

IDENTIFICATION OF SIBUTRAMINE IN ADULTERATED DIETARY SLIMMING SUPPLEMENTS BY GC-MS

Annotation: Nowadays the consumption of dietary slimming supplements has increased especially those which are marketed as being 100% natural products. Despite of the widespread use there is an increasing concern about their quality, as some of them have been found to be adulterated with pharmaco-active ingredients in order to increase their efficiency.

This research aimed at evaluating the safety of dietary slimming supplements by identification of sibutramine which is one of the most common adulterants detected in these products. In this study, 13 samples of dietary slimming supplements were examined after they were collected from public Syrian markets. At first a visual inspection was made on the label ,instructions leaflet and dosage forms' physical characteristics .Then, identification of sibutramin was carried out using the analytical technique GC-MS. The results of visual inspection showed that all samples, except one, were suspected to be adulterated. As far as identification of adulterated component is concerned, the results showed that 3 international samples contained sibutramine.

Keywords: Dietary supplements, weight loss, adulteration, sibutramine, Gas Chromatography-Mass Spectrometry.

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ИДЕНТИФИКАЦИЯ СИБУТРАМИНА В ФАЛЬСИФИЦИРОВАННЫХ ДИЕТИЧЕСКИХ ДОБАВКАХ ДЛЯ ПОХУДЕНИЯ МЕТОДОМ GC-MS

Аннотация: В настоящее время потребление диетических добавок для похудения увеличилось, особенно тех, которые продаются как 100% натуральные продукты. Несмотря на широкое применение, существует все большая озабоченность по поводу их качества, поскольку некоторые из них были обнаружены фальсифицированными фармакоактивными ингредиентами с целью повышения их эффективности.

Это исследование было направлено на оценку безопасности пищевых добавок для похудения путем идентификации сибутрамина, который является одним из наиболее распространенных адсорбентов, обнаруженных в этих продуктах. В этом исследовании 13 образцов пищевых добавок для похудения были исследованы после того, как они были собраны с публичных сирийских рынков. Сначала был проведен визуальный осмотр этикетки, инструкции и физических характеристик лекарственных форм. Затем, идентификация сибутрамин был проведен с использованием аналитического метода ГХ-МС. Результаты визуального осмотра показали, что все образцы, кроме одного, были заподозрены в фальсификации. Что касается идентификации фальсифицированного компонента, то результаты показали, что 3 международных образца содержали сибутрамин.

Ключевые слова: Биологически активные добавки, похудение, фальсификация, сибутрамин, газовая хроматография-масс-спектрометрия.

1-Introduction: Obesity, defined as abnormal or excessive fat accumulation and a body mass index (BMI) above 30 kg/m², is considered a serious chronic disease and a risk factor of metabolic syndrome (MS), type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). At least 2.8 million obese people die globally each year due to obesity or obesity-related diseases [1,C.1]. The proportion of overweight or obese adults has already surpassed 70% in the USA and 50% in Europe [2,C.205]. Due to the mentioned facts ,dietary slimming supplements have become an attractive and easy options for losing weight .It is also have turned to be a good alternative for synthetic drugs, especially because of consumers' false impression that these products are all natural, harmless and with no side effects [3,C.167][4,C.51]. Dietary supplements are categorized as food in most countries so unlike standard prescription drugs, the quality of these products is not well studied [5,C.268]. and their manufacturers are not required to obtain FDA approval before producing, nor are they required to conduct preclinical and clinical tests before selling [6,C.19]. Since these products are not regulated they can be misleading and the patient can be confronted with adulterated products [7,C.38].Recent studies claim that dietary supplements often contain pharmaco-active ingredients or undeclared drug substances with a potential health risk [8,C.733] .Possible types of illegal substances encountered in adulterated dietary slimming supplements in order to achieve fast effects and better efficiency are classified in different pharmacological classes which include: Anorectics , Stimulants, Antidepressant, Laxative and Diuretics [4,C.51][9,C.260]. Out of these adulterants,the most frequently used are anorexics derived from amphetamines. Studies showed that about 80% of slimming products- illegally available in the market- contain these materials. Sibutramine which is considered an anorexic structurally related to

amphetamines is the most commonly detected in these products. It acts as a neurotransmitter reuptake inhibitor, reducing the reuptake of serotonin, norepinephrine, and noradrenalin, resulting in higher concentration of these compounds at the synaptic clefts, leading to a reduction in appetite. This compound was approved by FDA in 1997 and was legally prescribed and sold for the treatment of obesity until 2010 when Abbott Laboratories voluntarily withdrew sibutramine from the market due to the high risk of heart attacks and strokes, especially in patients with a history of cardiovascular disease [10,C.43][11,C.15]. Analysis of adulterated dietary supplements is one of the most important area of scientific research within the fields of quality control [12,C.117]. Therefore the purpose of the present study was to evaluate the safety of dietary slimming supplements by identification of sibutramine as a prohibited drug and common adulterant in dietary slimming supplements using Gas Chromatography-Mass Spectrometry technique.

2- MATERIALS AND METHODS

- Samples collection

Samples of dietary slimming supplements were collected randomly from the local market. These samples were divided into 5 categories according to the following symbols: A,B,C,D,E . Each product had 3 different batch numbers except for the sample E which had only one batch number. All pharmaceutical dosage forms were capsules. The samples A,B,C were Locally Manufactured while both samples D and E were internationally manufactured.

- Chemicals

Reference standard of sibutramine was kindly obtained from the Directorate of Quality Control and Scientific Research, Methanol(from Merck).

- visual inspection

A visual inspection of the products suspected to be adulterated, is the first analysis to be performed without using advanced equipment. The focus could be on the absence or deterioration of packaging or leaflets. Mandatory information must be present on the sample's label and leaflets (e.g. batch number, expiry date). Therefore the absence of or the inconsistency between the information of labeling and leaflets makes the product suspicious. Finally we examine the physical and chemical characteristics of pharmaceutical dosage forms (shape, color, size, dissolution, disintegration) compared to, if possible, a genuine product [13,C.268].

- **Sample Preparation**

We randomly selected one capsule from each slimming product. A small amount of each capsule's homogenized content, was extracted with 10 mL methanol for 30 min in round bottom test tube using laboratory rotator. The extract was centrifuged (10 min at 1000 rpm). The supernatant was collected for examination. Then, about 1 μ L of the top layer of the compound was injected to GC-MS device.

- **Chromatographic conditions**

GC-MS (Gas Chromatography-Mass Spectrometry) analysis was performed using a capillary column [HP-5ms, 25 m (length), 0.25 mm (diameter), 0.25 μ m (film)] as the stationary phase. The column oven temperature was initially held at 40 $^{\circ}$ C for 1 min, then programmed to reach 280 $^{\circ}$ C, at a rate increase of 15 $^{\circ}$ C/min, and then held for 2 min at 280 $^{\circ}$ C. The total run time was 19 min. The temperatures of the injector and detector were set at 250 and 280 $^{\circ}$ C, respectively. The carrier gas was helium, at a working flow rate of 1 ml/min and the injection volume was 1 μ L. The MS conditions were: ionization energy 70 eV, mass range 25-1000 amu and ionization technique was electron impact.

3- Results and DISCUSSION

Results of visual inspection showed that all samples, except one, were suspected to be adulterated. This sample was the only one accompanied by an internal instruction leaflet, and all the information that should be mentioned on the label existed. Examination of capsules' physical characteristics showed the presence of differentiation of capsules' color of the samples- whether they were of different batch numbers or even of the same batch number.

In spite of the manufacturer's claim that their products contained only natural components mentioned on the label, in this study the presence of synthetic drug in 3 internationally manufactured slimming products commercialized in the Syrian market was investigated.

Identification of sibutramine was performed based on two criteria: Retention time and molecular ions observed in mass spectrum analysis. The retention time of the pure reference standard adulterant (13,36 min) was compared with those of investigated slimming products. For further ensuring the reliability of results, and after assuring the similarity in retention times of the standard material to the investigated slimming products, Fragmented masses m/z of investigated slimming supplements were compared to standard mass spectrum those in the NIST libraries installed on the GC-MS linked computer (Figure 1), (Figure 2).

The increase of adulteration reports in the past years all over the world, (as shown in this paper) should prompt governmental healthcare authorities to set protocols to control the production and market surveillance of dietary supplements in order to protect the public from potential harm [14,C.28][15,C.1]. Unlike medications, dietary supplements are poorly monitored by regulatory agencies for quality and safety .So pharmacists should advise consumers who prefer to use supplements more than prescription drugs , despite of their beliefs, that these products are mostly less effective than medications and could be more dangerous than them to their health

[15,C.1][16,C.44]. Economically motivated adulteration of "dietary supplements" with undeclared synthetic drugs, meant to increase the efficacy of respective products in the claimed indications, is a common and dangerous phenomenon. Therefore, the development of new reliable, fast and accurate analytical methodologies for the detection and structural identification of adulterants is critically important to protect public health and ensure the quality of dietary supplements [17,C.1661][18,C.177].

Unfortunately, continuous consumption of dietary slimming supplements which are illegally adulterated with synthetic materials, may cause severe harm to patients and could be considered as a threat to individuals' health and even a reason for mortality [9,C.260]. Depending on the results of the visual inspection all samples, except one, were suspected to be adulterated. However we only found 3 adulterated samples. So in order to fight adulteration phenomena we often need to conduct confirmatory tests using advanced analytical techniques [13,C.268].

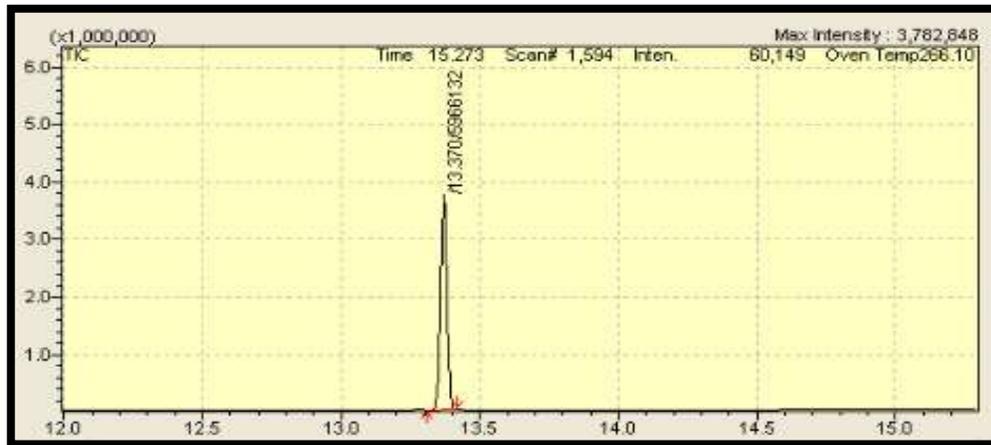


Figure 1: Spectrum of sibutramine Reference standard at retention time 13,36 min

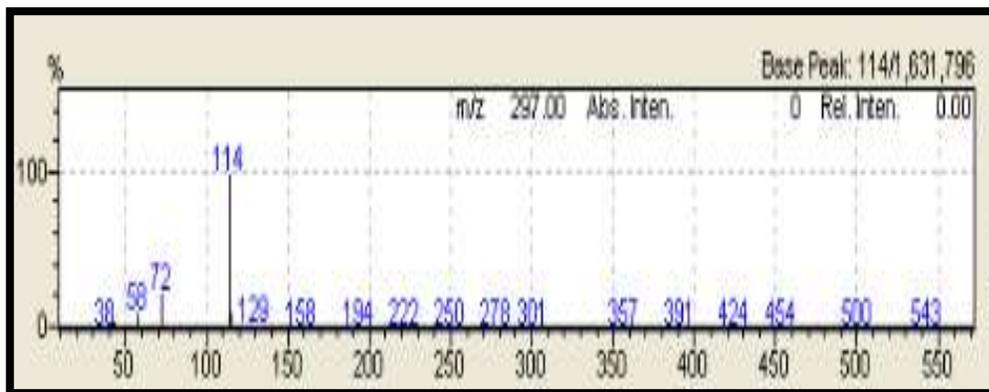


Figure 2: Fragmented mass of sibutramine 58m/z, 72 m/z and 114 m/z

- Conclusion

Despite of the growing use of dietary supplements the fact that 23% of the analyzed products were adulterated with prohibited pharmaceutical substance proved the necessarily to conduct premarketing safety tests. Adulterated Samples that claim to be 100% natural are a clear evidence about the manufacturers' attempts to achieve rapid results in weight reduction in order to encourage consumers to purchase their products continuously leading to very serious health consequences. The Ministry of Health should warn people about these products and a strict governmental control and regulations of their marketing and sales on the Syrian market are recommended.

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