



Microwave-assisted extraction and differential scanning calorimetry in the chemical identification of slimming agents apprehended in the south region of Brazil

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ABSTRACT

Over the past decades, consume of slimming agents considerably increased in several countries, including Brazil, due to weight-loss and stimulant properties. Since these drugs are controlled to prevent illicit and indiscriminate use, there is a parallel illegal market that uses the Internet and irregular pharmacies in order to distribute these formulations. Slimming agents produced by these illegal sources are known for being manufactured with little or none quality control resulting in uncertain and unknown formulations. For forensic purposes, apprehended pharmaceuticals have to undergo a process of chemical identification that can be difficult due to its complex matrix. In this sense, application of assisted energies in the extraction step such as microwave irradiation can be a promising method to increase the recuperation of the target molecules of the sample. Therefore, the aim of this research was to identify four slimming agents apprehended in Brazil by means of visual inspection, Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry and Gas Chromatography – Mass Spectrometry. Moreover, the efficiency of solid-liquid extraction and microwave-assisted extraction was compared. It should be noted that our work was one of the few to use Differential Scanning Calorimetry and the application of microwave irradiation in the analysis of apprehended materials. Results showed that the majority of the samples was counterfeit being composed of one or several adulterants or contaminants. Initially, visual inspection resourcefully screened the slimming agents for possible signs of falsification, however it failed to detect fraudulent products that were very similar to veridical medicines. Sequentially, Fourier Transform Infrared Spectroscopy detected functional groups present in the samples while the presence or absence of the alleged active ingredients were successfully measured with Differential Scanning Calorimetry and, thus, providing a full chemical screening of the apprehended materials. Gas Chromatography- Mass Spectrometry confirmed the presence of adulterants such as caffeine, fluoxetine and phenolphthalein as well as contaminants such as sulfuroil in the falsified samples. Finally, comparison of extraction procedures indicated that microwave-assisted extraction increased the recovery of compounds detected in chromatographic analysis to a greater extent than solid-liquid extraction.

1. Introduction

Obesity and overweight are a serious worldwide public health concerns linked to several risk factors that can decrease quality and span of life [1,2]. In order to treat these conditions, slimming drugs such as mazindol, sibutramine, amfepramone, phentermine and cathine have been extensively employed therapeutically [3]. According to previous researches, the United States has the highest number of

anorectic drugs users while Germany, Chile and Brazil have the highest rate of consumption for these compounds [4,5].

Although its considerable usage within the population, the abusive consumption of these drugs is related to several side effects, which may include heart attacks, increased blood pressure, high pulse rate, strokes, pulmonary hypertension, nervousness, headache and paresthesia [2,6,7]. Since slimming agents are associated with undesirable effects, they have been withdrawal from innumerable markets including, for

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instance, the European Union, the United States and Brazil. [3]. However, these drugs continued to be distributed and used through illegal sources [8].

Nowadays, the trade of drugs through the Internet or the illicit market is considerably increasing and has become a major problem since a great portion of products may be falsified. According to the World Health Organization (WHO), a pharmaceutical product that is falsely labelled, has different compounds or concentrations does not have its active ingredients or is falsely packed can be defined as a counterfeit drug [9]. These products are potentially dangerous to the health of the user since they can contain substances that may lead to several unknown side effects [10].

In the context of anorectic agents, the major concerns regarding falsification may include the fact that these drugs can have toxic substances or impurities in its composition. Besides, the alleged pharmaceuticals may have dangerous concentrations of active or unexpected ingredients as long as do not have any active substance [8]. It is known that > 30% of all the pharmaceutical drugs sold in sub-industrialized countries are counterfeit while it reaches 1% in industrialized nations. Thus, analytical techniques that can identify falsified drugs are of foremost importance in order to control and reduce the illicit market [10].

According to the literature, appetite suppressant agents can be detected through several techniques such as Gas Chromatography (GC), Liquid Chromatography (LC) [11] and Capillary Electrophoresis (CE) coupled to Mass Spectrometry (MS) [12], Fourier Transform Infrared Spectroscopy (FT-IR) [13] and Nuclear Magnetic Resonance (NMR) [14]. However, most of these techniques are expensive and time-consuming which can be a problem in development countries such as Brazil that have higher incidences of falsification and contraband. Therefore, the development of new analytical tools and the optimization of conventional techniques are crucial to answer for the high demand of analysis [3].

Extraction is a step of analysis that can interfere considerably with the instrumental results [13]. Generally, interactions between the target molecule and its matrix are very intense so that extraction have to be assisted by microwaves. In general lines, microwave irradiation is a form of electromagnetic energy which can homogeneously increase the temperature of the extraction environment enhance the efficiency of the procedure using lower volumes of organic solvents and less amounts of time [15]. This assistance is also a considerable useful tool when the active ingredients are found in lower concentrations, which can hamper its detection [16].

As can be seen, in order to combat counterfeit drugs, there is an urgent need to determine fast, low-cost and sensitive procedures to detect its active ingredients or potential impurities and adulterants. Previously, these factors have already been extensively studied by our research group on the analysis of other apprehended pharmaceuticals such as phosphodiesterase type 5 inhibitors [17] and amphetamine-type stimulants [18]. Therefore, the aim of this work was to evaluate solid-liquid (SLE) and microwave-assisted extraction (MAE) applied to slimming drugs apprehended by the station of Pelotas, Rio Grande do Sul State of the Brazilian Federal Police and to identify these samples by GC-MS, FT-IR and DSC.

2. Materials and methods

2.1. Chemicals, materials and samples

HPLC-grade methanol (J.T. Baker, Phillipsburg, USA) and hexane (Panreac, Barcelona, Spain) were used in the extraction process while aluminum crucibles were acquired from Netzsch (Selb, Germany) for DSC analysis. Sibutramine hydrochloride was purchased in a school pharmacy of the Federal University of Pelotas and used as a reference material. The Brazilian Federal Police of Pelotas, Brazil kindly provided samples (Table 1) that were apprehended in the south region of the

country from 2016 and 2018. Before analysis, all the content tablets or capsules were retrieved and homogenized in order to have representative sampling since few samples were provided.

2.2. Extraction

2.2.1. Solid-liquid extraction

The content of the capsules and the pellets were macerated and the 500 mg of obtained solid was extracted with 15 mL of methanol. Samples were subjected to vortex agitation for 1 min and injected in a GC-MS equipment. A filtration process was required in cases were the solid partially solubilized in the solvent. For Dimagrir, after agitation, a clean-up with 20 mL *n*-hexane was performed. All analysis were executed in triplicate.

2.2.2. Microwave – assisted extraction

500 mg of the grounded content of the samples and 10 mL of methanol were introduced in a round-bottom flask and subjected to extraction in a microwave model Discover CEM (Matthews, USA) for 10 and 15 min under 200 W of potency and 55 °C of temperature. A filtration process was required in cases were the solid partially solubilized in the solvent. For Dimagrir, after agitation, a clean-up with 20 mL *n*-hexane was performed. All analysis were executed in triplicate.

2.3. GC-MS Analysis

After extraction, one μL of the methanolic phase was injected in a GC-MS model QP2010SE (Shimadzu, Kyoto, Japan) equipped with an Rtx-5MS capillary column (Restek, Bellefonte, USA) and using helium as the carrier gas. Split mode (1:20) was used with the temperature of the injection port at 280 °C. The initial temperature of the analysis was 100 °C for increasing 10 °C.min⁻¹ to a final temperature of 315 °C and holding for 10 min. The ion source operated from *m/z* 35 to 550 with the ion electron ionization at 70 eV. The identification of the peaks was conducted using a NIST-08 library.

2.4. FT-IR analysis

Approximately 5 mg of the pulverized samples were placed in a FT-IR model Shimadzu Prestige 21 (Shimadzu, Kyoto, Japan) equipped with Attenuated Total Reflection from 4000 cm⁻¹ to 600 cm⁻¹.

2.5. DSC analysis

Approximately 3 mg of the pulverized samples were introduced in aluminum crucibles and put in a DSC model 200 F3 Maia (Netzsch, Selb, Germany). The initial temperature of analysis was 30 °C increasing 10 °C.min⁻¹ to 250 °C under an atmosphere of nitrogen with a flow of 50 mL.min⁻¹. Before analysis, the equipment was calibrated employing a standard of indium.

3. Results

3.1. Visual inspection

Samples of slimming agents were initially evaluated by its package, label and appearance of the content according to guidelines to detect counterfeit pharmaceuticals indicated by WHO. In the case of package, the unnamed products from Chernobyl and Wuffingras laboratories were not properly sealed exposing the tablets to degrading conditions of the environment, which was not seen at the packages of Dimagrir and Dualid S.

As for the label, several inaccuracies were found for products from Chernobyl and Wuffingras laboratories such as lack of full address of the manufacturer, hologram, trade name, dosage form and number of units per container. Besides, the alleged active ingredient of both

Table 1
Information about the supposed slimming drugs apprehended by the Brazilian Federal Police.

Commercial name	Date of apprehension	Alleged active ingredient	Batch number	Expiry date	Dosage form
Dimagrir	September 2016	Mazindol	L26900	October 2017	Pellets
Dualid S	January 2018	Amfepramone	0906520	September 2019	Capsules
Wuffingras	June 2017	Sibutramine	W20151001	December 2017	Capsules
Chernobyl	June 2017	Sibutramine	SBT15	September 2019	Capsules

samples (sibutramine) was spelled incorrectly and written in a foreign language, which is prohibited in the Brazilian legislation. Although sibutramine is currently legal in Brazil, both producers did not have a health license to distribute this active ingredient in the country making the formulations illegal. On the other hand, information regarding expiry date, batch number, drug strength as well as name and logo of the manufacturer were found in all the studied samples.

In the case of Dimagrir, several required data were not found since the sample did not have its external package with the label. Finally, Dualid S had the majority of the required information for a genuine pharmaceutical. In Brazil, the supposed active ingredients of Dimagrir (mazindol) and Dualid S (amfepramone) are legal, however there is no laboratory licensed to distribute both substances in the country.

Analysis of the physical format of capsules and tablets indicated that the recipients were uniform in shape, size and color for all the samples. It should be noted that there were no markings, breaks or splits as well as presence of empty capsules in the apprehended materials. However, the size of the powder that constituted the content of the samples was visually not uniform with the presence of particles that did not adhere to one another point to signs of falsification. Table 2 summarizes the results of the visual inspection of the apprehended material.

3.2. Chemical analysis and evaluation of the extraction procedures

3.2.1. FT-IR analysis

After a visual inspection, one of the simplest procedures in a chemical identification process is FT-IR analysis [19]. Comparison of the FT-IR spectrum of the reference material of sibutramine hydrochloride (Fig. 1S) and the content of the capsules of the unnamed product from Wuffingras (Fig. 2S) revealed several discrepancies between the results. Typical molecular vibrations of sibutramine such as N–H (3418 cm^{-1}), C–N (1406 cm^{-1}) and C–Cl (1010 cm^{-1}) were not found in Wuffingras indicating that the material did not contain its alleged active ingredient and was only composed of excipients. On the other hand, comparison of

Table 2
Results of the visual inspection of the samples.

Parameter of evaluation	Wuffingras	Chernobyl	Dimagrir	Dualid S
Container	Not sealed	Not sealed	n.a.	Sealed
Information legible	Yes	Yes	Yes	Yes
Trade name registered	No	No	No	Yes
Manufacturer registered	No	No	No	No
Spelling of the active ingredient	Incorrectly	Correctly	Correctly	Correctly
Active ingredient	Legal ^a	Legal ^a	Legal ^a	Legal ^a
Manufacture's name/logo	Yes	Yes	n.a.	Yes
Presence of hologram	No	No	n.a.	No
Manufacture's address	No	No	n.a.	Yes
Presence of drug strength	Yes	Yes	Yes	Yes
Presence of dosage form	No	No	n.a.	Yes
Number of units stated	No	No	n.a.	Yes
Batch number and expiry date	Yes	Yes	Yes	Yes
Shape of tablets/capsules	Uniform	Uniform	Uniform	Uniform
Size of tablets/capsules	Uniform	Uniform	Uniform	Uniform
Damaged or empty capsules/tablets	No	No	No	No
Content	Variable	Variable	Uniform	Variable

Note: Non-available (n.a.)

^a Sibutramine, mazindol and amfepramone are currently legal in Brazil.

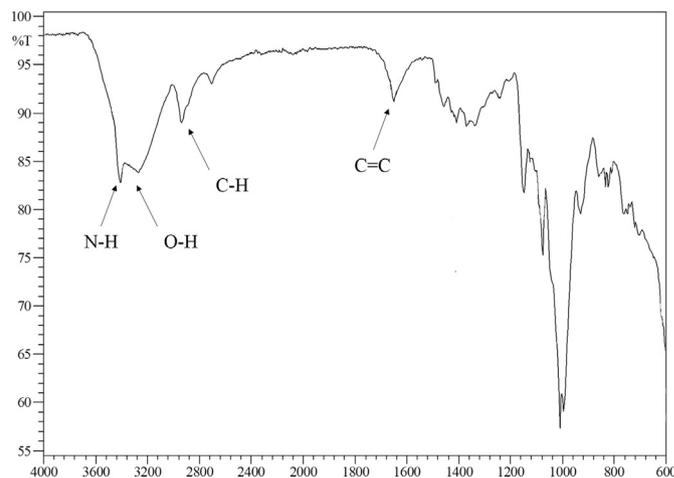


Fig. 1. FT-IR spectrum of the content of the unnamed product from Chernobyl.

the unnamed product from Chernobyl (Fig. 1) with the reference material had a similarity in the spectroscopic bands possibly indicating the presence of the alleged active ingredient sibutramine.

For Dimagrir, FT-IR analysis (Fig. 3S) indicated the presence of several molecular vibrations such as O–H (3300 cm^{-1}), C=N (1648 cm^{-1}) and C–Cl (668 cm^{-1}) that were linked with the presence of its supposed active ingredient mazindol. This was not the case of Dualid S (Fig. 4S), in which the majority of the bands of the spectroscopic spectra did not match the marketed substance amfepramone due to the presence of NH₂ (3292 cm^{-1}), C–N (1659 cm^{-1}) and C–F (1029 cm^{-1}) vibrations that are not related to the chemical structure of this particular compound.

3.2.2. DSC analysis

DSC analysis of the reference material of sibutramine hydrochloride (Fig. 5S) revealed the presence of a broad endothermic event ($T_{\text{peak}} 123.7\text{ }^{\circ}\text{C}$; $T_{\text{onset}} 117.6\text{ }^{\circ}\text{C}$; $\Delta H 90.98\text{ J.g}^{-1}$) related to the dehydration of the solid followed by another endothermic event ($T_{\text{peak}} 200.0\text{ }^{\circ}\text{C}$; $T_{\text{onset}} 197.3\text{ }^{\circ}\text{C}$; $\Delta H 102.3\text{ J.g}^{-1}$) that corresponded to the melting point of the analyte. On the other hand, calorimetric profile of Wuffingras (Fig. 2) indicated two exothermic events that were related to solid-solid

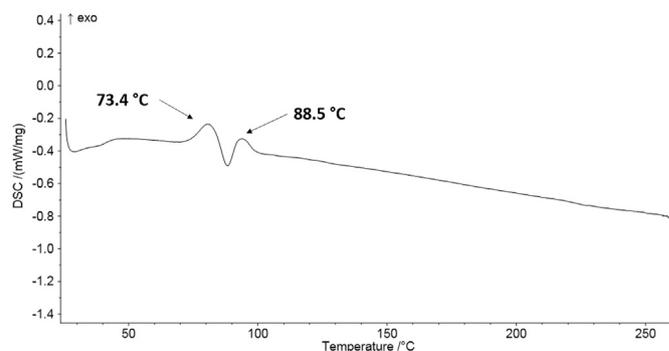


Fig. 2. DSC thermogram of the content of the unnamed product from Wuffingras.

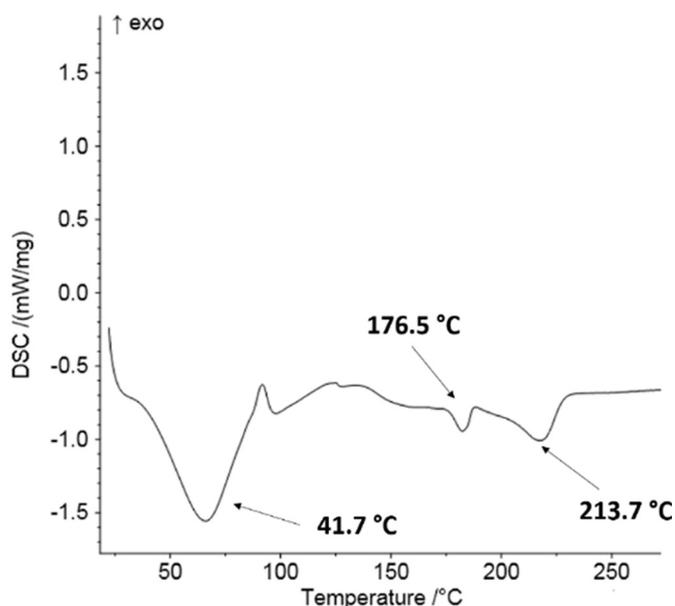


Fig. 3. DSC thermogram of the content of Dimagrir.

transitions ($T_{\text{peak}} 80.7\text{ °C}$; $T_{\text{onset}} 73.4\text{ °C}$; $\Delta H 10.34\text{ J.g}^{-1}$ and $T_{\text{peak}} 93.8\text{ °C}$; $T_{\text{onset}} 88.5\text{ °C}$; $\Delta H 6.34\text{ J.g}^{-1}$) in the material. As can be seen in the calorimetric profile of the unnamed product from Wuffingras, there were no events that could be related to sibutramine but only to excipients. Similarly, for the unnamed product from Chernobyl (Fig. 6S), there were two endothermic events that were not associated with the alleged active ingredient. Thus, DSC results did not corroborate with previous results of the FT-IR analysis, which indicated a possible presence of sibutramine in the formulation.

For Dimagrir (Fig. 3), there was two endothermic events ($T_{\text{peak}} 66.3\text{ °C}$; $T_{\text{onset}} 41.7\text{ °C}$; $\Delta H 156.95\text{ J.g}^{-1}$ and $T_{\text{peak}} 189.4\text{ °C}$; $T_{\text{onset}} 176.5\text{ °C}$; $\Delta H 5.32\text{ J.g}^{-1}$) possibly related to solid-solid transitions of the excipients. Subsequently, other endothermic event endothermic event ($T_{\text{peak}} 217.1\text{ °C}$; $T_{\text{onset}} 213.7\text{ °C}$; $\Delta H 31.44\text{ J.g}^{-1}$) was linked to degradation processes in the sample.

In the case of Dualid S (Fig. 4), the first endothermic event ($T_{\text{peak}} 147.7\text{ °C}$; $T_{\text{onset}} 144\text{ °C}$; $\Delta H 85.35\text{ J.g}^{-1}$) was related to the melting point of an excipient while the subsequent melting point ($T_{\text{peak}} 157.3\text{ °C}$;

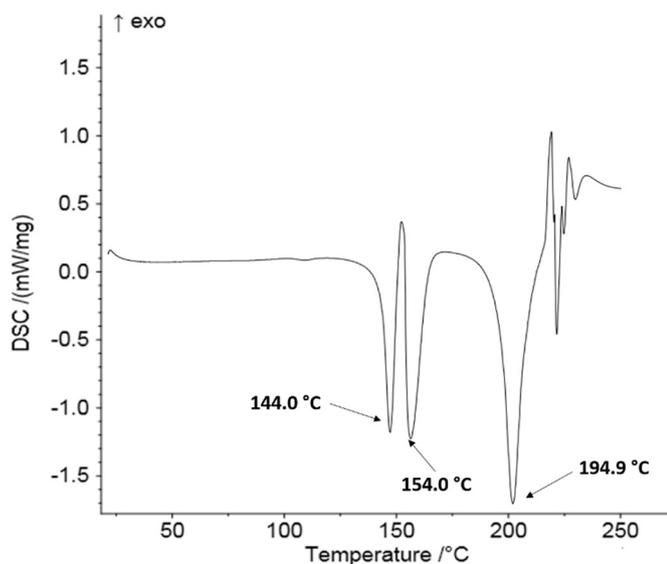


Fig. 4. DSC thermogram of the content of Dualid S.

$T_{\text{onset}} 154\text{ °C}$; $\Delta H 98.02\text{ J.g}^{-1}$) was related to fluoxetine hydrochloride. The final endothermic event ($T_{\text{peak}} 200.7\text{ °C}$; $T_{\text{onset}} 194.9\text{ °C}$; $\Delta H 167.68\text{ J.g}^{-1}$) was associated with the degradation of the sample.

3.2.3. GC–MS analysis

GC–MS analysis of the content of the unnamed product from Wuffingras (Fig. 7S) by SLE confirmed the absence of sibutramine in the sample and the presence of caffeine. Further analysis by GC–MS using MAE indicated the presence of other adulterants in the constitution of the sample. Results of Chernobyl (Fig. 8S) also confirmed the absence of the active ingredient with the sample being constituted of sulfuro, which was opposite to the preliminarily results of FT-IR that indicated the presence of sibutramine. The detected compounds were not described in the labels of both alleged pharmaceuticals making these substances adulterants.

Chromatographic results of Dimagrir (Fig. 9S) confirmed the presence of mazindol in the sample as well as several excipients. As can be seen in the chromatogram, although not removing all the possible interferes, the clean-up procedure was efficient to eliminate or diminish the presence of compounds that could elute at the same retention time as the analyte. This step was required since mazindol was found in lower concentrations and any possible interferer could mask its presence. For Dualid S (Fig. 10S), GC–MS analysis concluded the absence of amfepramone and the presence of the adulterant fluoxetine. Table 3 summarizes the experimental results obtained in the chemical analysis of the samples.

SLE and MAE were applied in order to evaluate the recovery of the compounds present in the samples. In general lines, MAE was more efficient than SLE recovering more substances from the matrix as well as enhancing the extraction compared to SLE. For the unnamed product from Wuffingras (Fig. 5), application of microwaves enabled the observation other adulterants in the sample such as phenolphthalein and benzoic acid as well as increased the concentration of caffeine, which were accomplishments not possible using SLE. In the cases of the unnamed product from Chernobyl (Fig. 11S) and Dimagrir (Fig. 12S) there was an increase in the chromatographic signal for the constituents of the samples which similarly happened for Dualid S (Fig. 13S) providing the extraction of both isomers of fluoxetine.

Spectrometric analysis of the samples confirmed the presence of compounds indicated in previous procedures. For mazindol, it was not possible to observe the molecular ion because of the disintegration of the hydroxyl group from the initial structure resulting in fragments of m/z 266 and 268 that are typical to the presence of chlorine in the molecule. In the cases of sibutramine hydrochloride and fluoxetine hydrochloride it was also not possible to detect the molecular ion since both substances have an inorganic aggregate, however further fragmentations confirmed the presence of both compounds in the analyzed samples. Table 4 summarizes the results obtained by the analytical techniques in the analysis of the formulation of slimming agents.

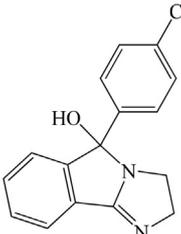
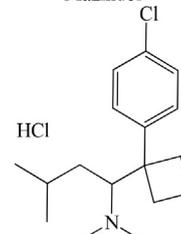
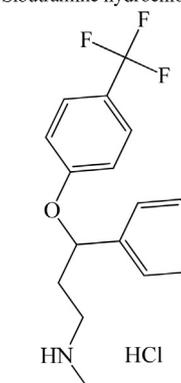
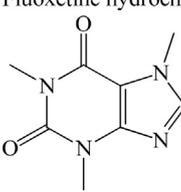
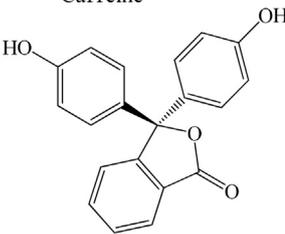
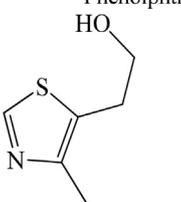
4. Discussion

4.1. Visual inspection

The first step in the analysis of suspected pharmaceuticals is visual inspection, which comprehends the examination of the package and the composition of the material. Although several fake preparations can meticulously appearance as genuine medicines, generally this process can assess if the studied sample is a substandard, counterfeit or degraded pharmaceutical. It should be noted that visual inspection is a complementary tool and that the chemical signature of the samples have to be determined in order to identify the components of the material [24–26].

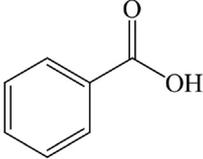
According to WHO, substandard pharmaceuticals are products of genuine manufacturers authorized to produce and distribute a certain drug in the market that do not meet the standard quality control set by

Table 3
Experimental and literature data of the compounds analyzed by DSC and GC–MS techniques.

Chemical structure	Experimental melting point (°C)	Literature melting point (°C)	Exact mass	Molecular fragmentation (m/z)
 Mazindol	176.5	198–199 [20]	284.07	268 (34%); 266 (100%); 231(27%);
 Sibutramine hydrochloride	197.3	193–195.5 [21]	315.15	114 (100%); 72 (12%)
 Fluoxetine hydrochloride	154.0	156–157 [22]	345.11	309 (2%); 104 (5%); 44 (100%)
 Caffeine	n.a.	238 [20]	194.08	194 (100%); 109 (44%); 55 (31%)
 Phenolphthalein	n.a.	258–262 [20]	318.09	274 (100%); 225 (59%); 181 (41%)
 Sulfurol	n.a.	≥ 25 [23]	143.04	143 (31%); 113 (46%); 112 (100%)

(continued on next page)

Table 3 (continued)

Chemical structure	Experimental melting point (°C)	Literature melting point (°C)	Exact mass	Molecular fragmentation (m/z)
 Benzoic acid	n.a.	122 [20]	122.04	122 (85%); 105 (100%); 77 (82%)

Note: Non-available (n.a.)

health regulatory agencies. Interaction of the material with temperature, humidity and other environmental factors may interfere with the composition of the medicine placing it as degraded sample. On the other hand, counterfeit medicines comprehend pharmaceuticals that are fraudulently mislabeled and have unknown or different ingredients than the stated in the label [24,26].

As could be seen in the results of the visual inspection of the studied samples, the unnamed products from Wuffingras and Chernobyl laboratories were placed as counterfeit pharmaceuticals since the respective labels did not have the required information, the package was not properly sealed and the content the tablets was not homogeneous. Dualid S was initially characterized as a probable genuine medicine even if it the manufacturer did not have a health license to distribute the active ingredient since it had the majority of the required features. Subsequently, in chemical analysis, this conclusion was refuted since there was no active ingredient in the sample. Visual inspection for Dimagrir was inconclusive since the sample was provided without the external package that contained the label. Similar patterns of falsification detected by visual inspection were also seen in previous researches that analyzed antimalarial drugs [27], pharmaceuticals and dietary supplements [28].

4.2. Chemical analysis and evaluation of extraction procedures

The consumption of slimming agents is growing worldwide due to the increasing levels of obesity, illicit use by professional drivers in order to keep awake at work and the misbelieve that these products are safe [2,29]. Given the growing number of samples required for forensic analysis and the fact that visual inspection can be inconclusive in some cases, there is a need to develop as well as optimize methods and techniques that can screen the products efficiently and in shorter time

[13,22]. Generally, preliminary analysis is performed using FT-IR [8] which is confirmed by GC-MS [14]. To the best of our knowledge, this one of the few researches to report the use of DSC and microwave irradiation as forensic tools to analyze apprehended slimming agents.

As seen in the spectroscopic results of the samples, it was possible to observe the presence or absence of the supposed active ingredient by the presence of characteristic chemical groups. However, excipients can masquerade the presence of the analyte as well as simulate its presence even when there is no active ingredient [8]. This was the case observed in the unnamed product from Chernobyl in which the bands could indicate the presence of sibutramine, however subsequent tests concluded that the product did not contain the active ingredient. Other drawbacks were observed by previous researches that employed this technique such as difficulties in discriminating bands [30] and necessity to analyze the samples by other analytical tools [13,18]. Therefore, FT-IR analysis only served as an initial screening of the apprehended material with the results having to be confirmed by other techniques [18].

For forensic purposes, DSC can be used as a quick evaluation to determine the presence or absence of the active ingredient by the identification of the melting point as well as to detect possible fraudulent formulations due to incompatibilities seen in the forms of thermal curves in the calorimetric profile [31]. Although, there are few researches linking this technique to forensic analysis, DSC could be efficiently employed area since it requires little sample preparation and lower analysis cost when compared to other conventional analytical tools such as GC-MS or LC-MS [32]. Besides, the application of MAE can also diminish the extraction time using lower volumes of organic solvents being environmental-friendly and, thus, optimizing the overall procedure.

The calorimetric profile of the samples indicated disparities between the reference material of sibutramine hydrochloride and the

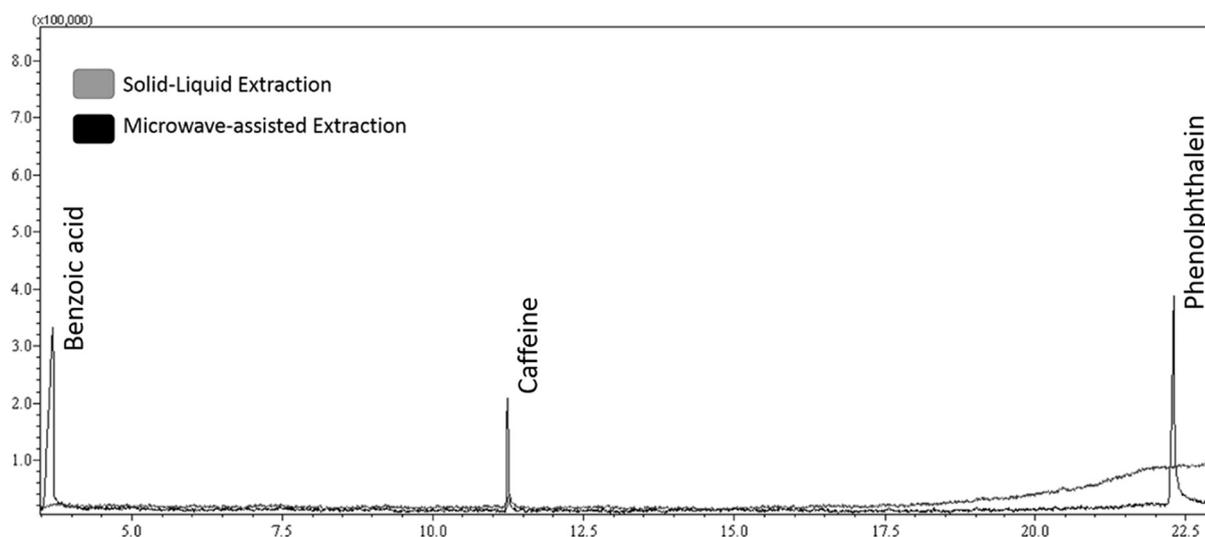


Fig. 5. Comparison of extraction procedures applied to the content of the unnamed product from Wuffingras.

Table 4
Summarized results of the analytical techniques applied in the analysis of the formulations of slimming agents.

Sample	Analytical technique		
	Visual inspection	FT-IR	DSC
Dimagrir	Package and label were not provided preventing the analysis. The capsule was not damaged and was uniform.	Bands related to the mazindol were found indicating its presence in the formulation.	Melting point of mazindol was not observed indicating its absence in the formulation.
Dualid S	Package was sealed, label had correct information and tablet was not damaged indicating that the sample was genuine.	Bands related to amfepramone were not found indicating its absence in the formulation	Melting point of amfepramone was not observed indicating its absence in the formulation
Wuffingras	Package was not properly sealed, label was inaccurate and the content was not uniform indicating that the sample was counterfeit	Bands related to the active ingredient were not found indicating its absence in the formulation	Melting point of sibutramine was not observed indicating its absence in the formulation
Chernobyl	Package was not properly sealed, label was inaccurate and the content was not uniform indicating that the sample was counterfeit.	Some bands related to the active ingredient were found in small absorbance indicating its presence in the formulation	Melting point of sibutramine was not observed indicating its absence in the formulation
			GC-MS
			Mazindol was identified. Therefore, the formulation was genuine. Fluoxetine was the only component detected. Therefore, the formulation was counterfeit. Caffeine, phenolphthalein and benzoic acid were detected. Therefore, the formulation was counterfeit. Sulfuroil was the only component detected. Therefore, the formulation was counterfeit.

Note: Fourier Transform Infrared Spectroscopy (FT-IR); Differential Scanning Calorimetry (DSC); Gas Chromatography – Mass Spectrometry (GC-MS).

products allegedly containing this active ingredient. Since the experimental value agreed with the literature that reports the occurrence of this process between 193 and 195.5 °C the DSC analysis indicated the absence of sibutramine hydrochloride in the unnamed products from Wuffingras and Chernobyl [21]. This thermal evaluation is in line with previous reports that distinguished genuine and counterfeit phosphodiesterase type 5 inhibitors with successful results [33].

On the other hand, DSC analysis for Dimagrir indicated the melting point of mazindol as 176. 5 °C, which was not close to the value reported of 198–199 °C giving the false impression of the absence of mazindol in Dimagrir. This variation between the melting points can be explained by the low concentrations of the active ingredient in the sample as well as to interactions with the excipients and other constituents that shifted the result. Analyzing several pharmaceuticals, previous researches already noticed a similar behavior of the influence of the formulation to the resulting calorimetric profile [31].

In the case of Dualid S, the presence of distinct melting points indicated the incompatibility of the constituents of the sample. This fact is linked to the inadequate composition of the supposed pharmaceutical that can have severe biopharmaceutical implications since the release and absorption of the compounds is changed [21]. It should be noted that DSC analysis indicated the absence of the alleged active ingredient amfepramone. Subsequent tests confirmed that the sample was composed of fluoxetine whose melting point was very close to the reported in the literature (156–157 °C) [22].

In Brazil, the alleged active ingredients of the studied samples sibutramine, amfepramone and mazindol are legal controlled drugs placed at B2 list (psychotropic anorectic substances) of the Sanitary Vigilance National Agency (ANVISA), which is a public autarchy responsible for the regulation of the distribution of pharmaceutical drugs in the country [34]. There is no manufacturer licensed in Brazil to distribute mazindol and amfepramone making products apprehended within borders with these active ingredients illegal formulations. Nonetheless, illicit manufactures produce these substances and commercialize by the Internet and by irregular pharmacies distributing these drugs across the country [8].

The analyzed illicit formulations of slimming agents had a considerable amount of adulterants or contaminants in the form of caffeine, sulfuroil, fluoxetine, benzoic acid or phenolphthalein. These substances not declared on the label have been reported on previous works that analyzed alleged pharmaceutical formulations of slimming agents [35] as well as in herbal products [1,14,29] and can be linked to a series of health hazards to the quality of life of its users [13]. According to the Brazilian legislation, production, distribution and commercialization of falsified or adulterated pharmaceuticals are typified as a crime that can be punishable for up to 15 years of incarceration and/or a fine. In this order, since the majority of the analyzed samples was considered a counterfeit they could be placed under this normative [36].

Caffeine is a stimulant drug that can act as a slimming agent and for this reason is widely used as an adulterant in illicit weight loss formulations and herbal products [1,14,29]. In considerable doses, this substance can cause hypertension, seizure, stroke and death [29]. On the other hand, sulfuroil is a food additive known for having a nutty, meaty and sulfury flavor description that is not linked with health hazards [37]. Since it was encapsulated, the presence of sulfuroil is probably related to contaminations at the local of the manufacturer of the alleged slimming products.

Fluoxetine have also been previously reported in slimming agents and is used in treatment of brain and dietary disorders due to its antidepressant properties [1,29,35]. Chemically, this compound does not have a close structure to tricyclic and tetracyclic antidepressants but it is a secondary amine linked to phenyl and tolyl groups [22]. The use of fluoxetine is linked to several side effects that include manic and suicidal behavior, seizures besides withdrawn and serotonin syndrome [35]. In Brazil, fluoxetine is a legal controlled drug placed at the C1 group with the retention of the prescription in order to be used [34].

Benzoic acid is a typical excipient found in pharmaceutical formulations since it contains antimicrobial and antifungal activities and has a low toxicity to humans [38]. On the other hand, phenolphthalein is an adulterant known for having a laxative effect that with a continuous use can lead to carcinogenic effects and, for this reason, it was removed from the pharmaceutical market [39]. Although banned from medicinal use, phenolphthalein continues to be employed in illicit herbal preparations or slimming formulations as reported in previous researches [14,29,39].

The matrix of pharmaceuticals are known for having excipients in the formulation that can difficult the extraction process of the active ingredient generating sub estimated results [9]. In the literature, there are few extraction methodologies applied to the analysis slimming agents in pharmaceutical formulations with the majority using a simple SLE [2,29] or an ultrasonic bath-assisted extraction [1,13,18]. Therefore, the development and optimization of extraction procedures is important in order to improve the accuracy and the sensitivity of the analysis of slimming agents [16].

As seen in the comparison of extraction methods, MAE was superior in the recovery of the formulation than SLE. This was possible since microwaves are a form of irradiation that enhance the contact between solvent and sample through dipolar rotation and ionic conduction mechanisms, which increase the extraction process [15]. Moreover, compared to ultrasonic bath-assisted extraction, MAE possibilities a superior control over the parameters of extraction such as power and temperature assuring reproducibility of the results. Despite the considerable importance, few researches employ microwaves in order to extract anorectic agents [40].

5. Conclusion

According to the obtained results, the majority of the analyzed samples were counterfeit composed of adulterants or cross-contaminated with another substance. Besides, the use of a single analytical process such as visual inspection, FT-IR, DSC or GC-MS was not suited for the analysis of the apprehended materials since it could lead to false interpretations. Thus, there is need for a sequence of several analytical tools in order to provide precise and accurate results for the overall analysis. It should be noted that Differential Scanning Calorimetry was not a powerful tool to detect the presence or absence of the alleged active ingredient, but could be used as a screening analysis to complement results obtained by other analytical approaches. As for the extraction procedures, the application of microwave irradiance proved to be a significant factor in the recovery of compounds from the matrix. Therefore, MAE could be an important tool in order to increase the recovery of active ingredients in low concentrations or adulterations that could not be extracted from the matrix employing conventional methodologies.

Declaration of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scijus.2018.11.001>.

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