Simultaneous LC-MS/MS Determination of Sildenafil and Related Analogues Added Illegally to Herbal Products Intended for the Treatment of Erectile Dysfunction

Martin Dušek¹, Miloslav Šanda², Petr Cuhra³, Sofiá Baršová¹

E-mail: martin.dusek@szpi.gov.cz

¹ Czech Agriculture and Food Inspection Authority (CFAIA), Prague, Czech Republic
² Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Overview

Purpose
- Develop an LC-MS/MS screening method for detection of designer analogs of sildenafil, tadalfil and vardenafil in a herbal dietary supplements.
- Develop an LC-MS/MS method for determination of sildenafil, hydromocitadin, acetildenafil, hydrochlorofiadil, tadalfil, ethyltidadil and yohimbine in a natural dietary supplements.

Method
- Extract herbal supplements with acetonitrile.
- Modify known LC solvent system (designated for UV detection) for use with positive ion electrospray LC/MS.
- Perform LC/MS analyses of “all-natural” herbal dietary supplements marketed to enhance sexual function.

Results
- Developed LC-MS method is applicable as a screening method for detection of PDE-5 inhibitors.
- Developed LC-ESI-MS/MS method is applicable for determination of eight PDE-5 inhibitors and yohimbine.
- Three herbal dietary supplements from twenty-four analyzed were found to contain illegally added PDE-5 inhibitors.

Introduction

Sildenafil (Viagra), vardenafil (Levitra) and tadalfil (Cialis) are phosphodiesterase-5 (PDE-5) inhibitors approved for the treatment of erectile dysfunction (ED). Viagra, Levitra and Cialis are prescription drugs and must be used under medical supervision. Despite the efficacy of PDE-5 inhibitors as a treatment for ED, their drawbacks are also notable. Adverse effects such as headache, visual disturbances, dyspepsia and muscle aches have been reported. Therewithal, patients with hypertension, hyperlipidemia and ischemic heart diseases, which using nitrate medication, should not take synthetic PDE-5 inhibitors. These patients may resort to herbal remedies and dietary supplement for treatment for ED. Nevertheless, some herbal products have been illegally adulterated not only with the three FDA approved ED drugs, but synthetic analogues of these drugs have been found as well. However, because herbal product are often considered to be “all natural” and inherently safer and then healthier are synthetic ingredients, there exists a risk for those individuals for whom synthetic PDE-5 inhibitors are contraindicated. The list of ED drugs analogous in herbal dietary supplements continues to grow and includes such compounds as homosildenafil⁴,⁵ hydroxymocitadin⁶,⁷ acetildenafil⁶,⁷, hydrosuccinylidil⁸,⁹ piperidino adamantidil⁸,⁹, amindimetadil⁸,⁹, piperidinobenzidil⁴,⁵ and methsildenafil¹⁰. Therefore, it is necessary to develop an analytical method to determine as wide as possible spectrums of synthetic PDE-5 inhibitors in herbal products marketed to enhance sexual function or efficacy.

Methods and Materials

Sample preparation and Extraction:
- Twenty four samples of natural dietary supplements were analyzed for PDE-5 inhibitors. Twenty one samples were in capsule dosage form, two were liquid and one was in two cases of capsule dosage form, a last portion was prepared from the content of either five capsules or one capsule depending on the number of capsules in the sample. 100 mg was added to 25 mL MeCN and ultrasonicated for 10 min. The suspension was centrifugated at 4500 rpm for 5 min, and a portion of the supernatant solution was diluted 50-fold by MeCN H₂O (1:1) three times prior LC-MS/MS analysis. The samples were diluted 50x by MeCN H₂O and filtered prior LC-MS analysis.

Liquid Chromatography/Mass Spectrometry:
- HPLC Conditions: Column: Phenomenex Gemini 3 µm C18 column (100 mm × 2.0 mm). Column oven temperature: 35 °C. Injection volume: 10 µL. Flow rate: 0.3 mL/min. Solvent A: 5 mM ammonium formate; Solvent B: acetonitrile. LC gradient: 0 → 0 min: 20%; 0 → 15 min: 50%; 15 → 20 min: 50% B; 20 → 25 min: 30% B; 25 → 30 min: 80% B; 30 → 30 min: 80% B.
- Precursor ion scan mode: precursor m/z 263, 450-550 amu, 0.2 s/m/z 263, 550-650 amu, 0.4 s/m/z 263, 350-450 amu, 0.6 s/m/z 312, 400-500 amu, 0.2 s.

Results and Discussion

LC-MS/MS screening method for detection PDE-5 inhibitors:
- Collision-induced dissociation (CID) MS in positive ion electrospray ionization (ESI) was optimized by direct injection of the MeCN-H₂O (1:1) solution of ED drugs (5 µg/mL) at 10 µL/min. The collision energy varied from 5 to 13 eV. The MS² product ion spectra are shown in Fig. 1. These pictures represent four groups of ED drug, and related analogues of them, with one (or more than one) common fragment in their fragmentation pattern. The MS² product ion mass spectrum of sildenafil is given in Fig. 1A. The fragment pattern of sildenafil (m/z 475) produced fragments at m/z 211, 209, 208, 206 and 204. The most abundant fragments at m/z 211 and 283 were also observed in MS² product ion mass spectrum of hydroxymocitadin and the structure of these fragments (see Fig. 1A) is independent of the modification of the sildenafil structure. These common fragments were observed for the other groups of ED drugs. Both MS² product ion spectra of acetildenafil (see Fig. 1B) and hydroxymocitadin include common fragment at m/z 287. The MS² product ion mass spectra of tadalfil (see Fig. 1C), ethyltidadil and amindimetadil include common fragment at m/z 269. Last fourth group that include vardenafil (see Fig. 1D) and piperidino adamantidil in common fragment at m/z 312 and 151. These fragments are unique for each group and over the group these fragments are independent of the substitution of molecules. These presently known common fragments could be used for the confirmative method based on the MS/MS precursor ion scan mode. In this method over the range 100 µm the precursor ion given fragments at m/z 263, 283, 297, 163, 329 were scanned.
- Chromatographic separation and LC/MS/MS analysis:
- The instrument was operated in positive ESI mode, with 18 MS/MS transitions monitored during one time segments in Multiple Reaction Monitoring (MRM) mode. Each compound was quantified and confirmed using at least two MRM transitions. Selection and tuning of transitions, as well as analyte dependencies parameters determined in the positive ESI mode, and collision energy (CE), were performed using infusion and mixing of individual erectile drug solution with the mobile phase by means of the T-piece. The mass transition with base linearity and lower LOD was used for quantification. Finally, m/z 475/283 (sildenafil), 481/151 (vardenafil), 555/478 (hydrosuccinylidil), 467/287 (acetildenafil), 453/257 (hydroxyadamidil), 386/286 (tadalfil), 404/282 (ethyltidadil) and 450/151 (piperidino adamantidil) transitions were selected. Fig. 4 showed SRM traces obtained from 0.5 µg/mL standard solution of ED drugs mixture.

Conclusion

In the study, liquid chromatography with MS detection was used originally for the determination of eight synthetic PDE-5 inhibitors and related analogues. The potential of the PDE-5 inhibitor method was extended by precursor ion scan mode using with fragments specific for each group of related analogues of PDE-5 inhibitors. Although precursor ion scan is not able to distinguish two groups of PDE-5 inhibitors from each (amindimetadil-ethyltidadil and homosildenafil-methsildenafil) due to their similar or the same molecular weight, this method could be very useful tool for scanning for presence of illegally added PDE-5 inhibitors. This method has been successfully applied for screening for PDE-5 inhibitors in herbal products marketed to enhance sexual function. Among the twenty-four samples, three were found to contain illegally added PDE-5 inhibitors.

References