

ANALYSIS OF DIETARY SUPPLEMENTS AND DRUGS FOR ERECTILE DYSFUNCTION BY HPLC-UV

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Abstract: The popularity of dietary supplements and drugs for erectile dysfunction on the internet, permissive legal framework in this domain and purchasing these drugs from pharmacy on prescription only, explain why they are often subject to counterfeit. A HPLC-UV method for the identification and quantification of sildenafil, tadalafil and vardenafil in dietary supplements and drugs is described. Separation of these substances was performed on a Phenomenex C₈ 5 μ m 150x4.6 mm column, using as mobile phase a mixture of phase A = 0.1% formic acid in water and phase B = 0.1% formic acid in acetonitrile, in a concentration gradient, phase A concentration decreasing from 80 to 20% in 15 minutes. The detection was set at 262 nm. The supplements purchased from pharmacies did not contain phosphodiesterase 5 inhibitors, but in two supplements purchased on the internet, sildenafil was quantified (13.87 \pm 1.79 mg/capsule and 18.34 \pm 2.16 mg/capsule). The method was used for the determination of sildenafil in Viagra[®] 100 mg tablets purchased online, their content was higher by 35.80% from the declared composition.

Cuvinte cheie: suplimente alimentare, HPLC-UV, sildenafil, tadalafil, vardenafil

Rezumat: Popularitatea de care se bucură pe internet suplimentele alimentare și medicamentele pentru disfuncție erectilă, cadrul legal permisiv în acest domeniu și achiziționarea medicamentelor din farmacie doar pe bază de prescripție medicală, fac ca acestea să fie adesea falsificate. Este descrisă o metoda HPLC-UV de identificare și cuantificare a sildenafilului, tadalafilului și vardenafilului din suplimente alimentare și medicamente. Separarea acestor substanțe s-a făcut pe o coloană Phenomenex C₈ 150x4,6 mm 5 μ m, folosind ca fază mobilă un amestec de A=0,1% acid formic în apă și B=0,1% acid formic în acetonitril în gradient de concentrație, faza A scăzând de la 80 la 20 % în 15 minute. Detecția s-a făcut la 262 nm. În suplimentele alimentare achiziționate din farmacii nu s-au pus în evidență substanțe din clasa inhibitorilor de fosfodiesterază 5 de tipul celor descrise, dar în două suplimente achiziționate de pe internet a fost determinat cantitativ sildenafilul (13,87 \pm 1,79 mg/capsulă și, respectiv, 18,34 \pm 2,16 mg/capsulă). Metoda a fost utilizată și pentru dozarea sildenafilului din tablete Viagra[®] de 100 mg achiziționate de pe internet, conținutul acestora fiind mai mare cu 35,80% față de compoziția declarată.

INTRODUCTION

Phosphodiesterase-5 inhibitors are first-line medication for the treatment of male erectile dysfunction and clinical studies show that they are effective and well tolerated by patients.(1) Sildenafil was initially introduced in therapy for pulmonary arterial hypertension, however, has proven effective in the treatment of erectile dysfunction and in 2003, the Food and Drug Administration (FDA) approved the marketing of vardenafil and tadalafil, as well.(2)

Their use in counterfeit dietary supplements for enhancing male potency is due primarily to permissive legislation in food supplements (3) and secondly, the fact that these substances are released from pharmacy and by prescription only.

The literature presents many studies on the sildenafil, vardenafil or tadalafil content of drugs or dietary supplements purchased on the "black market" or on the internet. Drugs purchased on the internet do not have a precise dosage of the active substance or contain in addition to the active substance

other substances and expose consumers to the risk of overdose, additive or synergistic effect.(4)

In addition to the substances as such, in literature, at least 46 different structural analogs of these compounds are identified in dietary supplements. Unlike structural analogues of phosphodiesterase 5 inhibitors, which are considered experimental substances without marketing authorisation, illegally introduced in what are considered harmless dietary supplements, sildenafil, vardenafil and tadalafil have a known safety profile and clinical efficacy.

FDA warns consumers that at least one third of dietary supplements for enhancing male potency, presumed to be of plant origin contain highly active substances not declared on the label, and the analysis of 17 such products purchased on the internet, 6 of these supplements contained sildenafil, vardenafil and analogues. These counterfeit dietary supplements expose consumers to hazardous potentiating interactions especially with antianginose drugs, such as organic nitrites and nitrates, as patients with erectile dysfunction, generally use polypharmacy

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for the treatment of other associated pathologies, such as hypertension, diabetes mellitus, hypercholesterolemia etc.(5)

METHODS

Analyzed dietary supplements and analytical method Standards and Reagents

Calibrators (sildenafil, vardenafil, tadalafil) were of at least 98.0% purity and were purchased from Sigma-Aldrich.

All solvents methanol, acetonitrile, formic acid were of HPLC grade and purchased from Merck (Merck KGaA, Darmstadt, Germany).

Preparation of standard solutions

Stock solutions of 1 mg/ml in methanol were prepared and the working solution was obtained by diluting the stock solution with methanol.

The chromatographic system

Measurements were performed on a Merck Hitachi chromatographic system consisting in: L-7100 binary pump with degasser L-7612; automatic injector L-7200 equipped with L-7360 thermostat and DAD detector 7455.

Equipment used:

AB54S balance (Mettler-Toledo), MP225 pH-meter (Mettler-Toledo), 2-15 centrifuge (Sigma), mixer 10 (Falc Instruments), water purifying apparatus Direct Q (Millipore), ultrasonic bath Transsonic T700H (Elma).

Chromatography column: Phenomenex C₈ 150x4.6 mm, 5µm

Mobile phase

The mobile phase consisted of phase A = 0.1% formic acid in water and phase B = 0.1% formic acid in acetonitrile in a concentration gradient, phase A decreasing from 80 to 20% in 15 minutes.

Detection was set at 262 nm.

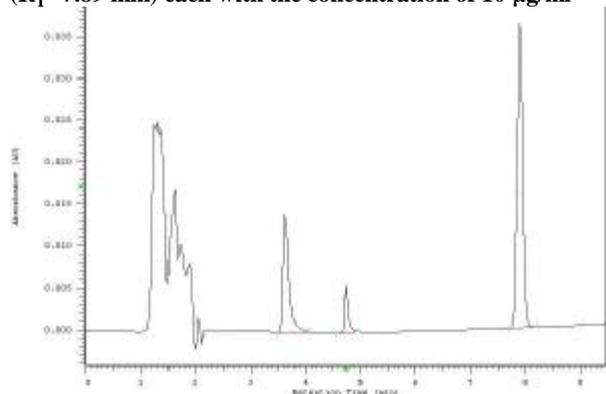
Dietary supplements and drugs purchased on the internet

A total of five dietary supplements were purchased from pharmacy and 5 supplements and a drug (Viagra[®] 100 mg) from the internet. Extraction of active substances was performed with mobile phase by stirring in an ultrasonic bath for 20 minutes at room temperature. The mixture was centrifuged at 3500 rpm for 15 minutes, filtered and the supernatant was injected into the chromatographic system.

RESULTS

The described method allows a good separation of sildenafil (R_T-7.89 min), tadalafil (R_T-3.77 min) and vardenafil (R_T-4.67 min) from the mixture (see figure no. 1).

Figure no. 1. Chromatogram of the standard mixture of tadalafil (R_T-3.77 min), vardenafil (R_T-4.67 min), sildenafil (R_T-7.89 min) each with the concentration of 10 µg/ml



Analysis of dietary supplements for the treatment of

erectile dysfunction in men purchased in pharmacy (N = 5 supplements) has not revealed the presence of sildenafil, tadalafil or vardenafil while in supplements purchased on the internet (N = 5 supplements) in two of them sildenafil was identified (see figure no. 2). The two alleged vegetal supplements contained 13.87 ± 1.79 mg sildenafil / capsule and respectively 18.34 ± 2.16 mg / capsule.

Figure no. 2. Chromatogram of a dietary supplement sample counterfeit with sildenafil (R_T-7.78 min)

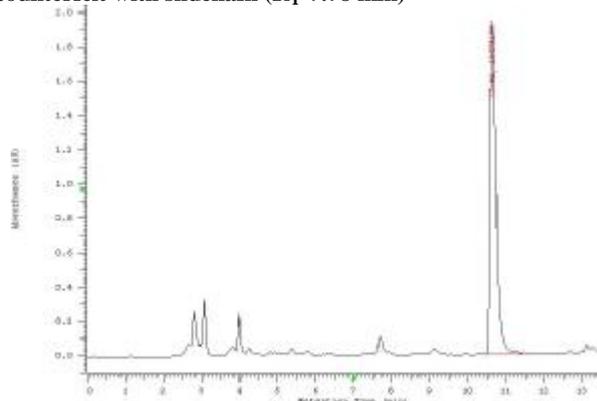


Table no. 1. Sildenafil content of dietary supplements purchased on the internet

Dietary supplement	Retention time (minutes)	Sildenafil (mg/pharmaceutical formulation)
S ₁	7.78	13.87 ± 1.79 mg/capsule
S ₂	7.72	18.34 ± 2.16 mg/capsule

Sildenafil containing product (Viagra[®] 100 mg) purchased from the internet presented an average of 135.80 ± 5.07 mg sildenafil/tablet (+35.80%).

Table no. 2. Sildenafil content of Viagra[®] tablets purchased on the internet

Sample no.	Declared content mg sildenafil/tablet	Real content mg sildenafil/tablet	Difference %
P ₁	100 mg	133.77	33.77
P ₂		141.24	41.24
P ₃		129.76	29.76
P ₄		138.43	38.43
Average value		135.80	35.80
SD		5.07	
CV%		3.73	

DISCUSSIONS

The developed analytical method is a simple and cheap alternative to determination by LC-MS.

After outlawing "spice shop" stores in Romania, selling counterfeit goods allegedly of vegetal origin is widely performed through the internet.(6)

Analyses show that dietary supplements purchased from pharmacy (5 supplements for erectile dysfunction) did not contain *phosphodiesterase-5* inhibitors such as sildenafil, tadalafil or vardenafil. Some supplements purchased on the internet (5 dietary supplements), in two of them have revealed small doses of sildenafil (13.87 mg/capsule respectively 18.34 mg/capsule). A study in Japan shows that sales of counterfeit dietary supplements or drugs on the black market exceed 2.5 times the legal sales.(7) Analysis of 538 counterfeit dietary supplements confiscated in the Netherlands between 2007-2010 shows that 98% contained *phosphodiesterase 5* inhibitors (72%

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containing sildenafil, 14% containing tadalafil and only 2% vardenafil), this study concluded that the use of these substances in food supplements for erectile dysfunction is intended to sell effective products despite the risk of jeopardizing health.(8) Besides sildenafil, tadalafil or vardenafil supplements may contain structural analogs without marketing authorisation, with unpredictable side effects due to lack of selectivity PDE-5/PDE-6.(9)

The sildenafil tablets (Viagra® 100 mg) purchased on the internet, and actually containing 135.80 ± 5.07 mg sildenafil (+35.80%), expose the consumers primarily to cardiovascular adverse effects. A 2010 meta-analysis of several studies show that cardiovascular diseases are a cause of erectile dysfunction in men and at the same time the use of *phosphodiesterase 5* inhibitors in these patients produce adverse cardiovascular effects (increased risk of myocardial infarction, palpitations, tachycardia).(10)

Another possible complication of therapy with *phosphodiesterase 5* inhibitors is priapism described in 2.5% of patients after taking 50 mg sildenafil and in 2.7% of patients after taking 100 mg sildenafil.(10) Sildenafil overdose (11) or concomitant medication (alpha-adrenergic antagonists, psychotropic substances such amitriptyline, nortriptyline and fluphenazine) can double the risk of priapism.(10)

This "overdose" of sildenafil in illicit purchased tablets is not a single fact. In 2008 VICTORY project analyzed 518 sildenafil tablets from the "black market", of which 284 (55%) contained the substance in the mentioned dose (100 mg) and 234 (45%) were counterfeit sildenafil tablets, their content varying between 24 -157 mg/tablet which was supposedly containing 100 mg sildenafil citrate.(4)

More recent studies show that tadalafil was identified in illicit supplements in the gelatin capsule composition, so that a routine analysis of the contents of the capsule reveals no falsification.(12)

CONCLUSIONS

10 dietary supplements were analyzed, 5 purchased from the pharmacy did not contain *phosphodiesterase-5* inhibitors such as sildenafil, tadalafil or vardenafil and 5 purchased on the internet, two of them containing a low but active dose of sildenafil. In case of Viagra® tablets purchased on internet, the dose stated on the package was exceeded significantly.

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