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Identification and Detection of Undeclared Herbal Slimming Adulterants

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Abstract

The Food and Drug Administration (FDA) is advising consumers to stop using multiple weight-loss products that contain the undeclared drug ingredients e.g. sibutramine, which was removed from the market in 2010 for safety reasons and may present significant risks for those with coronary artery disease and other heart issues. Sibutramine and similar undeclared ingredients in herbal medicines are a real challenge for the public health and safety. In recent years, the need for quality assurance tools to ensure the identity, purity, and quality of botanical material has risen dramatically. HPTLC has emerged as a versatile, high throughput, and cost-effective technology, that is uniquely suited to meet these requirements. Most separation techniques do not allow parallel analysis of numerous samples at the same time and they often face problems in separating complex mixtures of substances. However, visual evaluation of HPTLC plates allows for convenient comparison of many samples side by side, where similarities and differences can clearly be seen. The quality of raw materials is rapidly and easily determined by HPTLC. For the identification of herbal drugs and other naturally derived materials standardized HPTLC is the method of choice and recommended by pharmacopoeias worldwide, furthermore adulterated samples are reliably identified. The main objective of the present study is to check pharmaceutical analogue adulteration of nonprescription and prescription slimming products in the laboratory using chromatographic techniques and to discuss its side effects in the interest of consumers and public health safety. Since intentional adulteration of "natural herbalmedicines" with unknown synthetic drugs or chemicals is a common and dangerous phenomenon of alternative medicine, it is important to modify and validate analytical tools to monitor and evaluate *these* herbal drugs.

1. Introduction

Herbal medicines (HMs) are gaining popularity worldwide as an alternative approach to prescription drugs for many reasons including a general perception that they are safe. Recently, unscrupulous manufacturers have made it more difficult for the regulators to detect undeclared ingredients by incorporating pharmaceutical analogues into their products. Analogues are created by modifying the original chemical structure of a compound — for example, by adding a hydroxyl group. It is suspected that these analogues are developed to evade detection by the FDA and similar agencies, making the herbal products more difficult to regulate, and to reduce the risk of patent-infringement lawsuits, furthermore, may potentially cause serious toxic adverse effects.

The imperceptible use of these analogues is very dangerous because they have not been tested formally for efficacy and safety. In view of the potential harm to the public, more effective and proactive measures are required to guard against the illicit use of pharmaceutical analogues. There is also a need for increased awareness among the public and the medical professionals about this emerging threat.

Increasing global interest in herbal medicines creates a serious need for truly objective data, not only on their efficacy, but also their side effects and interactions. In the interests of public health safety and looking into severity of this epidemic, an emphasis for the regular laboratory checking of herbal medicines for their safety and quality is a need of the day. In continuation of our earlier studies (Kamil, 2010, 2011), the main objective of the present study is to check pharmaceutical analogue adulteration of nonprescription and prescription slimming products in the laboratory using chromatographic techniques and to discuss its side effects in the interest of consumers and public health safety. Since intentional adulteration of "natural herbalmedicines" with unknown synthetic drugs or chemicals is a common and dangerous phenomenon of alternative medicine, it is important to modify and validate analytical tools to monitor and evaluate *these* herbal drugs (Fig. 1).

Dozens of products being touted as dietary supplements, which actually contain a hidden prescription drug or compound that has not been adequately studied in humans, have been found to be sold across various herbal or natural product shops.

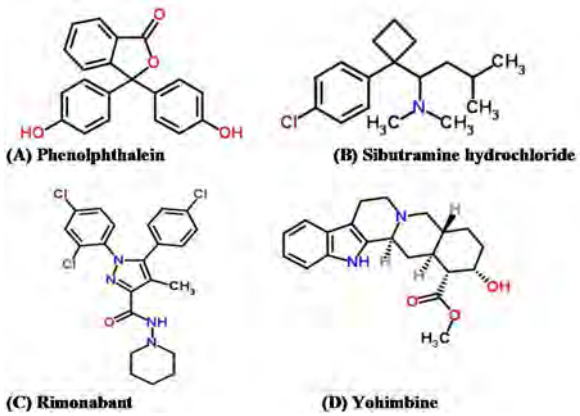

Fig. 1: Chemical structures of some undeclared drug ingredients

Most of the products are adulterated with sibutramine (B)-the drug substance which was banned long before, or phenolphthalein (A) (the chemical substance that was never allowed to be used in medicine). Some products were analyzed by other regulatory authorities like MHRA, FDA and found to contain high levels of heavy metals like lead or arsenic. The highest health risks associated with drinking slimming tea on a regular basis are dehydration and diarrhea which could lead to life-threatening potassium ion depletion. Since proper heart function is dependent on correct electrolyte balance, a severe deficiency of potassium ion can cause heart failure. The US Food and Drug Administration reported several cases of deaths and/or near deaths associated with the use of these slimming teas (FDA Medical Bulletin, 1994). Rimonabant (C) works differently from old-fashioned diet pills. Generic rimonabant is part of a new class of drugs called cannaboid-receptor-blockers, which work effectively on the brain's receptor sites that control the craving for food (Huestis *et al*., 2001; Kim *et al*., 2002).

Due to the advancement in hyphenated techniques like liquid chromatography tandem mass spectrometry (LC-MS/MS), gas chromatography-tandem mass spectrometry (GC-MS/MS), high performance thin layer chromatography (HPTLC) and other conventional tools, it has become possible to detect synthetic drugs and their structural analogues as adulterants even if they are present in small quantities.

High Performance Thin Layer Chromatography (HPTLC) is a chromatographic technique that utilizes the capillary action of a solvent and a stationary phase to separate compounds in a sample mixture. A thin layer of adsorbent material (i.e. the stationary phase) is coated on a sheet of glass, aluminum foil or even plastic. The sample mixture to be separated is placed near the bottom edge of the plate which is in the vertical position using an automated TLC sampler to accurately control the droplet size and position. The bottom edge of the plate is placed in mobile phase solvent which is drawn up the plate via capillary action. Different compounds or analytes ascend the plate at different rates resulting in separation. Different modes of detection such as fluorescent UV light exposure, white light exposure, staining reagents etc are then be employed to visualize the separated compounds which appear as horizontal lines or spots at various intervals up the plate. Reference standards are often used for comparison to the material of interest. HPTLC is the most powerful analytical version of this form of chromatography.

In this paper an attempt has been made to increase the herb, dietary supplements, and natural products industries' awareness of the need for enhanced scrutiny via laboratory analysis of herbal product ingredients or finished products being adulterated with illicit substances and/or prescription drugs using HPTLC. This research will definitely provide a unique and highly valuable service to the dietary supplement industry in addressing this serious problem. This paper also gives in brief health-related risks after consuming such spurious products and adulterants.

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Table 1: Some of the Herbal Medicines tested in the laboratory for checking adulteration

Sl. No.	Herbal Medicine	Laboratory test results-undeclared drugs and/or chemical ingredients (other than herbal ingredients
1	Seven Slim Capsule	Sibutramine hydrochloride
2	Zein Slimming Capsule	Sibutramine hydrochloride and Phenolphthalein
3	Magrim capsule	Sibutramine hychrochloride
4	Phytoshape capsule	Sibutramine hydrochloride
5	Meiziziaonang slimming capsule	Sibutramine hydrochloride
6	Super Slim capsule	Sibutramine hydrochloride and Phenolphthalein
7	Super fat burningcapsule	Sibutramine hydrochloride
8	Super Slim Green lean Body capsule	Phenolphthalein
9	LiziSlimming capsule	Sibutramine hydrochloride
10	Fat Magnet	Sibutramine hydrochloride
11	EUBE Quick Acting Slimming Capsules	Sibutramine HCl and Phenolphthalein.
12	Starvex Capsule (Slimming Supplement)	Sibutramine HCl
13	New Hydroxycut Hard Core Tablets	Sibutramine hychrochloride
14	Magrim Diet Capsules	Sibutramine hychrochloride
15	Zeinatat Capsule	Sibutramine hydrochloride
16	Dymaburn Xtreme	Yohimbine & Ephedrine
17	Quick Acting Slimming Capsules	Sibutramine hydrochloride& Phenolphthalein
18	Super Slim Capsules	Sibutramine hydrochloride and Phenolphthalein
19	Fat Burner Tablets	Sibutramine hydrochloride
20	Diet Capsules	Sibutramine HCl + Phenolphthalein
21	Hoodia Gordonii Capsules	Sibutramine HCl
22	Super Power Fatting	Cyproheptadiene
23	Herbal Mixture Powder	Dexa methasone
24	Sabr Plus Capsules	Sibutramine HCl + Phenolphthalein
25	Natural Max slimming Capsules	Sibutramine hydrochloride
26	Xenandrine RFA-X	Yohimbine
27	Hydroxy cut Max for Women	Yohimbine
28	Hydroxy cut Hardcore	Yohimbine
29	Lipo 6	Yohimbine
30	Atro-phex Energy & weight Management Breakthrough Capsules	Yohimbine
31	Diet Citrimax	Chromium Poly nicotinate & Chromium picolinate
32	Lipo 6 hers	Yohimbine
33	Lipo 6 X Advance Formula	Yohimbine
34	Redline 4fl.oz (120 ml)	Yohimbine
35	Lipo 6 Black	Yohimbine

2. Materials and Method

2.1. Chemicals

Phenolphthalein (A) Sibutramine hydrochloride (B) Rimonabant (C) Yohimbine (D) were received from the Sigma Aldrich and other recognized agencies. HPLC grade methanol, acetonitrile, and ammonia were obtained from Merck, USA. Formic acid was obtained from Sigma, St. Louis. High purity water was prepared by a Waters Milli Q plus purification system.

2.2. Equipment

HPTLC analysis for all samples and standards were applied on Merck HPTLC silica gel 60 F254 10 x 10 cm plate by means of micro capillary tubes using 8 µL each of standards and samples. The samples were developed with a toluene-ethyl format-formic acid (5:4:1) over a distance of 80 mm in at win trough tank. TLC plates were sprayed with Dragen Droff reagent. The plates' images were captured with the CAMAG Video Store 2 software.

2.3. Sample preparation

Approx. 1.0 g of sample was weighed and mixed with 5.0 mL of methanol. The samples were ultra-sonicated for 30.0 minutes and filtered using a 0.45 µm membrane. The volume of the filtered supernatant was completed to 10.0 mL with methanol.

3. Results and Discussion

Research analyses on about hundred herbal medicinal products(HMPs),it has been found that the undeclared active pharmaceutical ingredients are present in more than thirty five of these products include either sibutramine (a controlled substance), rimonabant (a drug not approved for marketing in the United States), phenolphthalein (a solution used in chemical experiments and a suspected cancer causing agent), phenmetrazine (a sympathomimetic drug used primarily as an appetite depressant-considered to have greater potential for addiction than the amphetamines), Yohimbinein weight loss products being sold in the market, besides caffeine and nicotine (Maldonado *et al*., 2006; Vander Stelt *et al*., 2001). The tainted products are listed here in Table 1 along with the undeclared drugs and / or chemical ingredients analyzed using chromatographic techniques.

HPTLC analysis was made using two different mobile phase solvent mixtures (toluene-ethylformate-formic acid with 5:4:1 and *n*-hexane-acetone with 70:30). Images of the developed TLC chromatograms were captured with CAMAG Video Store 2 software. The wavelengths used were 254 and 366 nm and one plate was sprayed with Dragon Dorffs reagent which results in capturing allimages in the visible region. Fig. 2 shows the HPTLC image taken for the two standard samples as well as the five tested herbal samples. As shown in Fig. 2, two samples (No. 3 & 5 in Fig. 2) were eluted with Rf values that are perfectly matching the one observed for the standard rimonabant sample (No. 1 in Fig. 2). Moreover, one sample (No. 7 in Fig. 2) is eluted with an Rf value that is similar to the Rf value observed for the eluted sibutramine standard solution (No. 2 in Fig. 2). As clearly shown in Fig. 2, the results obtained from the HPTLC is in good agreement with the results obtained using the optimized HPLC method developed in this work (Fig. 2).

3.1. Phenolphthalein and the associated risks

Phenolphthalein was an ingredient in some Over-the-Counter laxative products until 1999 when the FDA reclassified the drug as "not generally recognized as safe and effective" after studies indicated that phenolphthalein presented a potential carcinogenic risk. Phenolphthalein has also been found to be genotoxic in that it can damage or cause mutations to DNA.

3.2. Sibutramine and the associated risks

Sibutramine is a Schedule IV controlled substance and the active pharmaceutical ingredient in Meridia, an approved prescription drug to treat obesity. Taking more than 3 times the recommended daily dosage of sibutramine, may cause increased blood pressure, tachycardia, palpitations, and seizure. Populations who would be at increased risk of serious adverse health effects from consuming a standard dose of sibutramine include (Deadwyler *et al*., 2007): Patients with a history of hypertension, coronary artery disease, congestive heart failure, arrhythmias, or stroke, narrow angle glaucoma, history of seizure, predisposed to bleeding events and those taking concomitant medications known to affect hemostasis or platelet function, severe hepatic dysfunction and those concurrently taking the following medications: Sumatriptan, Dihydroergotamine, Dextromethorphan, Meperidine, Pentazocine, Fentanyl, Lithium, Tryptophan, MAO inhibitors.

3.3. Yohimbine and the associated risks

Side effects of yohimbine include difficulty breathing, chest pain, palpitations, anxiety, queasiness, sleeplessness, and vomiting. Normal doses of yohimbine can cause the blood pressure to go up. Large doses of yohimbine (40 mg per day or more) can cause the blood pressure to go down, and have been blamed for a few heart attacks and even deaths. Yohimbine can make heart disease or blood pressure problems worse. The less common side effects that do not usually require medical attention include dizziness, headache, flushing, nausea, nervousness, sweating, and tremors.

3.4. Alert

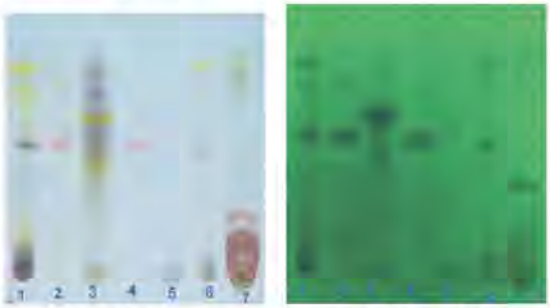
These tainted weight loss products pose a great risk to public health because they contain undeclared ingredients and, in some cases, contain prescription drugs in amounts that greatly exceed their maximum recommended dosages.

These weight loss products, some of which are marketed as "dietary supplements," are promoted and sold on various shops & pharmacies. Some of the products claim to be "natural" or to contain only "herbal" ingredients, but actually contain potentially harmful ingredients not listed on the product labels or in promotional advertisements. These products have not been approved by the FDA, are illegal and may be potentially harmful to unsuspecting consumers (2).

3.5. Side effects

Any time if a product that may harm public health should be withdrawn in cooperation with government authorities. Unfortunately, dozens of these products are used by the public and every day we see new products in the market. The issue is that these products are being touted as dietary supplements but actually contain

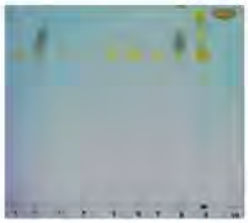
Thin Layer Chromatograms showing adulteration in Slimming Herbal Medicines



(A) 366nm **(B) 254 nm UV light**

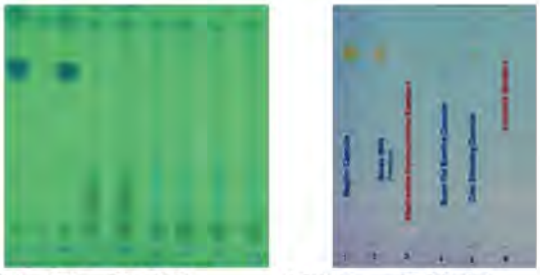
After Derivaization (A.D): Vanillin:Sulphuric acid
Mobile Phase: Toluene: Ethyl Format:Formic acid (5:4:1) Silica Gel Plates

Track 1 Zein slimming capsule.
Track 2 Super slim capsule
Track 3 Eube slim capsule
Track 4 Phenolphthalein (standard)
Track 5 Seven slim herbal capsule
Track 6 Quick acting slimming capsule
Track 7 Fat Magnet



A. D: Dragon Dorff's reagent (visible light)
Mobile Phase: Toluene: Ethyl formate: Formic acid (5:4:1)

Track 1 Super Slim capsule
Track 2 Fat Magnet
Track 3 Lizi Slim Capsule
Track 4 Meziggianang Capsule
Track 5 Magrim capsule
Track 6 Sibutramin HCl (standard)
Track 7 Seven Slim capsule
Track 8 Super Fat Burning Capsule
Track 9 Zein Slimming capsule
Track 10 Acomplia (standard)



(A) 254nmUV light **(B)366 nm UV light**

A.D: Dragon Dorff's reagent (visible light)
Mobile Phase: Toluene: Ethyl formate:Formic acid (5:4:1)

Track 1 Super Slim capsule
Track 1Magrim capsule
Track 2 Sibutramine HCl
Track 2 Seven Slim capsule
Track 3 (Track 1+2)
Track 3 Sibutramine HCl (standard)
Track 4 Super Fat Burning capsule
Track 4 Super Fat burning capsule
Track 5 (Track 4+2)
Track 5 Zein Slimming capsule
Track 6 Zein Slimming capsule
Track 6 Acomplia (standard)
Track 7 (Track 6 +2)
Track 8 Magrim capsule
Track 9 (Track 8+2)

Fig. 2: The HPTLC image of Sibutramine, Phenolphthalein, and the tested herb samples as chromatograms

hidden prescription drugs or compounds that have not been adequately studied in humans for their harm. The side effects that can result from use of these products depend on the substance of adulteration, which vary from one to another. However many serious adverse effects are expected to occur, namely, cardiac problems, stroke, high blood pressure, liver damage and kidney problems.

4. Conclusion

Nowadays, the consumption of herbal weight loss formulations has increased vastly, due to misleading advertisements for obesity treatment on internet and the media; weight loss products where adulterations with synthetic therapeutic substances can lead to severe side effects and/or potentially fatal interactions with conventional medicines.

Here we have identified an emerging trend where over-the-counter products, frequently represented as dietary supplements, contain hidden active ingredients that could be harmful. Consumers may unknowingly take products laced with varying quantities of approved prescription drug ingredients, controlled substances, and untested and unstudied pharmaceutically active ingredients. These deceptive products can harm public health! Hidden ingredients are increasingly becoming a problem in products promoted for weight loss.

Laboratory analysis confirmed that the above products contain sibutramine, phenolphthalein and yohimbine. Consumers should stop using these products and those who have experienced any negative side effects should consult a health care professional as soon as possible. This may cause severe health problems, and the health authorities should warn people about these deceptive products. The efficacy and safety of herbal weight loss drugs should be tested and strict government control and regulation of their marketing and sales are recommended.

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