

DETECTION OF ADULTERANTS BY FTIR AND GC-MS IN HERBAL SLIMMING FOOD SUPPLEMENTS

Anca Mihaela POPESCU¹, Gabriel Lucian RADU²

The main objective of this study was to detect the active pharmaceutical ingredients in herbal food supplements designed to reduce body weight using two analytical methods: Fourier Transform Infrared spectroscopy, and gas chromatography coupled with mass spectrometry. 10 dietary supplements were analyzed for the first time in Romania. Three of these food supplements were found to be adulterated with illegal substances such as sibutramine, and fluoxetine.

Keywords: Fourier Transform Infrared spectroscopy, gas chromatography-mass spectrometry, adulteration, herbal food supplements, body weight, sibutramine, fluoxetine

1. Introduction

Obesity became one of the contemporary civilisation diseases, having a negative impact on the quality of life, and moreover, the human lifespan [1]. Under this context, herbal slimming food supplements have become a good alternative for synthetic drugs, being preferred by consumers because of the false impression that there are all natural, with no side effects and not harmful to human health. The effects of herbal slimming food supplements are much slower compared to those of synthetic drugs [2]. For this reason, in order to increase efficiency, weight loss food supplements are frequently adulterated with active pharmaceutical ingredients such as sibutramine and its analogues (N-desmethylsibutramine, N-didesmethylsibutramine, N-formyldesmethyl-sibutramine, N-formyldidesmethylsibutramine) [3], rimonabant, benzodiazepines, fluoxetine, furosemide, phenolphthaleine [4].

In 2011, L.M. De Carvalho et al. have classified possible types of illegal substances encountered in adulterated herbal slimming food supplements in five different pharmacological classes: anorexics (amfepramone, femproporex, sibutramine, mazindol, fenfluramine); anxiolytics (benzodiazepines: diazepam, flurazepam, clonazepam, alprazolam, medazepam, midazolam, oxazepam, oxazolam, chlordiazepoxide); antidepressants (fluoxetine, paroxetine, sertraline,

¹ National Research and Development Institute for Food Bioresources – IBA Bucharest and Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest Romania, e-mail: anca_popescu34@yahoo.com; anca.popescu@bioresurse.ro

² Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest

bupropion); diuretics (furosemide, spironolactone, hydrochlorothiazide) and laxatives (phenolphthaleine) [5].

Sibutramine was approved as a drug to treat obesity by the Food and Drug Administration (FDA) in 1997 (Meridia manufactured by Abbott's). Because of the side effects (excitation of the central nervous system: nervousness, xerostomia, headache, numbness and paraesthesia; cardiovascular events: increased blood pressure and pulse rate and increased risk of heart attack and stroke), it was withdrawn from the market in January 2010 by the European Medicines Agency (EMA) [2], and in September 2010 by the FDA. Another substance that is used in the adulteration of herbal slimming food supplements is the laxative phenolphthaleine, a substance that was withdrawn by the FDA in 1997, due to the potential carcinogenic properties [6]. Moreover, fenfluramine, a drug with a function of full feeling and appetite losing that had side effects such as heart valve disease and pulmonary hypertension, including a condition known as cardiac fibrosis [7], was withdrawn from the market by FDA in 1997,.

Weight loss food supplements adulterated with sibutramine have led to side effects like headache, vertigo, numbness to serious cardiovascular effects, depending on the amount of product consumed [8].

According to literature data, analysis methods used until now for determination of undeclared substances in herbal slimming food supplements were: LC-PDA [9], GC-MS [10], LC-MS/MS [11, 7], NMR [12], X-ray powder diffractometry [1], TLC-image [8], IR [13, 14].

Fourier transform infrared spectroscopy was applied to detect sibutramine or other active pharmaceutical ingredients illegally added to herbal slimming food supplements, substances that play a crucial role in public health, and the results were confirmed by GC-MS technique. 10 food supplements from six countries were analyzed with these techniques as follows: 3 from China, 2 from Romania, 2 from Lebanon, and one from Spain, France, and USA, respectively.

2. Materials and method

2.1. Chemicals

Pure sibutramine was purchased from Biotrend Chemicals AG (Zurich, Switzerland). Acetone and methanol used as solvents were provided by Merck, Germany.

2.2. Commercial formulations of dietary supplements

Herbal food supplement samples were supplied by the National Office of Medicinal, Aromatic Plants and Bee Products, or have been bought from the Romanian market.

2.3. ATR-FTIR measurements

Nicolet 6700 FT-IR Spectrometer (Nicolet Instrument Co., Madison, USA) equipped with DTGS (Deuterated Triglycine Sulphate) detector, a universal attenuated total reflectance (ATR) sampling system with a single bounce ZnSe – diamond crystal was used. Measurements were made in absorption mode, and a small amount of sample was deposited on the ATR crystal, so that it covered the whole crystal. The pressure applied was the same for all measurements. Each spectrum consisted in 64 co-added scans at a spectral resolution of 4 cm^{-1} in the field of $4000\text{--}500\text{ cm}^{-1}$. The spectra were obtained using version 8 of Omnic software (Nicolet Instrument Co.). After each measurement, the crystal was cleaned with acetone and allowed to dry in ambient air. The background spectrum was obtained after each cleaning of the crystal, after cleaning and drying, using the same experimental conditions as for samples.

2.4. GC-MS analysis

GC-MS system consists in HP6890 GC, HP5973 MS-Agilent and 5% phenyl polysilphenylene-siloxane capillary column, TR-5MS (30 m x 0.25 mm i.d. $0.25\text{ }\mu\text{m}$ film thickness). $1\text{ }\mu\text{L}$ injection using splitless mode was performed on $280\text{ }^{\circ}\text{C}$ injector port with helium flow at 1 mL/min . The oven ramping temperature was held at 150°C for 20 min, and increased then with 10°C/min to 280°C , and hold for 15 minutes. The screening was performed on selected ion monitoring mode at m/z 58, 72, 114 (sibutramine), while identification was done on full scan mode ($40\text{--}500\text{ a.m.u.}$). The spectra obtained for the compounds were compared to the spectra of pure substances, or with the spectra of known compounds, using the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. This method used was adapted from the Laboratory and Scientific Section United Nations Office on Drugs and Crime Vienna ST/NAR/34 [15].

2.5. Sample preparation

2.5.1. FTIR analysis

Sample preparation requires minimal processing, consisting in empty capsules, and grinding them until obtaining a homogeneous powder. In order to analyze each of the samples using FTIR, a small amount of sample was deposited directly on the ATR crystal.

2.5.2. GC-MS analysis

Stock solutions contained sibutramine (1.0 mg/mL in methanol). The solutions of $100.0\text{ }\mu\text{g/mL}$ were prepared by mixing 1.0 mL of stock solution with 9 mL of absolute methanol.

All the 10 analyzed products were solid powders encapsulated, and each capsule was emptied. Every 100 mg of fine powder was mixed with 1 mL of absolute methanol. Samples were mixed thoroughly by vortexing, followed by 15 minutes of sonication and 5 minutes centrifugation at 4000 rpm . The supernatant was collected and filtered by $0.2\text{ }\mu\text{m}$ membrane filters for GC-MS analysis.

3. Results and discussions

3.1. ATR-FTIR spectroscopy

Because of the false impression that natural weight loss food supplements are safe and do not present health risks, their consumption has significantly increased. About 50 herbal slimming food supplements annually enter the market in Romania. Consequently, a rapid and efficient analysis method for the screening of these products is compulsory.

FTIR spectra were obtained for all of the 10 food supplements marketed for weight loss, but also for the sibutramine standard.

The area of interest for sibutramine, as is also shown in literature data, is located in the spectral region $2900\text{--}750\text{ cm}^{-1}$, because in this domain the absorption peaks of the characteristic groups can be observed [16]. In this spectral region, the domains of $750\text{--}1150/1350\text{--}1500/1850\text{--}1950/2600\text{--}2900\text{ cm}^{-1}$ are also included, where the characteristic region for sibutramine is situated, according to Maluf and Pontarolo (2010).

The sibutramine spectrum (Fig. 1) shows significant peaks at 821 and 833 cm^{-1} characteristic to aromatic CH groups deformation out of plane, at 1010 cm^{-1} in the plane, 1370 cm^{-1} for isobutyl group, 1407 and 1428 cm^{-1} for NCH_3 group, 1482 cm^{-1} for CH_3 group stretching vibration and CH_2 isobutyl scissor, and at 1491 cm^{-1} aromatic $\text{C}=\text{C}$ stretch [17].

By comparing the 10 spectra of food supplements with the spectrum of the pure sibutramine it was observed that one of them was adulterated with sibutramine. In the spectrum related to the sixth sample, the bands detected were characteristic for sibutramine (Fig. 2): 821 cm^{-1} to aromatic CH groups deformation out of plane, 1011 cm^{-1} in the plane, 1370 cm^{-1} characteristic isobutyl group, and 1428 cm^{-1} NCH_3 group. For all other supplements, data obtained from analyzing the spectrum are not relevant to the claims that these supplements is adulterated.

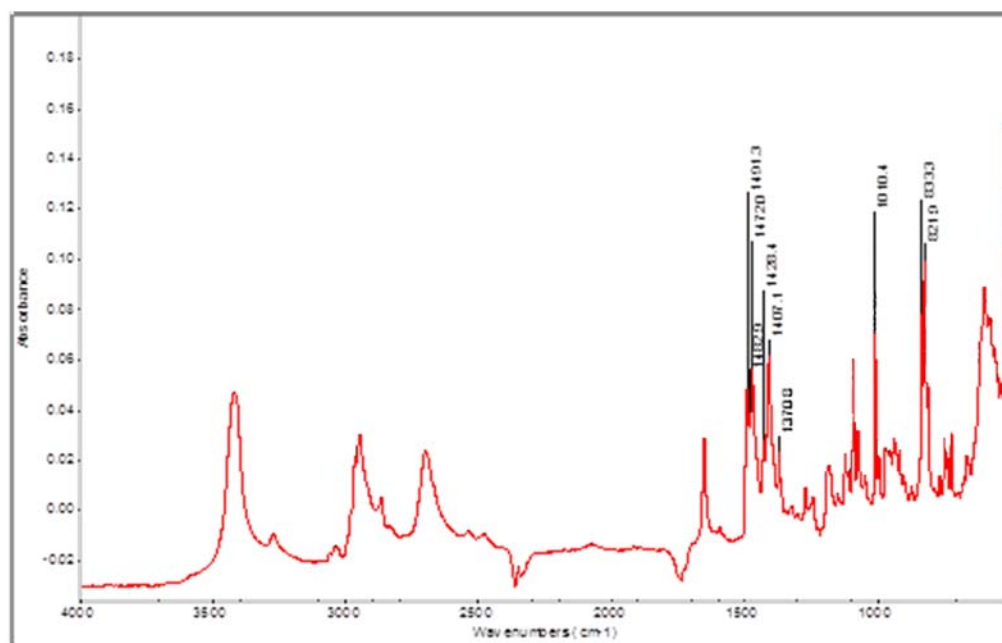


Fig. 1. FTIR spectrum for pure sibutramine

3.2. GC-MS

GC-MS was used to confirm the results obtained by FTIR. After analyzing the 10 weight loss food supplements, an efficient chromatographic separation was observed (it does not require hydrolysis or derivatization procedure): sibutramine was detected at retention time 8.86 min. In two of the products, a new compound appeared with a retention time of 9.862 min and m/z 44. After comparing this new compound with the existing data in the NIST library, it was found to be fluoxetine, a pharmaceutical substance from the class of antidepressants.

3 of the total number of products analyzed were found to be adulterated with banned substances, but also with active pharmaceutical ingredients. All 3 adulterate supplements were from China and they were labelled as food supplements that have in the composition only plants – “100% natural products”.

The mass spectrum for the food supplement which was found to have sibutramine, a substance banned in the U.S. and Europe in 2010, is shown in Fig. 3.

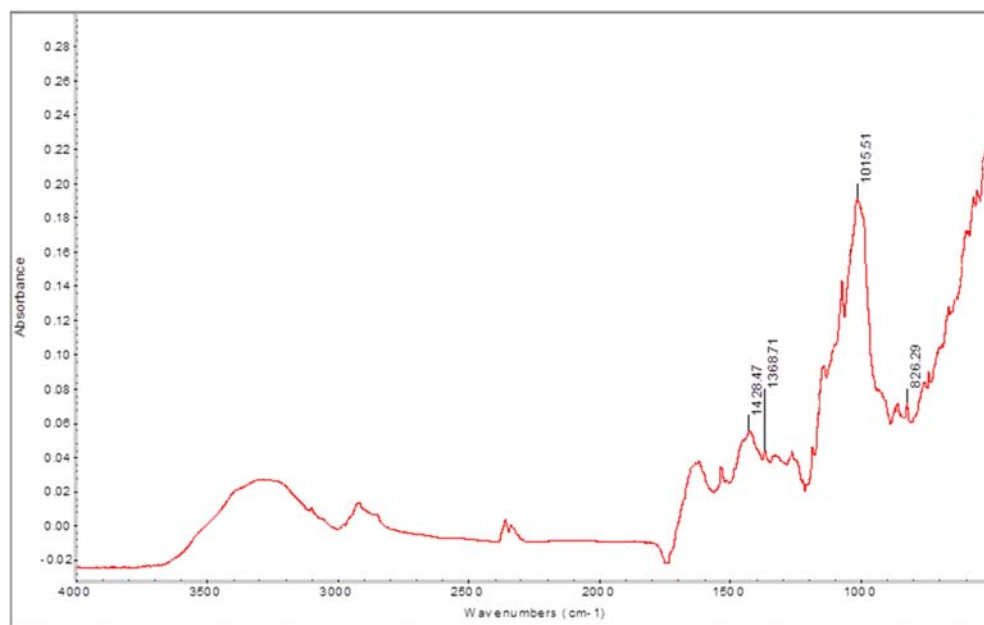


Fig. 2. FTIR spectrum for the product identified with sibutramine

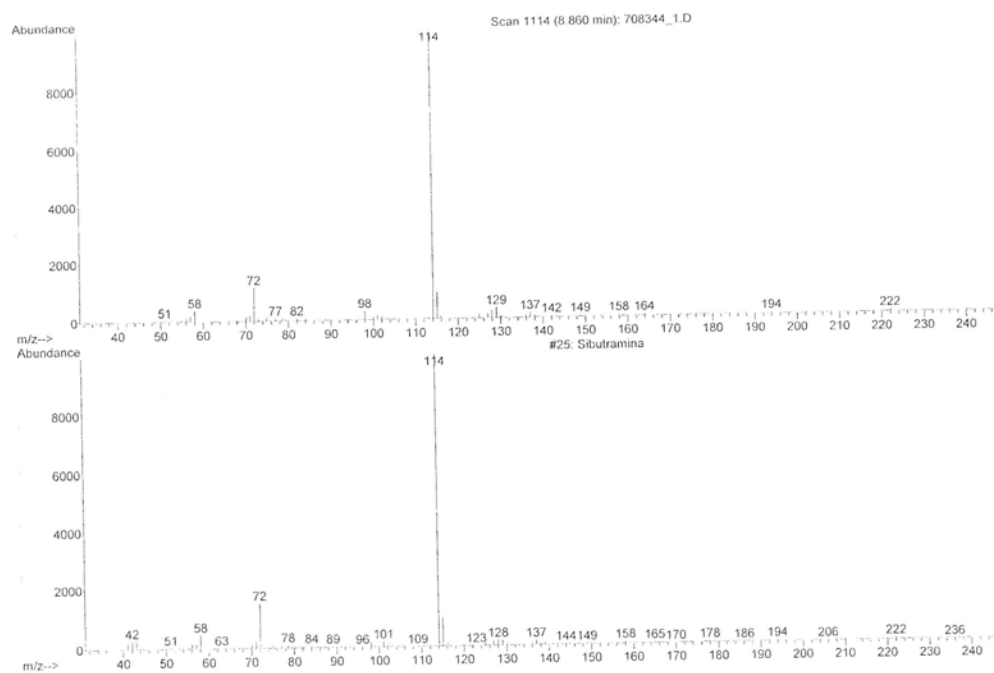


Fig. 3. Identification of sibutramine. The mass spectra have peaks at retention time 8.86 min.

The two products adulterated with fluoxetine have Chinese origin and were brought into the country by the same importer, but from two different manufacturers. The first analyzed product where fluoxetine was found was sold illegally in Romania, especially through the internet. The product was labeled as a natural product - only based on plants, but the label also specified that it received marketing right from the competent authority. In this food supplements, fluoxetine was detected at retention time 9.862 min., as Fig. 4 shows.

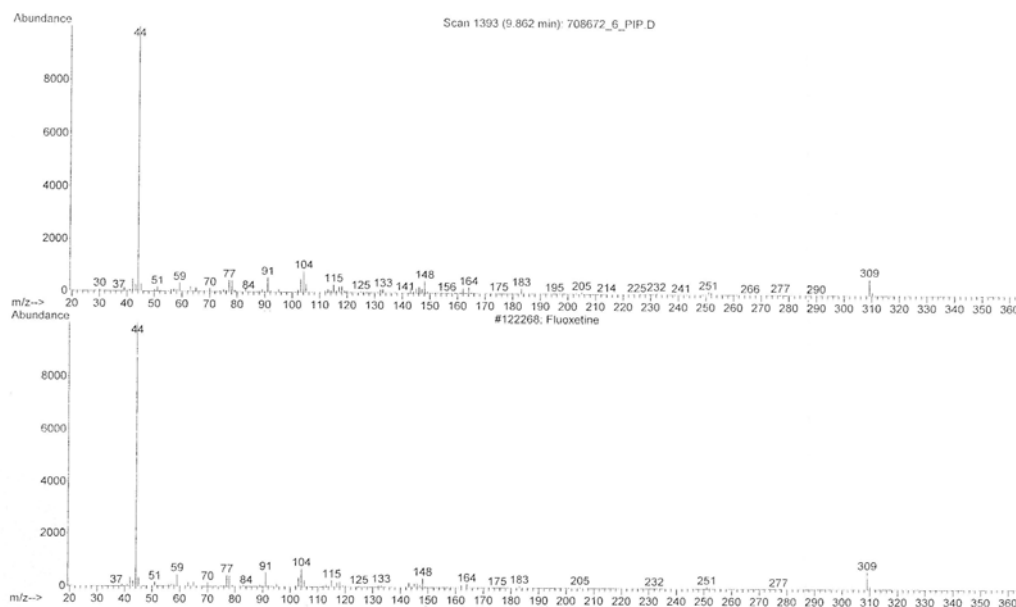


Fig. 4. Identification of fluoxetine (first sample). The mass spectra have peaks at retention time 9.862 min

The mass spectrum for the second food supplement in which fluoxetine has been detected is shown in Fig. 5.

This study represents the first screenig done in Romania, in which fluoxetine was identified in herbal slimming food supplements. Fluoxetine is the active ingredient in Prozac, a medicine used to treat depression, and especially food disorders, such as obsessive compulsive disorder and bulimia.

This substance has been intentionally added by the manufacturer to increase product efficacy and to have spectacular results, but not lastly to increase sales. Most commonly, fluoxetine may cause anorexia, thus explaining its presence in the herbal slimming food supplements.

Substances such as those identified in this study (sibutramine and fluoxetine) that are not declared on the label of weight loss food supplements can have very serious consequences on the quality of life of consumers. The results

obtained by GC-MS analysis were confirmed by Fourier transform infrared method. Dietary supplements intended to reduce body weight were detected in qualitative terms as "spiked" with active pharmaceutical ingredients such as: sibutramine and fluoxetine.

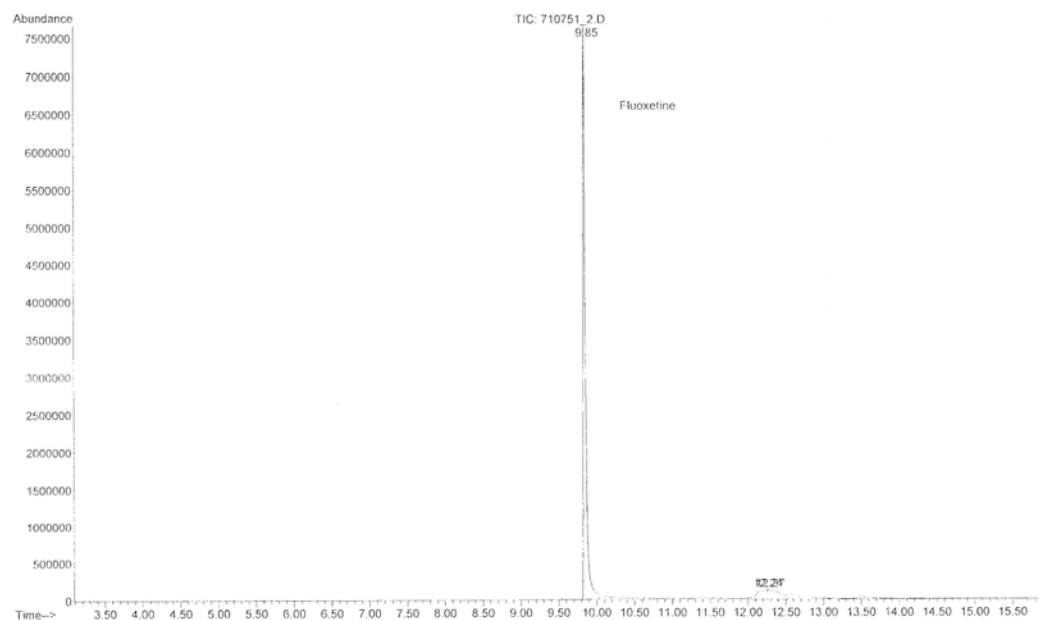


Fig. 5. Identification of fluoxetine (second sample). The mass spectrum has peaks at retention time 9.85 min

5. Conclusions

3 products adulterated with sibutramine and fluoxetine, substances that are prohibited in food supplements, were detected as a result of 10 weight loss food supplements screening. It is alarming the fact that 30% of the products analyzed were adulterated with pharmaceutical active substances and prohibited. Considering that all adulterated food supplements originate from China, particular attention should be paid to all products entering the Romanian market from Asian space.

When drug substances undeclared on the label are hidden in Herbal food supplements composition, they are dangerous for consumers' health. Pharmaceutical substances may interfere with the consumers' diet causing adverse reaction and side effects, as the consumers are not warned about the presumptive risks of the so called "natural" product consuming.

The screening method performed using Fourier transform infrared is very helpful, especially for the authorities with control responsibilities, as the minimal sample preparation requires a very short time, the analysis is done in about 5 minutes for a sample, special reagents are unnecessary, and the costs are minimal. Thus, this spectroscopic method can be a very useful tool, especially in situations where the number of samples to be analyzed is high. The screening of adulterated or suspected to be adulterated supplements can be achieved using the spectroscopic method, and these results can be confirmed by GC-MS.

These methods used in this study were very effective in identifying pharmaceutically active compounds sibutramine and fluoxetine in the composition of weight loss food supplements.

The screening of all herbal food supplements advertised as reducing body weight proved to be necessary for the detection of sibutramine or other substances before the products entries on the market

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