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Original Article

Determination of sildenafil and tadalafil adulteration by LC-MS/MS and 23 elements by ICP-MS in food supplements

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ABSTRACT

Background and Aims: Since the increasing prevalence of erectile dysfunction disease, the use of phosphodiesterase type 5 (PDE-5) inhibitors is widespread in the current era. The first and the most known molecule of PDE-5 inhibitors is sildenafil. It is widely used in the market. The second and more developed one is tadalafil. It is known that these substances are found in sexual stimulant food supplements as adulteration. In addition, food supplements can contain metals that can cause hazardous activity by accumulating. In our study, 11 randomly chosen sexual stimulant known products are studied which were bought from different stores in Edirne.
Methods: Adulteration with sildenafil and tadalafil was determined by LC-MS/MS because of the enhanced accuracy and precision, general applicability, and higher selectivity. Also, the determination of 23 metals, including known heavy metals, was made with ICP-MS.
Results: In the study, 7 of 11 samples detected at least 1 compound of sildenafil (up to 92.44 mg in one serving) or tadalafil (up to 23.62 mg in one serving). Neither of the samples exceeded the limits of metal daily intakes that are shown in Table 4.
Conclusion: In past studies, adulteration was determined in food supplements, and it is still detected today. The results we obtained are engrossing for the regulations of sexual stimulant food supplement sales. Their additional effects on total daily intake raise health concerns. The data obtained from this study will contribute to inform consumers and health professionals about the status of food supplements in the market.

Keywords: Adulteration, dietary food supplements, metals, PDE-5 inhibitors, sildenafil, tadalafil

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INTRODUCTION

Erectile dysfunction is a widespread disorder that affects quality of life (Kessler, Sollie, Challacombe, Briggs, & Van Hemelrijck, 2019). The incidence of erectile dysfunction increases with advancing age, and its incidence may increase due to the increasing world average age. The public is interested in food supplements that are stated to be natural because they think they are safe and reliable. Recently, the increase in the variety of supplements and fear of the undesirable effects of the drugs, and the ease of access with online shopping, ensure the growth of this food supplements market. Some brands are likely to adulterate their products for enhancing the sexual stimulant effect. The adulterating compounds are mostly Phosphodiesterase-5 (PDE-5) inhibitors (Tseng, & Lin, 2002; Tucker, Fischer, Upjohn, Mazzera, & Kumar, 2018).

PDE-5 is an enzyme that specifically cleaves and degrades cGMP to 5′-GMP in the corpus cavernosum smooth muscle. PDE-5 inhibitors have a structure that is comparable to that of cGMP; they bind to PDE-5 competitively and prevent cGMP hydrolysis, which improves the effects of nitric oxide. Prolongation of erection time is maintained by an increase in cGMP in smooth muscle cells. The relaxation of corpus cavernosum smooth muscles is not directly impacted by PDE-5 inhibitors. Therefore, for an erection to occur after ingestion of these drugs, there must be sufficient sexual stimulation (Huang & Lie, 2013).

Sildenafil and tadalafil the PDE-5 inhibitors (Figure 1) are used in the treatment of erectile dysfunction and pulmonary hypertension. Tadalafil is also used for the treatment of benign prostatic hyperplasia. Tadalafil causes inhibition of PDE-11 concentrated in the testis, prostate and skeletal muscle, causing pain and myalgia (Huang & Lie, 2013). Since PDE-5 inhibitors are metabolized by the CYP3A4 enzyme, they may interact with substances that cause CYP3A4 inhibition and induction. In addition, concomitant administration of alpha-blockers and PDE-5 inhibitors may cause additive vasodilator effects. Moreover, since the use of nitrates increases the production of cGMP, the use of PDE-5 inhibitors will reduce the degradation of cGMP and cause a synergistic vasodilator effect. Sildenafil could cause myocardial infarction in patients with unknown cardiac disease history; there have been some cases already reported (Feenstra, van Drie-Pierik, Lacle, & Stricker, 1998; Kekilli, Beyazit, Purnak, Dogan, & Atalar, 2005). Flushing, nasopharyngitis, headache, dyspepsia and nasal congestion are the most frequent adverse effects observed with PDE-5 inhibitors. PDE-5 inhibitors have also been linked to rare but dangerous cases of long lasting erections (about more than 4 hours) and priapism (erections lasting painfully more than 6 hours) (Huang & Lie, 2013).

Heavy metals are a type of contaminant that may be found in soil, water, air, plants, food, etc. Exposure to heavy metals may cause DNA damage, oxidative stress and lipid peroxidation on humans and animals (Wu et al., 2016). The presence of metals in food supplements can have significant impacts on human health. Moreover, some of these metals, such as lead, are toxic even at low levels of exposure. This is particularly concerning because food supplements are often taken over a long period

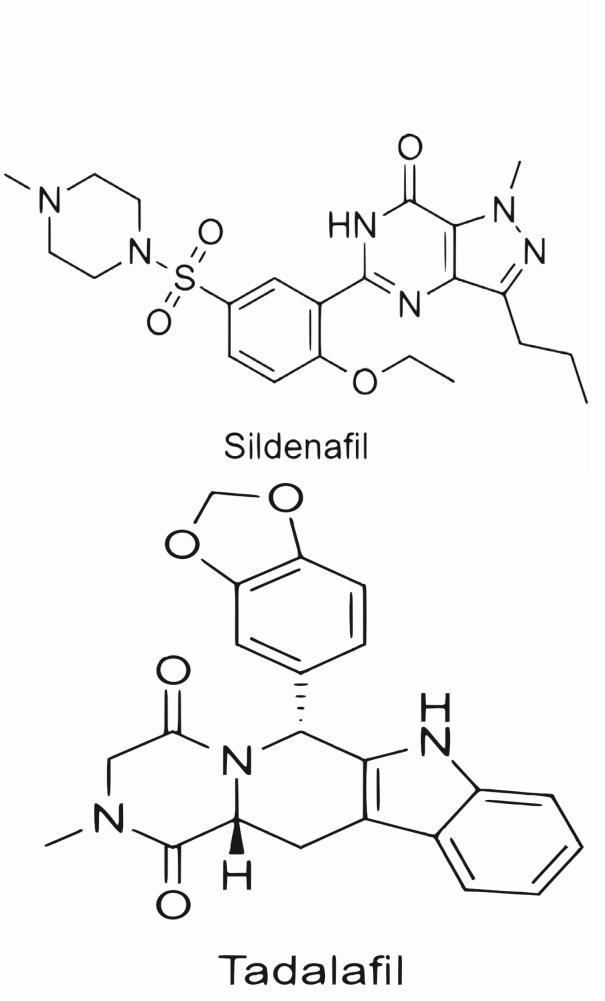

Figure 1. Chemical structures of sildenafil and tadalafil.

of time, which increases the risk of cumulative exposure. Accumulating evidence shows that exposure to heavy metals could raise the risks of obesity, diabetes, kidney dysfunction, cardiovascular diseases, neurodevelopmental disorders, and cancer (Wang et al., 2023). It is possible for dietary supplements that are advertised as herbal or natural to contain heavy metals (Clemens & Ma, 2016). This is because certain herbs and plants can absorb heavy metals from the soil in which they are grown, and thus, these metals can then be concentrated in the supplement. In addition, metals can be found in food supplements due to a variety of sources, such as contamination of raw materials, processing equipment or packaging materials. The public commonly believes that natural products pose no risk to health and that, even in the absence of sufficient evidence of medical benefits, there have no adverse effects.

ICP-MS was performed for the determination of 23 metals in 11 samples in this study. Elements of Li, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Ba, Tl, Pb and Bi were determined. Among them, heavy metals, particularly As, Cd and Pb are of main concern because of their hazardous activity. Maximum levels for certain contaminants in foods are set in Commission Regulation No. 1881/2006 by European Commission (European Commission, 2006). These regulations are

also available in the Turkish Food Codex Legislation (Turkish Food Codex, 2011).

In addition to setting maximum levels for other contaminants such as dioxins, mycotoxins, and nitrates, these regulations also specify limits for certain metals. Specifically, lead, cadmium, arsenic, mercury, and tin are among the contaminants primarily mentioned. Literature shows that high concentrations of these metals sometimes appear in food supplements (Dolan, Nortrup, Bolger, & Capar, 2003; Ernst, 2002).

Elements are usually determined by techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES) (Altundağ, Yildirim, & Altıntığ, 2019), atomic absorption spectrometry (Ahmad et al., 2019), and inductively coupled plasma mass spectrometry (ICP-MS) (Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010). For a convenient result, a sensitive analysis technique such as ICP-MS, which is more advanced than the generally used atomic absorption spectroscopy, is performed in this study.

This study aims to investigate the potential adulteration of sexual stimulant products with sildenafil and tadalafil, to determine the levels of 23 metals, including known heavy metals, and to assess the potential health risks associated with the use of these products. The objectives of the study will be achieved by identifying the presence and concentration of sildenafil and tadalafil in sexual stimulant products sold in Edirne, evaluating the levels of metals in these products, and informing regulators, health professionals, and consumers about the potential risks associated with the use of sexual stimulant food supplements in order to ensure their safety.

MATERIALS AND METHODS

ICP-MS analysis of heavy metals

Analysis of ICP-MS performed with an Agilent 7700 mass spectrometry (Agilent Technologies, Waldbronn, Germany). The EPA Method 200.8 guideline was followed to perform the analysis (U.S. EPA., 1994).

An amount of 0.5 grams of each sample was weighed using an analytical balance. The weighed samples were treated with 5 mL of concentrated HNO_3 (%65, Merck) and subjected to microwave digestion to dissolve the elements into an inorganic environment. The dissolved samples were then placed in Teflon microwave vials, sealed, and evenly placed in the microwave. Sensors were used for pressure and temperature adjustments, and the device parameters were set from the menu. After a 35-minute digestion process, the samples were transferred to storage containers and refrigerated until analysis. Then, a 100-fold dilution was made, and the samples were centrifuged. All dilutions were made with double-deionized water produced by the MilliQ water purification system (Millipore). The element standard solutions were created by diluting a 1000 mg/L stock solution (ICP standard CertiPUR, Merck, Germany). The samples were analyzed by using ICP-MS. Three readings were taken for each sample to obtain results with calculated standard deviations. The standard addition method was used to verify the accuracy of the analysis by preventing interference from the sample matrix.

ICP-MS conditions

The ICP-MS device has a power of 1550 watts and a sample acquisition rate of 0.3 revolutions per second (rps). It operates with an octupole radio frequency (OctP RF) at 180 volts and has a deviation of 13 volts and a depth of 8 millimeters. The maximum blank concentration is 100%, and it requires a stabilization time of 50 seconds. The device has an OctP slope of negative 8 volts, a nebulizer pump rate of 0.1 rps, and a matching voltage of 1.80 volts. It uses an energy separator of 5 volts and has a calibration curve confidence interval of 0.95. The Omega lens operates at 10 volts, and the carrier gas flow rate is 1.05 liters per minute (L/min). The sample acquisition time is 50 seconds, and the relative standard deviation is 5%. The device operates with a cell output of negative 50 volts, sample-to-cone (S/C) temperature of 2 degrees Celsius, layer slope of negative 40 volts, and a cell input of negative 30 volts.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of sildenafil and tadalafil

Reagents

All of the chemical solvents and standards used were of analytical quality. Sildenafil drug tablets were obtained from Biofarma (Istanbul, Türkiye), Tadalafil drug tablets were obtained from Nobel Türkiye (Istanbul, Türkiye), Hyper grade acetonitrile, methanol, dimethyl sulfoxide (DMSO), acetic acid, and formic acid were obtained from Merck (Darmstadt, Germany) for LC-MS.

Sampling

The samples were obtained from pharmacies, gas stations, and herbal stores located in the Edirne province of Türkiye. All the products were of Turkish origin. The samples were stored at room temperature until analysis. For liquid samples, the samples were directly diluted and prepared using the sample preparation procedure below. For solid samples, the drugs were pulverized by using a mortar and pestle to a powder form, and other samples were homogenized in a solvent medium. A total of eleven samples were analyzed, including eight solid and three liquid samples.

Extraction methods

A 1 g sample (liquid 1 mL) was taken into a falcon tube and 10 mL DMSO were added. The solution was vortexed 30 seconds and waited sonicator over 10 minutes at 50 ºC. Samples centrifuged at 13500 rpm for 5 min and 20 µL clear supernatant mixed with 980 µl methanol after extracting the samples filtered with a 0.22 micron nylon filter, and then injected to the LC-MS/MS system (dilution factor is 500).

LC-MS/MS conditions

The Agilent 1260 (Agilent Technologies, Waldbronn, Germany) LC system equipped with a Poroshell EC C18 4.6x150 mm 2.7micron (Agilent Technologies, Wilmington, DE, USA) column set at 40 ºC and a mobile phase flow rate of 0.6 mL/min was used for LC analysis. Gradient elution mobile phases consisted of 2 mM ammonium formate, 0.1 % formic acid in water (solvent A) and 1 % formic acid in methanol (solvent B). The gradient was started with a ratio of 90:10 (A:B). It was held constant for 1 minute. From 1 to 3 minutes, the ratio of A:B was decreased to 20:80. The ratio was kept constant at 20:80

for 6 minutes. At 6.1 minutes, the ratio was reduced to 5:95 and maintained for 10 minutes. At 10.1 minutes, the ratio was returned to the initial concentration of 90:10 (A:B).

The total method lasted 14 minutes. Sample temperature was stabilized at 4 ºC in the autosampler prior to analysis. A 5 µL sample volume was placed into the analytical column for analysis.

Analyses of MS/MS were successfully accomplished on an electrospray ionization (ESI) interface equipped Agilent 6460 triple quadruple LC-MS system (Agilent Technologies, Waldbronn, Germany). Electrospray ionization was executed in the positive ionization mode. The MS was performed with a 500 ms cycle time. For finding the optimal parameters of ion path and ion source of the studied compound, the quantitative optimization was applied by direct injection of standards utilizing an HPLC Agilent 1260. Multiple reaction monitoring (MRM) mode of the dominant product ion for each solution was realized using the optimal conditions. The ion source parameters were as follows: Gas Temperature: 275 ˚C; Gas Flow: 10 l/min; Nebulizer: 40 psi; Sheat Gas Heater: 375 Sheat Gas Flow: 10 l/min; Capillary (positive): 3000V. Multiple reaction monitoring mode consisted of comparison of pair ion (precursor and product ion m/z values) and LC retention times with standards served to confirm the identification of the analyte in the samples (Figure 3). Ion pairs were 475.0/100.0 and 475.0/50.0 for sildenafil, and 390.1/268.0 and 390.1/135.0 for tadalafil. MassHunter (Agilent LC-MS software) was used for both the data collecting and processing.

Calibration curve and quantification

All of the calibration curves were prepared in a 5 point calibration range 5, 10, 25, 50, 100 ng/g and injected 3 times for all points. Repeatability calculations were performed based on the standard deviation of three injections. Results within the range of 2-10% were obtained as repeatable. All the analytes curve linearity is $r^2 \geq 0.995$ (Figure 2). Limit of detection (LOD) and limit of Quantification (LOQ) values of the analytes (calculated over signal to noise ratio) are in Table 1. Because of the sugar content and chocolate samples, tadalafil and sildenafil standards were spiked with blank-chocolate, and recovery values were calculated. Since the standard deviation is below 5%, the precision value of the method is appropriate. When considering the recovery values and standard deviation, it has been determined that the method is highly accurate.

RESULTS AND DISCUSSION

The samples were quickly prepared for analysis because of the simple sample preparation technique that was developed in our study. Solid phase extraction (SPE) is an expensive method and takes a long time to perform. In our study, we applied only solid liquid extraction with DMSO. Our sample preparation time is nearly ten minutes. Song et al. have longer procedures and filtering stages in their study than ours (Song, El-Demerdash, & Lee, 2012). With the standard addition method, 25 and 75 ng/g recoveries were made at low and high concentrations on commercially purchased blank chocolates, and method verification was provided (Table 1). The developed method has been applied to matrices such as herbal products, food products and drug substances, and reproducible results have been obtained. In the literature, different sample preparation methods have been used for each matrix (Oh, Zou, Low, & Koh, 2006).

According to previous studies, several methods can be utilized to determine sildenafil and tadalafil in food supplement products. The most used methods are thin layer chromatog-

Table 1. The partial validation parameters of Tadalafil and Sildenafil in blank chocolate.

Compound	[M+H]+ m/z	R2 (Linearity)	LOD (ng/mL)	LOQ (ng/mL)	Recovery Low (%)	Recovery High (%)	%RSD
Tadalafil	390.1	0.9992	0.06	0.18	82	93	3.55
Sildenafil	475	0.9997	0.07	0.20	86	97	4.37

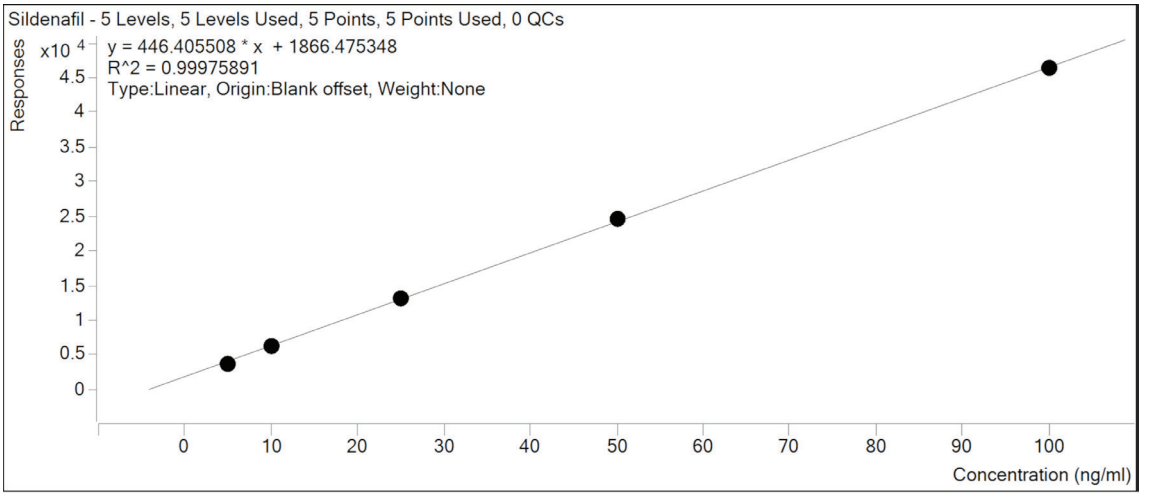

Figure 2. Linearity of sildenafil.

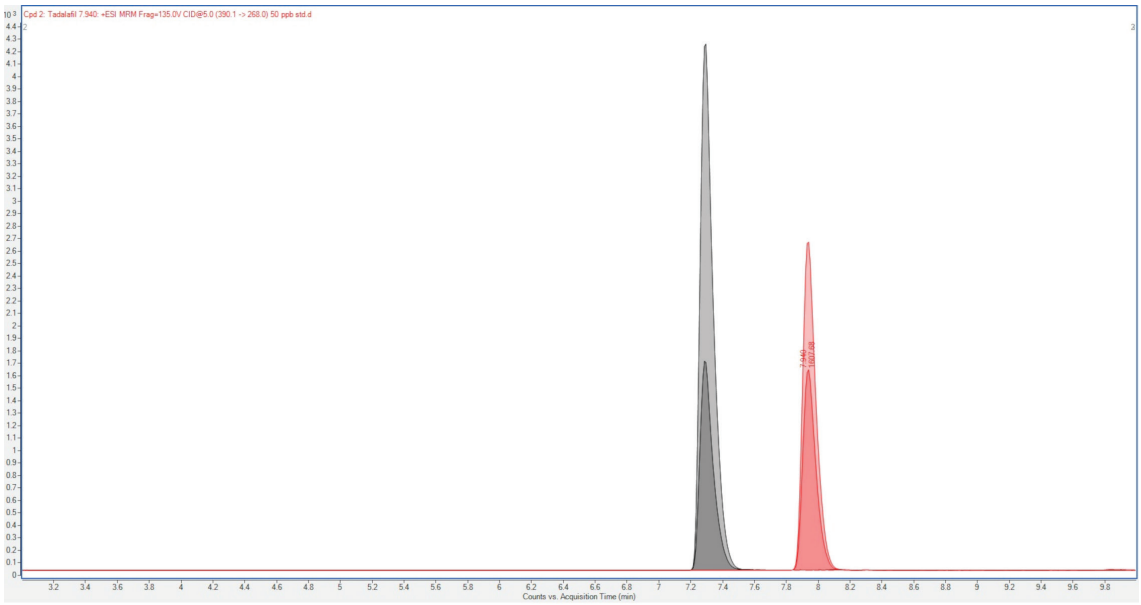

Figure 3. Chromatographic Separation of Tadalafil (Red, rt:7.29) and Sildenafil (Grey, rt:7.95).

Table 2. Quantitation results.

Sample no and Form	Compound	Final Concentration	Compound Dose Per Serving	One Serving Size
1 Liquid (Plant Based Drop)	SDF	0 ng/g	ND	5 mL
	TDF	0 ng/g	ND	
2 Liquid (Plant Based Drop)	SDF	0 ng/mL	ND	5 mL
	TDF	0 ng/mL	ND	
3 Liquid (Energy Drink)	SDF	206157.61ng/mL	30.91 mg	150 mL
	TDF	0 ng/mL	ND	
4 Paste (Herb and Honey Mixture)	SDF	3469060.12 ng/g	41.62 mg	12 g
	TDF	0 ng/g	ND	
5 Paste (Herb and Honey Mixture)	SDF	0 ng/g	ND	12 g
	TDF	0 ng/g	ND	
6 Paste (Herb and Honey Mixture)	SDF	4622334.76 ng/g	92.44 mg	20 g
	TDF	0 ng/g	ND	
7 Chocolate (Plant Based Chocolate)	SDF	1493053.67 ng/g	35.83 mg	24 g
	TDF	984203.35 ng/g	23.62 mg	
8 Chocolate (Plant Based Chocolate)	SDF	2152374.75 ng/g	38.74 mg	18g
	TDF	0 ng/g	ND	
9 Chocolate (Plant Based Chocolate)	SDF	2520986.17 ng/g	45.37 mg	18 g
	TDF	12660.51ng/g	0.22 mg	
10 Capsule (Plant Based Capsule)	SDF	8067844.26 ng/g	3.63 mg	450 mg
	TDF	575.81 ng/g	0.00025mg	
11 Effervescent Tablet (Multivitamin)	SDF	0 ng/g	ND	5.5 g
	TDF	0 ng/g	ND	

TDF, Tadalafil; SDF, Sildenafil; ND, Not Detected.

raphy (TLC) (Miller & Stripp, 2007; Moriyasu et al., 2001), micellar electrokinetic chromatography (MEKC) (Berzas, Rodrguez, Castaneda, & Rodrguez, 2002), nuclear magnetic resonance (NMR) (Venhuis, Blok-Tip, & de Kaste, 2008), flow injection analysis (Lopes Júnior et al., 2012), spectrophotometry (Harikrishna, Nagaralli, & Seetharamappa, 2008), high-performance liquid chromatography (HPLC)-ultraviolet (UV) (Daraghmeh, Al-Omari, Badwan, & Jaber, 2001; Miller & Stripp, 2007), HPLC-diode array detection (Venhuis, Blok-Tip, & de Kaste, 2008), gas chromatography (GC)-MS (Miller & Stripp, 2007), liquid chromatography (LC)-MS (Miller & Stripp, 2007; Venhuis, Blok-Tip, & de Kaste, 2008), electrospray tandem (ESI)-MS, and combinations of these methods can be applied together (Singh et al., 2009; Zhu et al., 2005).

TLC assays lack analytical sensitivity in typical assays due to extended analysis periods and quantification issues. GC-MS lacks analytical sensitivity because of its chemical characteristics, namely its low transition to the gas phase (Dural, 2020). Due to chemical interactions with molecules with similar chemical compositions in the absence of pre-separation using a column, spectrophotometric analysis might provide false positive results. Analytical procedures based on HPLC-UV give appropriate analytical sensitivity and repeatability to determine substances (Dural, 2020).

The developed and applied LC-MS/MS method provides higher accuracy and precision for LOD and LOQ value than the HPLC-UV systems (Table 1) (Dural, 2020). In the LC-MS/MS system that we used, calibration started at 5 ng/g, while in the HPLC-UV system used by Dural, it started at 20 ppb. Additionally, due to the possibility of matrix effect and interference, the LC-MS/MS system is a much more advanced analysis technique compared to the HPLC-UV system. Obtained method parameters are compatible with other methods developed in the literature (Lee et al., 2021).

It was determined that there was 30.91 mg of sildenafil in one serving (150 mL) in Sample 3 which is an energy drink (Table 2). The determination is 38.7 mg of sildenafil in Sample 8, which is a chocolate product for women as written on the package. The addition of an active ingredient approved only for men by the FDA to a product specifically marketed for women indicates a lack of pharmacological knowledge regarding adulteration by the producers of this product. Sample 4, which is a mixture of herbs and honey in paste form, has 41.62 mg sildenafil in one serving (12 g), making its effect close to the recommended daily dose of sildenafil. Sample 6, which is also a mixture of herbs and honey in paste form, has 92.44 mg of sildenafil in one serving (20 g), that is around the maximal dosage of sildenafil. Additionally, the presence of these active substances is not stated on the product packaging. The recommended daily dose of sildenafil is 50 mg as a single dose before sexual activity, and the recommended maximum daily dose is 100 mg (Bethesda, 2012). According to dosage information, Sample 3 and 4 are close to the recommended daily dose amount. Sample 6, on the other hand, has exceeded the recommended daily dose amount and approached the maximum daily dose of 100 mg (Bethesda, 2012).

Sample 7 is a chocolate bar containing 23.62 mg of tadalafil and 35.83 mg of sildenafil in one serving (24g). There is no information on the presence of these active substances on the package. The amount of tadalafil in one serving of this sample is above the maximum daily dose for erectile dysfunction treatment. In addition, 35.83 mg of sildenafil in its content will create a synergistic effect and potentially cause a hazardous activity. Due to the combination of these substances in high levels, this sample is the unhealthiest supplement among the samples. In sample 9, which is a chocolate bar, 45.37 mg of sildenafil and 0.22 mg of tadalafil were determined in one serving. In sample 10, which is an herb containing capsule, 3.63 mg of sildenafil and a trace amount of tadalafil were found in one serving. It should be noted that although the substances are at very small amounts, adulteration is still illegal. Tadalafil is recommended to be taken as a single 10 mg dose 1 hour before sexual activity for erectile dysfunction. Although the dose of tadalafil can be adjusted according to its effect and developing tolerance, it is recommended that the daily dose should not exceed 20 mg (Bethesda, 2012).

Data observed from ICP-MS (Table 3) has shown that none of the samples used in the metal content evaluation exceeded the daily metal limits (Table 4) in their one size serving. It is important to note that the size of the serving can impact the amount of metal present in a sample. Based on the results of the study, none of the samples exceeded the daily limit for metals when measured per serving size. However, it is still possible that there could be a risk of accumulating too much metal over time if the samples are consumed frequently or in larger quantities.

Na^+ and K^+ are the electrolytes to maintain blood volume and fluid. Still, consuming too much sodium and too little potassium may raise blood pressure. Sample 11 has a 187.95 mg of sodium and 0.43 mg of potassium in one serving (Table 3), which can be hazardous to an individual with hypertension on a sodium diet.

As, Cd, Cr and Pb are the most toxic heavy metals studied in this study. According to the data observed in the Table 3, the maximum values of these metals determined from the samples are 3.1514 µg, 0.6300 µg, 4.9701 µg and 6.3272 µg, respectively. These values can affect the daily heavy metal intake especially for arsenic and chromium in a certain sense. Using the food supplement several times, contaminated water and food consumption could increase the amount of arsenic and chromium intake. Therefore, total intake may exceed the daily usage limits and cause toxic effects (Table 4).

Additionally, effects of combined metal exposure on human health are still being studied. When metals are combined, their interaction can produce additive, synergistic or antagonistic effects due to the ability of certain metals to either facilitate or hinder the absorption of other metals (Xiao et al., 2021). Previous studies have suggested that the toxicity of multiple metals with the same or similar organ toxicity may be additive, but it still needs to be studied (Hong, Jin, & Zhang, 2004; Madden, & Fowler, 2000). Currently, there is insufficient information to establish science-based limits for specific products based on the combined exposure to metals with similar toxicities. Therefore, in this study, metal restrictions will be evaluated separately, given the lack of conclusive evidence on the effects of combined exposures.

Table 3. Total amount of metals in each sample (µg).

Samples	Li	B	Na	Mg	Al	K	Ca	V	Cr	Mn	Fe
1	6.9840	4.2614	755.1255	41.4579	2.9421	521.0734	42.1655	0.0193	0.2047	0.1485	15.8606
2	6.6354	2.6533	837.9692	60.0059	1.9259	1271.1705	16.6941	0.0406	0.1609	0.1249	6.9382
3	148.0858	101.0386	21998.2178	4756.1353	350.8711	3378.3841	4748.6677	0.5958	4.9701	0.6379	58.8998
4	10.2785	42.6031	1283.1488	2802.1419	58.2739	13914.6667	1223.6129	0.1780	0.6352	28.5669	100.1891
5	12.0561	25.0213	1558.8974	1888.8992	47.7539	10988.9256	789.3358	0.3056	0.6347	9.7524	89.1118
6	12.2510	22.0481	417.5935	1120.0842	53.5080	4942.4064	460.6529	0.1174	1.3322	9.5444	58.5257
7	18.8839	77.4876	1289.0675	22577.4927	49.8642	59665.6799	1852.9611	0.2449	3.9881	62.2178	294.2305
8	11.6535	46.2160	9634.0883	10873.0405	87.7392	50145.9809	2800.9252	0.2681	4.5369	25.7412	204.0241
9	14.6336	36.2459	10350.3930	8339.9514	47.9354	34156.1806	2002.5262	0.2190	3.2233	20.3172	154.8425
10	0.2835	0.8499	51.6186	187.4495	1.5047	402.4890	337.1979	0.0179	0.0143	1.3743	5.2842
11	9.5994	1.7393	187953.3994	693.5467	11.0453	439.6487	252.6896	0.1946	0.4221	0.9843	6.6853

Li, Lithium; B, Boron; Na, Sodium; Mg, Magnesium; Al, Aluminum; K, Potassium; Ca, Calcium; V, Vanadium; Cr, Chromium; Mn, Manganese; Fe, Iron.

Samples	Ni	Co	Cu	Zn	As	Sr	Cd	Sb	Ba	Tl	Pb	Bi
1	3.5752	0.0095	1.1184	6.1986	0.0921	0.3310	0.0148	0.3684	0.5689	0.0029	0.2331	10.9755
2	2.3969	0.0147	0.3491	2.1761	0.1199	0.3688	0.0171	0.0567	0.5455	0.0026	0.1965	10.9131
3	54.2766	0.2638	6.3977	20.1586	3.1514	59.6470	0.4258	0.8153	48.7270	0.0545	6.3272	331.5763
4	6.4942	0.2849	9.8288	27.8385	0.4526	22.0836	0.1359	0.1772	18.1771	0.0175	1.2112	32.3222
5	5.7549	0.3330	7.9699	27.9315	0.8579	9.1445	0.0806	0.1341	6.8655	0.0050	0.7746	21.9694
6	6.6812	0.0870	3.2459	7.3262	0.4350	4.5277	0.0949	0.1455	6.0016	0.0080	1.1405	46.6552
7	29.3361	2.5960	105.0657	161.0723	0.3973	46.4253	0.6300	0.5656	48.4686	0.0326	1.0039	50.8705
8	13.9507	1.3154	38.0474	66.8492	0.2779	25.8148	0.5336	0.6673	26.3749	0.0184	0.9218	39.0154
9	11.1158	0.8524	29.1357	45.4345	0.4584	19.1460	0.2732	0.7816	18.4763	0.0149	0.7191	35.4141
10	0.1595	0.0104	0.7047	2.1596	0.0058	1.1065	0.0041	0.0190	0.4268	0.0001	0.0274	1.0030
11	0.9742	0.0820	0.3719	1.9498	0.1033	5.2018	0.0267	1.2485	4.8535	0.0012	0.2272	9.9099

Ni, Nickel; Co, Cobalt; Cu, Copper; Zn, Zinc; As, Arsenic; Sr, Strontium; Cd, Cadmium; Sb, Antimony; Ba, Barium; Tl, Thallium; Pb, Lead; Bi, Bismuth.

Table 4. Minimum risk levels/recommended dietary allowances/no observed adverse effect levels of the elements/day (Doses may differ between authorities).

Elements	AI/RDA/NOAEL/ MRL/DI/PTDI	References
Li	RDA: 1 mg/day for adults	(Voica, Roba, & Iordache, 2021)
B	Estimated DI: 13mg	(Nielsen, 1997)
Na	AI: 1500 mg for adults	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Mg	RDA: 420 mg for males and 320 mg for females	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Al	DI: 0.10-0.12 mg of Al/kg/day for adults	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
K	AI: 4700 mg for adults	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Ca	AI: 1000 mg for adults	(Meyers, Hellwig, & Otten, 2006)
V	MRL: 210 µg for adults	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Cr	RDA: 35 µg for males and 25 µg for females	(Meyers, Hellwig, & Otten, 2006)
Mn	RDA: 2.3 mg for males and 1.8 mg for females	(Meyers, Hellwig, & Otten, 2006)
Fe	PTDI: 48 mg	(Akinyele & Shokunbi, 2015)
Co	DI: 0.005-1.8 mg	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Ni	DI: 100-300 µg	(Akinyele & Shokunbi, 2015)
Cu	RDA: 900 µg for adults	(Meyers, Hellwig, & Otten, 2006)
Zn	AI: 11 mg for males and 8 mg for females	(Meyers, Hellwig, & Otten, 2006)
As	MRL: 21 µg	(Meyers, Hellwig, & Otten, 2006)
Sr	DI: 2-4 mg	(Nielsen, 2004)
Cd	MRL: 14 µg	(Avula, Wang, Smillie, Duzgoren-Aydin, & Khan, 2010)
Sb	MRL: 0.4 and 6 µg/kg/d	(Raab, Stiboller, Gajdosechova, Nelson, & Feldmann, 2016)
Ba	MRL: 0.2 mg/kg/d	(Raab, Stiboller, Gajdosechova, Nelson, & Feldmann, 2016)

Table 4. Continue.

Elements	AI/RDA/NOAEL/ MRL/DI/PTDI	References
Tl	DI: <5 µg	(Filipov, 2015)
Pb	DI: 490 µg	(Kumar et al., 2020)
Bi	RDA: 0.6-0.8 g	(Bradley, Singleton, Po, 1989)

Abbreviations: AI, Adequate intake; DI, Daily intake; RDA, Recommended dietary allowance; PTDI, Provisional tolerable daily intake; NOAEL, No observed adverse effect level; MRL, Minimum risk level. Non-specified intakes are for healthy adults of 70kg.

In our study, we analyzed 23 metals, and some of them have not been assigned limit values by official authorities. The Turkish Food Codex Regulation on contaminants specifies limit values for Sn, Pb, Hg, and Cd, while the European Union Commission Regulation on contaminants specifies limit values for Sn, Pb, Hg, As, and Cd. Although EFSA suggests limit values, it is not legally obliged.

CONCLUSION

The levels of sildenafil, tadalafil and 23 metals in 11 food supplements from a Turkish market were studied, and their potential risks to health are discussed. The results show that the amounts of the concerning toxic metals in the studied food supplements were not as high as to set an individual hazardous activity. However, their mechanism of additive, synergistic and potentiation effects requires further studies. The result of the sildenafil and tadalafil determination analysis is a more serious concern. 7 of 11 samples were found to contain sildenafil and tadalafil separately or in combination.

The active pharmaceutical substances and heavy metals in food supplements with unknown concentrations can be a risk to health of an individual. Unintentional misusing, overusing, or interaction of the supplement with other medications, existing health problems, or other pharmaceuticals within the same food supplement have the potential to cause significant adverse effects on health. Our results represent the fact that there must be frequent and developed control to these products.

It is important to note that the lack of official limit values for certain metals does not necessarily mean that they are safe or that they do not pose a risk to human health. Further research is needed to determine safe exposure levels for these metals, and regulatory authorities should consider establishing limit values for them.

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