Detection of corticosteroid compounds and *phosphodiesterase* inhibitors (PDH-5) as counterfeit in herbal products available in Iranian market by HPLC method

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A B S T R A C T

Weight-gain and potency enhancing drugs are the most popular herbal products in Iran which are easily available via the internet and through illegal markets. The content of some of these popularly purchased products were investigated for the presence of illegal substance like *phosphodiesterase* inhibitors (PDH-5) and corticosteroids by HPLC method. For this study, ten samples of both kind of herbal medicine were obtained from Iranian markets, then chromatographic analysis of corticosteroids was achieved isocratically on a C18 column (C18, 5μm , 150 mm x 4.6 mm) by utilizing a mobile phase of methanol/water (55:45, v/v, pH 7.0) at a flow rate of 1.5 mL/min with UV detection at 250 nm, while analysis of *phosphodiesterase* inhibitors followed by a C18 column (C18, 5μm, 150 mm x 4.6 mm) using a mobile phase of methanol/water (65:35 v/v, diethyl amine(100μl/l, pH 3.5) at a flow rate of 1.5 mL/min. UV detector operated at 290 nm. After quantitative analysis, different contents of sildenafil and tadalafil in 3 cases of enhancing herbal remedies and dexamethazone in 3cases of weight gaining herbal medicine have been identified.

Introduction

In recent years, the overt production of counterfeit herbal medicines has led to a major issue in the public health. The impact of using the counterfeit products sometimes could be irreversible and in some unfortunated and dire cases has led to the death of the user, may be due to the lack of awareness [1,2]. The international medical products anti counterfeiting task force (IMPACT) claims that about 30% of salable medicines in some areas in Africa, Asia and Latin America may be counterfeit [3]. According to the definition of the World Health Organization, counterfeit medicine can be some kind of products without active ingredients, with incorrect amount of active ingredients, with wrong ingredients, with fake packaging, with large amount of impurities and contaminants [4, 5]. Nowadays, the whole world is concerned about the impact of this problem over the global health. In order to fight this phenomenon, urgent and drastic measures have been proposed and many analytical methods for the detection of medicine counterfeits have been developed [6-9]. Furthermore, phytotherapies have been successful in capturing the market under the impression that they were safe and without any side effects. Although, synthetic compounds have been found in many herbal preparations. The most encountered herbal therapeutic categories in Iran were weight-gain drugs and aphrodisiac drugs widely sold outside regular pharmacies, on the internet, in fitness clubs, cosmetic salons or on the street corners [10]. most of these products contain large amount of synthetic compounds for instance, *phosphodiesterase* inhibitors and corticosteroids which are ordered in special cases and their consumption irregularly causes disorders which are often serious and even lead to the death [11-14]. It seems that we need more proceeding to fight against the rise of the counterfeits in weight-gain and enhancement of sexual function medicine which are used increasingly in Iran as herbal products. On the other hand, we don't have exact evaluations or reports about quality and composition of drugs imported to our country illegally. In this study, we tried to prepare suitable package for authorities and society in general about chemical composition of such herbal medicines by using of fast analytical method to distinguish between genuine herbal drugs and products which contain synthetic compounds. For this purpose, ten sample of each group of drugs were gleaned from outside of regular pharmacies, then by comparing between genuine sample and suspect drugs chromatogram, counterfeit sample were identified.

Materials and Methods

The reference standards of Sildenafil citrate (batch 904958), Tadalafil (batch RS0480) were purchased from Chennai, India and Dexamethasone, Prednisolone, was purchased from Bal Pharma, India. 20 types of herbal weight-gaining and aphrodisiac products were purchased from the irregular markets; on the internet and on the street corner. HPLC-grade methanol was purchased from Merck. Water was glass-double distilled, and further purified for using HPLC with the aid of a maxima purification system (USF ELGA, England). Stock solutions of all standards and herbal products were prepared in methanol and stored at 4ºc. The used HPLC system (Shimadzu, Kyoto, Japan) comprised two pumps of Shimadzu LC-10A solvent delivery system controller (SCL10AD), a UV/Vis spectrophotometric detector (SPD-10AD), a column oven (CTO-10A) and a data processor (C-R4A).

Sample preparation

Ten weight-gain herbal products (Ginsing, Carbo, Metan, Mega mass, G-fast, Fat fast, Easy fat, anonymous, Max fat, Fat face) and Ten sexual potency enhancing herbal products (Vigarex, SX, Max man, Spermax, King man, Golden eagle, Green Viagra, anonymous, Toos kimiagaran , Magna RX)

were purchased from market. Each sample obtained from dissolving one pill or capsule in 8 ml methanol then all samples centrifuged at 4000 rpm for 5min. Supernatant solutions were then used for quantitative analysis (see below).

Validation

The rectilinear relationship between concentrations of the analyzes and the peak-area ratio as UV detector response was evaluated. The concentrations used ranged from 0.0031 to 12 mg/ml for sildenafil; from 1.25 to 25µg/ml of tadalafil and from 0.125 to 250µg/ml for dexamethazone. Control samples that used in method validation prepared with the standard solutions at three different concentrations as described above (sildenafil: 3.12, 62.5 and 2500 µg/ml; tadalafil: 1.25, 3.12 and 25 µg/ml; dexamethazone 0.125, 0.5 and 2µg/ml. Three different preparations of the analytical standard were analyzed in triplicate on the same day for the determination of intra-day assay precision. These determinations were repeated using freshly prepared standard solutions on three separate days to determine inter-day precision of analysis. The LOD and quantification LOQ were defined as the concentration of the drug giving a signal-to-noise ratio of 3:1 and 10:1, respectively.

HPLC Analysis

To analysis of weight-gain products; a mixture of water and methanol (45:55) was used as mobile phase and analytical separation was achieved on a RP 18 HPLC column (15×25 mm ID). The column oven temperature was set at 50ºc. UV detector operated at 250 nm. For aphrodisiac products; mobile phase composed of mixture of triethylamine (100µL/L; pH 3.5; adjusted with phosphoric acid) and methanol (35:65). The

detection wavelength was set at 290nm. The other separating conditions were the same as weight-gain products analysis.

Results

As Figure 1 shows, the validated methods demonstrated excellent chromatographic specificity, with no interference at the retention times of sildenafil and talafile and dexamethazone and prednisolone. The detection limits for sildenafile, tadalafil, dexamethazone were 0.02 µg/ml, at a signal-to-noise ratio of 3:1 and the quantification limit corresponding to a coefficient of variation of less than 20% were 0.05 µg/ml for sildenafil, tadalafil, dexamethazone. The calibration curves were linear over the concentration range mentioned in section 2.3. The linearity of the results is expressed by the coefficient of determination ($r2$). For all calibration curves coefficient of the linear regression analysis were >0.997. The performance characteristics and validation data are summarized in Table 4. Results of the analysis of the herbal weight-gain and aphrodisiac products are shown in Tables5 and 6. As mentioned in Table 5 the results relating to the presence of the prednisolone were no fraud in all samples.
Dexamethazone was contained in Mega max (66.52 µg/ml per pill), Fat fast (68.65 µg/ml per pill), Easy fat (50.99 µg/ml per pill), anonymous (145.42 µg/ml per capsule), Max fat (37.58 µg/ml per pill), fat face (95.42 µg/ml per pill). Sildenafil was contained in King man (10 mg/ml per pill), Max man (9 mg/ml per pill) and green Viagra (8mg/ml per pill). Tadalafil was contained in Max man (8µg/ml per pill) and Magna RX (5µg/ml per pill).
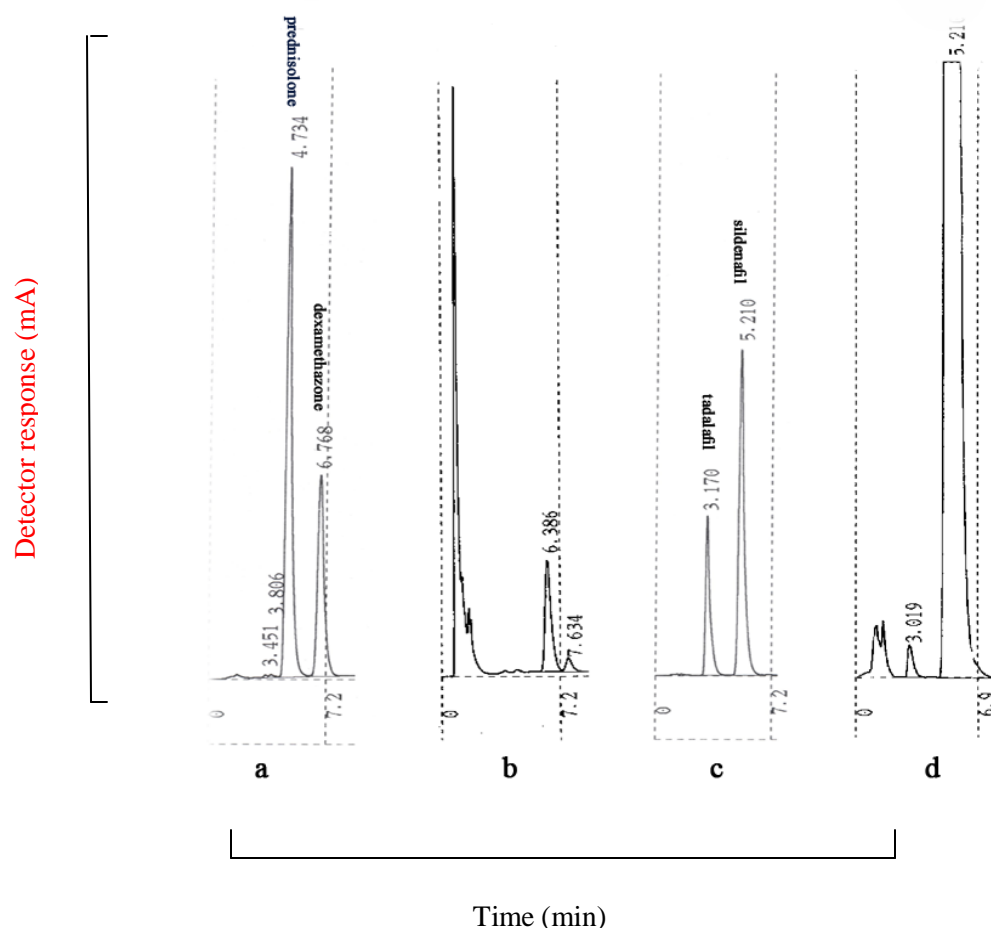
Fig. 1. Typical chromatogram of dexamethazone and prednisolone obtained by applying a mobile phase composed of A mixture of water and methanol (60:40), other analytical condition described in section2.3. (a), chromatogram of real sample (b:Fast fat), Representative chromatogram of the separation of the sildenafil and tadalafil using the conditions described in Section 2.3 with a mobile phase composed of: mixture of triethylamine (100µL/L; pH 3.5; adjusted with phosphoric acid) and methanol (40:60). (c), chromatogram of real sample (d:Max man).

Table1. Inter- and intra-day precision and accuracy for determination of Tadalafil with HPLC method.

Con.c of tadalafil(µg/ml)	Concentration found Mean±SD	CV(٪)	Accuracy(%mean deviation)
Inter day(n=6)			
1.25	1.6±0.01	0.006	128
3.12	3.9±0.08	0.021	125
25	24.5±0.6	0.025	98
Intra day(n=6)	1.55±0.01	0.006	124
1.25	3.4±0.08	0.023	180
3.12	23±0.65	0.028	92
25			

Table2. Inter- and intra-day precision and accuracy for determination of Sildenafil with HPLC method.

Con.c of sildenafil (µg/ml)	Concentration found (Mean±SD)	CV(٪)	Accuracy(%mean deviation)
Inter day(n=6)			
3.12	3.48±0.2	0.068	111
62.5	61.1±1.35	0.022	97
2500	2613±130.47	0.049	104
Intra day(n=6)	3.2±0.5	0.15	102
3.12	62±1.6	0.0258	99
62.5	2400±136.3	0.056	96
2500			

Table3. Inter- and intra-day precision and accuracy for determination of Dexamethasone with HPLC method.

Con.c of dexa(µg/ml)	Concentration found (Mean±SD)	CV(٪)	Accuracy(%mean deviation)
Inter day(n=6)			
0.125	0.14±0.01	0.077	112
0.5	0.56±0.15	0.26	112
2	2.6±0.36	0.138	130
Inter day(n=6)	0.132±0.02	0.15	105
0.125	0.52±0.18	0.34	104
0.5	2.3±0.4	0.17	115
2			

Table4. Validation data for analysis of sildenafil, tadalafil and dexamethasone.

Com	LOD(µg/ml)	LOQ(µg/ml)	Equation	r^2
Sild	0.41	1.25	Y=4421.1x+14119	0.999
Tada	1.04	3.12	Y=13362x+1700 3	0.998
Dexa	0.0208	0.0625	Y=6615x-52752	0.998

Table 5. Amount of sildenafil and tadafil in ten types of sexual potency enhancing drugs.

Brand	sildenafil (µg/ml) per pill or capsule	Tadalafil (µg/ml) per pill or capsule
Vigarex (pill)	-	-
SX(pill)	-	-
Max man (pill)	1×10^4	8.0
Spermax (pill)	-	-
King man (capsule)	9×10^3	-
Golden eagle (capsule)	-	-
Green Viagra (pill)	-	-
Anonymous (capsule)	8×10^3	-
Toos kimiagaran (capsule)	-	-
Magna RX (pill)	-	5.0

Table 6. Amounts of dexamethasone and prednisolone and tadafil in 10 weight-gain herbal medicines.

Brand	Dexamethazone (µg /ml) per Pill or Capsule	Prednisolone (µg/Ml) per Pill or Capsule
Ginsing (capsule)	-	-
Carbo (pill)	-	-
Metan (pill)	-	-
Mega mass	66.52	-
G-fast (pill)	64.12	-
Fat fast (capsule)	68.65	-
Easy fat (capsule)	50.92	-
Anonymous (capsule)	145.42	-
Max fat(pill)	37.58	-
Fat face (pill)	98.42	-

Conclusion

Counterfeiting in pharmaceutical industry is a global concern. There are numerous reports about identification of synthetic compounds in herbal products as adulterants. Most of these compounds could be dangerous for human health even in low concentrations. The Whole world tries to apply effective methods for fighting this phenomenon. The first cases of adulterants medicine were detected in 1990s [15]. In developing countries, one of the most counterfeited medicines is sexual potency enhancing drugs. In 2008, a case of presence of phosphodiesterase type 5 inhibitors (PDE5-i) reported as adulterant herbal product presented in Singapore [16]. Similar clinical trials have been reported in India, USA, Taiwan and Belgium [17-20]. In 1991, Goldman and coworkers reported the presence of prednisolone in herbal medicine [21]. In 1992, there was a case of potent topical steroid addition used for having a round face and truncal obesity in a Chinese herbal medicine [22]. In this study simple and rapid chromatographic techniques have been employed for determination of synthetic compounds. Results showed the presence of sildenafil and tadalafil in aphrodisiac products and dexamethasone in weight-gain herbal medicine which are being used by Iranians. These products were illegally imported into the country and had no licensed label from the Iranian Ministry of Health. Up to now, there have been no comprehensive reports about the presence of synthetic compounds in sexual potency and weight-gain products offered in the Iranian market. The unexpected side effect of illegally sold herbal medicine is increasing. Thus, there is an urgent need to raise public awareness to the possible health risk of using the counterfeit herbal products which are claimed that they are natural and safe. In this paper we tried to illustrate presence of synthetic compounds in some unlicensed products to appreciate authority's attention to this phenomenon and consequences in the public health.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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