Detection of hazardous weight-loss substances in adulterated slimming formulations using ultra-high-pressure liquid chromatography with diode-array detection

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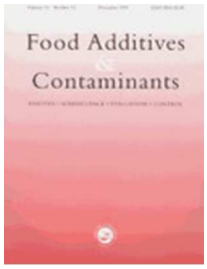

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Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2011-382.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	28-Oct-2011
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Methods/Techniques:	HPLC
Additives/Contaminants:	Additives general, Caffeine
Food Types:	Dietary supplements, Nutritional supplements
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Abstract

The presence on the market of illegal products for slimming purposes or the treatment of overweight is a public health issue. These products may illicitly contain chemicals in order to improve their effectiveness. Some of these weight loss compounds are responsible for adverse events including fatal outcomes. A general strategy for the analysis of any suspect formulation begins with a large screening for the general search of a wide range of compounds. A methodology for the qualitative and quantitative determination of 34 compounds in slimming preparations (such as dietary supplements or medicinal products) was used for the control of slimming formulations from the market, including over the internet. The fast liquid chromatography system (UHPLC) used a gradient of solvent (phosphate buffer and acetonitrile), a C18 end-capped column and a diode array detector. This system allows dual identification based on retention time and UV spectra. The analytical method is simple, fast and selective since 34 weight-loss compounds can be detected in a 15 min run time. Thus, 32 commercial slimming formulations were analysed using this method, allowing the detection and quantification of hazardous active substances: caffeine, clenbuterol, nicotinamide, phenolphthalein, rimonabant, sibutramine, didesmethylsibutramine, synephrine and yohimbine.

Keywords: Dietary supplement, slimming preparation, weight-loss, adulterants, screening, sibutramine, ultra-high pressure liquid chromatography

Introduction

Nowadays, slimming products are, with erectogenic drugs, one of the most life style health products sold outside the pharmaceutical distribution network (Biesemeier et al. 2008). Such products can be easily purchased as food supplement or medicinal products in some retail stores as well as in beauty salons or over the internet. These products have not been assessed and approved by the authorities, resulting in a health risk to unsuspecting consumers, including fatal outcomes (Tang et al. 2011; Kerrigan et al. 2005). Indeed, in November 2008, the French Agency for the Safety of Health Products issued an alert (AFSSAPS, 2008) concerning a young woman dead after having taken dietary supplement capsules named “Best life”. It was demonstrated that these capsules contained sibutramine, a regulated pharmaceutical substance that should be taken under medical follow up of patients because of possible side effects.

The aim of the presence of synthetic substances in slimming preparations is to increase efficacy in the treatment of obesity or weight-loss purposes. These adulterants are more and more present in slimming products on the worldwide marketplace, as related by other national health authorities (US Food and Drug Administration, National Institute for Public Health and the Environment of the Netherlands, Health Canada, Swiss Medic...): dietary supplements and herbal ingredients adulterated with potentially noxious chemical ingredients (Carvalho et al. 2011; Jung et al. 2006) or counterfeit medicines (FDA, 2010),

After a risk analysis based on the study of warnings and reports from medicines agencies (FDA, 2009; Venhuis et al. 2009), 34 weight-loss substances have been selected (Table 1) to be screened in suspect slimming formulations. They belong to different pharmacological categories: anorectics (sibutramine, rimonabant, fenfluramine, amfepramone, phentermine) used to reduce appetite, stimulants (amphetamine, ephedrine, metformine, synephrine, caffeine, yohimbine) used to induce temporary improvements in either mental or physical function, antidepressants (phenobarbital, fluoxetine, penfluridol) used to alleviate anxiety disorders, laxatives (phenolphthalein) used to raise intestinal transit, diuretics (bumetanide,

furosemide, spironolactone, triamterene, althiazide) used to increase loss of water, and also vitamins (nicotinamide) or amino-acids.

Slimming formulations (medicines, dietary supplements and instant coffee powders) were collected from different sources (over the internet, inspectorate sampling or following pharmacovigilance alerts) to be tested using an in-house chromatographic method. For the screening of the selected substances, literature describes the use of conventional liquid or gas chromatography (LC-DAD, LC-MS, GC-MS) (Saka et al. 2008; Bogusz et al. 2006; Zou et al. 2007), capillary electrophoresis (Cianchino et. 2008) and also NMR (Vaysse et al. 2010). However few methods have been developed for the simultaneous analysis of a large extent of compounds (Carvalho et al. 2011).

This paper proposes a screening method designed to be simple and fast, using a modern system more and more available in control laboratories: fast chromatography with UV detection. Ultra High Pressure Liquid Chromatography (UHPLC) is based on the use of columns with small diameter (2.1 mm) packed with sub-2 µm particles. Compared with conventional HPLC, UHPLC provides significant advantages concerning peak capacity, selectivity, resolution and run time. These good separation efficiencies are particularly appreciated for multi-analytes screening (Klose et al. 2010; Wang et al. 2008; Murray et al. 2009; Badoud et al. 2009). Working up to 1000 bar, close to the optimal flow-rate, 100 mm columns offer high peak capacity. Moreover, run time and solvent consumption are drastically reduced. Unfortunately, no UHPLC method was dedicated to the screening of weight-loss substances. Compared with existing HPLC methods, this new UHPLC method is fast, simple and selective for the detection of adulterants in slimming formulations. This article reports the results of the analysis of 32 slimming formulations using the UHPLC screening method. The description of the screening methodology has been focused on six of them, and the presence of hazardous weight-loss substances is finally discussed.

Materials and methods

Chemicals, reagents and samples

Amphetamine, 2,4-dinitrophenol, metformine hydrochloride, usnic acid, amfepramone (or diethylpropion) hydrochloride, bergenin monohydrate, bumetamide, clenbuterol hydrochloride, dantoin, ephedrine hydrochloride, fluoxetine hydrochloride, furosemide, levothyroxine, liothyronine (or 3,3',5 triiodo L,thyronine), nicotinamide, penfluridol, phenobarbital, pseudoephedrine, salicin, sibutramine hydrochloride monohydrate, spironolactone, triamterene, fenfluramine hydrochloride, caffeine, phenolphthalein, phenylalanine and synephrine (or axedrine) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Althiazide, oxethazaine, phenformin hydrochloride, phenothiazine were purchased from Fluka (Saint-Quentin Fallavier, France). Phentermine was purchased from Supelco (Saint-Quentin Fallavier, France). Yohimbine hydrochloride was purchased from Extrasynthèse (France). Rimonabant was kindly obtained from Sanofi-Aventis (Gentilly, France). The purity of all those standards is known and greater than 98.0% (w/w). Acetonitrile and methanol (Carlo Erba-SDS, France) were HPLC grade. Sodium dihydrogen phosphate dihydrate and phosphoric acid (VWR, France) were analytical grade. Water was ultra pure HPLC grade (Milli-Q, Millipore, France).

Thirty two slimming products (Table 2) were tested using the UHPLC method.

Chromatographic conditions

The method was developed on an Acquity UPLC/DAD system with Empower software (Waters, France) and a trifunctional C-18 column, fully endcapped, bonded to ethylene bridged hybrid substrate (Acquity BEH C18, 100 x 2.1 mm, 1.7 µm, Waters France) at 30°C (Table 3). The mobile phase was composed of (A) phosphate buffer 50 mM solution (7.8 g/l sodium dihydrogen phosphate dihydrate) adjusted to pH 3.8 with phosphoric acid 10% (v/v) and (B) acetonitrile. A gradient was applied from 5% (v/v) to 65% (v/v) of mobile phase B. The mobile phase was delivered at a flow rate of 0.35 ml/min. Samples were stored at 6°C in the autosampler prior to the injection. The injection volume was 1 µl. The detection was set in "maxplot" mode between 210 and 400 nm. UHPLC conditions were similar for screening, confirmatory step and quantification.

Preparation of solutions

Individual standard stock solutions of each of the 34 substances were prepared at 0.5 mg/ml in methanol. Standard working solution was prepared by appropriate dilution of each standard stock solution with methanol in order to obtain a mixture of compounds at the nominal concentration of 12.5 µg/ml.

For the preparation of sample solutions, a single dilution solvent was used. Mobile phase at initial proportions has been chosen as dilution solvent in order to minimise chromatographic interferences, and enhance the detection of compounds eluting in the beginning of the chromatogram (such as metformine and synephrine). These conditions were tested with all the substances of the study and shown suitable solubility (except for rimonabant). However some problems may exist with the sample matrix and an alternative solvent was used for some particular products (Fat Cut and Riomont). Examples of sample preparation are described hereafter:

- For Lida, Hyperdrive and Ephedrine tablets, one capsule content or one tablet was finely powdered and dispersed in 20 ml with a dilution solvent prepared by mixing 5 volumes of acetonitrile with 95 volumes of mobile phase A (phosphate 50 mM buffer solution) adjusted to pH 3.8,
- Fat Cut sample was prepared adding 10 ml of pure acetonitrile to 1 g of powder (the choice of acetonitrile was motivated because of the formation of a colloidal suspension when the previous dilution solvent (acetonitrile/phosphate 50 mM buffer solution, 5/95 v/v) was used,
- For Riomont, one tablet was finely powdered and dispersed in a mixture of solvents (ethanol, acetonitrile, water) according to the indications of the Market Authorization file of the reference medicine,

In all cases, the suspension obtained was then mechanically stirred during 15 minutes, sonicated 15 minutes and then centrifuged during 15 minutes at 3 500 r/min (5 minutes at 13500 r/min for Fat Cut). The clear supernatant was filtered through a 0.45 µm pore size GHP membrane filter (Pall-Gelman) discarding the first millilitre, and suitably diluted before analysis.

Methodology of analysis

A two step methodology was implemented for the 32 slimming products. The first step is dedicated to the screening for the detection of active substances. Screening sample solutions were injected and the presence of weight-loss substances was suspected on the basis of both retention time and UV spectrum compared with reference data obtained from standard working solution containing the 34 substances together. After this screening step, the confirmation of the identity of the detected substance and the assay were carried out. The detection wavelength was adjusted to the maximum of absorption for the assay of the analyte. With the diversity and complexity of matrices (medicines, herbal products, vitamins mixture, instant coffee powder…), the method of standard addition has been used for the confirmation step. A known quantity of the standard detected in the screening step was added directly on the sample powder before the addition of the dilution solvent, in order to obtain twice the estimated concentration in the screening step. The confirmation of the presence of the weight-loss compound was effective when the spiked peak stayed with a symmetrical shape and the area was proportional to the added quantity. The use of peak purity tests allows to ensure of the specificity of the method. The quantification was realized using the standard addition methodology, and the extraction recovery was evaluated calculating the recovery factor (RF) between spiked sample and standard solution.

Results and discussion

Validation criteria of the UHPLC method

The chromatographic method is able to screen 34 weight-loss compounds potentially present in slimming formulations, in less than 15 min. These compounds exhibit rather different physico chemical characteristics, and the difficulty of the method development was to be able to detect substances with distant polarities (from metformine to rimonabant). Figure 1 shows the separation of the 34 weight-loss compounds using optimized chromatographic parameters reported in Table 3.

The main difficulty in the detection and the quantification of adulterants in slimming products is that matrices are often very different. Since a conventional method validation according ICH guidelines could not be strictly performed, we have carried out elements of validation using the 34 standard compounds. For all individual compounds, standard working solution was used to determine resolution and symmetry according to the European Pharmacopoeia (Ph. Eur. 7th ed.). Two solvent peaks appeared in the profile of the blank injection without any interference with analysed substances in the working standard solution chromatogram. They were attributed to the phosphate buffer solution. It could be noted that a good resolution is obtained for most of the 34 peaks, although some critical pairs of peaks are not fully separated. Except for the racemic mixture ephedrine and pseudo-ephedrine, all other compounds could be easily identified on the basis of UV spectral data. Good asymmetry was observed for all peaks ($0.8 < As < 1.5$), except for metformine ($As = 2.1$) at the beginning of the chromatogram (Table 1).

After appropriate dilutions of standard working solution, limits of quantification were evaluated for each analyte at a signal-to-noise ratio (S/N) of 10 according to ICH recommendations (ICH, 2005). A linearity study was also performed for 12 analytes (amfepramone, caffeine, clenbuterol, ephedrine, fenfluramine, nicotinamide, phenolphtaleine, pseudoephedrine, rimonabant, sibutramine, synephrine and yohimbine) chosen for their hazardous nature, their occurrence in suspicious samples and their different partition coefficient (octanol/water) ranging from -0.4 (synephrine) to 30.9 (rimonabant). For each selected substance, standard calibration

curve in methanol was established ranging from the limit of quantification to the nominal concentration (around 12.5 µg/ml) or more. Limits of quantification of compounds ranged from 0.1 µg/ml to 5.0 µg/ml depending of the analysed compound. Those values are acceptable regarding active therapeutic concentrations. Moreover it has been demonstrated that the 12 selected substances had a linear response (r^2>0.999) on the studied concentration range (Table 1).

Application to samples

Results from the analysis of the 32 samples (Table 2) using the UHPLC method leads to several comments. Different batches of the same product do not contain the same ingredients: Lida with or without sibutramine, Metabodrene with or without yohimbine, Fat Cut with sibutramine or its derivative. Moreover, the amount of active substance is not always the same: Hyperdrive with 166 mg or 327 mg of caffeine per capsule. Caffeine is currently present in dietary supplement at amounts between 4 mg and 327 mg per unit. Several formulations contain a combination of 2 or 3 active substances. The simultaneous presence of some of these substances is particularly worrying when a sample contains several active substances for which the drug interaction is not known.

The description of the screening methodology has been focused on six of the 32 slimming products. Following the methodology proposed in the *Materials and methods* section, several peaks were detected in the chromatograms of samples (Figure 2) and were identified on the basis of retention times and UV spectra comparisons with standard data:

- Rimonabant was identified in Riomont (medicine designed as a white round tablet, manufactured in India, purchased over the internet and labelled with 20 mg of rimonabant),
- Sibutramine was identified in Lida #1, and synephrine and caffeine were identified in Lida #2. Lida #1 and Lida #2 (capsules presented as food supplement without any chemical compound declared in the composition) were two batches purchased over the internet on two different web sites,

- Clenbuterol was identified in Ephedrine tablet (medicine presented as white round scored tablet manufactured in China, purchased over the internet and labelled with 50 mg of ephedrine hydrochloride per tablet),
- Caffeine was identified in Hyperdrive (capsule presented as food supplement labelled with a mixture of vitamins and amino acids, purchased over the internet),
- Caffeine, phenolphthalein and an unknown peak were identified in Fat Cut (12 grams of instant coffee powder in a sachet manufactured in China and coming from a sampling by French health authorities).

Standard addition quantification was used for the confirmation and assay of all compounds. Recovery factors (Table 4) ranging from 90% to 111% were evaluated to be quite acceptable, and did not highlight matrix interference. Three independent assays of those substances were performed and RSD values were also considered quite acceptable ranging from 2.1% to 10.1 % suggesting a homogeneity problem of capsules.

Rimonabant was found in Riomont® at the strength of 19 mg per tablet. It is a regulated pharmaceutical substance that could be taken under medical survey of patients because of possible side effects such as depression and suicide. For those reasons, the European Medicines Agency has recommended the withdrawal of the marketing authorization of Acomplia® (rimonabant) in the European Union the 16 January 2009 (EMA, 2009).

Concerning Lida #1, the presence of sibutramine was confirmed at the strength of 30 mg per capsule which represents two times the amount of a single dose of Sibutral® 15 mg, authorized medicine on the French market until 2009. As rimonabant, sibutramine is a regulated pharmaceutical substance that should be taken under medical follow up because of possible side effects such as blood pressure increase, tachycardia or palpitations. For those reasons, the European Medicines Agency has recommended the suspension of marketing authorizations for sibutramine-containing medicines the 21 January 2010 (EMA, 2010).

The analysis of Lida #2 (same denomination, same packaging and different lot number) showed a chromatographic profile fully different from the Lida #1 chromatogram: absence of sibutramine and detection of synephrine (19 mg per capsule) in combination with caffeine (10 mg per capsule). Synephrine (or oxedrine), an adrenergic compound, is a stimulant more and more used since the ban of ephedrine in several countries in 2003 (AFSSAPS, 2003). It could be deduced for such set of results that the manufacturer do not proposed a single formulation on the market in order to pass through authorities' controls.

Using the screening methodology, ephedrine was not detected in Ephedrine tablets. A limit of quantification in the sample has been estimated at 1 mg per tablet, which represent 1/50 of the labelled dose. Instead of the labelled compound, clenbuterol, a β-agonist molecule, has been detected with an amount of 15 µg per tablet which represent a therapeutic amount for humans. Clenbuterol was used few years ago for veterinary indications (respiratory treatment in horses) and is now often used for weight-loss purposes or body-building activities. This compound belongs to the list of prohibited substances issued by the World Anti-Doping Agency (WADA, 2011).

The active substance found in Hyperdrive was caffeine with an amount of 327 mg per capsule. Considering usual caffeine contents of dietary supplements (Andrews et al. 2007) and advices from official agencies (Health Canada, 2010) recommending a maximal caffeine daily dose of 400 mg, the intake of 2 capsules once a day (as suggested on the sample label) may endanger consumers. Literature reports fatal issues due to caffeine intoxications (Kerrigan et al. 2005).

Fat Cut sample was characterized with the presence of both caffeine and phenolphthalein. It should be underlined that caffeine was not quantified because this sample being sold as an instant coffee preparation, it was an evidence to find this substance. Phenolphthalein, found at the strength of 60 mg per sachet, is a laxative drug forbidden for over-the-counter sales in US and in Europe (EMA, 1997) because of carcinogenicity concerns. An unknown compound was detected in this sample with UV spectra very similar to the one of sibutramine. A mass

spectrometry investigation allowed to identify this unknown compound as N,N-didesmethylsibutramine, a structural analogue of sibutramine. This kind of molecule is structurally close to the original, but its pharmacological properties (including adverse effects) have not been assessed. It could be believed that fraudulent manufacturers may adulterate their slimming products with analogues molecules instead of original ones in order to bypass regulatory agencies.

Conclusion

An UHPLC/DAD method was used and found to be adequate and highly suitable for the screening of 34 weight-loss compounds in complex matrices in less than 15 min. The use of a photodiode array detector allowed weight-loss compounds identification by comparison with reference data. It allows a quick detection and quantification of active substances among the most commonly used for slimming indication, and then to determine the composition of suspect samples in order to assess their hazardous character. The UHPLC/DAD method is simple, fast and selective for the determination of forbidden and harmful chemical compounds in slimming preparations. This method allows also the detection of active substances which, once they are fully characterized (using mass spectrometry for example), could lead to updates of the UHPLC screening method database. This was so far for example experienced with sulbutiamine and N,N-didesmethylsibutramine.

The analysis of 32 slimming formulations using the UHPLC/DAD method allowed the detection and the quantification of 9 hazardous active substances at a therapeutic content: caffeine, clenbuterol, nicotinamide, phenolphthalein, rimonabant, sibutramine, N,N-didesmethylsibutramine, synephrine and yohimbine. Most of them are regulated compounds because of side-effects or toxicological concerns. Those substances were found as single active substance or in combination, with added potential hazard considering that drug interaction and synergenic side effects are not known. Data also show that samples from different batches were of inconsistent formulation, with different active ingredients depending of the batch number.

Results of this study highlight the potential danger of slimming products available outside the pharmaceutical supply chain. Results also demonstrate the importance of analytical controls of slimming products for the safety of consumers, and the UHPLC/DAD method is very helpful for this purpose.

Acknowledgement

The authors want to thank Pr. M. Malet-Martino (Université Paul Sabatier, Toulouse, France) for the supply of samples and for helpful discussion.

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Table 1

Chromatographic criteria determined on weight-loss reference standards

N°	Compound	λ max (nm)	Retention time (min)	Resolution (maxplot)	Symmetry (maxplot)	Linearity range (µg/ml, n=5)	r²	LOQ (µg/ml)
1	Metformine	232	0.73	-	2.1	-	-	1.2
2	Synephrine	222-273	0.92	7.5	1.8	0.1 – 10.7	0.999	0.1
3	Nicotinamide	214-261	1.20	6.4	1.2	0.5 – 50.0	1.000	0.1
4	Phenylalanine	257	1.55	4.9	1.2	-	-	1.4
5	Salicine	211-267	2.79	21.9	1.2	-	-	0.1
6	Bergerin	216-272	2.97	5.2	1.3	-	-	0.1
7	Ephedrine	256	3.08	1.6	1.3	0.1 – 50.0	1.000	0.1
8	Pseudo-ephedrine	256	3.08	0	1.3	0.1 – 50.0	1.000	0.1
9	Caffeine	272	3.24	3.8	1.3	0.1 – 10.6	0.999	0.1
10	Amphetamine	257	3.37	3.4	1.3	-	-	1.6
11	Amfepramone	252	3.71	9.4	1.3	0.1 – 21.0	0.999	0.1
12	Phenformin	233	3.71	0	1.3	-	-	0.1
13	Phentermine	257	3.71	0	1.3	-	-	1.2
14	Triamterene	215-249-358	3.75	0.9	1.3	-	-	0.1
15	Clenbuterol	243-297	4.34	2.4	1.4	1.3 – 10.0	0.999	1.3
16	Yohimbine	220-271	4.65	6.8	1.3	0.2 – 10.2	0.999	0.2
17	2.4 Dinitrophenol	213-257-358	4.84	3.7	1.4	-	-	0.8
18	Phenobarbital	/	5.03	5.0	1.2	-	-	1.3
19	Fenfluramine	263	5.24	3.9	1.3	5.0 – 20.1	1.000	5.0
20	Furosemide	233-274-336	5.41	6.4	1.2	-	-	1.3
21	Liothyronine T3	224-296	5.62	4,8	1.2	-	-	1.3
22	Althiazide	226-271-314	5.92	5.5	1.1	-	-	1.3
23	Dantoin	265	5.96	0	1.2	-	-	1.2
24	Levothyroxine T4	223-301	6.11	2.9	1.2	-	-	1.3
25	Phenolphthalein	229-275	6.22	3.4	1.2	1.0 - 50.0	1.000	0.8
26	Fluoxetine	227-257	6.64	8.7	1.3	-	-	1.3
27	Sibutramine	223	6.88	5.9	1.4	0.3 – 21.6	0.999	0.3
28	Bumetanide	224-266-340	6.88	0	1.2	-	-	1.3
29	Spironolactone	239	7.57	2.9	1.1	-	-	0.1
30	Oxethazaine	258	7.94	4.0	1.2	-	-	1.3
31	Penfluridol	265	8.25	5.9	1.7	-	-	1.2
32	Phenothiazine	252-315	8.68	6.5	1.1	-	-	1.3
33	Usnic acid	232-282	10.32	12.2	1.3	-	-	0.1
34	Rimonabant	232-282	11.05	1.5	1.0	1.0 - 50.0	0.999	0.8

Table 2. Results of the screening method performed on different kind of samples. The underlined sample names correspond to the 6 examples described in the article.

	Caffeine (mg/unit)	Clenbuterol (µg/unit)	Nicotinamide (mg/unit)	Phenolphthalein (mg/unit)	Rimonabant (mg/unit)	Sibutramine (mg/unit)	Synephrine (mg/unit)	Yohimbine (µg/unit)	Other
Dietary supplement									
3x slimming power	-	-	-	-	-	6	-	-	-
Dyma burn xtrem	225	-	-	-	-	-	29	63	-
EA fit minceur	4	-	-	-	-	-	-	-	-
ECA Xtrem	211	-	28	-	-	-	25	-	-
Elan sil	-	-	21	-	-	-	-	-	-
Hyperdrive 3.0+ #1	327	-	-	-	-	-	-	-	Sulbutiamine = 65 mg/capsule
Hyperdrive 3.0+ #2	166	-	-	-	-	-	-	-	Sulbutiamine = 122 mg/capsule
Lida dai dai hua #1	-	-	-	-	-	30	-	-	-
Lida dai dai hua #2	10	-	-	-	-	-	19	-	-
Lida dai dai hua #3	-	-	-	-	-	33	-	-	-
Metabodrene 356 #1	47	-	-	-	-	-	27	-	-
Metabodrene 356 #2	47	-	-	-	-	-	24	900	-
Nojo	-	-	21	-	-	-	-	-	-
Ronaxil #1	145	-	-	-	-	-	-	-	-
Ronaxil #2	152	-	-	-	-	-	-	-	-
Royal slimming formula	-	-	-	-	-	9	-	-	-
Stack rush	55	-	-	-	-	-	-	-	-
Thermadrol	197	-	-	-	-	-	-	-	-
Zantrex-3 #1	310	-	-	-	-	-	-	-	-
Zantrex-3 #2	191	-	-	-	-	-	-	-	-
Coffee powder									
Café minceur	Presence	-	-	51	-	21	-	-	-
Coffee weight loss	Presence	-	-	-	-	19	-	-	-
Fat Cut #1	Presence	-	-	90	-	traces	-	-	N,N-Didesmethylsibutramine *
Fat Cut #2	Presence	-	-	49	-	18	-	-	-
Fat Cut #3	Presence	-	-	60	-	23	-	-	-
Medicine									
Acomplia® 20 mg	-	-	-	-	20	-	-	-	-
BP20	-	20	-	-	-	-	-	-	-
Clenbuterol tablet	-	15	-	-	-	-	-	-	-
Ephedrine tablet	-	15	-	-	-	-	-	-	-
Riomont® 20 mg	-	-	-	-	19	-	-	-	-
Reductil® 10 mg	-	-	-	-	-	10	-	-	-
Sibutral® 10 mg	-	-	-	-	-	9.3	-	-	-

*compound identified by mass spectrometry

Table 3. UHPLC chromatographic conditions

Column	Acquity BEH C18 1.7 µm, 100x2.1 mm		
Mobile phase A	Sodium dihydrogen phosphate dihydrate 50 mM pH 3.8 buffer		
Mobile phase B	Acetonitrile		
Gradient	Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
	0 - 1	95	5
	1 - 8	95 → 35	5 → 65
	8 - 13	35	65
	13 - 14	35 → 95	65 → 5
	14 - 15	95	5
Flow rate	0.35 ml/min		
UV detection	Maxplot		
Injection volume	1 µL		
Column temperature	30°C		
Sample temperature	6°C		
Run time	15 min		
Dilution solvent	Acetonitrile / mobile phase A (5 volumes / 95 volumes)		

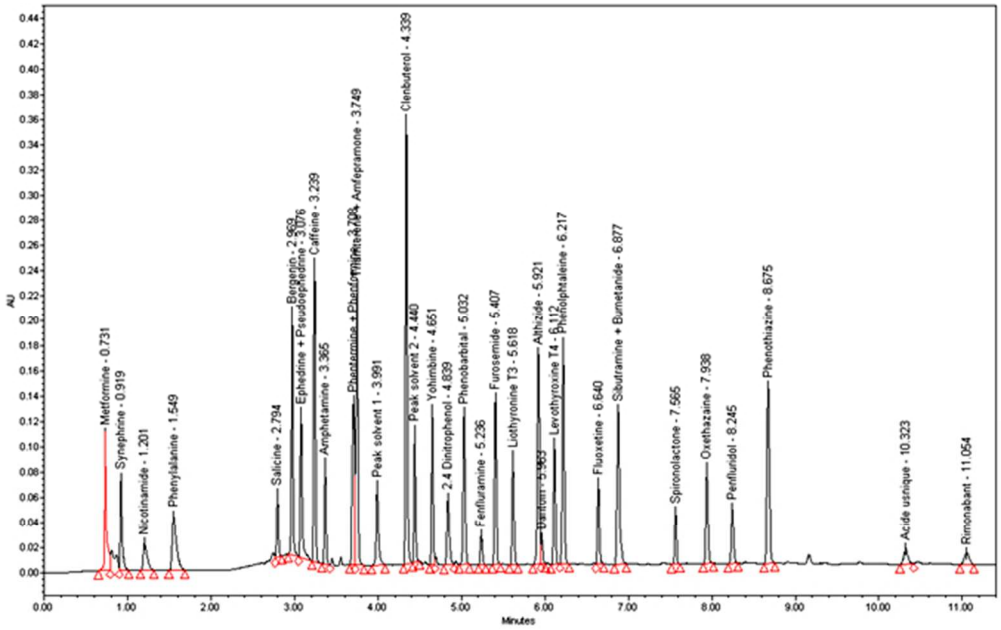
Table 4. Analytical results of assay of the 6 selected samples

Slimming products	Compounds	Extraction (n=6)		Assay (n=6)	
		Recovery (%)	RSD (%)	mg/unit	RSD (%)
Lida #1	sibutramine	110	4.2	30.1	5.0
Lida #2	caffeine	/	/	10	/
(*estimated values*)	synephrine	/	/	19	/
Hyperdrive	caffeine	90	7.8	327.0	10.1
Ephedrine tablet	ephedrine	/	/	ND	/
	clenbuterol	109	3.9	0.015	4.4
Fat Cut	phenolphthalein	111	4.1	89.8	5.1
Riomont	rimonabant	102	1.3	19.2	2.1

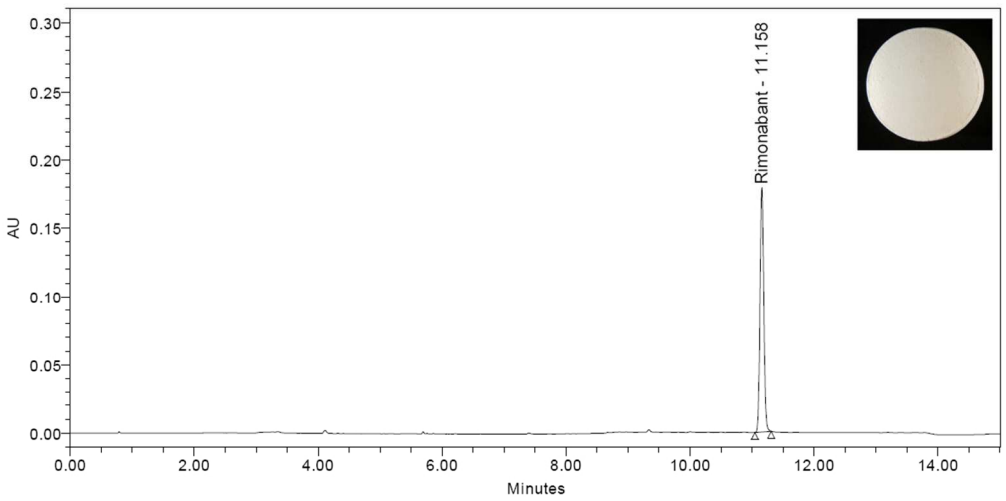
Captions for figures

Figure 1. UHPLC/DAD chromatogram of 34 weight-loss substances (under chromatographic conditions of Table 3)

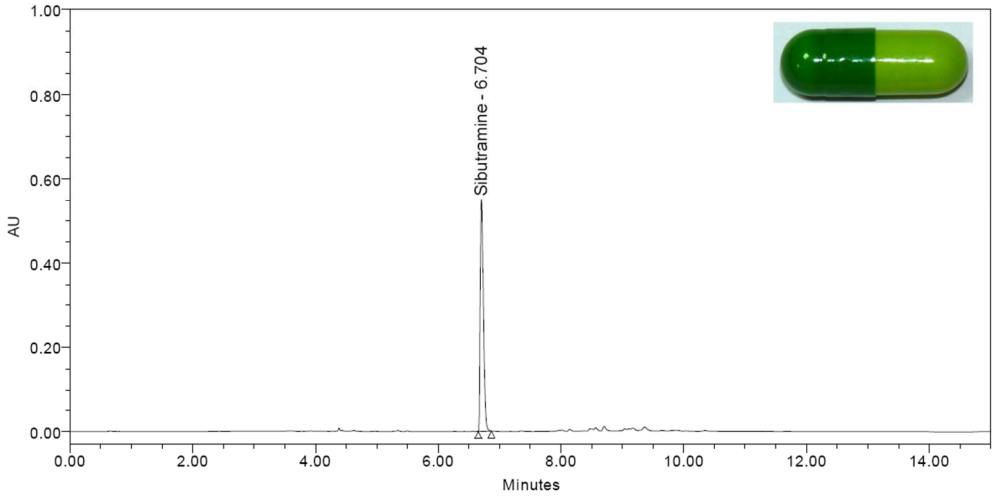
Figure 2. UHPLC-DAD chromatograms of screening sample solutions: (**2a**) Riomont at λ=246 nm, (**2b**) Lida #1 at λ=223 nm, (**2c**) Lida #2 at λ=223 nm, (**2d**) Hyperdrive at λ=272 nm, (**2e**) Ephedrine tablet at λ=243 nm and (**2f**) Fat Cut in maxplot mode



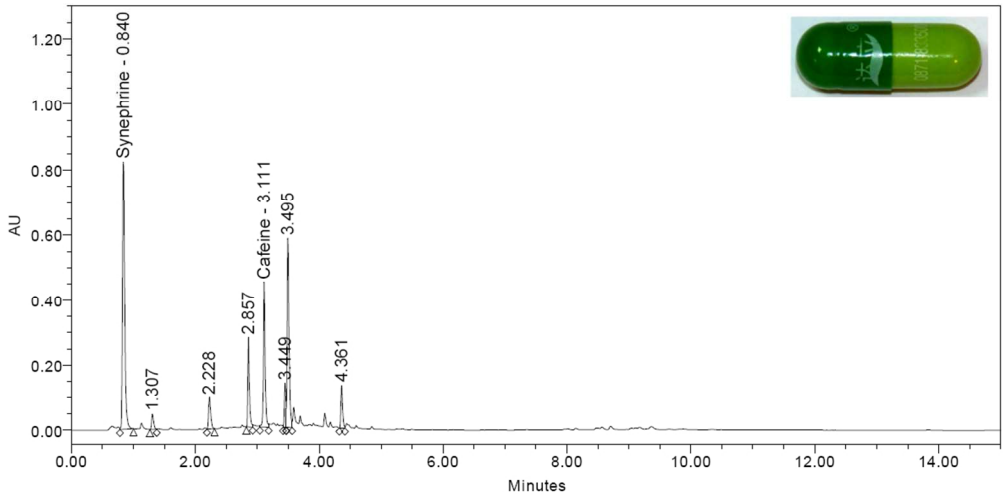
UHPLC/DAD chromatogram of 34 weight-loss substances (under chromatographic conditions of Table 3)
238x148mm (72 x 72 DPI)



416x205mm (72 x 72 DPI)



421x208mm (72 x 72 DPI)



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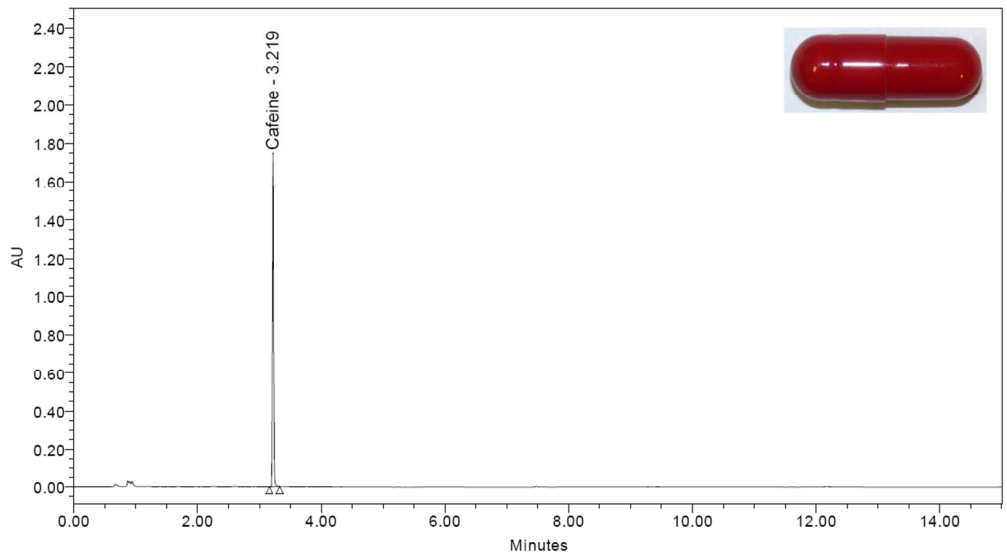

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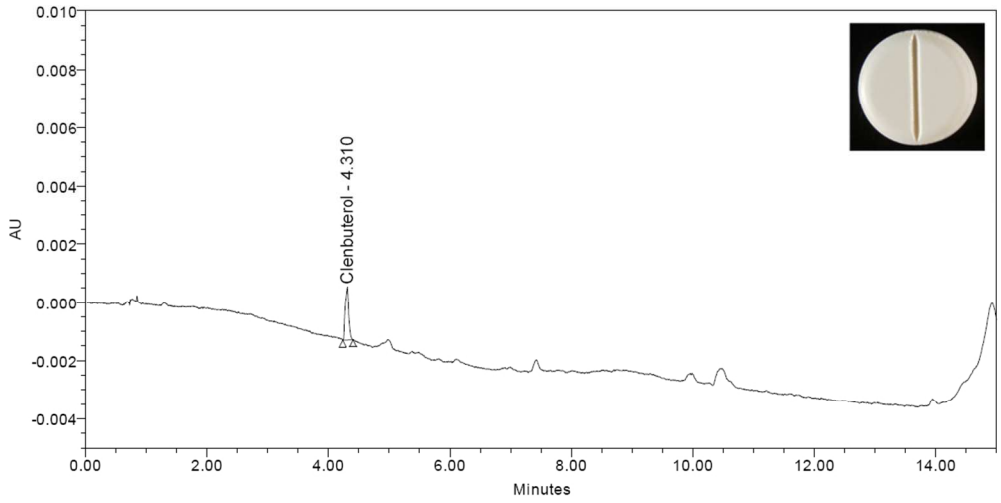
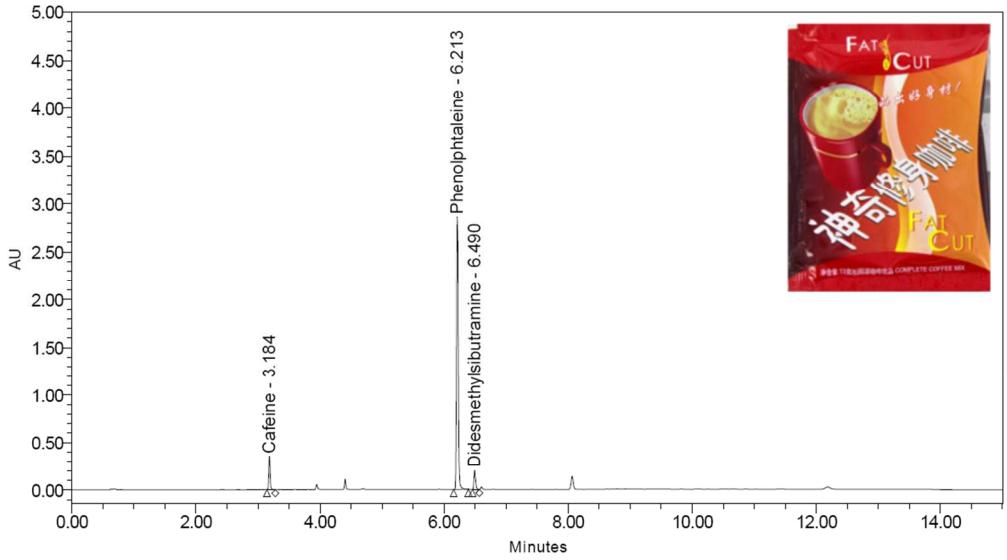


419x207mm (72 x 72 DPI)



416x229mm (72 x 72 DPI)