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Rapid-screening detection of acetildenafils, sildenafils and avanafil by ion mobility spectrometry☆

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ABSTRACT

Ion mobility spectrometry was used as a rapid screening tool for the detection of acetildenafils, sildenafils and avanafil within adulterated herbal supplement matrices. Acetildenafils show a tendency for partial fragmentation during the desorption/ionization process affording two peaks in the ion mobility spectrum in addition to the intact compound. The fragmentation appears to occur α to the carbonyl group along the C—N bond attaching the piperazine moiety, producing a common fragment (K_o = 1.0280 $cm^2 V^{-1} s^{-1}$) along with the respective piperazine fragment. The sildenafils and avanafil afford one molecular ion peak per compound.

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1. Introduction

Erectile dysfunction is a condition that affects about 30 million men in the United States and may result from psychological, neurological, hormonal, arterial, or cavernosal impairment or from a combination of these factors [1–3]. Traditionally a physician would diagnose the condition after evaluating a thorough history (medical, sexual and psychological) along with physical examination and appropriate laboratory tests. Treatments for erectile dysfunction include psychosexual therapy, vacuum constriction devices, vascular surgery, alprostadil dosing or prescription oral type 5 phosphodiesterase (PDE-5) inhibitors. The relatively low-cost, effectiveness and pain-free therapy offered by PDE-5 oral medication has allowed it to become a recommended first-line treatment [3].

FDA-approved PDE-5 inhibitors include sildenafil (Viagra®, introduced by Pfizer in 1998), vardenafil (Levitra®, Bayer, 2003), tadalafil (Cialis®, Eli Lilly, 2003) and the recently approved avanafil (Stendra™, Vivus, Inc., 2012). PDE-5 inhibitors work in the presence of sexual stimulation by inhibiting phosphodiesterase type 5, which is responsible for hydrolyzing cyclic guanosine monophosphate (cGMP) to GMP. Through inhibition of this hydrolysis a return to the flaccid state is blocked and an erection is maintained [3].

PDE-5 inhibitors are legally obtained with a physician prescription, but now the Internet provides discreet alternatives marketed as dietary or herbal supplements. The popularity of the PDE-5 inhibitor drugs has led to extensive counterfeiting in the herbal supplement market, which has become a major arena for numerous generic knockoffs and designer analogs intended to avoid detection by regulatory agencies [4–36]. Since dietary supplements do not need FDA approval for marketing, a large and ever changing list of suppliers increase the possibility for adulteration with a drug or drug analog. Further, trafficking fake pharmaceuticals is very profitable: counterfeit Viagra® boasts a profit margin ten times higher than the street drug heroin [37]. Additionally, trafficking pharmaceuticals has lacked the severe penalties associated with Class I drugs (heroin, LSD), though the recently signed Food and Drug Administration Safety and Innovation Act aims to address this topic [38].

In many cases of herbal supplements the illegally added drug is not declared on the product label in an effort to conceal their addition from regulators and the consumer. This practice promotes the appearance of an "all-natural proprietary blend" that gives rise to the product's efficacy. This deception can have hazardous consequences on consumers who also utilize muscle-building supplements that increase nitric oxide production or those who take nitrates for heart disease, as the combination can lead to a sudden drop in blood pressure followed by heart attack. For those supplements which contain drug analogs the risk is unknown as these compounds have not undergone the

☆ The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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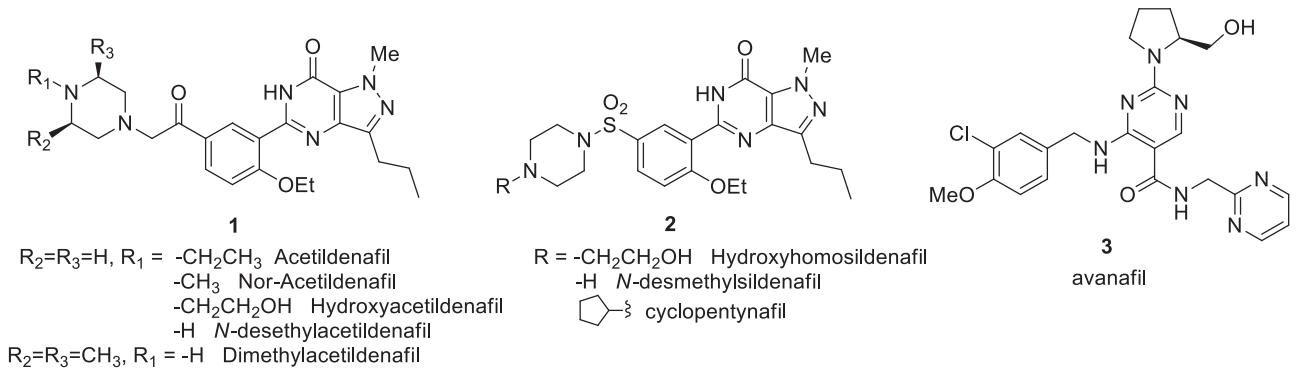

Fig. 1. Study analytes.

clinical trials necessary for approval and no toxicity data has been obtained.

Given the sheer volume of herbal supplement products arriving in the U.S. a rapid screening method for detecting PDE-5 inhibitors and their analogs is needed. A pass/fail approach to screening products allows a reduction in time and resources used through traditional lab analysis (HPLC, GC, etc.) and speeds up turnaround times for health alerts issued by the FDA. Previous work in our laboratory has shown ion mobility spectrometry (IMS) to be a useful rapid screening approach to the analysis of herbal supplements for adulteration by sildenafil, vardenafil, tadalafil and five known sildenafil analogs [10]. Herein we expand the scope of IMS for the detection of the acetildenafil family of analogs, along with previously untested sildenafil analogs and the recently approved avanafil in herbal supplements (Fig. 1). Acetildenafils (**1**) differ from sildenafils (**2**) via replacement of the SO_2 group with an acetyl group, a structural difference that affects their physical properties as well as the number of observed peaks in the ion chromatograms. Avanafil (**3**) adds a whole new structural motif to the list.

2. Experimental

2.1. Materials

Reference standards of acetildenafil (94.4%), hydroxyacetildenafil (97.2%), dimethylacetildenafil (98.1%), *N*-desethylacetildenafil (86.3%), nor-acetildenafil (96.6%), cyclopentynafil (98.5%), *N*-desmethylsildenafil (99.6%) and hydroxyhomosildenafil (98.0%) were synthesized in-house according to the literature and characterized using nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) [5,7,13,27,29,39–42]. Purities were assessed by high-low chromatography and were used for the estimation of limit of detection [43]. Avanafil reference standard (100.0%) was obtained from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). 1-Methyl piperazine, 1-ethylpiperazine, 1-(2-hydroxyethyl)piperazine and 2,6-dimethylpiperazine were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Piperazine was received from Acros Organics (Morris Plains, NJ, USA). 1-Cyclopentylpiperazine was purchased from Matrix Scientific (Columbia, SC, USA). HPLC-grade isopropyl alcohol (IPA) was obtained from EM Science (Gibbstown, NJ, USA) and hydrochloric acid (HCl) was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and used as received. Water was purified to 18 MΩ cm using a Milli-Q water system from Millipore (Bedford, MA, USA). Five herbal supplements previously analyzed [10] and determined to be free of PDE-5 inhibitors were used as matrices for spiking in this work.

2.2. Reference standards, samples and piperazines preparations

A diluent of 75:25 IPA:0.1 N HCl was used to dissolve standards using sonication for 10 min to produce solutions at concentrations near 200 μg/ml, which were then diluted 1:1 with IPA to afford ~100 μg/ml stock solutions. Stock solutions were diluted further with IPA to afford 0.5, 1.0, 2.5, 5.0 and 10 μg/ml in order to estimate limit of detection (LOD). Composites of each unadulterated herbal supplement were made by mixing the contents of 2–3 capsules. IMS analysis showed close uniformity across the 5 composites, so one composite was selected to represent all five for spiking. For spiking experiments approximately 1.5 mg of representative composite was added to a centrifuge tube and diluted with 50 ml of diluent, then centrifuged for 15 min at 3100 rpm. The composite extract solution was decanted and portions were spiked with standard stock solution to afford target concentrations of ca. 2.0 μg/ml of drug or drug analog for sample analysis. For the piperazine study, piperazines were dissolved in diluent at approximately 200 μg/ml and diluted 1:1 with IPA to afford ca. 100 μg/ml stock solutions. A portion of the stock solution was then mixed with the composite extract solution prepared above to give target concentrations of ca. 2 μg/ml.

2.3. Ion mobility spectrometry

An IONSCAN®-LS (Smiths Detection, Danbury, CT, USA) ion mobility spectrometer operated in positive ion mode was used for analysis. Operating conditions are as follows: drift heater temp. (°C) = 232; inlet heater temp. (°C) = 289; desorber temp. (°C) = 291; calibrant block heater temp. (°C) = 78; drift flow (cc/min) = 300; analysis duration (s) = 13.75; shutter grid width (ms) = 0.2; scan period (ms) = 50; number of co-added scans per segment = 28; sampling period (μs) = 50. Isobutyramide served as the internal calibrant with a reduced mobility $K_o = 1.5022\ cm^2\ V^{-1}\ s^{-1}$. Spectra were recorded using the IONSCAN®-LS IM-Station Software version 5.389. For analysis 1 μl sample was deposited on a Teflon substrate using an autosampler and the volatiles were allowed to evaporate. Analyte bands were identified visually and user-selected for analysis by the IM-Station Software. The software fit the selected bands to Gaussian band shapes and reported the band peak drift time, full width at half maximum (FWHM), amplitude, area and computed the reduced ion mobility from the band peak drift time.

2.4. Mass spectrometry

Solutions of the analogs in diluent at concentrations of 1 μg/ml were prepared and analyzed by direct infusion using a Thermo

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