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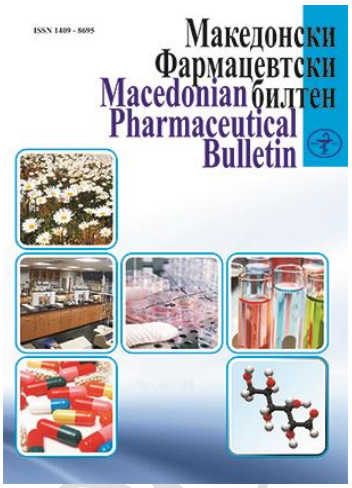

Title: Detection of sibutramine adulteration in slimming teas using HPLC-DAD

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Detection of sibutramine adulteration in slimming teas using HPLC-DAD

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Abstract

The consumption of slimming teas has been increasing due to the widespread belief in their safety as natural products. However, the adulteration of natural products for losing weight has become a continuously more significant issue, particularly when they are found to contain undeclared and potentially harmful substances. The aim of the current study was to investigate the possible presence of sibutramine, one of the most common adulterants in slimming teas, randomly selected from the market in the Republic of North Macedonia. Identification and quantification of sibutramine were performed using an HPLC-DAD method, with a mobile phase consisted of acetonitrile and water containing 0.3% triethylamine (TEA) (pH adjusted to 7.0) (75:25, *v/v*), for a total runtime of 10 min. The analysis results revealed the presence of sibutramine in three of the six tested products, with quantified levels ranging from 1.68 to 2.51 mg/g. These products pose health risks, as sibutramine can increase the risk of heart attack, stroke, high blood pressure, and other cardiovascular problems. Therefore, increased laboratory inspection of natural slimming products using suitable analytical methods is crucial for protecting public health. In this regard, the applied method was validated for accuracy, precision, linearity, specificity, sensitivity, and robustness. The validation results demonstrated that the method is suitable for the routine analysis of sibutramine adulteration in slimming teas.

Keywords: sibutramine, adulteration, HPLC-DAD, slimming teas

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Introduction

Obesity is recognized as a public health issue. It's a medical condition that increases the risk of many other diseases such as type 2 diabetes, cardiovascular diseases, abnormalities in lipid metabolism, and it can contribute to the development of cancer (Fitch, 2022). Considering these health concerns, weight loss and treatment of obesity are of high importance. Various methods such as dietary changes, increased physical activity, pharmacologic strategies, and surgical intervention have been used to lose weight (Firozian et al., 2021). Natural slimming products are highly popular due to their low cost, ease of use, and the recognition of minimal or no side effects (Mathon et al., 2014). Slimming coffees or teas are widely marketed and numerous preparations are available to consumers through several distribution channels (over the counter in pharmacies, supermarkets and herbalist's shops), and particularly easy to obtain via the Internet (Ekor, 2014). These products are generally accepted as safe, but in recent years, adulteration has become a very serious problem. Adulteration encompasses the illegal addition of synthetic drugs in weight loss products labelled to contain natural ingredients only, for providing faster slimming effects and increased financial profit for the seller (Rocha et al., 2016). It was reported that consumers are likely to quit using slimming products if they don't realize any initial slimming effects (Jairoun et al., 2021; Rocha et al., 2016). Slimming products containing illegal additives pose significant risks, including side effects, adverse events such as intoxication or drug interactions, and even life-threatening reactions, as consumers are unaware of the substances they are administering (Muschietti et al., 2020). Numerous reports for "completely natural" slimming products being adulterated with undisclosed synthetic drugs to strengthen their biological effects have been published (Firozian et al., 2021; Jairoun et al., 2021; Pratiwi et al., 2021; Zhivikj et al., 2020). As reported, sibutramine and its metabolites were the most prevalent adulterants (Deconinck et al., 2014). The adulteration of slimming products with sibutramine is facilitated by their classification as foods, allowing them to enter the market without undergoing additional quality and safety assessments (Kemsawasd et al., 2023). Sibutramine, a serotonin-norepinephrine reuptake inhibitor, used as an antidepressant, exhibits anorexic effects through a combination of mechanisms, including appetite suppression, enhanced feelings of satiety, and possibly inducing thermogenesis (Luque and Roy, 2002). Since 2002, several cardiovascular adverse events (hypertension, tachycardia, arrhythmias, myocardial infarction) were reported in sibutramine-

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treated patients, which led to a contraindication of its use as an antiobesity drug in patients with established coronary heart disease, previous stroke, heart failure, or cardiac arrhythmias (Scheen, 2011). In 2010, sibutramine was withdrawn from the US and European markets following clinical trial data that revealed adverse effect on the cardiovascular system associated with an increased risk of heart attacks and strokes (Csupor et al., 2012; EMA, 2010). Additional side effects including nervousness, headache, anxiety, constipation, xerostomia, numbness, and paraesthesia are also possible (Ariburnu et al., 2012). Significant health risks associated with the undeclared presence of sibutramine in slimming products are emphasizing the necessity for enhanced post-market surveillance of such products. To address this safety concern, six slimming teas available on the Macedonian market were analyzed for sibutramine adulteration using an HPLC-DAD method.

Materials and methods

Materials

Referent standard of sibutramine was purchased from HPC Standards GmbH, Germany. Six teas for reducing body weight that were commercially accessible in the Republic of North Macedonia were chosen at nearby stores. All the teas were labeled to contain natural ingredients only. The samples were identified using numbers 1 to 6 (Table 1).

Table 1.

For the analysis, following chemicals were used: HPLC grade acetonitrile (ACN) (Carlo Erba Reagents, Italy), triethylamine, (99.0%, Carolina Chemical, United States), orthophosphoric acid, (85%, Fisher Scientific, Pennsylvania, United States), and methanol, HPLC grade (99.9%, Fisher Scientific, Pennsylvania, United States). All solutions were prepared using redistilled pure water DIRECT Q5 UV (Millipore SAS, France).

Instrumental and analytical conditions

The HPLC-DAD method used for determination of undeclared sibutramine in slimming teas was based on the method reported by Silva et al. (2009) with certain modifications. The analysis was carried out on Shimadzu LC-20 HPLC system, working software: CLASS-VP 7.4 SP

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2 (Shimadzu Corporation, Kyoto, Japan) using a LiChrocart Purospher RP-8 endcapped column (150 mm x 4.6 mm, 5μm). The mobile phase consisted of ACN and water (75:25 *v/v*). Triethylamine (0.3%) was added previously to the water phase and its pH was adjusted to 7.0 using an orthophosphoric acid. Injection volume of the sample was 20 μL. The column oven was set at 25 °C. The detection wavelength was set to 225 nm and the flow rate was maintained at 1.5 mL/min. For confirmation of the identity of sibutramine, LC-MS/MS instrumentation (Agilent technologies, Inc., CA, USA) was used employing the conditions of the high-pressure liquid chromatography-electrospray tandem mass spectrometry method developed by Krivohlavek et al. (2016).

Preparation of stock standard solution

Sibutramine solution was prepared in 10 mL volumetric flask. A 10 mg sample of the sibutramine standard was accurately weighed using a Sartorius Quintix analytical balance (Germany). The sample was then dissolved in methanol and sonicated using an AC-150H ultrasonic bath (MRC Laboratory Equipment, Israel). The working solution of sibutramine was prepared from the corresponding solution to obtain a concentration of 60 mg/L. Prior to analysis, the standard solution was filtered using a 0.45 μm cellulose acetate membrane filter (Labfil, China).

Sample preparation

To simulate real consumption conditions, 2.5 g of each slimming tea was dissolved in 250 mL of hot deionized water (85 °C) following the preparation instructions on the packaging. The prepared solutions were cooled to room temperature and then vortexed and sonicated for 15 minutes in an ultrasonic bath. Each sample was prepared in triplicate, filtered through a 0.45 μm cellulose acetate membrane filter, and transferred to glass vials for further analysis.

Method validation

The method was validated according to ICH guideline (EMA, ICH Q2(R2), 2022), including validation parameters such as specificity, linearity, accuracy, precision, sensitivity, and robustness.

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Specificity of the method was evaluated by analyzing chromatograms of mobile phase (diluent), blank, and sibutramine standard solution at a concentration of 60 mg/L.

To assess the linearity of the method, standard solutions of sibutramine were prepared by diluting a stock standard solution with deionized water to obtain different exact concentrations of sibutramine: 15.63 mg/L, 31.25 mg/L, 62.50 mg/L, 125.00 mg/L, 250.00 mg/L, 500.00 mg/L. Three injections from each concentration were performed. The linearity of the calibration curve was evaluated using a linear regression analysis.

Limit of detection (LOD) and limit of quantification (LOQ) of sibutramine were determined by analysing different concentrations of the analyte and measuring the signal-to-noise ratio. The LOD is the concentration that gives a signal-to-noise ratio of approximately 3:1, while the LOQ is the concentration that gives a signal-to-noise ratio of approximately 10:1.

The accuracy of the method was determined by applying the standard addition method and determining the analytical yield. A sample of instant tea proven to be free of sibutramine was used and spiked with exact amounts of sibutramine at three concentration levels, corresponding to 25%, 100% and 150% of the target concentration, i.e., 15 mg/L, 60 mg/L and 90 mg/L. From each concentration level, three replicates were prepared and injected. The percentage recovery of added sibutramine and the relative standard deviation (RSD) were calculated for each replication. System precision was assessed by six injections of a sibutramine standard solution with a concentration of 125 mg/L. Method precision was evaluated by five injections of Sample 1 (Table 1), which was prepared by dissolving 0.5 g in 50 mL mobile phase. Intermediate precision was verified by preparing the same samples for repeatability testing and injecting it into the HPLC system at an interval of approximately 10 days. The individual analyte concentration data obtained were used to calculate reproducibility (R), standard deviation of reproducibility (SRw), and relative standard deviation of reproducibility (RSDRw).

To verify the robustness of the method, deliberate, small changes to the experimental parameters were applied, such as flow rate, and column temperature.

Results and discussion

HPLC-DAD method

A liquid chromatography (LC) method with UV detection, reported by Silva et al. (2009) for studying the stability of sibutramine in soft gelatin capsules, was slightly modified to

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investigate the adulteration of sibutramine in slimming teas randomly sampled from the market in the Republic of North Macedonia. Method optimization included adjustment of the flow rate to 1.5 mL/min in order to reduce the retention time. The specificity of the method was assessed by comparing chromatograms of the mobile phase, a blank sample, and a standard sibutramine solution (Fig. 1). For the identification of sibutramine, a diode-array detector (DAD) was used, providing for spectral analysis of the compound of interest and providing peak purity information. Chromatograms demonstrated no interference between the sibutramine peak and the solvent. Furthermore, good resolution was achieved between matrix components and sibutramine, confirming that the method is specific and selective. Additional confirmation of peak identity was achieved by comparing the spectra of the analyte peak with those obtained from a standard sibutramine solution. The results indicated no co-elution of other peaks with sibutramine at its retention time (Rt) of 8.12 minutes, as confirmed by peak purity analysis.

A linear correlation was observed between the peak area and the concentration of sibutramine over the working range of 15.63–500 mg/L, corresponding to 15.63 to 500 µg of sibutramine per gram of test sample. The linear relationship between peak area and concentration was established using least squares regression analysis, resulting in the regression equation $y = 20864x – 29889$, with an R^2 value of 1. The LOD for sibutramine was 0.94 mg/L, while the LOQ was found to be 1.90 mg/L. Both LOD and LOQ were determined based on multiple injections of Sample 1 (Table 1), which was diluted to provide sibutramine concentrations ranging from 0.47 to 15 mg/L. The results for accuracy and precision are presented in Table 2. Recovery rates for sibutramine at three concentration levels ranged from 95.1 to 102.1%. Precision testing included repeatability (within-day precision), intermediate precision (between-day precision), and system precision. The repeatability of the method, expressed as the RSD, was 9.55%, while the intermediate precision was 10.4%. Additionally, the RSD for injection sensitivity was determined to be 0.5%. The obtained values are in accordance with the criteria established in ISO/IEC 17025 (Rivera and Rodriguez, 2011). Method robustness was demonstrated by deliberately altering chromatographic conditions, including flow rate (±0.5 mL/min) and column temperature (±5 °C), which resulted in no significant changes in system suitability parameters.

Table 2.

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Determination of sibutramine in real samples

Six slimming teas marketed in the Republic of North Macedonia were examined for their safety consumption regarding the presence of sibutramine. Before being analyzed, the samples were prepared as directed on the packaging, which involved dissolving them in hot water. This approach was chosen to mimic the actual conditions under which consumers prepare and consume these teas, rather than dissolving the samples in a mobile phase. The objective was to determine the actual amount of sibutramine that dissolves in water, as opposed to the amount extracted using a mobile phase, which would not reflect real consumption. By using this method, the true concentration of sibutramine present in the teas as consumed by users was accurately assessed.

Fig. 1-3

As presented in Table 1, three of the six examined teas (50%) used for reducing body weight were found to contain sibutramine. These samples displayed the same UV spectra and peaked at the same retention time as the standard sibutramine (Figure 1 and 2). Confirmation of sibutramine was performed using LC-MS in multiple reaction monitoring (MRM) mode, targeting the transition from the precursor ion *m/z* 280.2 to the product ion *m/z* 125.0 (Krivohlavek et al., 2016) (Figure 3). Quantitative study revealed that Sample 1 contained 2.47 mg/g or 6.26 mg in a tea bag sibutramine, Sample 2 contained 2.51 mg/g or 12.56 mg in a tea bag sibutramine, and Sample 3 contained 1.68 mg/g or 6.82 mg in a tea bag sibutramine. According to our knowledge, the first investigation of slimming products available on the Macedonian market was conducted by Zhivikj et al. (2020). In that study, nineteen weight loss supplements were simultaneously screened for the presence of five potential adulterants (sibutramine, fluoxetine, caffeine, theobromine, and phenolphthalein) using an HPLC-DAD method. The analysis revealed that two products were adulterated with fluoxetine, each containing 20 mg per dosage unit, while one product contained undeclared theobromine at a level of 4.8 mg per dosage unit. Fluoxetine has been considered potentially useful for weight loss due to its appetite-suppressing side effect observed during depression treatment (Melaré et al., 2023), and theobromine has been associated with weight loss because of its diuretic properties (Martínez-Pinilla et al., 2015). An increasing number of studies highlight the concerning trend of adulteration in slimming products (Gheorghiu et al., 2025), with several reports specifically detecting the presence of sibutramine as an adulterant. Kim et al. (2014)

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using an LC-MS/MS method detected sibutramine in 62 of the totals out of 188 weight loss foods and supplements collected between 2009 and 2012 in South Korea, ranged in levels from 0.03 to 132.40 mg/g (2010), from 0.88 to 76.2 mg/g (2011), and from 0.07 to 0.24 mg/g (2012). Mathon et al. (2014) reported that half of the 52 investigated herbal slimming supplements using an HPTLC-UV densitometric method were adulterated with sibutramine at amounts reaching up to 35 mg per capsule. In Croatia, a study by Krivohlavek et al. (2016) identified sibutramine in 20% of 123 tested slimming food supplements, with concentrations reaching up to 26.41 mg/g, as determined by LC-ESI-MS/MS. An investigation by Hachem et al. (2016) using H NMR spectroscopy analyzed 160 herbal food supplements and found sibutramine in 43 samples, with amounts ranging from 0.1 to 22 mg per dosage. Recently, Carles et al. (2025) have reported that all examined 12 weight loss supplement samples (capsules, tea, and coffee bags) using LC-HRMS contained sibutramine with concentrations ranging from 7.5 mg to 15.4 mg per unit. Our method yielded sibutramine levels that are comparable to those found in other investigations that revealed sibutramine adulteration.

For obesity treatment, sibutramine was prescribed at doses of 10–15 mg daily. However, even at these doses, it significantly increased blood pressure and heart rate in some patients (Tziomalos et al., 2009). Doses exceeding 20 mg per day may increase risks of severe cardiovascular events, including heart attacks and strokes, particularly in individuals with pre-existing heart conditions (Scheen, 2011). At doses of 25 mg daily and above, serious adverse effects, including palpitations, arrhythmias, elevated blood pressure, and even death, may occur (Wooltorton, 2002; Tziomalos et al., 2009). It is relatively simple to reach the health-compromising dosage given the levels of sibutramine included in the examined slimming teas and the standard recommendation to take it three to four times a day. Additionally, the dangerous levels of sibutramine adulteration in slimming products vary depending on individual tolerance, underlying health conditions, and drug interactions. Sibutramine adulteration in slimming products remains a critical public health issue, requiring coordinated efforts from regulatory agencies, researchers, and consumers to prevent serious health risks. Raising public awareness about the risks of sibutramine-adulterated products is essential to prevent adverse health effects. However, health agencies must also strengthen regulations, conduct market surveillance, and implement laboratory testing using advanced analytical methods.

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Conclusion

This study demonstrated that certain slimming teas marketed in the Republic of North Macedonia are adulterated with sibutramine. It was detected in three out of six slimming teas analyzed, at concentrations of 1.68, 2.51, and 2.47 mg/g, despite being marketed as natural products. The employed HPLC-DAD method with a total runtime of 10 minutes showed high sensitivity and specificity, combined with a simple extraction procedure, making it a practical and efficient approach for routine laboratory analysis of sibutramine adulteration in slimming teas. Regulatory agencies and other relevant authorities should be prompted to increase market inspections and enforce mandatory quality control of natural slimming products to mitigate the risks posed by adulterated slimming products.

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Резиме

Определување на сибутрамин во чаеви за слабеење со примена на HPLC-DAD метод

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Клучни зборови: сибутрамин, адултерирање, HPLC-DAD, чаеви за слабеење

Употребата на чаеви за слабеење е во пораст како резултат на верувањето дека овие производи се природни и безбедни. Меѓутоа, адултерирањето на природните производи за слабеење претставува сериозен проблем, особено кога во нив ќе се откријат недекларирани и потенцијално штетни супстанции. Целта на ова истражување е да се испита евентуалното присуство на сибутрамин како еден од најчестите адултеранти во чаеви за слабеење што се набавени од пазарот во Република Северна Македонија по случаен избор. Идентификација и определување на сибутрамин во анализираните примероци беше направено со HPLC-DAD метод, со користење на мобилна фаза составена од ацетонитрил и вода (75:25 *v/v*), со 0,3% триетиламин додаден претходно во водената фаза и pH подесена на вредност 7, за вкупно време на анализа од 10 минути. HPLC анализата на избраните примероци (вкупно шест) покажа присуство на сибутрамин во три примероци, со содржина на сибутрамин од 1,684 до 2,511 mg/g. Ваквите производи претставуваат ризик за здравјето бидејќи сибутрамин може да го зголеми ризикот од хипертензија, миокардијален инфаркт и други кардиоваскуларни проблеми. Затоа е потребно да се засили лабораториската контрола на природните производи за слабеење, со примена на погодни аналитички методи, како есенцијален пристап за заштита на јавното здравје. Во оваа смисла, применетиот хроматографски метод беше валидиран за точност, прецизност, линеарност, специфичност,

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осетливост и робустност. Добиените резултати покажаа дека методот е соодветен за рутинска анализа на сибутрамин во адултерирани чаеви за слабеење.

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Table 1. Sibutramine adulteration in six slimming teas supplied from the market in the Republic of North Macedonia

Slimming tea	Ingredients	Country of origin	Sibutramine adulteration
1	Guarana, chitosan, honey plant, L-carnitine, pine tree, lemon, red ruby, rosemary, marjoram, green tea, vitamin A, tannin extract	Turkey	2.474 mg/g
2	Ginger, L-carnitine, green tea, mate tea, chamomile, rosehip	Turkey	2.511 mg/g
3	Instant coffee, ginger, ginseng, L-carnitine, cinnamon, flax seed, safflower, Lepidium, collagen I-III, hyaluronic acid, L-ascorbic acid, caffeine	Turkey	1.684 mg/g
4	Senna leaf 45%, frangula bark 25%, chamomile, flower 15%, peppermint leaves 15%	Bosnia and Herzegovina	ND
5	Buckthorn bark 20%, restharrow root 20%, dandelion root, liquorice root, chamomile flower, sweet fennel fruit	North Macedonia	ND
6	Birch leaf, wild thyme, hibiscus flower, nettle leaf, peppermint leaf, dandelion root, parsley leaf, horsetail, savory, lemon balm leaf, rosehip	North Macedonia	ND

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Table 2. Accuracy and precision of HPLC-DAD method for determining sibutramine adulteration of slimming teas

Parameters	Accuracy Recovery + RSD%			Precision RSD%
Concentration/ concentration level	15 mg/L (25%)	60 mg/L (100%)	90 mg/L (150%)	
Sibutramine (%)	97.8 + 11.3	95.1 + 10.6	102.1 + 1.7	9.55

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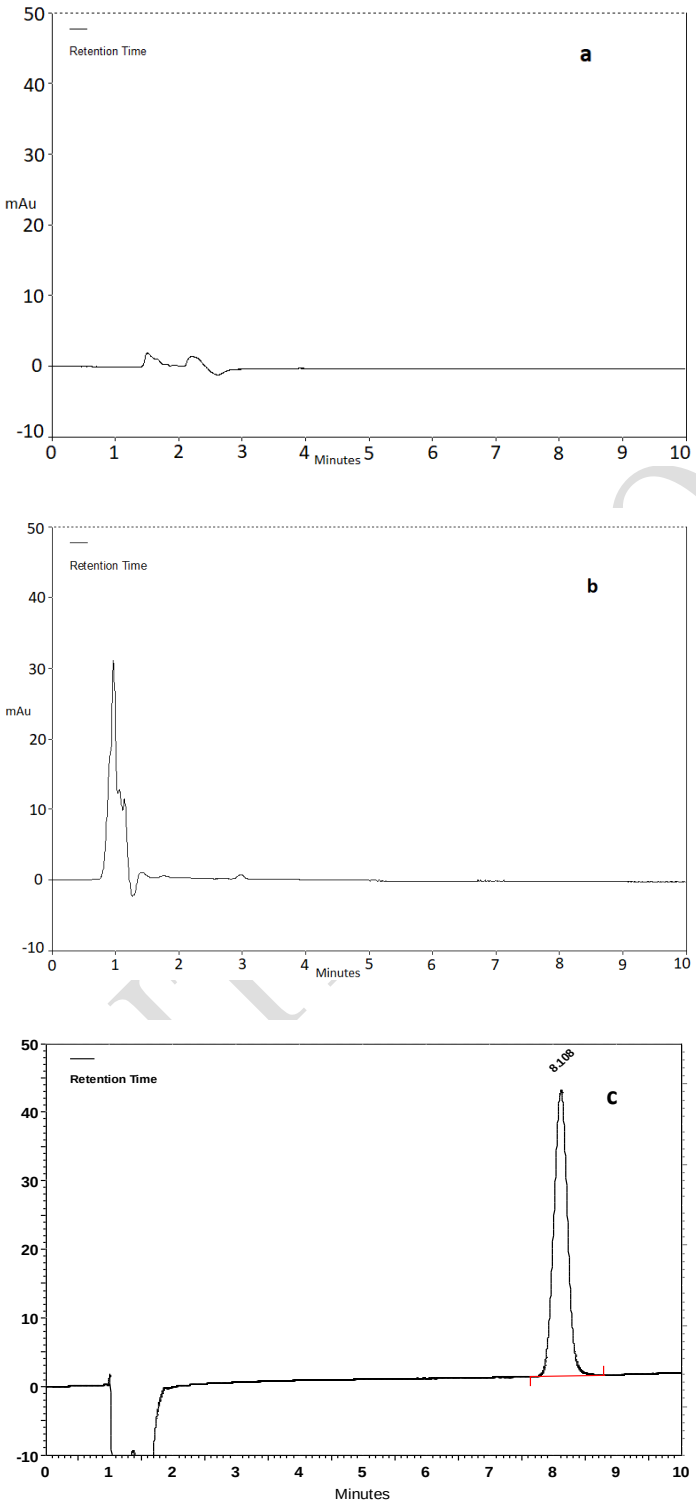

Fig. 1. HPLC chromatograms of: a) mobile phase (diluent); b) blank; c) standard solution of sibutramine (30 mg/L).

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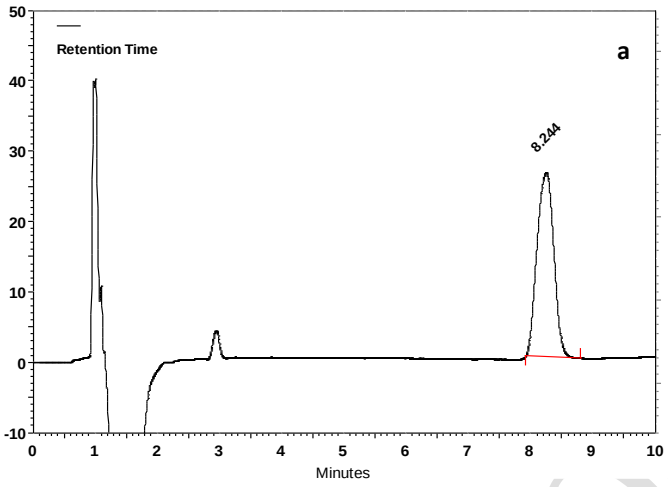

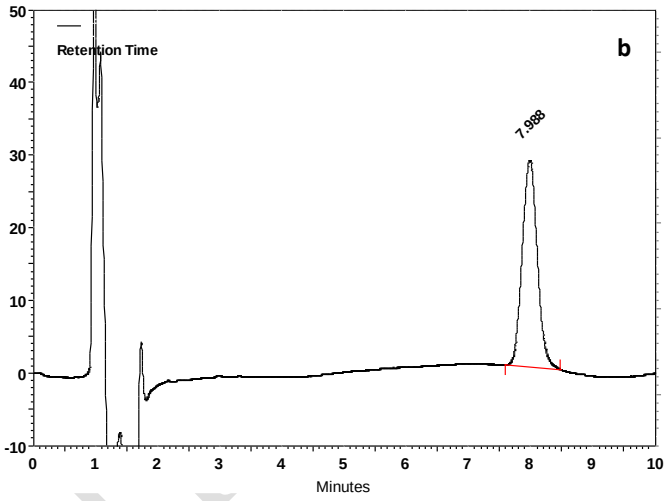

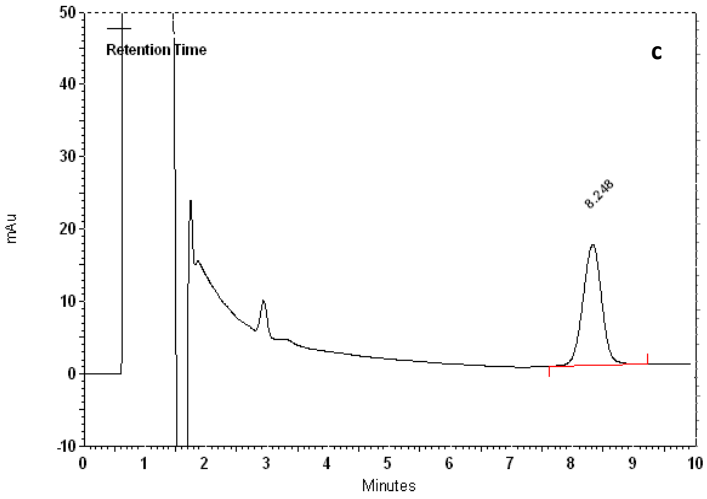

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Fig. 2. HPLC chromatograms of sibutramine-adulterated samples: a) Sample 1 containing 2.474 mg/g sibutramine; b) Sample 2 containing 2.511 mg/g sibutramine; and c) Sample 3 containing 1.684 mg/g sibutramine.

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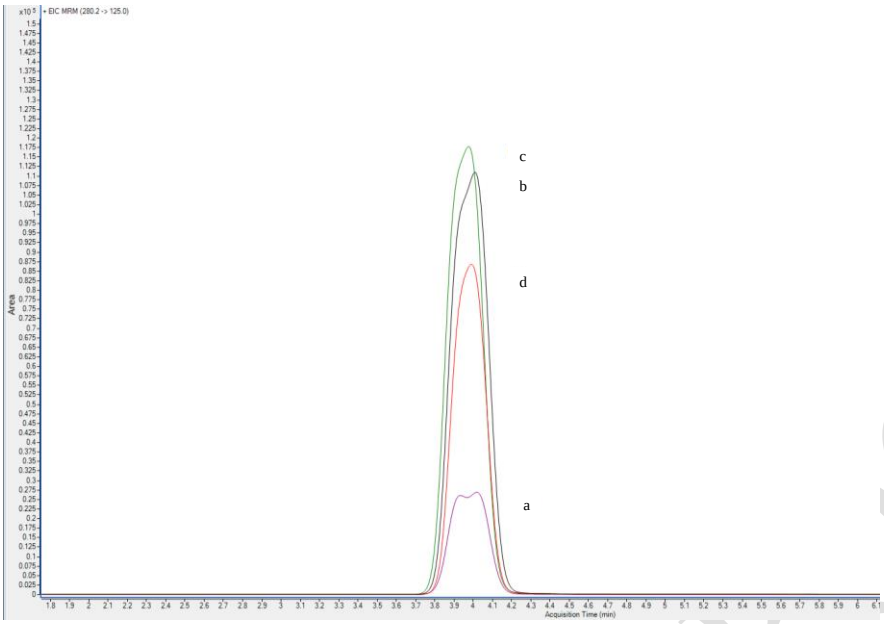

Figure 3. Multiple reaction monitoring (MRM) ion chromatogram, with the sibutramine precursor ion at *m/z* 280.2 and the product ion at *m/z* 125.0: a) Sibutramine standard, b) Sample 1, c) Sample 2, d) Sample 3.

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