboratories throughout the world for identity testing, because it is simple, rapid, robust and inexpensive. In confirmation experiments, we conducted a qualitative analysis.

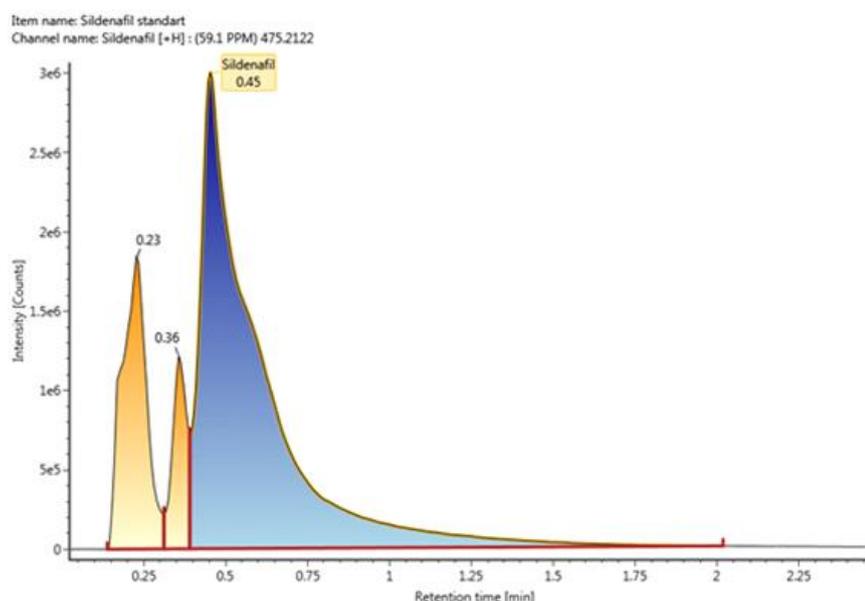


Fig. 2. UPLC chromatogram of sildenafil standard

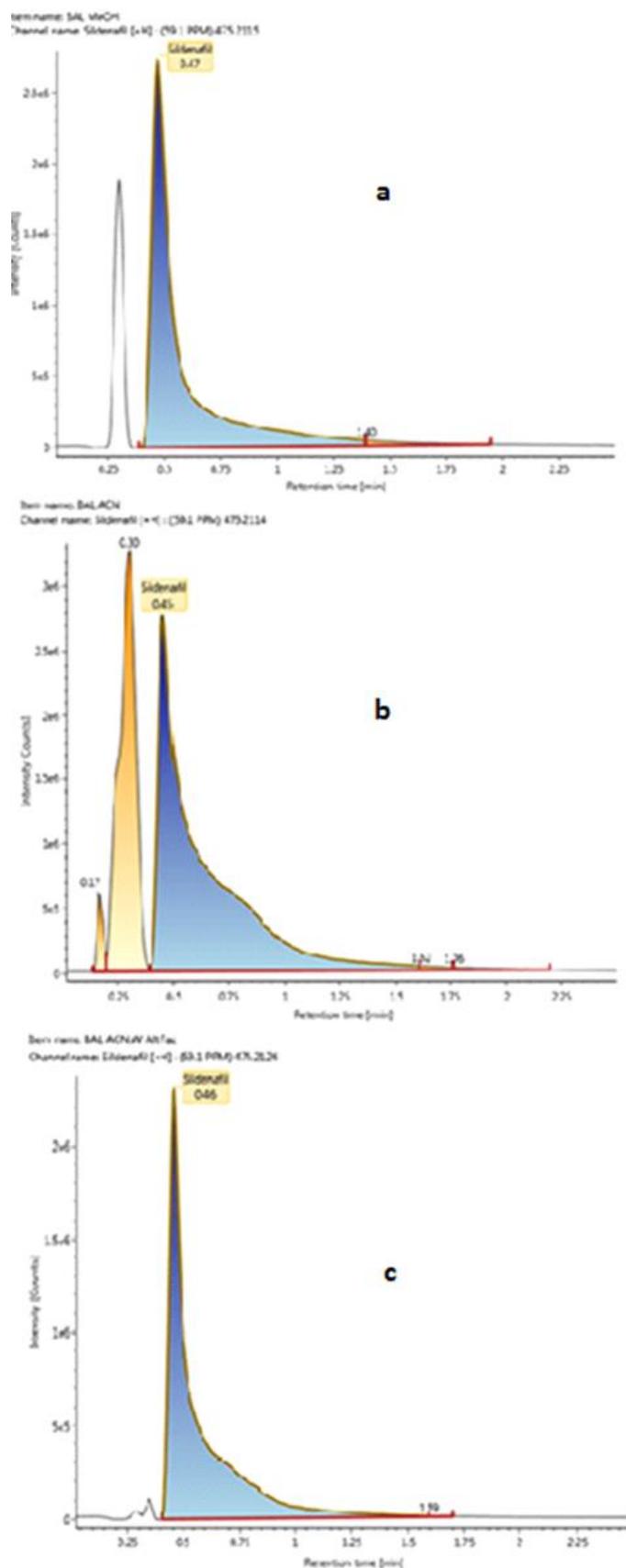


Fig. 3. Representative chromatograms of sildenafil (SIL; $m/z = 474.5$). SIR, single-ion recording; ES+, electrospray positive ion mode. Chromatograms of product (a), (b) and (c) extracts. Peaks correspond to peak of sildenafil. Chromatogram (a) sample extractant; Methanol, (b) sample extractant; Acetonitrile, (c) sample extractant; Acetonitrile/Water (50:50, v:v)

Various methods have been employed in detection of sildenafil and related compounds in various media. High performance liquid chromatography (HPLC) method is developed for the determination of the amount of sildenafil in pharmaceutical preparations and for evaluation of pharmacokinetic parameters of sildenafil. Thin layer chromatography (TLC) methods are used for the identification of sildenafil as an illegal additive in roborant soft drinks and foods (Al-Tahami, 2014).

The sample examined in the present study is a herbal honey mixture used as an immune system booster and the composition of honey is rather complicated. The presence of the active substance has been confirmed in the tested sample. In qualitative terms, there is a high incidence of adulteration of dietary supplements in the Turkish market.

The high incidence of adulteration with sildenafil is due to the fact that it is likely to be discovered first, for that matter being the cheapest in procurement. This way the manufacturer will be able to reduce the cost of the production as well as to increase profit margin (Al-Tahami, 2014).

Conclusions

The derived data confirmed that the multi-residual ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-Tof) method is a fast and reliable method for the routine analysis of honey products adulterated with of PDE-5 inhibitors. The influence of different solvents and different extraction was examined, and different extraction media responded very similarly. To our knowledge, this is the first time that sildenafil illegally mixed into a herbal honey mixture has been found by ultra performance liquid chromatography- quadrupole time of flight mass spectrometry using Waters UNIFI software in Turkey.

These findings confirm the phenomenon of adulteration of food supplements in the world market, which is increasing in recent years. This represents a serious risk to public health considering that the synthetic analogues of approved PDE-5 inhibitors are often not characterized for their efficacy and toxicity, and that their mechanism of action might involve other receptors. Their presence in the market represents an important challenge for the scientific investigation and for the subsequent judicial process. Consequently, the authorities should have a programme for screening adulterants in

counterfeit herbal products, quickly spot new trends in which drugs are being counterfeited and stop distribution of fake medicines.

Acknowledgements

The authors would like to thank Novagenix Bioanalytical R&D Centre for their support of this work and for supplying the authors with reference standard.

References

- Alkharfy, K.M. (2009): Simple and sensitive LC-ESI-MS method for the quantitation of sildenafil in plasma samples. *J. Sep. Science.* 32, 3866–3870. <https://doi.org/10.1002/jssc.200900469>.
- Al-Tahami, K. (2014): Determination of sildenafil, vardenafil and tadalafil in dietary supplements sold in the Yemeni market. *Int. J. Scient. Res. (IJSR)* 3.4, 403-405. <https://doi.org/10.15373/22778179/APR2014/143>.
- Damiano, F., Silva, C., Gregori, A., Vacondio, F., Mor, M., Menozzi, M., Di Giorgio, D. (2014): Analysis of illicit dietary supplements sold in the Italian market: Identification of a sildenafil thioderivative as adulterant using UPLC–TOF/MS and GC/MS. *Sci. Justice* 54 (3), 228-237. <https://doi.org/10.1016/j.scijus.2014.02.009>.
- Maryam A. (2016): Some Aspects of the Problem of Adulterated Herbal Medicines by the Illegal Addition of Active Pharmaceutical Ingredients. *J. Clin. Toxicol.* 6, e124. <https://doi.org/10.4172/2161-0495.1000e124>.
- Ortiz, R. S., de Cássia Mariotti, K., Holzschuh, M. H., Romão, W., Limberger, R. P., Mayorga, P. (2013): Profiling counterfeit Cialis, Viagra and analogs by UPLC–MS. *Forensic sci. int.* 229 (1), 13-20. <https://doi.org/10.1016/j.forsciint.2013.03.024>.
- Ren, Y., Wu, C., Zhang, J. (2012): Simultaneous screening and determination of 18 illegal adulterants in herbal medicines and health foods for male sexual potency by ultra-fast liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Sep. Sci.* 35, 2847-257. <https://doi.org/10.1002/jssc.201200280>.
- Rendell, M. S., Rajfer, J., Wicker, P. A., Smith, M. D., Sildenafil Diabetes Study Group. (1999): Sildenafil for treatment of erectile dysfunction in men with diabetes: a randomized controlled trial. *Jama*, 281 (5), 421-426. <https://doi.org/10.1001/jama.281.5.421>.
- Umrani, D.N., Ramesh, K.G. (1999): Pharmacology of sildenafil citrate. *Indian J. Physiol. Pharmacol.* 43, 160-164.

Received: September 18, 2017

Accepted: October 18, 2017