
Determination of Undeclared Chemicals in Herbal Slimming Medicines using HPTLC

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Abstract

Unfortunately many of the licensed herbal slimming products have been found to contain non-prescription or even prescription drugs such as Sibutramine HCl, diethyl-propion, phenolphthalein, orlistat, xenical, pencillamine, ribomant etc. The unapproved presence of these substances may have originated from inadvertent substitution & contamination or intentional adulteration.

Agencies like FDA, MHRA have been issuing press releases warning consumers about the herbal weight loss products, which are believed to be widely available over in the market and especially over the internet. Quite a large numbers of herbal medicines or dietary supplements marketed as natural slimming products were analyzed in our laboratory. The method enables the screening of 2 weight loss compounds in fourteen herbal medicines in a very short time. Elements of validations on standards in solvent and co-chromatography have been carried out to ensure the reliability of the results.

Key Words - Tainted Herbal Slimming Products, Sibutramine HCl, Phenolphthalein, High Performance Thin Layer Chromatography, Public Health Safety.

Introduction

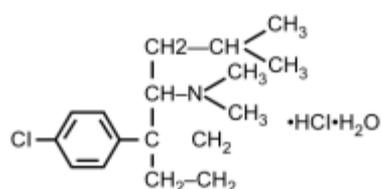
The adulteration by synthetic therapeutic substances of traditional medicines has been reported on various occasions and has been a global public health concern over the past several years. The U.S. Food and Drug Administration is expanding its international alert to consumers about tainted weight loss pills that contains undeclared, active pharmaceutical ingredients.

The use of complementary and alternative medicines has recently increased, thereby enhancing the market for herbal products worldwide (Budeker and Kronenberg, 2002; Zollman and Vickers, 1999). However, the purity of these herbal formulations exposes the human population to multiple risks and creates major concerns for various health agencies on both national and international levels (Fang, 2002). Adulteration of herbal formulations with undeclared synthetic drugs or by mixing the analogues of prescription drugs that are created by replacing or adding functional groups to the original chemical are the recent major problems since they may cause adverse side effects (Yeun *et al.*, 2007). As a result, analogue generation is a common strategy used in creating investigational drugs for most pharmaceutical companies. For example, 3, 4-methylenedioxymethamphetamine and methamphetamine are used by various drug companies as analogues of amphetamine (Wee *et al.*, 2005).

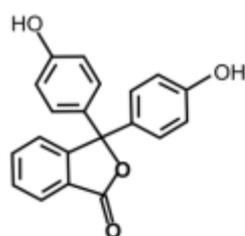
As a result, the World Health Organization (WHO) and the European Union (EU) issued several

guidelines concerning safe and appropriate use of herbal medicines (WHO regional publications, 1998; WHO regional publications, 2004). Some traditional Chinese medicine (TCM) slimming aids have been found to contain Sibutramine (see structure A), a medicine that should be used only under medical supervision as it may raise blood pressure (Nisoli *et al.*, 2001; Binkley and Knowles, 2002; Cordeiro and Vallada, 2002; Tafllinski and Chojnacka, 2001; Vidal and Quandte, 2006; Jung *et al.*, 2006). Others contain an amphetamine-like stimulant called methylphenidate that may lead to high blood pressure and adversely affect the efficacy of antidepressant and antipsychotic drugs (Volkow *et al.*, 2002; Konrad *et al.*, 2004; Faraone *et al.*, 2004). Traditional Chinese herbal medicine is generally both safe and effective, and there are many patients who have experienced dramatic benefits to their health from treatment. Despite the fact that most TCMs are assessed in terms of their risk benefit ratio, it should not be surprising that Chinese herbal medicines may also cause significant adverse effects. For example, consumers were warned to avoid the potentially dangerous and illegal TCM product, Shubao Slimming Capsules, which was responsible for a case of irreversible liver failure in the UK (Herbal Safety News, 2004). The Medicines and Healthcare Products and Regulatory Agencies have suggested that caution should be exercised when using a TCM slimming aid. The agencies said that the safety, quality, and efficacy of these unlicensed medicines could not be assured due to the possible illegal adulteration or contamination with pharmaceutical drugs or toxic herbal ingredients.

The highest health risks associated with drinking slimming tea on a regular basis are



(A) Sibutramine Hydrochloride



(B) Phenolphthalein

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 & Drug Administration reported several cases of deaths and/or neardeaths associated with the use of these slimming teas (FDA Medical Bulletin, 1994).

The main objective of this study is to devise a method for the detection of most common and logic synthetic adulterants in slimming herbal remedies using thin layer and high performance thin layer chromatography (HPTLC). Since intentional adulteration of “natural herbal medicines” 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.

Materials and Method

Chemicals

Sibutramine hydrochloride (A) and Phenolphthalein (B) were purchased from the Sigma-Aldrich (Cat.No.S9948 & P9750 respectively). HPLC grade methanol, toluene, ethyl formate, were obtained from Merck, USA. Formic acid was obtained from Sigma, -Aldrich. High purity water was prepared by a Waters Milli RO & Milli Q plus purification system.

Equipment

HPTLC analysis for all samples and standards were applied on Merck HPTLC silica gel 60 F 254 10 x 10cm and 10 x 20 cm plates by means of micro capillary tubes using 6 μ L each of standards and samples. The samples were developed with a toluene-ethyl formate-formic acid (5:4:1) over a distance of 15cm in a twin trough tank. TLC plates were sprayed with Dragendroff's reagent. The plate's images were captured using Photo Documentation System with the CAMAG Video Store 2 software (Switzerland).

Sample preparation

Approx. 1.0 g of sample was weighed and mixed with 5.0 ml of methanol. The samples were Ultrasonicated for 30.0 minutes and filtered using a 0.45 μ m membrane. The volume of the filtered supernatant was completed to 10.0 ml with methanol.

Results and Discussion

Table 1: A summary of the tested samples for both standards and herbal medicinal samples, The tainted products are listed here in the table along with the undeclared drugs and / or chemical ingredients analyzed:

S.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	Megrim capsule	Sibutramine hydrochloride
4	Phytoshape capsule	Sibutramine hydrochloride
5	Meizijiaonang slimming capsule (Chinese)	Sibutramine hydrochloride
6	Super Slim capsule	Sibutramine hydrochloride and Phenolphthalein
7	Super fat burning capsule	Sibutramine hydrochloride
8	Super Slim Green lean Body capsule	Phenolphthalein
9	Lizi Slimming capsule	Sibutramine hydrochloride
10	Fat Magnet	Sibutramine hydrochloride
11	EUBE Quick Acting Slimming Capsules	Sibutramine HCl and Phenolphthalene .
12	Starvex Capsule (Slimming Supplement)	Sibutramine HCl
13	Magrim Diet Capsules	Sibutramine Hydrochloride (Very high conc.)
14	Zein AlAtat Slimming Capsule	Sibutramine hydrochloride

HPTLC analysis was made using mobile phase solvent mixtures (toluene-ethylformate-formic acid with 5:4:1). Images of the developed TLC chromatograms were captured with CAMAG Video Store 2 software. The wavelengths used were 254 nm and 366 nm and one plate was sprayed with Dragondorffs reagent which results in capturing all images in the visible region. Figure 1 shows the HPTLC image taken for a standard sample Phenolphthalein (No. 4 in Fig.1) as well as the six tested herbal samples, out of which three contain phenolphthalein (No.1, 2 and 6) as evident both in UV light 254nm as well as after development with Vanillin - Sulphuric acid reagent. As shown in Figure 2, eight samples (No. 1-5,7-9 in Figure 2) were eluted with Rf values that are perfectly matching the one observed for the standard Sibutramine hydrochloride sample (No.6 in Figure 2). Moreover, four samples (No1,4,6 &8 in Figure 3) that are similar to the Rf value observed for the Sibutramine HCl standard solution (No. 2 in Figure 3), and are further confirmed when co-chromatographed with Sibutramine HCl (3, 5, 7 & 9).

Conclusion

The developed HPTLC conditions were successfully applied for the determination of undeclared herbal slimming adulterants. The methods provide an accurate screening method of slimming pharmaceutical ingredients in herbal remedies. Using the modified HPTLC conditions, both Phenolphthalein and Sibutramine were isolated with high linearity and selectivity with low detection limits. This study shows that these herbal products have been altered by synthesized compounds that actually promote weight loss. Phenolphthalein and Sibutramine are medicines which are banned. As a result it would be harmful to one's health to attempt to take these ingredients in herbal slimming medicines.

What is Phenolphthalein and what are 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.

What is Sibutramine and what are 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:

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.

What can consumers do to help protect themselves from harm?

Consult with your health care professional before taking herbal medicines or dietary supplements to treat obesity or other diseases.

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 (fda.gov).

Two pharmacological classes drugs used for weight loss, namely Sibutramine hydrochloride (A) and Phenolphthalein (B), were investigated in various adulterant slimming drugs.

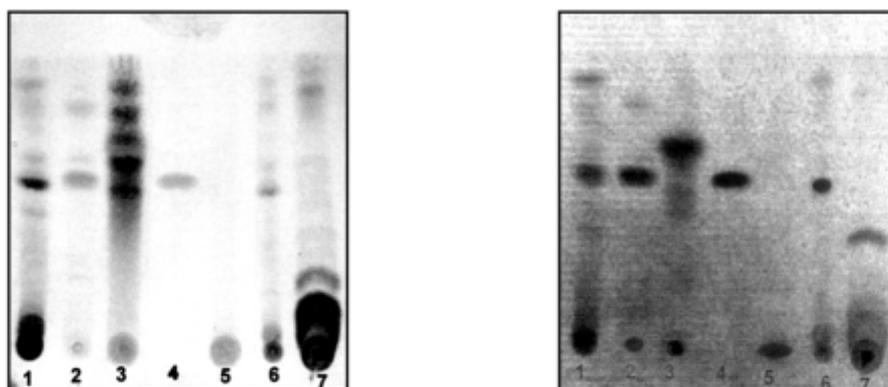


Figure 1: After Development(A.D): Vanillin -Sulphuric acid 254 nm UV light Mobile Phase (M. B): Toluene : Ethyl Formate : Formic acid (5:4:1) Silica Gel Plates

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

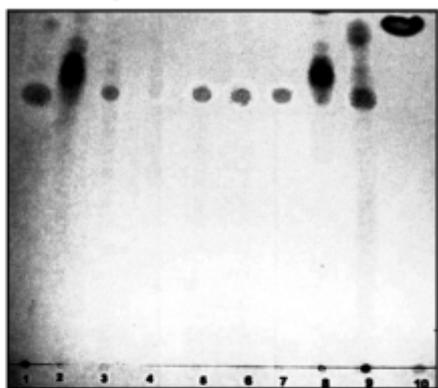


Figure 2: A. D: Dragon Dorff's reagent (visible light) M.B: Toluene : Ethyl formate : Formic acid (5:4:1)

Track 1 Starvex capsule

Track 2 Fat Magnet

Track 3 Lizi Slim Capsule

Track 4 Meziggianang Capsule

Track 5 Eube Quick Acting capsule

Track 6 Sibutramin HCl (standard)

Track 7 Seven Slim capsule

Track 8 Phyto Shape Capsule

Track 9 Zeinitat capsule

Track 10 Acomplia (standard)

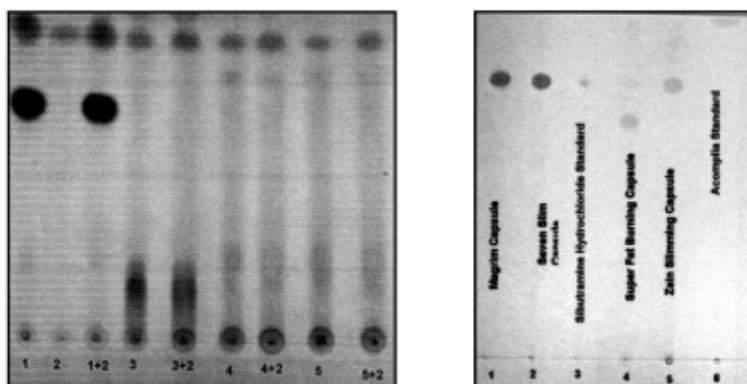


Figure 3: Polyamide plate – Co Chromatogram Silica Gel plate 254 nm UV light A.D:Dragon Dorff's reagent (visible light) M.B: Toluene : Ethyl formate : Formic acid (5:4:1)

Track 1 Super Slim capsule

Track 1 Magrim 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)

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