



**HAL**  
open science

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

Herve Rebiere, Pauline Guinot, Corinne Civade, Pierre Antoine Bonnet, Alain Nicolas

### ► To cite this version:

Herve Rebiere, Pauline Guinot, Corinne Civade, Pierre Antoine Bonnet, Alain Nicolas. Detection of hazardous weight-loss substances in adulterated slimming formulations using ultra-high-pressure liquid chromatography with diode-array detection. Food Additives and Contaminants, 2011, pp.1. 10.1080/19440049.2011.638676 . hal-00762899

**HAL Id: hal-00762899**

**<https://hal.archives-ouvertes.fr/hal-00762899>**

Submitted on 9 Dec 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-382.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	28-Oct-2011
Complete List of Authors:	REBIERE, HERVE; French Agency for the Safety of Health Products, AFSSAPS, Laboratories and Controls Directorate GUINOT, PAULINE; French Agency for the Safety of Health Products, AFSSAPS, Laboratories and Controls Directorate CIVADE, CORINNE; French Agency of the Safety of Health Products, AFSSAPS, Laboratories and Controls Directorate BONNET, PIERRE ANTOINE; French Agency of the Safety of Health Products, AFSSAPS, Laboratories and Controls Directorate NICOLAS, ALAIN; French Agency of the Safety of Health Products, AFSSAPS, Laboratories and Controls Directorate
Methods/Techniques:	HPLC
Additives/Contaminants:	Additives general, Caffeine
Food Types:	Dietary supplements, Nutritional supplements
Abstract:	The presence on the market of illegal products for slimming purposes or the treatment of obesity 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 endcapped 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 minutes run time. Thus, 32

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

	commercial slimming formulations were analysed using this method, allowing the detection and quantification of hazardous active substances: caffeine, clenbuterol, nicotinamide, phenolphthalein, rimonabant, sibutramine, N,N-didesmethylsibutramine, synephrine and yohimbine.

SCHOLARONE™  
Manuscripts

For Peer Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

4  
5 H. Rebiere\*, P. Guinot, C. Civade, P.-A. Bonnet, A. Nicolas

6  
7 The French Agency for the Safety of Health Products, AFSSAPS – Laboratories and Control  
8 Directorate – 635 rue de la Garenne – 34748 Vendargues cedex – France

9  
10 \* Corresponding author. Tel.: +33(0)467913900; fax: +33(0)467913983

11 *E-mail address:* [herve.rebiere@afssaps.sante.fr](mailto:herve.rebiere@afssaps.sante.fr)

12  
13 **Abstract**

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

29  
30  
31  
32

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

For Peer Review Only

## 33 Introduction

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

46 The aim of the presence of synthetic substances in slimming preparations is to increase efficacy  
47 in the treatment of obesity or weight-loss purposes. These adulterants are more and more  
48 present in slimming products on the worldwide marketplace, as related by other national health  
49 authorities (US Food and Drug Administration, National Institute for Public Health and the  
50 Environment of the Netherlands, Health Canada, Swiss Medic...): dietary supplements and  
51 herbal ingredients adulterated with potentially noxious chemical ingredients (Carvalho et al.  
52 2011; Jung et al. 2006) or counterfeit medicines (FDA, 2010),

54 After a risk analysis based on the study of warnings and reports from medicines agencies (FDA,  
55 2009; Venhuis et al. 2009), 34 weight-loss substances have been selected (Table 1) to be  
56 screened in suspect slimming formulations. They belong to different pharmacological  
57 categories: anorectics (sibutramine, rimonabant, fenfluramine, amfepramone, phentermine)  
58 used to reduce appetite, stimulants (amphetamine, ephedrine, metformine, synephrine,  
59 caffeine, yohimbine) used to induce temporary improvements in either mental or physical  
60 function, antidepressants (phenobarbital, fluoxetine, penfluridol) used to alleviate anxiety  
61 disorders, laxatives (phenolphthalein) used to raise intestinal transit, diuretics (bumetanide,

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

64  
65 Slimming formulations (medicines, dietary supplements and instant coffee powders) were  
66 collected from different sources (over the internet, inspectorate sampling or following  
67 pharmacovigilance alerts) to be tested using an in-house chromatographic method. For the  
68 screening of the selected substances, literature describes the use of conventional liquid or gas  
69 chromatography (LC-DAD, LC-MS, GC-MS) (Saka et al. 2008; Bogusz et al. 2006; Zou et al.  
70 2007), capillary electrophoresis (Cianchino et. 2008) and also NMR (Vaysse et al. 2010).  
71 However few methods have been developed for the simultaneous analysis of a large extent of  
72 compounds (Carvalho et al. 2011).

73  
74 This paper proposes a screening method designed to be simple and fast, using a modern  
75 system more and more available in control laboratories: fast chromatography with UV detection.  
76 Ultra High Pressure Liquid Chromatography (UHPLC) is based on the use of columns with small  
77 diameter (2.1 mm) packed with sub-2  $\mu\text{m}$  particles. Compared with conventional HPLC, UHPLC  
78 provides significant advantages concerning peak capacity, selectivity, resolution and run time.  
79 These good separation efficiencies are particularly appreciated for multi-analytes screening  
80 (Klose et al. 2010; Wang et al. 2008; Murray et al. 2009; Badoud et al. 2009). Working up to  
81 1000 bar, close to the optimal flow-rate, 100 mm columns offer high peak capacity. Moreover,  
82 run time and solvent consumption are drastically reduced. Unfortunately, no UHPLC method  
83 was dedicated to the screening of weight-loss substances. Compared with existing HPLC  
84 methods, this new UHPLC method is fast, simple and selective for the detection of adulterants  
85 in slimming formulations. This article reports the results of the analysis of 32 slimming  
86 formulations using the UHPLC screening method. The description of the screening methodology  
87 has been focused on six of them, and the presence of hazardous weight-loss substances is  
88 finally discussed.

89

**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.

107

*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  $\mu$ m, 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  $\mu$ l. The detection was set in "maxplot" mode between 210 and 400 nm. UHPLC conditions were similar for screening, confirmatory step and quantification.



119

120 *Preparation of solutions*

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

125

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

134 - For Lida, Hyperdrive and Ephedrine tablets, one capsule content or one tablet was finely  
135 powdered and dispersed in 20 ml with a dilution solvent prepared by mixing 5 volumes of  
136 acetonitrile with 95 volumes of mobile phase A (phosphate 50 mM buffer solution) adjusted  
137 to pH 3.8,

138 - Fat Cut sample was prepared adding 10 ml of pure acetonitrile to 1 g of powder (the choice  
139 of acetonitrile was motivated because of the formation of a colloidal suspension when the  
140 previous dilution solvent (acetonitrile/phosphate 50 mM buffer solution, 5/95 v/v) was used,

141 - For Riomont, one tablet was finely powdered and dispersed in a mixture of solvents  
142 (ethanol, acetonitrile, water) according to the indications of the Market Authorization file of  
143 the reference medicine,

144 In all cases, the suspension obtained was then mechanically stirred during 15 minutes,  
145 sonicated 15 minutes and then centrifuged during 15 minutes at 3 500 r/min (5 minutes at  
146 13500 r/min for Fat Cut). The clear supernatant was filtered through a 0.45 µm pore size GHP  
147 membrane filter (Pall-Gelman) discarding the first millilitre, and suitably diluted before analysis.

1  
2 1483  
4 149 *Methodology of analysis*

5  
6 150 A two step methodology was implemented for the 32 slimming products. The first step is  
7  
8 151 dedicated to the screening for the detection of active substances. Screening sample solutions  
9  
10 152 were injected and the presence of weight-loss substances was suspected on the basis of both  
11  
12 153 retention time and UV spectrum compared with reference data obtained from standard working  
13  
14 154 solution containing the 34 substances together. After this screening step, the confirmation of the  
15  
16 155 identity of the detected substance and the assay were carried out. The detection wavelength  
17  
18 156 was adjusted to the maximum of absorption for the assay of the analyte. With the diversity and  
19  
20 157 complexity of matrices (medicines, herbal products, vitamins mixture, instant coffee powder...),  
21  
22 158 the method of standard addition has been used for the confirmation step. A known quantity of  
23  
24 159 the standard detected in the screening step was added directly on the sample powder before  
25  
26 160 the addition of the dilution solvent, in order to obtain twice the estimated concentration in the  
27  
28 161 screening step. The confirmation of the presence of the weight-loss compound was effective  
29  
30 162 when the spiked peak stayed with a symmetrical shape and the area was proportional to the  
31  
32 163 added quantity. The use of peak purity tests allows to ensure of the specificity of the method.  
33  
34 164 The quantification was realized using the standard addition methodology, and the extraction  
35  
36 165 recovery was evaluated calculating the recovery factor (RF) between spiked sample and  
37  
38 166 standard solution.

39  
40 16741  
42 168  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 169 **Results and discussion**

3  
4 170 *Validation criteria of the UHPLC method*

5  
6 171 The chromatographic method is able to screen 34 weight-loss compounds potentially present in  
7  
8 172 slimming formulations, in less than 15 min. These compounds exhibit rather different physico  
9  
10 173 chemical characteristics, and the difficulty of the method development was to be able to detect  
11  
12 174 substances with distant polarities (from metformine to rimonabant). Figure 1 shows the  
13  
14 175 separation of the 34 weight-loss compounds using optimized chromatographic parameters  
15  
16 176 reported in Table 3.

17  
18 177

19  
20 178 The main difficulty in the detection and the quantification of adulterants in slimming products is  
21  
22 179 that matrices are often very different. Since a conventional method validation according ICH  
23  
24 180 guidelines could not be strictly performed, we have carried out elements of validation using the  
25  
26 181 34 standard compounds. For all individual compounds, standard working solution was used to  
27  
28 182 determine resolution and symmetry according to the European Pharmacopoeia (Ph. Eur. 7<sup>th</sup>  
29  
30 183 ed.). Two solvent peaks appeared in the profile of the blank injection without any interference  
31  
32 184 with analysed substances in the working standard solution chromatogram. They were attributed  
33  
34 185 to the phosphate buffer solution. It could be noted that a good resolution is obtained for most of  
35  
36 186 the 34 peaks, although some critical pairs of peaks are not fully separated. Except for the  
37  
38 187 racemic mixture ephedrine and pseudo-ephedrine, all other compounds could be easily  
39  
40 188 identified on the basis of UV spectral data. Good asymmetry was observed for all peaks ( $0.8 <$   
41  
42 189  $A_s < 1.5$ ), except for metformine ( $A_s = 2.1$ ) at the beginning of the chromatogram (Table 1).

43  
44 190

45  
46 191 After appropriate dilutions of standard working solution, limits of quantification were evaluated  
47  
48 192 for each analyte at a signal-to-noise ratio (S/N) of 10 according to ICH recommendations (ICH,  
49  
50 193 2005). A linearity study was also performed for 12 analytes (amfepramone, caffeine,  
51  
52 194 clenbuterol, ephedrine, fenfluramine, nicotinamide, phenolphthaleine, pseudoephedrine,  
53  
54 195 rimonabant, sibutramine, synephrine and yohimbine) chosen for their hazardous nature, their  
55  
56 196 occurrence in suspicious samples and their different partition coefficient (octanol/water) ranging  
57  
58 197 from -0.4 (synephrine) to 30.9 (rimonabant). For each selected substance, standard calibration  
59  
60

1  
2 198 curve in methanol was established ranging from the limit of quantification to the nominal  
3  
4 199 concentration (around 12.5 µg/ml) or more. Limits of quantification of compounds ranged from  
5  
6 200 0.1 µg/ml to 5.0 µg/ml depending of the analysed compound. Those values are acceptable  
7  
8 201 regarding active therapeutic concentrations. Moreover it has been demonstrated that the 12  
9  
10 202 selected substances had a linear response ( $r^2 > 0.999$ ) on the studied concentration range (Table  
11  
12 203 1).

#### 14 204 *Application to samples*

15  
16 205 Results from the analysis of the 32 samples (Table 2) using the UHPLC method leads to several  
17  
18 206 comments. Different batches of the same product do not contain the same ingredients: Lida with  
19  
20 207 or without sibutramine, Metabodrene with or without yohimbine, Fat Cut with sibutramine or its  
21  
22 208 derivative. Moreover, the amount of active substance is not always the same: Hyperdrive with  
23  
24 209 166 mg or 327 mg of caffeine per capsule. Caffeine is currently present in dietary supplement at  
25  
26 210 amounts between 4 mg and 327 mg per unit. Several formulations contain a combination of 2 or  
27  
28 211 3 active substances. The simultaneous presence of some of these substances is particularly  
29  
30 212 worrying when a sample contains several active substances for which the drug interaction is not  
31  
32 213 known.

33  
34 214  
35  
36 215 The description of the screening methodology has been focused on six of the 32 slimming  
37  
38 216 products. Following the methodology proposed in the *Materials and methods* section, several  
39  
40 217 peaks were detected in the chromatograms of samples (Figure 2) and were identified on the  
41  
42 218 basis of retention times and UV spectra comparisons with standard data:

- 44 219 - Rimonabant was identified in Riomont (medicine designed as a white round tablet,  
45  
46 220 manufactured in India, purchased over the internet and labelled with 20 mg of rimonabant),
- 47  
48 221 - Sibutramine was identified in Lida #1, and synephrine and caffeine were identified in Lida  
49  
50 222 #2. Lida #1 and Lida #2 (capsules presented as food supplement without any chemical  
51  
52 223 compound declared in the composition) were two batches purchased over the internet on  
53  
54 224 two different web sites,

1  
2 225 - Clenbuterol was identified in Ephedrine tablet (medicine presented as white round scored  
3  
4 226 tablet manufactured in China, purchased over the internet and labelled with 50 mg of  
5  
6 227 ephedrine hydrochloride per tablet),  
7  
8 228 - Caffeine was identified in Hyperdrive (capsule presented as food supplement labelled with a  
9  
10 229 mixture of vitamins and amino acids, purchased over the internet),  
11  
12 230 - Caffeine, phenolphthalein and an unknown peak were identified in Fat Cut (12 grams of  
13  
14 231 instant coffee powder in a sachet manufactured in China and coming from a sampling by  
15  
16 232 French health authorities).  
17  
18 233  
19  
20 234 Standard addition quantification was used for the confirmation and assay of all compounds.  
21  
22 235 Recovery factors (Table 4) ranging from 90% to 111% were evaluated to be quite acceptable,  
23  
24 236 and did not highlight matrix interference. Three independent assays of those substances were  
25  
26 237 performed and RSD values were also considered quite acceptable ranging from 2.1% to 10.1 %  
27  
28 238 suggesting a homogeneity problem of capsules.  
29  
30 239  
31  
32 240 Rimonabant was found in Riomont® at the strength of 19 mg per tablet. It is a regulated  
33  
34 241 pharmaceutical substance that could be taken under medical survey of patients because of  
35  
36 242 possible side effects such as depression and suicide. For those reasons, the European  
37  
38 243 Medicines Agency has recommended the withdrawal of the marketing authorization of  
39  
40 244 Acomplia® (rimonabant) in the European Union the 16 January 2009 (EMA, 2009).  
41  
42 245  
43  
44 246 Concerning Lida #1, the presence of sibutramine was confirmed at the strength of 30 mg per  
45  
46 247 capsule which represents two times the amount of a single dose of Sibutral® 15 mg, authorized  
47  
48 248 medicine on the French market until 2009. As rimonabant, sibutramine is a regulated  
49  
50 249 pharmaceutical substance that should be taken under medical follow up because of possible  
51  
52 250 side effects such as blood pressure increase, tachycardia or palpitations. For those reasons, the  
53  
54 251 European Medicines Agency has recommended the suspension of marketing authorizations for  
55  
56 252 sibutramine-containing medicines the 21 January 2010 (EMA, 2010).  
57  
58 253  
59  
60

1  
2 254 The analysis of Lida #2 (same denomination, same packaging and different lot number) showed  
3  
4 255 a chromatographic profile fully different from the Lida #1 chromatogram: absence of sibutramine  
5  
6 256 and detection of synephrine (19 mg per capsule) in combination with caffeine (10 mg per  
7  
8 257 capsule). Synephrine (or oxedrine), an adrenergic compound, is a stimulant more and more  
9  
10 258 used since the ban of ephedrine in several countries in 2003 (AFSSAPS, 2003). It could be  
11  
12 259 deduced for such set of results that the manufacturer do not proposed a single formulation on  
13  
14 260 the market in order to pass through authorities' controls.

15  
16 261

17  
18 262 Using the screening methodology, ephedrine was not detected in Ephedrine tablets. A limit of  
19  
20 263 quantification in the sample has been estimated at 1 mg per tablet, which represent 1/50 of the  
21  
22 264 labelled dose. Instead of the labelled compound, clenbuterol, a  $\beta$ -agonist molecule, has been  
23  
24 265 detected with an amount of 15  $\mu$ g per tablet which represent a therapeutic amount for humans.  
25  
26 266 Clenbuterol was used few years ago for veterinary indications (respiratory treatment in horses)  
27  
28 267 and is now often used for weight-loss purposes or body-building activities. This compound  
29  
30 268 belongs to the list of prohibited substances issued by the World Anti-Doping Agency (WADA,  
31  
32 269 2011).

33  
34 270

35  
36 271 The active substance found in Hyperdrive was caffeine with an amount of 327 mg per capsule.  
37  
38 272 Considering usual caffeine contents of dietary supplements (Andrews et al. 2007) and advices  
39  
40 273 from official agencies (Health Canada, 2010) recommending a maximal caffeine daily dose of  
41  
42 274 400 mg, the intake of 2 capsules once a day (as suggested on the sample label) may endanger  
43  
44 275 consumers. Literature reports fatal issues due to caffeine intoxications (Kerrigan et al. 2005).

45  
46 276

47  
48 277 Fat Cut sample was characterized with the presence of both caffeine and phenolphthalein. It  
49  
50 278 should be underlined that caffeine was not quantified because this sample being sold as an  
51  
52 279 instant coffee preparation, it was an evidence to find this substance. Phenolphthalein, found at  
53  
54 280 the strength of 60 mg per sachet, is a laxative drug forbidden for over-the-counter sales in US  
55  
56 281 and in Europe (EMA, 1997) because of carcinogenicity concerns. An unknown compound was  
57  
58 282 detected in this sample with UV spectra very similar to the one of sibutramine. A mass  
59  
60

1  
2 283 spectrometry investigation allowed to identify this unknown compound as N,N-  
3  
4 284 didesmethylsibutramine, a structural analogue of sibutramine. This kind of molecule is  
5  
6 285 structurally close to the original, but its pharmacological properties (including adverse effects)  
7  
8 286 have not been assessed. It could be believed that fraudulent manufacturers may adulterate their  
9  
10 287 slimming products with analogues molecules instead of original ones in order to bypass  
11  
12 288 regulatory agencies.

13  
14 289

## 15 16 290 **Conclusion**

17  
18 291 An UHPLC/DAD method was used and found to be adequate and highly suitable for the  
19  
20 292 screening of 34 weight-loss compounds in complex matrices in less than 15 min. The use of a  
21  
22 293 photodiode array detector allowed weight-loss compounds identification by comparison with  
23  
24 294 reference data. It allows a quick detection and quantification of active substances among the  
25  
26 295 most commonly used for slimming indication, and then to determine the composition of suspect  
27  
28 296 samples in order to assess their hazardous character. The UHPLC/DAD method is simple, fast  
29  
30 297 and selective for the determination of forbidden and harmful chemical compounds in slimming  
31  
32 298 preparations. This method allows also the detection of active substances which, once they are  
33  
34 299 fully characterized (using mass spectrometry for example), could lead to updates of the UHPLC  
35  
36 300 screening method database. This was so far for example experienced with sulbutiamine and  
37  
38 301 N,N-didesmethylsibutramine.

39  
40 302

41  
42 303 The analysis of 32 slimming formulations using the UHPLC/DAD method allowed the detection  
43  
44 304 and the quantification of 9 hazardous active substances at a therapeutic content: caffeine,  
45  
46 305 clenbuterol, nicotinamide, phenolphthalein, rimonabant, sibutramine, N,N-  
47  
48 306 didesmethylsibutramine, synephrine and yohimbine. Most of them are regulated compounds  
49  
50 307 because of side-effects or toxicological concerns. Those substances were found as single  
51  
52 308 active substance or in combination, with added potential hazard considering that drug  
53  
54 309 interaction and synergistic side effects are not known. Data also show that samples from  
55  
56 310 different batches were of inconsistent formulation, with different active ingredients depending of  
57  
58 311 the batch number.

1  
2 312

3  
4 313 Results of this study highlight the potential danger of slimming products available outside the  
5  
6 314 pharmaceutical supply chain. Results also demonstrate the importance of analytical controls of  
7  
8 315 slimming products for the safety of consumers, and the UHPLC/DAD method is very helpful for  
9  
10 316 this purpose.

11 317

12  
13  
14 318 **Acknowledgement**

15  
16 319 The authors want to thank Pr. M. Malet-Martino (Université Paul Sabatier, Toulouse, France) for  
17  
18 320 the supply of samples and for helpful discussion.

19  
20 321  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



322 **References**

- 323 Andrews KW, Schweitzer A, Zhao C, Holden JM, Roseland JM, Brandt M, Dwyer JT, Picciano  
324 MF, Saldanha LG, Fisher KD, Yetley E, Betz JM, Douglass L, 2007, The caffeine contents of  
325 dietary supplements commonly purchased in the US : analysis of 53 products with caffeine-  
326 containing ingredients, *Anal. Bioanal. Chem* 389:231-239  
327
- 328 AFSSAPS, press release. November 2008. Mise en garde sur les gélules « Best life ». Available  
329 from: [http://www.afssaps.fr/Infos-de-securite/Communiqués-de-presse/Mise-en-garde-sur-les-](http://www.afssaps.fr/Infos-de-securite/Communiqués-de-presse/Mise-en-garde-sur-les-gelules-Best-life)  
330 [gelules-Best-life](http://www.afssaps.fr/Infos-de-securite/Communiqués-de-presse/Mise-en-garde-sur-les-gelules-Best-life).  
331
- 332 AFSSAPS press release. December 2003. Available from: [http://www.afssaps.fr/Infos-de-](http://www.afssaps.fr/Infos-de-securite/Communiqués-Points-presse/EPHEDRA-MA-HUANG-et-EPHEDRINE-decision-du-8-octobre-2003)  
333 [securite/Communiqués-Points-presse/EPHEDRA-MA-HUANG-et-EPHEDRINE-decision-du-8-](http://www.afssaps.fr/Infos-de-securite/Communiqués-Points-presse/EPHEDRA-MA-HUANG-et-EPHEDRINE-decision-du-8-octobre-2003)  
334 [octobre-2003](http://www.afssaps.fr/Infos-de-securite/Communiqués-Points-presse/EPHEDRA-MA-HUANG-et-EPHEDRINE-decision-du-8-octobre-2003).  
335
- 336 Badoud F, Grata E, Perrenoud L, Avois L, Saugy M, Rudaz S, Veuthey JL, 2009, Fast analysis  
337 of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight  
338 mass spectrometry – I. Screening analysis, *J. Chromatogr. A* 1216:4423-4433.  
339
- 340 Biesemeier CK, Cummings SM, 2008, Ethics Opinion: Weight Loss Products and Medications,  
341 *J. Am. Diet. Assoc.* 108: 2109-2113.  
342
- 343 Bogusz MJ, Hassan H, Al-Enazi E, Ibrahim Z, Al-Tufail M, 2006, Application of LC–ESI–MS–MS  
344 for detection of synthetic adulterants in herbal remedies, *J. Pharm. Biomed. Anal.* 41:554-564.  
345
- 346 Cianchino V, Acosta G, Ortega C, Martinez LD, Gomez MR, 2008, Analysis of potential  
347 adulteration in herbal medicines and dietary supplements for the weight control by capillary  
348 electrophoresis, *Food Chem.* 108:1075-1081  
349
- 350 De Carvalho LM, M. Martini M, Moreira APL, De Lima APS, Correia D, Falcao T, Garcia SC, De  
351 Bairros AV, Do Nascimento PS, Bohrer D, 2011, Presence of synthetic pharmaceuticals and  
352 adulterants in slimming phytotherapeutic formulations and their analytical determination,  
353 *Forensic Sci. Int.* 204:6-12.  
354
- 355 European Medicine Agency. December 1997. Position paper on the genotoxic and carcinogenic  
356 potential of phenolphthalein. Available from:  
357 [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Position\\_statement/2009/09/WC5000](http://www.ema.europa.eu/docs/en_GB/document_library/Position_statement/2009/09/WC500003148.pdf)  
358 [03148.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Position_statement/2009/09/WC500003148.pdf)  
359  
360

- 1  
2 360 European Medicine Agency press release. January 2010. European Medicines Agency  
3 361 recommends suspension of marketing authorization for sibutramine. Available from:  
4 362 [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Press\\_release/2010/01/WC50006999](http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/01/WC50006999)  
5 363 [5.pdf](#).  
6 364  
7  
8 365 European Medicine Agency. January 2009. Acomplia - Withdrawal of the marketing  
9 366 authorization in the European Union. Available from:  
10 367 [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Public\\_statement/2009/11/WC50001](http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2009/11/WC50001)  
11 368 [2189.pdf](#)  
12 369  
13  
14 370 European Directorate for the Quality of Medicines and Healthcare. European Pharmacopoeia 7<sup>th</sup>  
15 371 edition, 2011.  
16 372  
17 373 FDA news release. Marsh 2009. FDA uncovers additional tainted weight loss products.  
18 374 Available from:  
19 375 <http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm149547.htm>.  
20 376  
21 377 FDA warning. January 2010. Counterfeit ALLI. Available from:  
22 378 <http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm198557.htm>.  
23 379  
24 380 Health Canada. March 2010. It's your health: Caffeine. Available from: [http://www.hc-](http://www.hc-sc.gc.ca/hl-vs/alt_formats/pdf/iyh-vsv/food-aliment/caffeine-eng.pdf)  
25 381 [sc.gc.ca/hl-vs/alt\\_formats/pdf/iyh-vsv/food-aliment/caffeine-eng.pdf](http://www.hc-sc.gc.ca/hl-vs/alt_formats/pdf/iyh-vsv/food-aliment/caffeine-eng.pdf)  
26 382  
27 383 International Conference on Harmonization of Technical Requirements for Registration of  
28 384 Pharmaceuticals for Human Use Q2(R1). 2005. Validation of Analytical Procedures: Text and  
29 385 Methodology.  
30 386  
31 387 Jung J, Hermanns-Clausen M, Weinmann W, 2006, Anorectic sibutramine detected in a  
32 388 Chinese herbal drug for weight loss, Forensic Sci. Int. 161:221-222.  
33 389  
34 390 Kerrigan S, Lindsey T, 2005, Fatal caffeine overdose: two case report, Forensic Sci. Int.  
35 391 1153:67-69.  
36 392  
37 393 Klose Nielsen MK, Johansen SS, Weihe Dalsgaard P, Linnet K, 2010, Simultaneous screening  
38 394 and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC–TOF–  
39 395 MS, Forensic Sci. Int. 196:85-92.  
40 396  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 397 Murray GJ, Danaceau JP, 2009, Simultaneous extraction and screening of diuretics, beta-  
3 398 blockers, selected stimulants and steroids in human urine by HPLC-MS/MS and UPLC-MS/MS,  
4 399 J. Chromatogr. B 877:3857-3864.  
5  
6 400  
7  
8 401 Saka K, Konuma K, Asai S, Unuma K, Nakajima M, Yoshida K, 2008, Identification of active  
9 402 ingredient in dietary supplements using non-destructive mass spectrometry and liquid  
10 403 chromatography-mass spectrometry, Forensic Sci. Int. 191:e5-e10.  
11 404  
12  
13 405 Tang MHY, Chen SPL, Ng SW, Chan AYW, Mak TWL, 2011, Case series on a diversity of illicit  
14 406 weight-reducing agents: from well known to the unexpected, Br. J. Clin. Pharmacol. 71:250-253.  
15  
16 407  
17  
18 408 Vaysse J, Balayssac S, Gilard V, Desoubzdanne D, Malet-Martino M, Martino R, 2010, Analysis  
19 409 of adulterated herbal drugs marketed for weight loss by DOZY <sup>1</sup>H NMR, Food Addit. Contam.  
20 410 27:903-916  
21  
22 411  
23  
24 412 Venhuis BJ, Zwaagstra ME, Van Der Berg JDJ, Wagenaar HWG, Van Riel AJHP, Barends DM,  
25 413 De Kaste D, 2009, Trends in drug substances detected in illegal weight-loss medicine and  
26 414 dietary supplements – A 2002-2007 survey and health risk analysis, National Institute for Public  
27 415 Health and the Environment, RIVM Report 370030002/2009. Available from:  
28 416 <http://www.rivm.nl/bibliotheek/rapporten/370030002.pdf>.  
29  
30 417  
31  
32 418 Wang J, Chen B, Yao S, 2008, Analysis of six synthetic adulterants in herbal weight-reducing  
33 419 dietary supplements by LC electrospray ionization-MS, Food Additives and Contaminants, 25:  
34 420 822-830.  
35  
36 421  
37  
38 422 World Anti-Doping Agency, January 2011. The 2011 prohibited list. Available from:  
39 423 [http://www.wada-ama.org/Documents/World\\_Anti-Doping\\_Program/WADP-Prohibited-  
41 425 list/To\\_be\\_effective/WADA\\_Prohibited\\_List\\_2011\\_EN.pdf](http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-<br/>40 424 list/To_be_effective/WADA_Prohibited_List_2011_EN.pdf)  
42  
43 426 Zou P, Sze-Yin Oh S, Kiang KH, Low MY, Chen B, 2007, Detection of sibutramine, its two  
44 427 metabolites and one analogue in a herbal product for weight loss by liquid chromatography  
45 428 triple quadrupole mass spectrometry and time-of-flight mass spectrometry, Rapid Commun.  
46 429 Mass Spectrom. 21: 614–618.  
47  
48 430  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Table 1

Chromatographic criteria determined on weight-loss reference standards

N°	Compound	$\lambda$ max	Retention time	Resolution	Symmetry	Linearity range		LOQ
		(nm)	(min)	(maxplot)	(maxplot)	( $\mu\text{g/ml}$ , n=5)	$r^2$	( $\mu\text{g/ml}$ )
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	Spirolactone	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
<b>Dietary supplement</b>									
3x slimming power	-	-	-	-	-	6	-	-	-
Dyma burn xtrem	225	-	-	-	-	-	29	63	-
EA fit minceur	4	-	-	-	-	-	-	-	-
ECA Xtrem	211	-	28	-	-	-	25	-	-
Elan sil	-	-	21	-	-	-	-	-	-
<u>Hyperdrive 3.0+ #1</u>	327	-	-	-	-	-	-	-	Sulbutiamine = 65 mg/capsule
Hyperdrive 3.0+ #2	166	-	-	-	-	-	-	-	Sulbutiamine = 122 mg/capsule
<u>Lida dai dai hua #1</u>	-	-	-	-	-	30	-	-	-
<u>Lida dai dai hua #2</u>	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	-	-	-	-	-	-	-	-
<b>Coffee powder</b>									
Café minceur	Presence	-	-	51	-	21	-	-	-
Coffee weight loss	Presence	-	-	-	-	19	-	-	-
<u>Fat Cut #1</u>	Presence	-	-	90	-	traces	-	-	N,N-Didesmethylsibutramine *
Fat Cut #2	Presence	-	-	49	-	18	-	-	-
Fat Cut #3	Presence	-	-	60	-	23	-	-	-
<b>Medicine</b>									
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 $\mu$ m, 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 $\rightarrow$ 35	5 $\rightarrow$ 65
	8 - 13	35	65
	13 - 14	35 $\rightarrow$ 95	65 $\rightarrow$ 5
	14 - 15	95	5
Flow rate	0.35 ml/min		
UV detection	Maxplot		
Injection volume	1 $\mu$ L		
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	/
( <i>estimated values</i> )	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

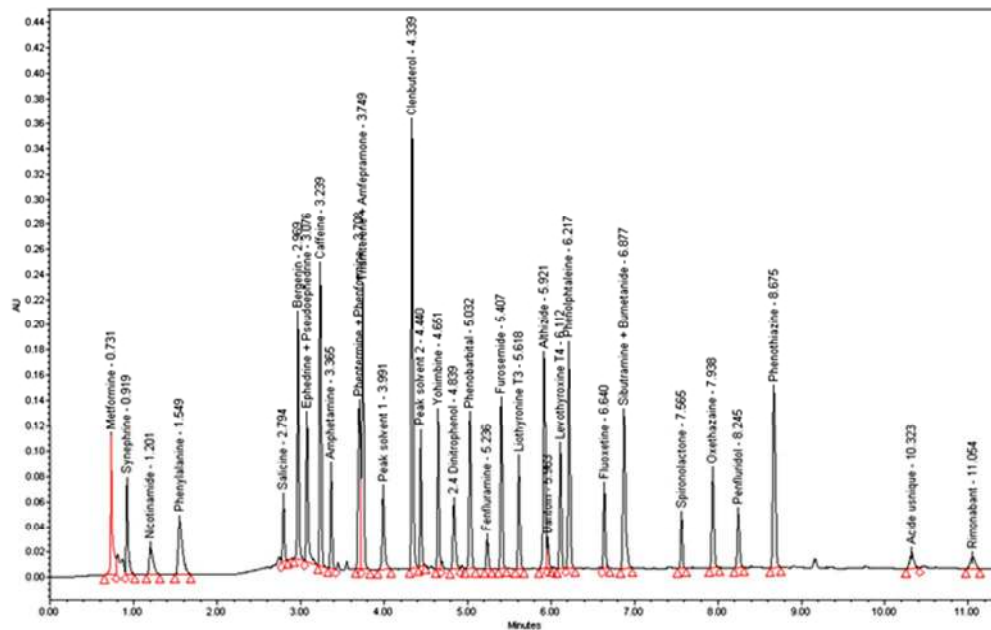
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Captions for figures**

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

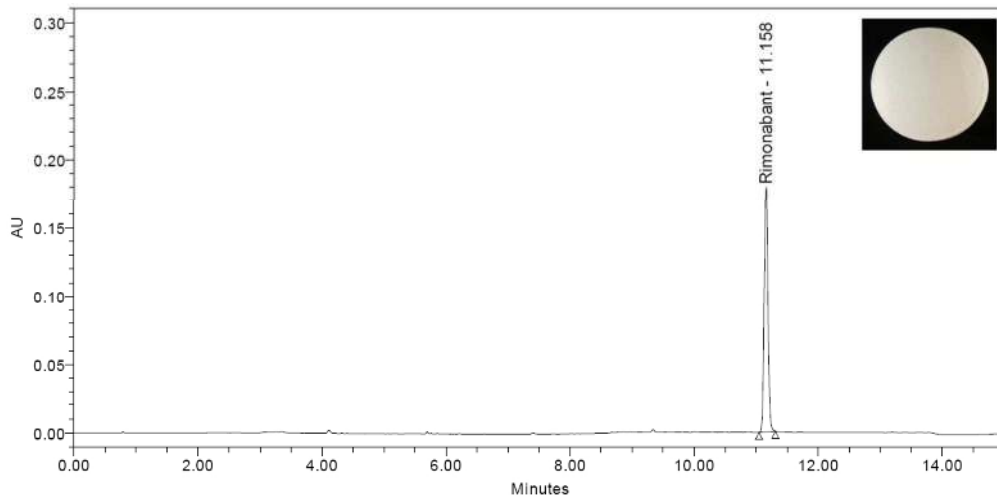
**Figure 2.** UHPLC-DAD chromatograms of screening sample solutions: **(2a)** Riomont at  $\lambda=246$  nm, **(2b)** Lida #1 at  $\lambda=223$  nm, **(2c)** Lida #2 at  $\lambda=223$  nm, **(2d)** Hyperdrive at  $\lambda=272$  nm, **(2e)** Ephedrine tablet at  $\lambda=243$  nm and **(2f)** Fat Cut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





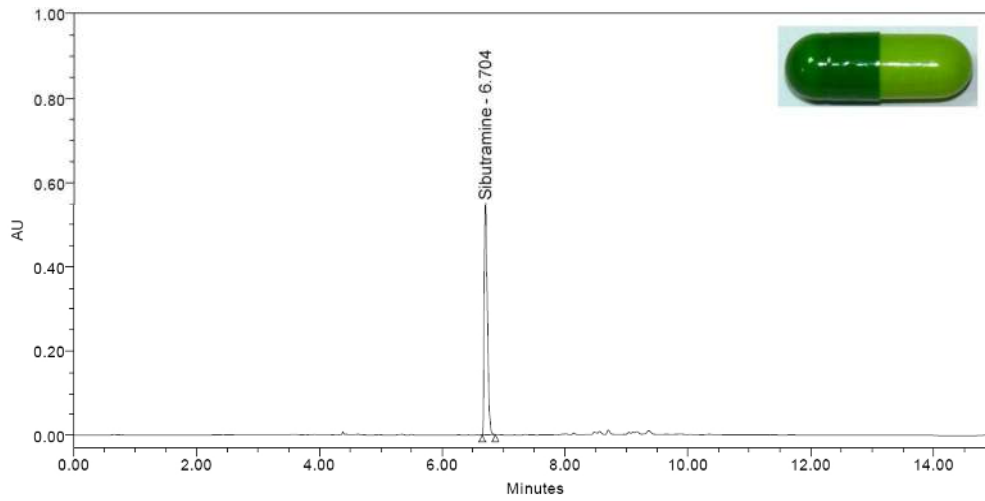
UHPLC/DAD chromatogram of 34 weight-loss substances (under chromatographic conditions of Table 3)  
238x148mm (72 x 72 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



416x205mm (72 x 72 DPI)

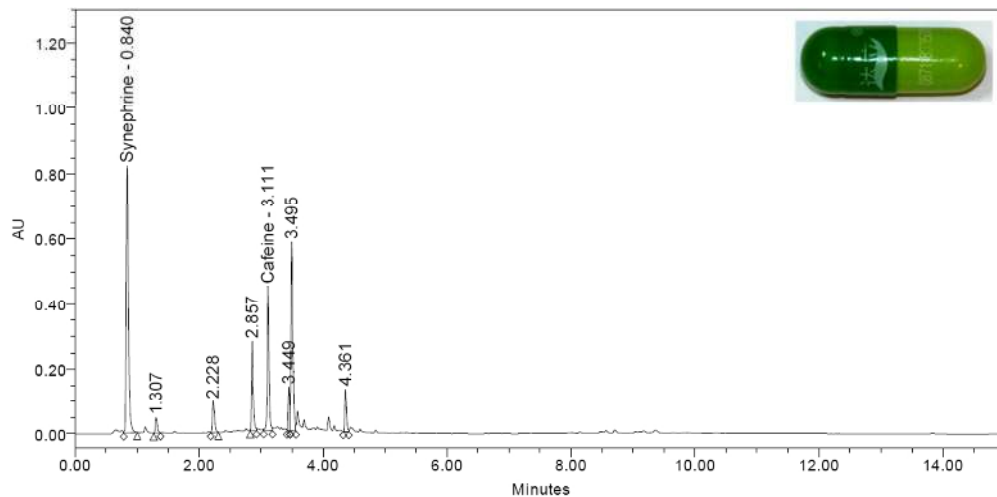
Review Only



421x208mm (72 x 72 DPI)

Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

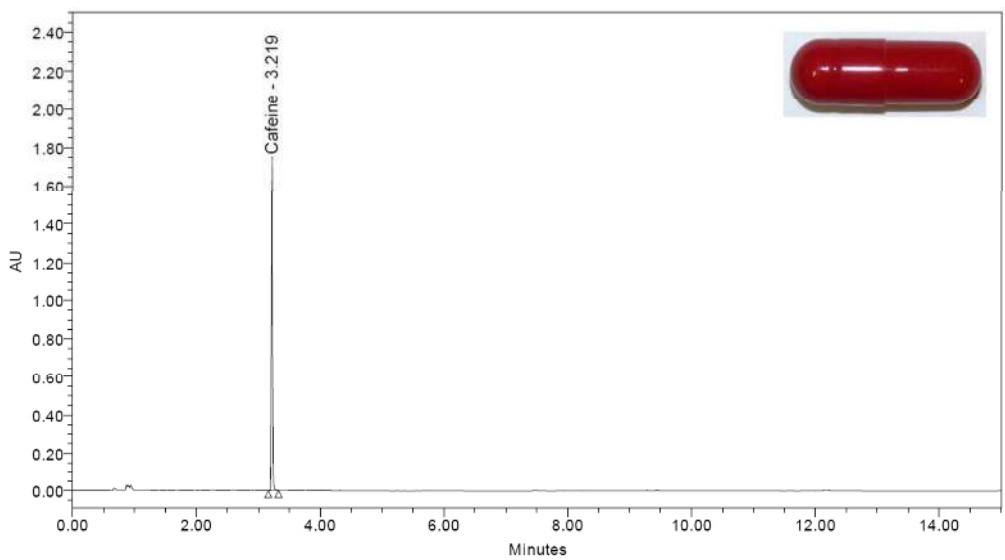


417x205mm (72 x 72 DPI)

Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

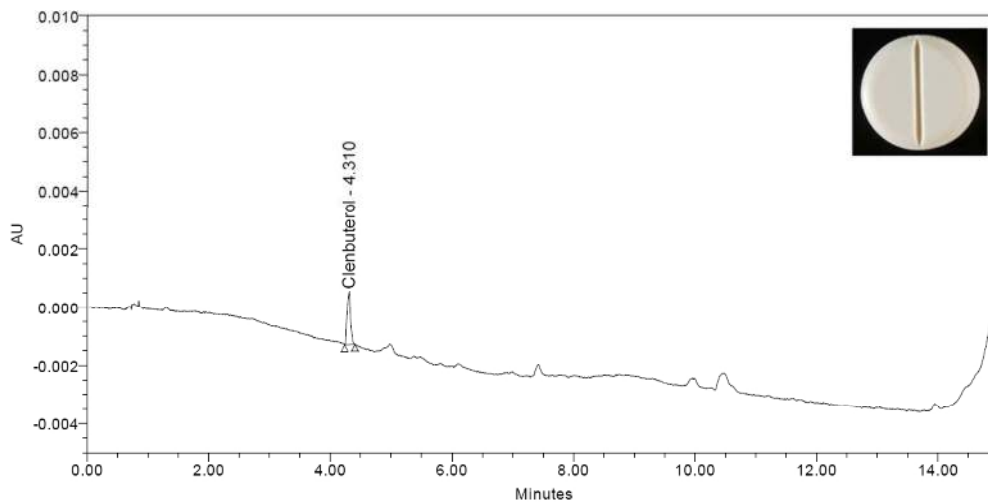
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



417x229mm (72 x 72 DPI)

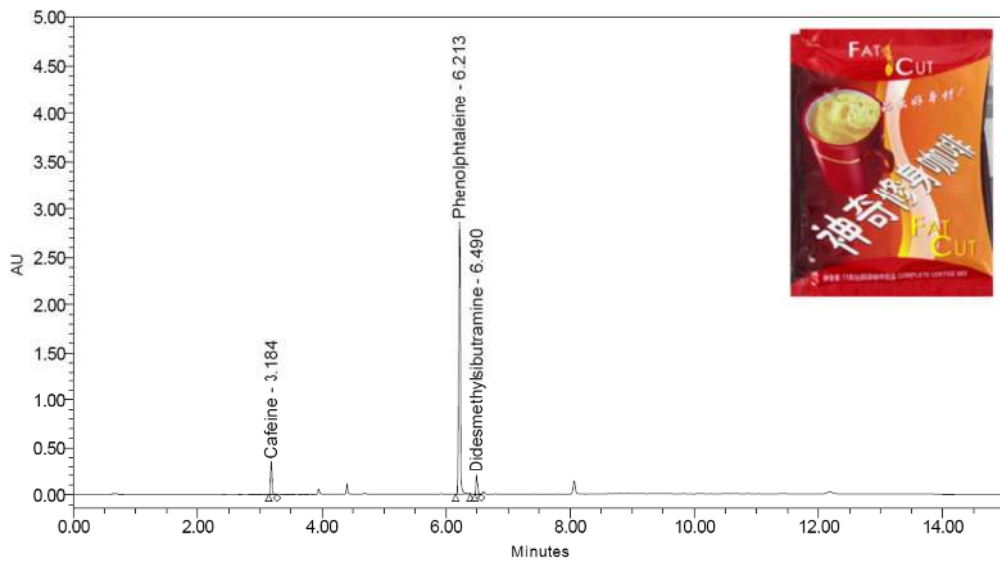
Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



419x207mm (72 x 72 DPI)

Review Only



416x229mm (72 x 72 DPI)

Review Only