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Determination of synthetic pharmaceutical adulterants in herbal weight gain supplements sold in herb shops, Tehran, Iran

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Abstract

Background Nowadays with the growing popularity of herbal remedies across the world, large sections of population rely on herbal drug practitioners for their primary care. Therefore there is a need to ensure about the safety of herbal drugs and to detect adulteration with undeclared active pharmaceutical ingredients. Herbal drugs are used as first-line drug therapy in some instances. Unfortunately even if there are claims as to be natural, undeclared active pharmaceutical ingredients have been detected in these supplements.

Objectives The purpose of the present study was to analyse herbal weight gain drugs collected from herb shops located in Tehran, Iran to detect hidden pharmaceutical ingredients using UHPLC and GC/MS instrumentations.

Methods Sixty herbal drugs advertised as weight gain supplements were gathered from herb shops Tehran province, Iran. All samples were analysed from analytical toxicology point of view to detect undeclared active pharmaceutical ingredients. Method was validated for quantitative analysis of cyproheptadine and dexamethasone.

Results Method validity parameters showed good results for quantitative analysis of pharmaceutical ingredients. Cyproheptadine, dexamethasone, sildenafil, tramadol, caffeine and acetaminophen were detected in herbal weight gain drugs. Analysed dosage forms contained cyproheptadine and dexamethasone in concentrations higher than therapeutic doses. Quantitative analysis of contaminated drugs showed that the content of pharmacologic ingredients were 0.2–67 and 5.5–10.1 mg/tablet or capsule for cyproheptadine and dexamethasone respectively.

Conclusions Despite natural supplements producers' claim, herbal weight gain drugs were not natural at all. Undeclared active pharmaceutical ingredients can predispose patients to health problems and even life-threatening situations.

Introduction

In the last few years use of natural drugs especially those labeled as plant origin supplements is experiencing a considerable growth in Iran and some countries [1]. Since ancient times, herbal drugs had been used for many purposes, to maintain well-being or as medicines in many situations such as addiction treatment, sexual performance enhancing, bodybuilding, athletic performance enhancement and obesity treatment [2–7]. There is a need to evaluate herbal drugs and natural supplements to gain a proven degree of efficacy and safety in many countries [8, 9]. In spite of the need for registration and marketing authorization for all drugs before entering drug market in Iran under the supervision of Iran Food and Drug Administration (IFDA) [10], local herb shops do not obey this law and there is no control for the production and distribution of their hand-made products. Unfortunately hand-made herbal drugs are not screened from efficacy and safety perspectives and this issue doesn't have priority for manufacturers due to high cost [8]. Counterfeit and substandard drugs constitute a global problem affecting health care systems in low and

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middle-income countries and also industrialized world [11]. This problem is an interest especially for those who are health professionals and policy makers for community safety issues [12]. Evaluating the content and quality of pharmaceutical dosage forms is among the integral parts of the quality control (QC) and Good Manufacturing Practices (GMP) [11]. Unfortunately herb shops do not control their herbal formulations and add active pharmaceutical ingredients to hand-made dosage forms to enhance pharmacologic activity and to get more profit out of their business [7]. World Health Organization (WHO) has definitions for substandard, spurious, falsely labeled, falsified and counterfeit (SSFFC) medicinal products. One of these definitions is a product with wrong active pharmaceutical ingredients (APIs) [13]. Recent reports have raised the suspicious of the presence of APIs in herbal drugs advertised for bodybuilding and athletics performance enhancement [4, 14]. There are many formal legal complaints that had been set out the facts and reasons to court outlining the presence of many side effects after the use of herbal drugs. Also we have noticed some adverse drug reactions with several clinical manifestations in cases referred from gym clubs to jurisdiction authorities to be investigated. Forensic medicine practitioners are responsible to evaluate these claims in professional commissions with regard to medical examination and forensic toxicology analysis of suspicious drugs.

Analysis of fake and adulterated herbal supplements is one of the important area of scientific research within the fields of quality control, complementary medicine, forensic and analytical toxicology. There are some reports concerning the forensic toxicology analysis of herbal drugs in Iran [5–7, 15, 16]. But contaminated and adulterated herbal weight gain drugs have seldom been discussed. A few reports with small sample sizes had been performed to detect APIs in bodybuilding herbal drugs in Iran. Jalili et al. (2015), in their study analysed three samples of herbal weight gain drugs and found dexamethasone as adulterant in the samples. Also they found sildenafil and tadalafil in enhancing herbal remedies [16]. Cho et al., confirmed the presence of dexamethasone, cortisone-21-acetate, prednisone-21-acetate and dexamethasone as adulterants in food supplements advertised for the treatment inflammatory diseases [17]. Contamination of dietary supplements with pharmacologic ingredients was verified by Odoardi et al. (2015). In their study methandienone, stanozolol and testosterone were explored as anabolic agents in dietary supplements [18]. As there are few reports about the analysis of fake herbal drugs used as athletics performance enhancer or bodybuilders we designed the present study to identify and quantify active pharmaceutical ingredients in adulterated herbal weight gain drugs. To gain the goal of the study herbal drugs advertised as body builder or weight gain aid were analysed from analytical toxicology point of view.

Materials & methods

Materials and reagents

Acetonitrile, chloroform, methanol (HPLC grade solvents), phosphoric acid, potassium dihydrogen phosphate (KH_2PO_4), hydrochloric acid, boric acid and sodium hydroxide were purchased from Merck Chemical Co. (Darmstadt, Germany). Buffers, mobile phase for ultra high performance liquid chromatography (UHPLC) system and eluents were prepared with HPLC grade water for chromatography (Merck Millipore). Drug standards for cyproheptadine, dexamethasone and prednisolone were prepared from European Pharmacopoeia, Strasbourg (France) and Sigma Chemical Co. respectively. Helium gas (99.99% purity) was supplied by Roham Co. (Tehran, Iran). Amitriptyline was supplied by Daru Pakhsh Pharmaceutical Chemical Co.

Methods

Method validation procedures

Standards were run on a daily and 3 day basis for assay calibration and integration the areas of peaks by the EZChrom Elite software.

In the present study the most common detected APIs (cyproheptadine and dexamethasone) were evaluated quantitatively in counterfeit herbal drugs. In the present study prednisolone and amitriptyline were adopted as internal standards because their structure, retention time and extraction efficiency are similar to those of dexamethasone and cyproheptadine respectively (Figs. 1 and 2). Linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and repeatability of the method were evaluated.

Linearity

When obtained test results of an analysis are proportional to the amount of the desired analyte in experiment medium, the quantitative analysis is linear [15].

Fifty μL of methanolic stock (1 μg/mL) of prednisolone and amitriptyline were used as internal standards for quantitative analysis of dexamethasone and cyproheptadine. The linearity of cyproheptadine was evaluated using seven concentrations of 250, 500, 750, 1000, 2000, 2500 and 3000 ng/mL of cyproheptadine. Each concentration was analysed three times. Regression line was drawn and expressed as correlation coefficient ($R^2 = 0.9956$) using least square method for the assessment of correlation between area under the curve (AUC) and cyproheptadine concentration.

Fig. 1 UHPLC chromatogram of dexamethasone and its internal standard, prednisolone
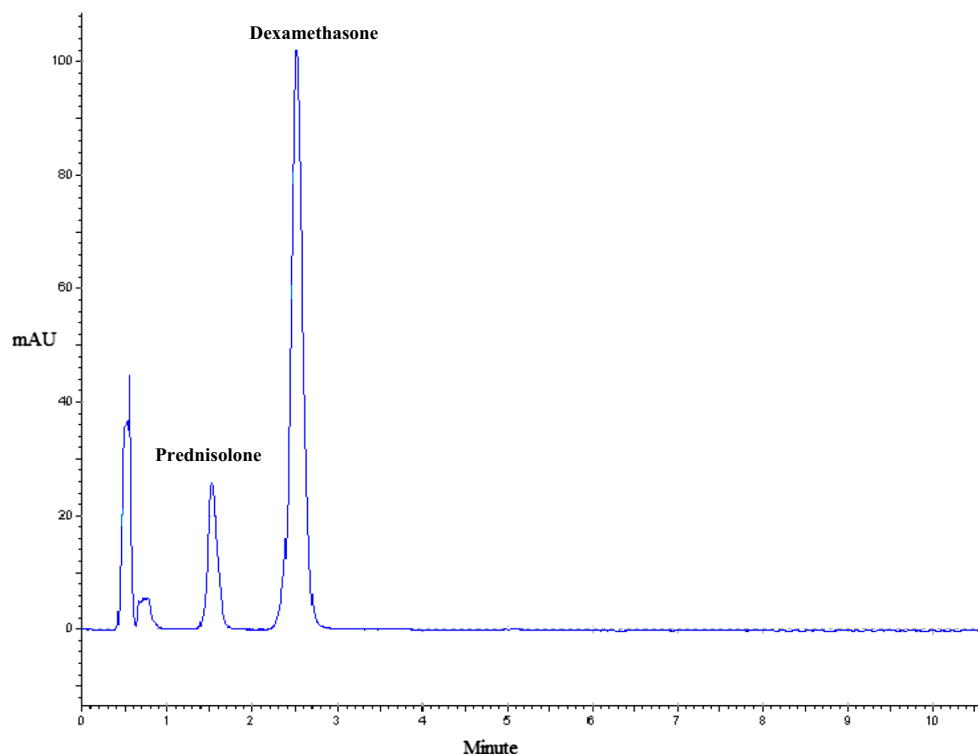
Regression line was drawn for dexamethasone at 500, 750, 1000, 1500, 2000, 3000 and 4000 ng/mL concentrations after triplicate analysis ($R^2 = 0.991$).

Limit of detection (LOD) and limit of quantitation (LOQ) measurement

LOD and LOQ of cyproheptadine and dexamethasone were calculated by the proposed method as the concentration with signal/noise = 3 and signal/noise = 10 respectively. For LOD determination, low and decreasing concentrations of cyproheptadine and dexamethasone were spiked in distilled water until signal/noise of about 3 was achieved. LOQ was evaluated in the same manner using ChemStation software.

Intra and inter-day accuracy, precision and repeatability

Accuracy indicates that the observed results of an analytical method is compatible with the true concentration of the analyte in the same sample under the same operating conditions and over a short interval of time. Precision and repeatability are suitable indicators of random errors [19]. Inter and intra-day study was carried out for the precision assessment. Three different concentrations of cyproheptadine (500, 1000, 2500 ng/mL) were spiked in distilled water for the preparation of quality control (QC) samples. The same QC samples were prepared for dexamethasone at 750, 2000 and 3000 ng/mL concentrations. Three different drug concentrations were

analysed three times on the same day ($n = 9$) at an interval time of 1 h for intra-day study. For inter-day study three drug concentrations were analysed three times on three consecutive days ($n = 27$).

Data are summarized as mean ± SD. To analyse the data statistically, we performed a one-way analysis of variance (ANOVA) for repeated measurements of the same concentration using SPSS software (Chicago, USA).

Herbal drugs analysis

Sample collection procedures

For scientific sample collection, Tehran, the capital of Iran, was divided into five regions (south, north, east, west and center). Sixty hand-made and factory-made body builder and weight gain herbal drug samples in different pharmaceutical dosage forms (tablets, capsules, powders and syrups) were bought from herb shops over 1 year study period, 1st of March 2017 till 30th February 2018. All of the samples had labels indicating "herbal supplements and natural products" and were advised by sellers to promote muscle mass and increase weight.

Physical characteristics of pharmaceutical dosage forms

Tablets, capsules and powders were characterized for some properties such as size, shape, color and odor.

Sample preparation steps

Dispersive liquid liquid microextraction (DLLME) is an efficient sample preparation technique. DLLME is a triple system including an aqueous sample containing analytes and a mixture of extracting and dispersing solvents for the isolation of active pharmaceutical ingredients from biologic and non-biologic matrices [20].The content of gelatin capsules were emptied. All tablets were grinded to fine powders. Extraction procedure was prevalidated in the laboratory [5–7].

One mg of prepared samples (triturated tablets, powders and capsules content) were mixed with one mL water. Two mL 0.1 M borate buffer (pH = 9.2) was added to the mixture. The pH of the analysis medium was adjusted to pH = 12 due to the alkaline characteristics of most drugs that are important in forensic toxicology analysis. Also extraction procedure was repeated at pH = 2–3 and pH = 8–9 for efficient extraction of drugs with acidic structure and opium alkaloids respectively. A mixture of 2.5 mL methanol+30 μL chloroform (dispersing + extracting solvents) was pushed rapidly to one mL of prepared samples in borate buffer in a 10 mL conical test tube. The mixture was stirred and ultrasonicated for 5 min followed by centrifugation. Chloroform was collected from the end of conical test tube and evaporated to dryness under gentle stream of nitrogen gas. Residue was dissolved in 30 μL of methanol and prepared to be analysed using gas chromatography/mass spectrometry (GC/MS) and UHPLC instrumentations. We should say that other possible herbal ingredients such as alkaloids, flavonoids and inert substances were not analysed in the present study due to little interest in this study context.

Apparatus and analytical conditions

UHPLC was performed using KNAUER photodiode array (PDA) detector equipped with cooling autosampler (PDA-1, 6 channels). Separation of analytes was performed using a Eurospher II 100–3 C-18 (100 mm × 3 mm) column. Two pumps were operated the system; first with degasser module and the second with mixing chamber. High pressure gradient mode with 10 mL/min and 750 bar maximum pressure was used in both pumps. Autosampler AS-1 with 10 μL loop volume, 15 μL tubing volume and 250 μL syringe volume was used. Tray configuration was 48 vials with tray cooling system. Ezchrom chromatographic software was used. Mobile phase consisted of phosphate buffer (pH = 2.32) and acetonitrile (63:37).

The GC/MS method for the detection of many drugs was prevalidated in our laboratory. GC/MS analysis was carried out on an Agilent model gas chromatograph (Agilent model 7890 A, Agilent technologies, Sdn Bhd, Selanger, Malaysia). The injector was fitted with split/splitless injector and a HP5-MS capillary column (30 m length, 0.25 mm ID, 0.25 μm film thickness, cross-linked 5% methyl phenyl silicon). Mass analyser (MS 5975 C, Agilent Technologies) was operated electron impact (70 eV) in full scan mode (50–550 m/z). The chromatographic conditions were as follows: Helium carrier gas (99.999%) was maintained at a

Fig. 2 UHPLC chromatogram of cyproheptadine and its internal standard, amitriptyline
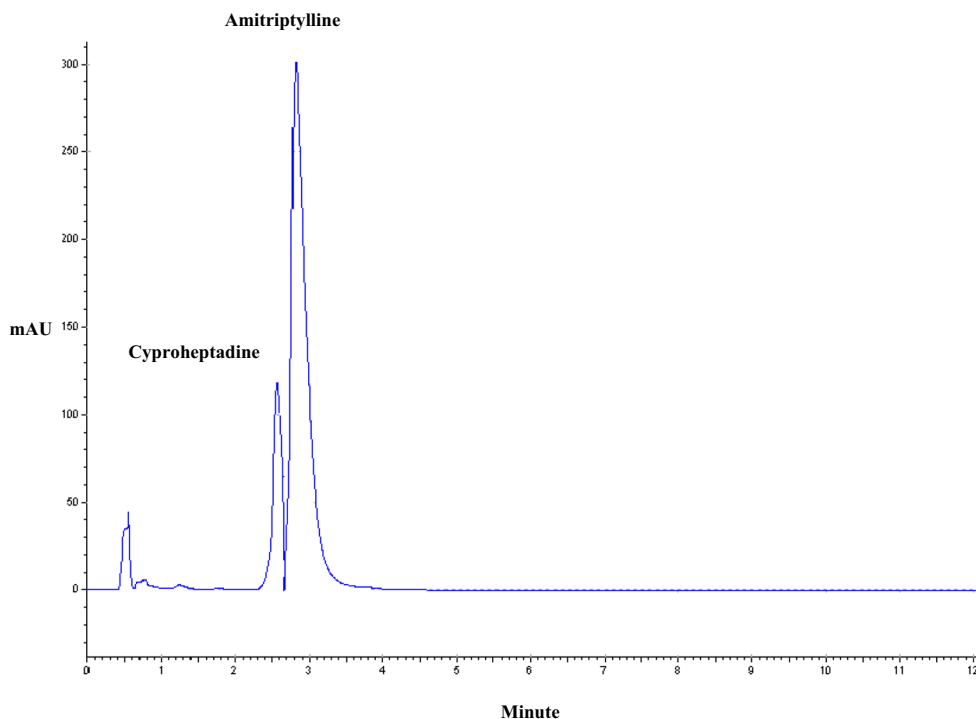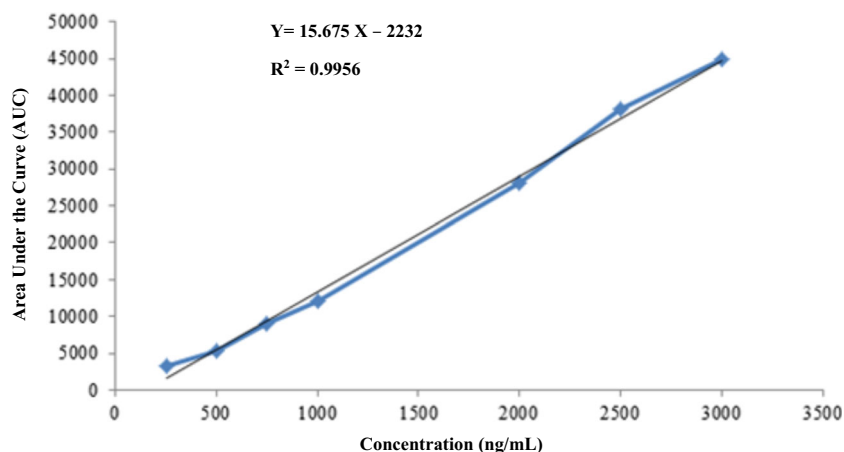
Fig. 3 Linearity plot for seven different concentrations of cyproheptadine using described UHPLC instrumentation

$$Y = 15.675\ X - 2232$$
$$R^2 = 0.9956$$

constant flow of 1.5 mL/min. Inlet and interface temperatures were set at 250 and 280 °C respectively. Injection volume was equal to one μL in splitless mode. Oven temperature was programmed at 60 °C as initial temperature hold for 1 min.

Temperature program rate was 2 °C/min and final temperature was set at 280 °C holding for 15 min. Qualitative and quantitative determination of drugs were done using NIST, Wiley and MPW 2011 libraries. Sample preparation steps and Instrumental conditions were set as general method for the detection of drugs with acidic and basic structures. Tramadol, sildenafil, acetaminophen and caffeine were detected using developed methods.
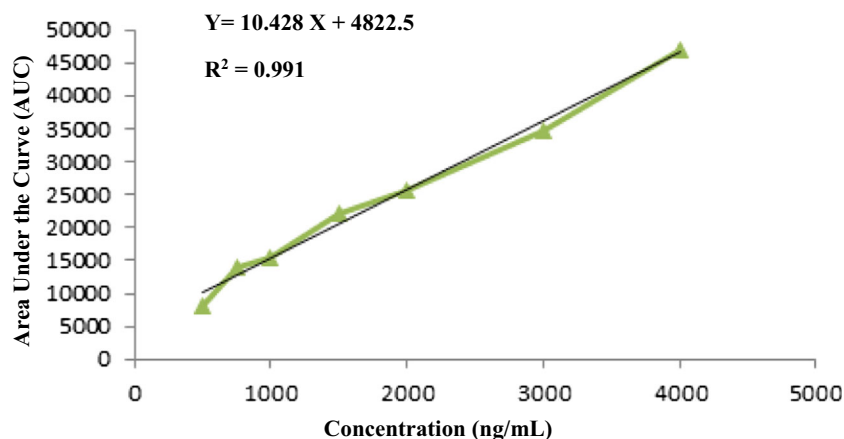
Results

Results for method validation

Linearity for cyproheptadine

Linear calibration curve was obtained for cyproheptadine at seven different concentrations with correlation coefficient

(R^2) equal to 0.9956 and CV < 10%. Linear regression equation was y = 15.675×-2231.5. Figure 3 shows the calibration curve for the concentration ranges of 250–3000 ng/mL of cyproheptadine.

Linearity for dexamethasone

Linear calibration curve for concentration ranges of 500–4000 ng/mL of dexamethasone was achieved with correlation $R^2 = 0.991$ and CV < 10%. Linear equation was y = 10.428× + 4822.5. Figure 4 shows the calibration curve for the concentration ranges of 500–4000 ng/mL of dexamethasone.

Detection and quantitation limits

Table 1 shows LOD and LOQ for cyproheptadine and dexamethasone using UHPLC instrumentation.

Inter and intra-day accuracy and precision

In both inter and intra-day precision study for cyproheptadine and dexamethasone, % coefficient of variation

Fig. 4 Linearity plot for seven different concentrations of dexamethasone using described UHPLC instrumentation

$$Y = 10.428\ X + 4822.5$$
$$R^2 = 0.991$$

Table 1 Limit of detection (LOD) and limit of quantitation (LOQ) of cyproheptadine and dexamethasone acquired using UHPLC instrumentation

Drug name	Limit of detection (LOD) ng/mL	Limit of quantitation (LOQ) ng/mL
Cyproheptadine	100	250
Dexamethasone	100	500

(%CV) were not more than 25% indicating good precision. Tables 2 and 3 show a summary of intra and inter-day validation parameters for cyproheptadine and dexamethasone respectively.

Analysis results for herbal supplements

Herbal supplements were purchased in different dosage forms such as capsules ($n = 27$), powders ($n = 23$), tablets ($n = 6$) and syrups ($n = 4$). Capsules were the most prevalent dosage forms (38.33%). All drugs smell like herbs. Capsules were made with hard gelatin covers encapsulated with white, beige or green color powders similar to dried different parts of herbs. Powders were packed in plastic or paper bags (20 g/bag). As tablets broken down, they exuded different colors ranging from yellow to brown. Labels of containers indicated that the product contained herbs such as Ginseng, Ginger, Ziziphora, Alfalfa, Malt, Gentian root, Wheat germ, Barley sprouts and other food supplements (protein, creatine, vitamins and zinc). None of the labels had standard logo indicating identity statement, manufacturer, producers and even license from IFDA. There were some statements on the labels such as: "*Natural product for fat face*", "*Gain 8-10 kilogram in one month*", "*With no adverse effects*" and "*Maximum strength*".

Capsules and tablets were the most prevalent dosage forms containing APIs. Qualitative analysis of samples showed that 26 (43.3%) of all pharmaceutical dosage forms contained at least one active pharmaceutical ingredient. Quantitative analysis of tablets and capsules showed that cyproheptadine content was in the range of 0.2–67 mg/tablet or capsule with mean ± SD equal to 17.28 ± 20.7 mg/tablet or capsule. Dexamethasone content of dosage forms was equal to 7.39 ± 3.2 mg/tablet or capsule in the range of 5.5–10.1 mg/tablet or capsule. Cyproheptadine was the only API detected in 16 samples. Dexamethasone was detected in four samples with cyproheptadine. One pack of unlabeled blue tablets contained dexamethasone. Brand names and active pharmaceutical ingredients detected in some brands are shown in Table 4.

It was evidenced that cyproheptadine, tramadol and sildenafil were detected with each other in two samples (Figs. 5 and 6).

Acetaminophen and caffeine were two APIs detected with cyproheptadine in one powder and one capsule dosage forms. Figure 7 shows "Dragon" tablet that contained cyproheptadine, tramadol and sildenafil.

Discussion

Results of the present study showed that method validation parameters exhibited acceptable results for linearity, LOD, LOQ, precision and accuracy for quantitative analysis of cyproheptadine and dexamethasone in herbal drugs. According to the results of the present study about 43% of analysed samples contained at least one active pharmaceutical ingredient.

Detecting and proving adulteration of drugs is one of the important tasks for forensic toxicologists and pharmaceutical

Table 2 Intra and inter-day precision and accuracy of cyproheptadine QC samples using described UHPLC instrumentation

Theoretical concentration (ng/mL) Intraday assay		Calculated concentration (ng/mL) (mean ± SD, $n = 3$)	Precision CV (%)	Accuracy recovery (%)
Day 1	500	483.69 ± 13.44	3.98	94.65
	1000	963.17 ± 174.36	21.24	98.03
	2500	2779.77 ± 261.22	9.9	98.81
Day 2	500	480.93 ± 2.59	0.76	95.09
	1000	902.52 ± 98.77	15.91	98.97
	2500	2669.46 ± 58.54	2.23	97.43
Day 3	500	483.38 ± 6.12	1.23	93.23
	1000	949.89 ± 51.09	6.32	97.77
	2500	2760.4 ± 54.76	2.09	97.9
Interday assay	500	482.70 ± 9.12	0.45	99.57
	1000	938.51 ± 32.4	4	97.07
	2500	2766.84 ± 40.38	0.42	99.43

Table 3 Intra and inter-day precision and accuracy of dexamethasone QC samples using described UHPLC instrumentation

Theoretical concentration (ng/mL) Intraday assay		Calculated concentration (ng/mL) (mean ± SD, $n = 3$)	Precision CV (%)	Accuracy recovery (%)
Day 1	750	1053 ± 137.02	15.04	97.87
	2000	1952.42 ± 75.81	4.18	89.90
	3000	2258.42 ± 172.55	8.15	95.29
Day 2	750	1235.64 ± 139.01	13.30	81.81
	2000	1909 ± 126.69	8.78	92.14
	3000	2225.95 ± 150.27	7.08	93.90
Day 3	750	1067 ± 72.85	8.21	99.43
	2000	1538.49 ± 48.36	3.10	85.77
	3000	2226.24 ± 33.67	1.61	93.84
Interday assay	750	1104.48 ± 63.21	11.52	92.64
	2000	1800.03 ± 37.05	13.72	98.21
	3000	2237.4 ± 20.53	0.86	94.34

Table 4 Brand names and active pharmaceutical ingredients detected in adulterated herbal weight gain drugs obtained from herb shops, Tehran, Iran

Product brand name	Dosage form	Detected active pharmaceutical ingredients	Quantity of drugs in formulations
Fat Fast	White capsules	Cyproheptadine	1.8 ± 2.2 mg/capsule
Fat Fast	Round creamy tablets	Cyproheptadine	0.5 ± 1.9 mg/tablet
Exir	Powder	Cyproheptadine	51.9 mg/g
Exir	Blue tablets	Cyproheptadine	22.8 ± 12.7 mg/tablet
Angel	Capsules	Cyproheptadine, Tramadol, Sildenafil	39.2 ± 24.5 mg/capsule
Dragon	Oval dark green tablets	Cyproheptadine, Tramadol, Sildenafil	19.5 ± 6.5 mg/tablet
Royal	Powder	Cyproheptadine	63.3 mg/g
Aflatoon (Herbal protein)	Powder	Cyproheptadine	44.1 mg/g
Red gelatin capsules in unlabeled box	Capsules	Cyproheptadine, Acetaminophen, Caffeine	41.9 ± 2.7 mg/capsule
Tablets in unlabeled box	Blue tablets	Dexamethasone	9.7 ± 2.8 mg/tablet
Tablets in unlabeled box	White tablets	Cyproheptadine	67 ± 0.3 mg/tablet
Promed Fat Face	White tablets	Cyproheptadine	21.5 ± 4.7 mg/tablet
V.A.M	White tablets	Cyproheptadine	0.2 ± 0.1 mg/tablet
Full Fat Body	Creamy tablets	Cyproheptadine, Dexamethasone	CYP[a]: 23.3 ± 9.1 mg/tablet DEX[b]: 7.3 ± 1.9 mg/tablet
Gain Up	White powder	Cyproheptadine, Dexamethasone	CYP:50.8 mg/g DEX:9.5 mg/g
Bomba	White powder	Cyproheptadine	56.3 mg/g
Yotam	Green powder	Cyproheptadine	39.7 mg/g
Miracle	Beige powder	Cyproheptadine	41.4 mg/g
Power Apple	Green capsules	Cyproheptadine	0.3 mg/g
Green hexagon tablets in unlabeled box	Green tablets	Cyproheptadine, Dexamethasone	CYP: 2 ± 0.6 mg/tablet DEX: 5.3 ± 0.8 mg/tablet
Tiar	Green Powder	Cyproheptadine	11.8 mg/g
FDA G-Fast	Tablets	Cyproheptadine, Dexamethasone	CYP: 23.8 ± 13.3 mg/tablet DEX: 9.1 ± 4 mg/tablet
Barley Sprout Powder	Beige powder	Cyproheptadine, Acetaminophen, Caffeine	60.7 mg/g
Barley Malt Powder	Creamy powder	Cyproheptadine	49.3 mg/g
Wheat Germ Powder	Yellow powder	Cyproheptadine	55 mg/g

[a] *CYP* Cyproheptadine, [b] *DEX* Dexamethasone

analysts [21]. Consistent with the results of the previous studies the method was well validated for quantitative analysis of cyproheptadine [21, 22]. The difference in obtained LOD and LOQ were attributed to difference in the sample preparation methods and analysis conditions. Maham et al., 2013 validated a method for the qualitative determination of cyproheptadine in urine sample using DLLME-HPLC method [22]. However they had used acetonitrile as dispersing solvent.

The method was validated for quantitative analysis of dexamethasone as undeclared API in weight gain supplements too. LOD and LOQ for dexamethasone were 100 and 500 ng/mL respectively. Friedrich et al., 2009 in their study validated a rapid UV spectrophotometric method for dexamethasone analysis in tablets. They stated that LOD and LOQ for dexamethasone were found to be 0.52 and 1.56 μg/mL respectively [23]. The discrepancy between our results and the results stated by these authors may be due to the use of different techniques in the method validation processes.

The main and second aim of the study was to analyse herbal weight gain drugs used by bodybuilders, athletics and geriatric population to promote muscle and body strength.

Results of the present study demonstrated that herbal weight gain drugs in Tehran, Iran were laced with cyproheptadine, dexamethasone, sildenafil, tramadol, acetaminophen and caffeine. In view of great tendency for herbal drugs all over the world, it is necessary to analyse counterfeit herbal drugs. WHO reported that 80% of patients in developing countries choose herbal drugs as the first-line therapy [24]. This high demand and economic incentives are among the most important factors that encourage herbal drug

manufacturers to adulterate drugs [25]. Also the desire to get fast and effective results in combination with widespread availability of food supplements via internet sites encourages people to use these drugs [7]. A potential recurring theme was the APIs found in herbal drugs labeled as natural supplements, tailored to deliver effectiveness and therapeutic outcomes. Some studies highlighted the presence of undeclared APIs in weight gain drugs [4, 16]. Cyproheptadine was the most prevalent adulterant in herbal weight gain drugs in the present study. In line with the results of the present study, cyproheptadine and dexamethasone were the two contaminants in herbal drugs used for the treatment of back pain in previous studies [4].

Many drugs are listed by other scholars as to be obesogenic and promote weight gain as their side effects including cyproheptadine, corticosteroids and many other drug categories [26]. This effect can arise from different drugs mechanisms. Appetite stimulation is one of the side effects of cyproheptadine that cause increase in food intake and weight gain [27]. In accordance with the results of the present study, dexamethasone was the most common contaminant in dietary supplements in Korea advertised for the treatment of bone ache, arthritis and joint pain [17]. Dexamethasone induces weight gain even if low doses used. Sodium retention and edema are among the most common side effects of dexamethasone [28]. Also dexamethasone exerts its obesogenic activity via increasing appetite [26]. That is why these drugs are added deliberately to herbal weight gain drugs.

It was observed that sildenafil, tramadol, caffeine and acetaminophen in some formulations. To the best of our
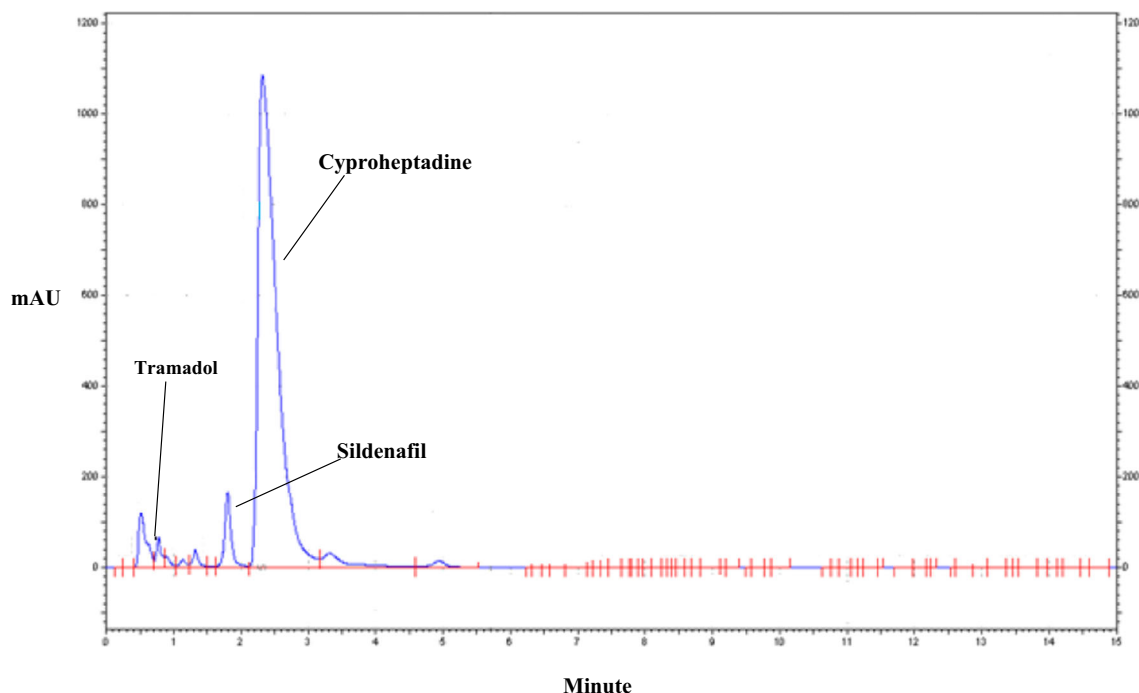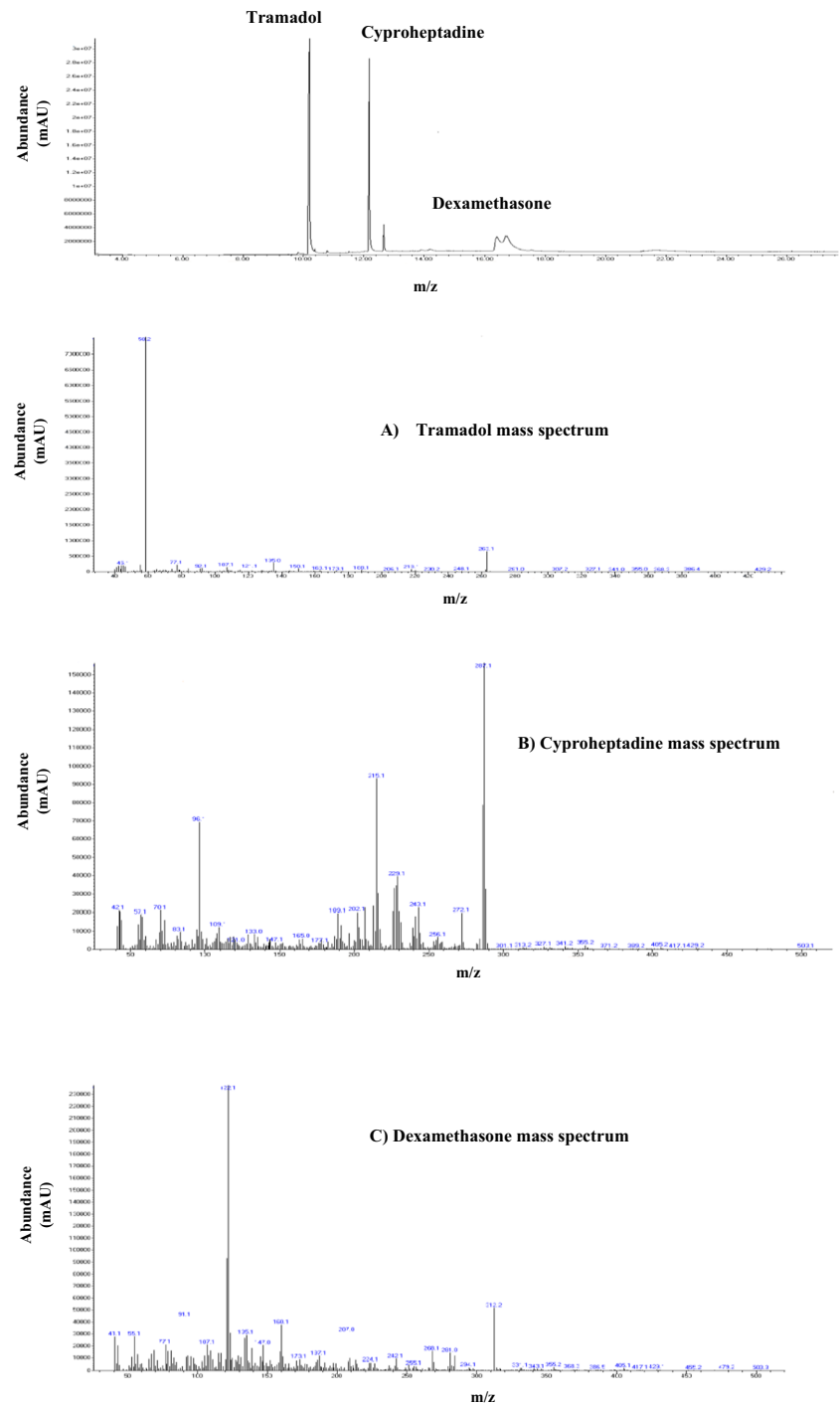

Fig. 5 UHPLC chromatogram of cyproheptadine, tramadol and sildenafil detected in adulterated herbal weight gain capsule

Fig. 6 GC/MS chromatograms (**a**) and mass spectra of cyproheptadine, dexamethasone and tramadol (**b**, **c**, **d**) separated from contaminated herbal weight gain drugs

knowledge and literature review this is the first report of detecting sildenafil and tramadol as adulterant in herbal weigh gain drugs. The role of tramadol, acetaminophen and caffeine is obscure for us. One possible explanation for adding sildenafil to herbal drugs is that sildenafil shows viable pharmacologic interventions to increase muscle function [29]. Skeletal muscle function reduces in situations such as aging as well as cancer cachexia and bed rest [29]. Body builders and athletics

favor to improve muscle function for racing. Changes in mass and quality of muscle affect skeletal muscle function. Therefore protein synthesis is one of the ways to improve muscle function [29]. Sildenafil causes protein synthesis, alteration in protein expression, nitrosylation and reduction in muscle fatigue via augmentation of nitric oxide-cyclic guanosine monophosphate signaling. Sildenafil improves skeletal muscle oxygenation during exercise in subjects with

Fig. 7 One adulterated herbal weight gain tablet labeled "Dragon" containing cyproheptadine, tramadol and sildenafil

intermittent claudication [30]. Another assumption for adding multiple drug classes to natural products is to solve many difficulties such as decreased libido or pain in elderly population. Also it is assumed that tramadol and acetaminophen were added to herbal drugs to relieve pain in elderly patients. Sildenafil can resolve loss of sexual desire in older subjects [31]. It is well recognized that some drugs such as caffeine may be added to herbal supplements as athletics performance enhancer, stimulant or energetics [7].

In some instances, contaminated herbal supplements vary widely in APIs concentrations and contain drugs much greater than prescription strength [32]. Cyproheptadine was detected in higher amounts of therapeutic doses in Traditional Chinese Medicines [29]. In the present study quantitative analysis of dosage forms showed that cyproheptadine and dexamethasone content were higher than therapeutic doses (more than tenfold) and also dosage forms had not content uniformity for active ingredients. The typical dose for cyproheptadine is 2–4 mg and each standard dexamethasone tablet contains 0.5 mg of dexamethasone as active ingredient. The worst scenario is that these dosage forms are prescribed as two or three capsules or tablets/day. However the adverse effects of added pharmacologic ingredients should be considered even they are present in low concentrations in final product [33].

Conclusion

Results of the present study showed that herbal drugs that are advised for weight gain and bodybuilding in Tehran, Iran are not natural whatsoever. They contain active pharmaceutical ingredients in higher doses than therapeutic amounts. Validated method for quantitative analysis of dexamethasone and cyproheptadine proved to be sensitive and had shown enough precision and repeatability. Although synthetic drugs cannot be produced except by permission of the licensing authorities, there is no regulation for the production of herbal

drugs. Therefore the quality and safety of natural supplements must be assured for patients' health.

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