

Detection of sibutramine in herbal food supplements by UHPLC/HRMS and UHPLC/MS-MS.

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Abstract

Recent studies have announced that many food supplements for weight loss contained undeclared sibutramine. We have analysed 10 samples of herbal weight loss food supplements (FS) by HPLC/HRMS and UHPLC/MS-MS. We have established that two of the analysed samples contained sibutramine: respectively 5 µg/per capsule and 20 µg/per capsule. Illegal inclusion of this substance in FS could cause serious side effects and long term health consequences. The regulatory requirements for FS should be enhanced for more comprehensive consumers' protection. The need for mandatory quality control of these products and public awareness is undeniable.

Keywords: Sibutramine, HPLC, Food supplements, Analysis, Undeclared substances, WADA.

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Introduction

Sibutramine or 1-(1-(4-chlorophenyl) cyclobutyl)-N, N, 3-trimethylbutan-1-amine (Figure 1) is a serotonin-noradrenaline re-uptake inhibitor which was used as a weight loss drug in the recent past [1]. It was approved as an anti-obesity drug in 1997 by FDA and in 1999 by the European Medicines Agency (EMA). It has been established that sibutramine intake increased risk of heart attacks and strokes [2,3]. Since 2010, when EMA concluded that the benefits of sibutramine as weight loss drug do not outweigh its risks, sibutramine drugs have been suspended throughout Europe [1]. Drugs containing sibutramine were withdrawn voluntarily by the manufacturer from the U.S. market in 2010 [2]. Sibutramine has been withdrawn also from the pharmaceutical market of many other countries like: Australia, New Zealand, Canada, China, India, Mexico and others [4-6]. Nowadays there are many cases of undeclared sibutramine in herbal food supplements (FS) for weight loss [7-18]. A variety of techniques could be used successfully for detection of sibutramine: HPLC/MS, HPLC-ESI-MS, LC-MS/MS and HPTLC. Illegal inclusion of this substance in FS could cause serious health consequences for consumers like non-fatal myocardial infarction and nonfatal stroke [3]. Sibutramine intake is also associated with numerous side effects: agitation; problems with vision, speech, or balance; dry mouth; upset stomach; flu symptoms, insomnia, skin rash and others.

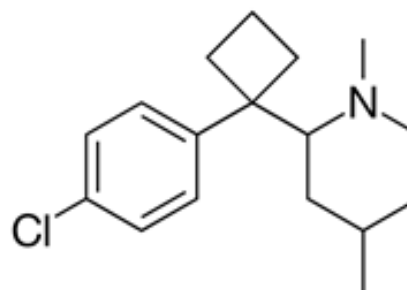


Figure 1. Sibutramine.

Material and Methods

Materials

Reference standard: Sibutramine hydrochloride monohydrate with purity >98%.

Reagents: Acetonitrile for HPLC, (Sigma Aldrich), distilled water.

Samples: 10 herbal food supplements for weight-loss. Samples were purchased from dietary supplement stores, pharmacies and online.

Methods

UHPLC/HRMS and UHPLC/MS-MS.

Instrumentation: “Accela UHPLC” with mass spectral detection HRMS “Q-Exactive” with H-ESI-interface (“Thermo Fisher Scientific”, Waltham, MA, USA) and column EVO C18 100 × 3 mm 2.6 μm.; ultrasonic bath (Branson Wilmington, NC, USA); apparatus for ultra-pure water: “Milli-Q”, “Milipore” (Bedford, MA, USA) and “Elga” (VWR International, Randor, PA, USA).

Sample preparation

The content of one capsule (or one ground pill) was introduced into a polypropylene 15 ml test-tube and extracted with 10 ml, 80% acetonitrile in water and treated by ultrasound and centrifuging. An aliquot part of the extract was filtered through a 0.45 μm membrane filter and the filtrate was diluted 100-fold with 50% acetonitrile/50% water. The obtained extracts of the submitted samples were analyzed up to 3 h after their preparation.

Mobile phase

Mobile phase A: 95% water/5% acetonitrile (pH=4.3; 10 mm ammonium formate).

Mobile phase B: 95% acetonitrile/5% water (pH=4.3; 10 mm ammonium formate).

Gradient: 50% mobile phase A/50% mobile phase B.

Speed: 600 μL/min.

Results and Discussion

According recent studies most customers consider FS as safe products, even safer than conventional drugs. Customers expect no side effects from FS use and many beneficial effects like: slimming or weight-control, increasing immunity and others. Most customers accept FS as pharmaceutical products with high quality. Most customers also trust FS label and the “Health Claims” of these products.

In fact the analytical control for FS is not obligatory. Most researchers and health specialists support that the legislation on FS is liberal and not strict enough. It is undeniable that for the ultimate protection of the consumers, quality control should be applied throughout all processing stages of FS—from the raw material to the finished product [19].

The results from our analyses showed that two of the studied ten FS contained the undeclared ingredient sibutramine—sample No 5 and sample No 8.

Figure 2 presents an extracted chromatogram of 1 μl extract of sample No 5 at scanning range of the mass spectrometric detector from 279.68 to 280.68 m/z (above) and mass spectrum of the peak with retention time 1.48 min (below).

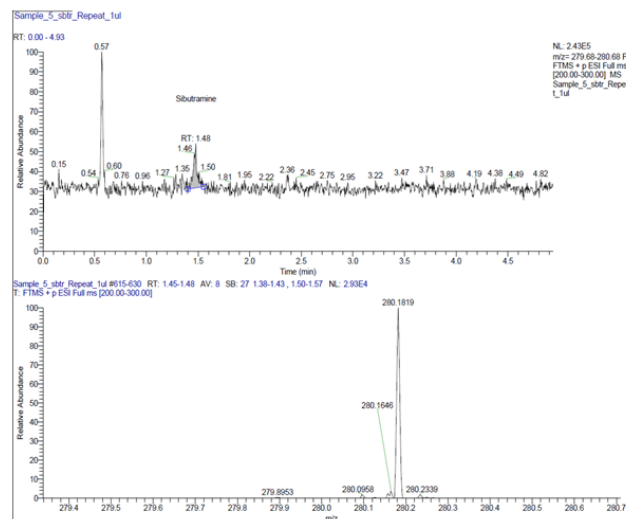


Figure 2. Chromatogram of referent sibutramine and sample No 5.

Figure 3 presents the data for sample No 5 as a comparison between total ion chromatography in scanning range 200–300 m/z (above), extracted chromatogram of the analyte (second line), the electronic signals transmissions 280>125 m/z and 280>139 m/z, characteristic for the analyte (line three and four) and ESI-mass spectrum (below), respectively—on the left for the referent solution (standard) and on the right for the analyzed sample (sample No 5).

The retention times and the areas of the respective peaks are marked on the analyte peaks (sibutramine with monoisotopic mass 279.175 Da).

The analytical results enable the conclusion that sample 5 contains 5 μg/per capsule sibutramine.

Figure 4 presents the data for sample No 8 as a comparison between total ion chromatography in scanning range 200–300 m/z (above), extracted chromatogram of the analyte (second line), the electronic signals transmissions 280>125 m/z and 280>139 m/z, characteristic for the analyte (line three and four) and ESI-mass spectrum (below), respectively—on the left for the referent solution (standard) and on the right for the analyzed sample (sample No 8).

The retention times and the areas of the respective peaks are marked on the analyte peaks (sibutramine with monoisotopic mass 279.175 Da).

The amount of Sibutramine in sample 8 was established to exceed 20 mg per tablet.

Medicinal products containing 10 mg Sibutramine under various trade names have been recommended for once-per-day intake by patients older than 16 y.

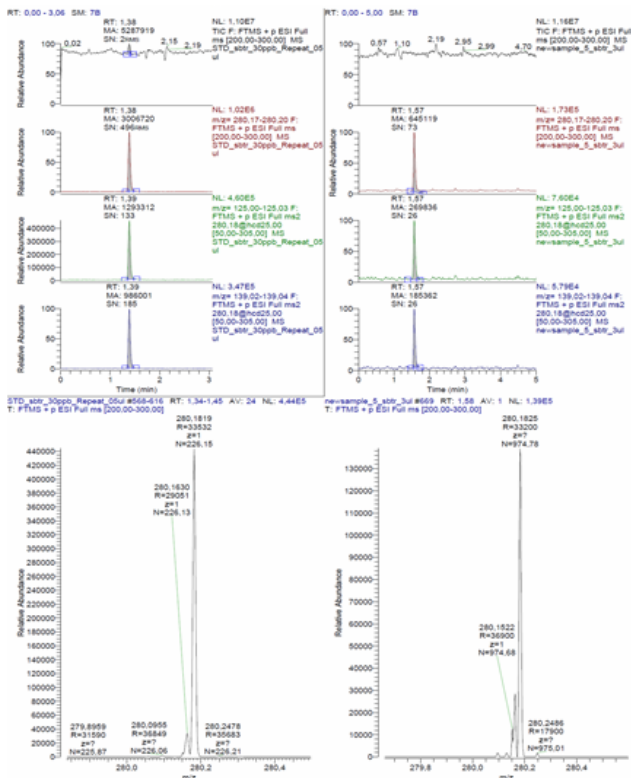


Figure 3. Chromatogram of standard sibutramine (left side) and the analyzed sample No 5 (right side).

The internet commercials for sample 8 state that the product is innovative, specially formulated to help in the weight loss process without forcing the consumers to change their everyday life. Health claims-“the product will reduce the weight with fewer efforts; the effect will be permanent as it acts extremely precisely, in a way that is natural and safe for the organism. Its effect is realized due to a special combination of absolutely neutral synergically bound extracts and herbs.

It suppresses the appetite in a natural way. Recommended intake: in the morning on an empty stomach with a glass of water. Absolutely safe product, without side effects; the food supplement sample No 8 contains an undeclared ingredient in concentration (>20 mg), twice greater than the concentration in a drug, containing Sibutramine (10 mg) (banned for intake in 2010).

Samples 5 and 8 are extremely hazardous FS and should not be marketed ad FS. The analytical results confirmed the theses of other researchers that numerous food supplements contained undeclared substances.

Globally the studies have revealed that about 20% of the food supplements contained undeclared substances. It has been established that the declared quantitative and qualitative content frequently did not correspond to the real formulation [20-27].

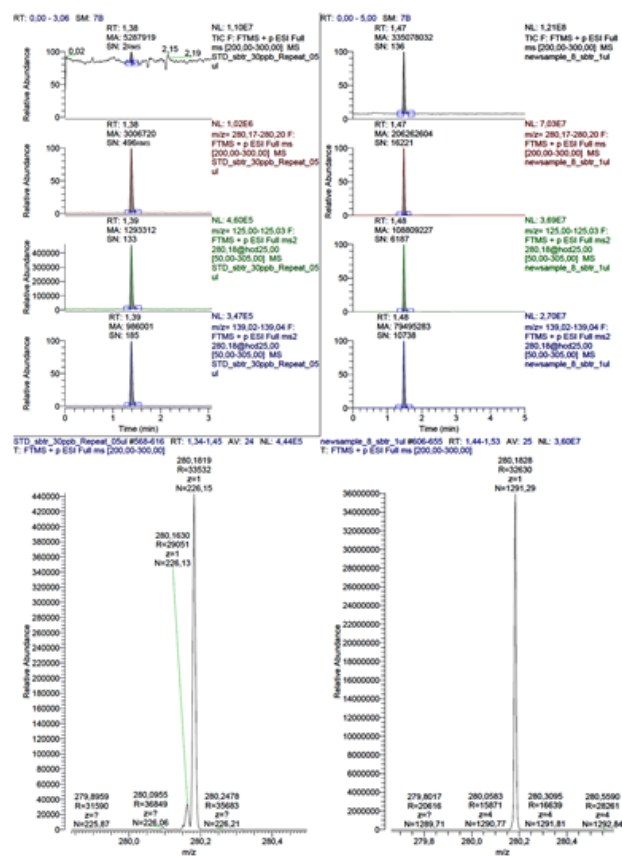


Figure 4. Chromatogram of standard sibutramine and analyzed sample No 8.

Conclusion

Most customers consider FS as safe products without side effects and contraindications. Customers do not expect a presence of a medicinal product in FS. For a patient with cardiovascular disease, the intake of herbal FS containing undeclared sibutramine could cause serious and even fatal consequences. According to recent studies more than 20% of herbal FS contain undeclared sibutramine. We have organized a screening of different weight loss FS for sibutramine by UHPLC/HRMS and UHPLC/MS-MS: 20% of analyzed samples contained undeclared sibutramine. The need for better quality control of FS and public awareness is undeniable.

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