

Sibutramine in slimming food supplements on the Croatian market determined by validated high-pressure liquid chromatography-electrospray tandem mass spectrometry method

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Summary

People are trying to solve their obesity problem with easy solutions like using herbal products. The most dangerous are those to which medicines, like sibutramine, are added, since patient believes that the supplement is mostly herbal-based and thus safe for use. Our study aimed at helping to control sibutramine in slimming food supplements on the Croatian market. Analysis of sibutramine in such products was done by using high-pressure liquid chromatography-electrospray tandem mass spectrometry. For sibutramine, and *N,N*-didesmethylsibutramine as an internal standard, mass spectrometry detection was performed in positive mode using Multiple Reaction Monitoring. The drugs were isolated using simple methanol extraction and were separated using reversed-phase liquid chromatography on a Zorbax SB C₁₈ column (150 mm × 2.1 mm, particle size 3.5 μm; Agilent Technologies, Santa Clara, California, USA) with a gradient elution using 0.1% formic acid in acetonitrile and 0.1% formic acid in water as a mobile phase at a flow rate of 0.2 ml·min⁻¹. The study showed that one-fifth of the samples tested over a period of six years contained sibutramine. Regular market control using the described method could prevent health risk to population.

Keywords

sibutramine; food supplements; liquid chromatography-electrospray tandem mass spectrometry

Obesity is a complexed condition, with serious social and psychological dimensions, that affects virtually all age and socioeconomic groups and threatens to overwhelm both developed and developing countries. In 1995, there were an estimated 200 million of obese adults worldwide and another 18 million under-five children classified as overweight. As of 2000, the number of obese adults has increased to over 300 million. Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Overweight and obesity, as well as their related non-communicable diseases, are largely preventable [1]. A drug therapy might be considered if obesity persists despite diet, physical exercise and life-style modification. Drugs used as weight loss aid can be divided

into two categories, based on their mechanisms of action: appetite suppressants and lipase inhibitors [2].

Sibutramine (sibutramine hydrochloride monohydrate, SIB) and its metabolites (mono-desmethylsibutramine and di-desmethylsibutramine; both are more pharmacologically active than sibutramine) share noradrenalin and serotonin reuptake inhibition and secondary receptor modification effects, such as down-regulation of β-adrenoceptors that were common among effective antidepressants of the time. Increasing the levels of noradrenalin and serotonin in the brain helps patients to feel full after eating and reduce their food intake [3, 4]. Based on the data from the sibutramine cardiovascular outcomes trial [5] in 2010, SIB was removed from European mar-

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ket. SIB demonstrated an increased risk of non-fatal cardiovascular events, such as stroke or heart attack. Therefore, the Agency's Committee for Medicinal Products for human use has concluded that any benefit brought by the use of SIB does not compensate the risks imposed to patients [6]. By 2011, SIB was withdrawn from all major markets globally. Slimming products are one of the most sold over-the-counter and food supplement products in most European countries connected with the increasing problem of obesity as well as culture promoting "ideal body figure". The most dangerous are the food supplements to which medicines, like SIB, are added as adulterants, since the patient believes he or she is taking a supplement that is mostly herbal-based and thus safe for use [7].

European Pharmacopoeia does not indicate an official method for quantification of SIB, but a few methods have been described in the literature using high performance liquid chromatography with UV photometric [8] and mass spectrometric detection [9, 10], capillary electrophoresis with UV detection [11], X-ray powder diffractometry and liquid chromatography [12], high performance liquid chromatography and high performance thin layer chromatography methods [13], as well as thin layer chromatographic image analysis method [14].

Since the number of slimming products increases, it is important, for regulatory purposes, to check these products for their quality and composition in order to evaluate population health risk. Therefore, in present work, a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for SIB determination was developed and validated. Additionally, the method was applied to analyse various slimming products on the Croatian market, which were categorized as weight loss dietary supplements.

MATERIAL AND METHODS

Materials studied

One hundred and twenty-three slimming products categorized as herbal food supplements (green tea, coffee, chocolate powder, tablets, capsules and various syrups) were collected on the Croatian market in the period from 2009 till 2014 by sanitary inspection (59%) and private distributors (41%). These herbal supplements were sampled in 2009 ($n = 10$), 2010 ($n = 39$), 2011 ($n = 10$), 2012 ($n = 23$), 2013 ($n = 16$) and 2014 ($n = 25$). Collected samples consisted mainly of

capsules, tablets and sachets with powder. They were analysed immediately after arrival to the laboratory.

Preparation of samples and standard solutions

Analytical standards sibutramine hydrochloride monohydrate (SIB) *N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N,N'*-dimethylamine hydrochloride was from Abbott Laboratories (Irving, Texas, USA) and *N,N*-didesmethylsibutramine hydrochloride (DDSIB) was from LGC standard (Wesel, Germany). Methanol and acetonitrile were purchased from J. T. Baker (Deventer, The Netherlands), both of purity suitable for liquid chromatography-mass spectrometry.

A SIB standard stock solution was prepared by dissolving solid SIB in methanol and this solution was further diluted with methanol obtaining working standard solutions of 1, 5, 10, 25, 50 and 100 ng·ml⁻¹, respectively. An internal standard of DDSIB at constant mass concentration of 10 ng·ml⁻¹ was used in all standard solutions and samples. All solutions were stored in a cool (at approx. 4 °C), dark place when not in use.

Calibration curve at six concentration levels was prepared by injecting 10 µl SIB standard solutions at the following concentrations: 1, 5, 10, 25, 50 and 100 ng·ml⁻¹ with constant concentration of internal standard, 10 ng·ml⁻¹ DDSIB. A calibration curve with standards was made every day. SIB was quantified by a calibration curve constructed by plotting peak area ratios (SIB/DDSIB) versus SIB concentrations.

The drug, sibutramine, was isolated from herbal remedies using simple methanol (liquid chromatography-mass spectrometry grade, J. T. Baker) extraction. Since there was no information about the content of SIB and/or its analogues in analysed samples, the sample was weighed into a 100 ml Erlenmeyer flask, and then internal standard DDSIB was added into 30 ml of methanol to obtain final concentration of 10 ng·ml⁻¹. One dosage unit of capsules, tablets or sachets with powder was always analysed. For capsules, the husks were removed first. The tablet contents were placed in a mortar and rubbed into fine powder. All the fine powder was transferred into a vessel and extracted with methanol by hand shaking for 1 min and centrifuged for 15 min at 4600 ×g. Before injection, supernatants were filtered through a polytetrafluoroethylene membrane filter (pore size, 0.2 µm) into an autosampler vial.

Equipment and LC-ESI-MS/MS conditions

Liquid chromatography (LC) analyses were

performed on a Zorbax SB C₁₈ narrow bore column (150 mm × 2.1 mm, 3.5 μm; Agilent Technologies, Santa Clara, California, USA) using an Finnigan Surveyor (Thermo Electron, San Jose, California, USA) series liquid chromatograph equipped with a quaternary pump and autosampler. Data acquisition and quantification were conducted using Xcalibur software version 2.4 (Thermo Electron). The column was thermostated at 40 °C. Chromatographic separation was performed using gradient elution with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) starting with a ratio of 80:20 (v/v); then at 1–10 min, 60% decreased to 10% of A; at 10–11 min stayed at 10% A; and then at 11–15 min at 60%. The flow was set at 0.2 ml·min⁻¹, column temperature was 40 °C, and the injection volume was 10 μl. Scan time for each transition reaction was 500 ms with scan width of $m/z = 1.0$.

Mass spectrometry (MS) analyses were performed on a Finnigan TSQ Quantum Ultra EMR triple stage quadrupole mass spectrometer (Thermo Electron) equipped with a heated-electrospray interface. The electrospray capillary temperature was 350 °C and the capillary voltage was 4500 V. Argon was used as a collision gas and nitrogen was used as sheath and auxiliary gas. MS detection was performed in positive mode using Multiple Reaction Monitoring (MRM). SIB monitoring ion was $m/z = 280.2$, and product ion was $m/z = 125.0$. For DDSIB, monitoring ion was $m/z = 252.4$ and product ion was $m/z = 125.0$.

RESULTS AND DISCUSSION

The purpose of slimming food supplements control was to determine the sibutramine presence in these products placed on the Croatian market in order to warn consumers on potential risk of serious cardiovascular diseases. This is the reason why the LC-MS/MS method was developed and validated. Based on different papers describing the analytical methods for detection and quantification of the adulterant, sibutramine drug, in slimming products [8–14], LC-MS/MS method was chosen because of its selectivity, qualitative, quantitative and confirmative performances.

A MRM procedure was applied. For SIB and DDSIB, one transition was monitored, from parent ion $m/z = 280.2$ to daughter ion $m/z = 125$ at retention time of 7.15 min; and from parent ion $m/z = 252.4$ to daughter ion $m/z = 125$ at retention time 6.14 min. Method validation was performed using both standard solution and samples of spiked food supplements.

Selectivity of the method was checked by the preparation and analysis of blank and spiked food supplements samples to verify the absence of potential interfering compounds in the sample. No interference was observed around SIB and DDSIB retention times in samples. Figs. 1A–1C show LC-ESI-MS/MS chromatograms of coffee extract in MRM mode of a blank, of a spiked coffee extract and of an appropriate standard solution (10 ng·ml⁻¹).

The limit of detection (LOD) and limit of quantification (LOQ) were estimated by injecting decreasing concentrations of matrix-matched calibrators and measuring the response at a signal-to-noise (S/N) ratio of ≥ 3 and ≥ 10 for LOD and LOQ, respectively. Using this approach, LOD was 1 ng·ml⁻¹ and LOQ was 5 ng·ml⁻¹. The internal standard-based quantification method was used for SIB. In this study, we chose to use DDSIB as an internal standard, which was accompanied by matrix-matched calibrators to minimize possible matrix effects. Good linear relationship ($r^2 > 0.998$) was achieved over the working range of 1–1000 ng·ml⁻¹, which corresponds to a content

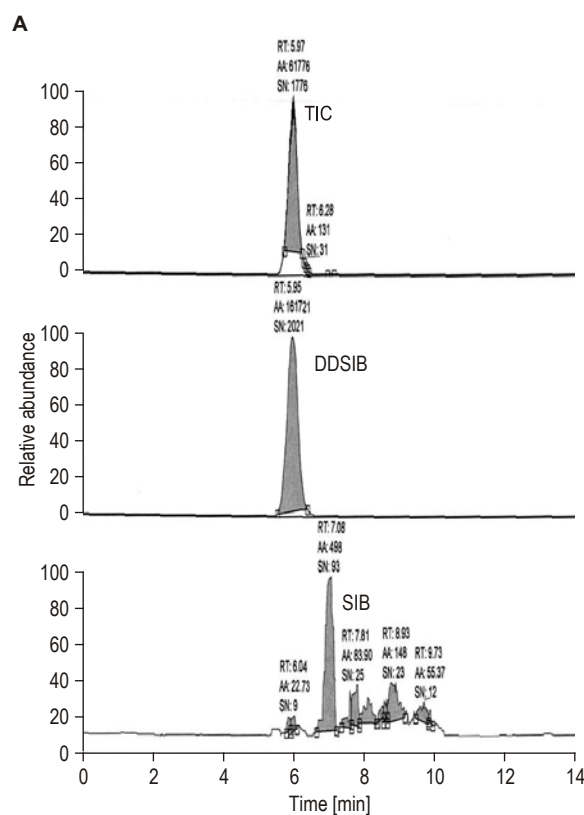


Fig. 1A. LC-ESI-MS/MS chromatogram of blank coffee extract.

TIC – total ion current, SIB – sibutramine, DDSIB – *N,N*-didesmethylsibutramine used as an internal standard.

of 1–100 μg SIB per kilogram of test sample. This was satisfactory for SIB analysis in herbal slimming food supplements.

In order to analyse the recovery of the extraction procedure, known amounts of standard solution of SIB were added to the tested slimming product sample (3 concentration levels in triplicate) for coffee and for capsule samples. Mean value for the capsule slimming sample was 82.9 % with the relative standard deviation (*RSD*) of 4.1 %, while for the green coffee it was 114.7 % with *RSD* of 9.8 %. Furthermore, robustness of extraction was also tested by spiking of capsule samples in six replicates of each slimming product using filtration at once, after 30 min and then after 120 min. Results showed good stability of the sample with mean recovery of 86.0 % with *RSD* of 5.4 %. *RSD* value decreased by prolonged time of stabilization.

Stability of prepared capsule samples spiked at three concentration levels was also investigated. The analysis was performed on the same day as extraction and results were compared with those obtained after 30 days of keeping solutions in the at

approx. 4 °C. Mean recovery was 85.0 % with *RSD* of 6.2 % on the same day of analysis and, after 30 days, recovery was 86.9 % with *RSD* of 2.7 %. These results confirmed stability of the samples at keeping in the dark at approx. 4 °C.

The validated LC-MS/MS method was used for routine analysis of SIB in 123 slimming products categorized as herbal food supplements (green tea, coffee, chocolate powder, tablets, capsules and various syrups) obtained from the Croatian market in the period from 2009 till 2014. (Tab. 1, Tab. 2). Results showed that 22.0 % of samples were non-compliant with declaration, containing 0.014 $\text{mg}\cdot\text{kg}^{-1}$ SIB or more. Most of non-compliant samples were found in 2009 and 2010, and the number of non-compliant samples decreased over following years (Tab. 1). After 2010, when SIB was forbidden as active substance in medical slimming products, the percentage decreased gradually down to 11.4 %. Similar results were obtained for products on the Dutch market during the period 2004–2013, and for the South Korean market during the period 2009–2012, 34 % and 26 %, respectively [15, 16]. Additionally, SIB

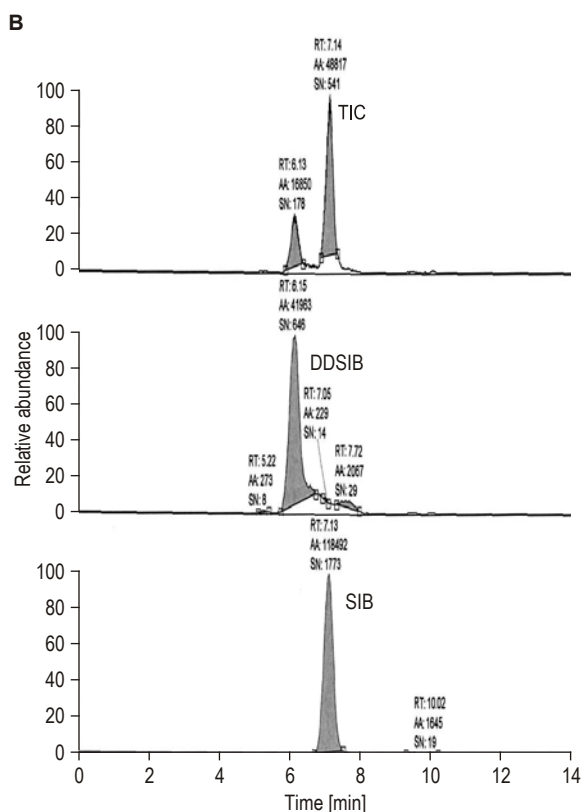


Fig. 1B. LC-ESI-MS/MS chromatogram of spiked coffee extract.

TIC – total ion current, SIB – sibutramine, DDSIB – *N,N*-didesmethylsibutramine used as an internal standard.

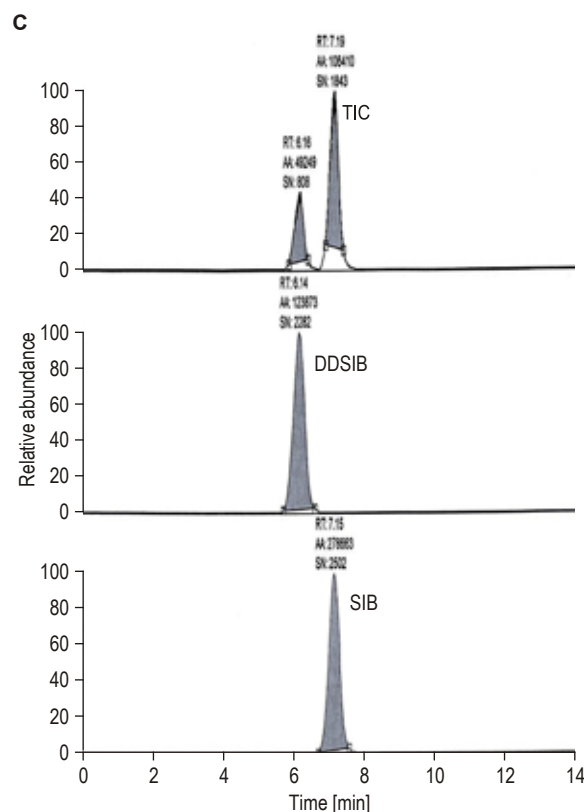


Fig. 1C. LC-ESI-MS/MS chromatogram of standard solution 10 $\text{ng}\cdot\text{ml}^{-1}$.

TIC – total ion current, SIB – sibutramine, DDSIB – *N,N*-didesmethylsibutramine used as an internal standard.

Tab. 1. Slimming food supplements analysed for sibutramine in a six-years period.

Year	Number of samples			Percentage of non-compliant samples [%]
	Total	Compliant	Non-compliant	
2009	10	6	4	40.0
2010	39	24	15	38.5
2011	10	9	1	10.0
2012	23	21	2	8.7
2013	16	13	3	18.8
2014	25	23	2	8.0
Total	123	96	27	22.0

and SIB analogues were the most frequently detected active pharmaceutical ingredients (representing 34%) in a joint market surveillance study focused on slimming dietary supplements performed by the Official Medicines Control Laboratories Network in 2012 [17]. The number of non-compliant samples increased to 18.8% in 2013, but levels of detected SIB were much lower than the highest values in 2009 and 2010. SIB was detected in the range from less than 0.010 mg·kg⁻¹ to 26410 mg·kg⁻¹, with 0.010 mg·kg⁻¹ being taken as the threshold of compliance (Tab. 2). The most contaminated groups of products were green coffee, capsules and tablets. SIB contents in green coffee samples were 0.014–2438 mg·kg⁻¹, while the highest content of SIB was found in one capsule sample in 2009 (26410 mg·kg⁻¹).

The results of this study demonstrate a real problem concerning the safety of slimming food supplements and herbal products. A portion of 22.0% of the analysed preparations declared as “natural and herbal” did not comply with dec-

laration, because they contained forbidden synthetic chemical, sibutramine, and thus could cause damage to health. Percentage of non-compliant samples ranged from 0%, in chocolate and tea, to 37.5% in green coffee (Tab. 2). All non-compliant slimming products originated from Asia and none was from European countries. Although a decreasing trend in the percentage of non-compliant products was noted also by European Rapid Alert System for Food and Feed [18], the number of slimming products ordered online is constantly increasing on European market [19]. The study of MATHON et al. [20] showed that, during two surveys, half of 52 weight loss supplements bought online were adulterated with SIB. Generally, it is important for regulatory purposes to check all these products for their quality and composition in order to prevent consumption of preparations of unknown or doubtful origin, which are dangerous for human health or even life-threatening. Any distribution of such products (of unknown origin or dubious quality) should be strictly forbidden.

The problem with SIB is not only that it was withdrawn from European market [6], but that it was also forbidden in slimming food supplements in EU [21] and also by Croatian national regulation [22]. The herbal food supplements in which SIB was found were not properly declared. They were contaminated and/or adulterated intentionally, to improve their effect on weight loss. Besides of the reasons why SIB was withdrawn, unknown substances contained in so called harmless products could affect health because of serious drug-drug and drug-herbal interactions. It is known that SIB acts like a prodrug with even more active metabolites (mono-desmethysibutramine and di-desmethysibutramine). Biochemical metabolism involves cytochrome P450 enzymes, CYP3A4, CYP2B6, CYP3A5 and CYP2C19

Tab. 2. Type and number of analysed slimming food supplements and range of determined sibutramine.

Samples	Number of samples			Percentage of non-compliant samples [%]	Sibutramine in non-compliant samples [mg·kg ⁻¹]
	Total	Compliant	Non-compliant		
Green coffee	32	20	12	37.5	0.014–2438
Tea	6	6	0	0.0	–
Chocolate powder	4	4	0	0.0	–
Tablets and capsules	59	48	11	18.6	0.400–26410
Syrup	11	10	1	9.1	0.250
Other	11	8	3	27.3	0.660–815
Total	123	96	27	22.0	

Other samples: gel capsules, chewing chocolate cubes, powder for preparing slimming drinks of various tastes and food supplement powder for sportsmen.

[4, 23] that are also parts of oxidative metabolism of more than 50% of all prescription medications and also some herbs [24]. Co-administration could lead to drug toxicity or no effective therapy, depending on the type of interaction. Also, content of SIB as unknown constituent in a slimming product could increase and worsen its effect [25, 26]. Moderate increase of SIB was caused by inhibitors of CYP3A4 such as cimetidine, ketoconazole, erythromycin, simvastatin, omeprazole and cyclosporine [25, 26]. The most commonly prescribed antidepressants, selective serotonin reuptake inhibitors (SSRIs) [27], when taken with SIB, cause the serious serotonin syndrome [25, 26]. The same happened with one of the top 10 best-selling herbs, St John's wort (*Hypericum perforatum*), that is typically used to treat depression [24, 25]. Co-administration of α_2 adrenergic blockers, such as the herb yohimbine (used for sexual dysfunction), with SIB has been recognized as potentially life-threatening due to potential sympathetic side effects resulting in hypertension and tachycardia [25]. Additionally, grapefruit juice that is used as a dietary intervention to lose weight and improve cardiovascular health, inactivates CYP3A4 and reduces SIB metabolism, increases bioavailability and thus its effect [24, 25]. Regular market control of slimming food supplements on SIB, for example by the method for its identification and quantification described in our study, could prevent population health risk.

CONCLUSION

The study showed that one-fifth of herbal slimming food supplements believed to be „healthy and harmless,, were contaminated or adulterated with active substance SIB, which was withdrawn from the market due to an associated risk of serious cardiovascular events. In order to prevent health risk by taking undeclared SIB in such products, which could also interact with other herbs and prescribed drugs, LC-ESI-MS/MS method was validated and performed in routine laboratory work. To the best of our knowledge, this was done for the first time with samples from the Croatian market.

REFERENCES

1. Global status report on noncommunicable diseases 2014. Geneva: World Health Organization, 2014. ISBN: 9789241564854.
2. Snow, V. – Barry, P. – Fitterman, N. – Qaseem, A. – Weiss K.: Pharmacologic and surgical management of obesity in primary care: a clinical practice guideline from the American College of Physicians. *Annals of Internal Medicine*, 142, 2005, pp. 525–531. DOI: 10.7326/0003-4819-142-7-200504050-00011.
3. Luque, C. A. – Rey, J. A.: The discovery and status of sibutramine as an anti-obesity drug. *European Journal of Pharmacology*, 440, 2002, pp. 119–128. DOI: 10.1016/S0014-2999(02)01423-1.
4. Hwang, I. C. – Park, J. Y. – Ahn, H. Y. – Kim, K. K. – Suh, H. S. – Ko, K. D. – Kim, K. A.: Effects of CYP3A5, CYP2C19, and CYP2B6 on the clinical efficacy and adverse outcomes of sibutramine therapy: a crucial role for the CYP2B6*6 allele. *Clinica Chimica Acta*, 428, 2014, pp. 77–81. DOI: 10.1016/j.cca.2013.11.007.
5. Caterson, I. D. – Finer, N. – Coutinho, W. – Van Gaal, L. F. – Maggioni, A. P. – Torp-Pedersen, C. – Sharma, A. M. – Legler, U. F. – Shepherd, G. M. – Rode, R. A. – Perdok, R. J. – Renz, C. L. – James, W. P. T. – and on behalf of the SCOUT Investigators: Maintained intentional weight loss reduces cardiovascular outcomes: results from the sibutramine cardiovascular OUTcomes (SCOUT) trial. *Diabetes, Obesity and Metabolism*, 14, 2012, pp. 523–530. DOI: 10.1111/j.1463-1326.2011.01554.x.
6. EMA/808179/2009, EMEA/H/A-107/1256: Questions and answers on the suspension of medicines containing sibutramine : Outcome of a procedure under Article 107 of Directive 2001/83/EC. London: European Medicines Agency, 2010. <<http://www.nhs.uk/news/2010/01January/Documents/Sibutramine-QA.pdf>>
7. Tang, M. H. Y. – Chen, S. P. L. – Ng, S. W. – Chan, A. Y. W. – Mak, T. W. L.: Case series on a diversity of illicit weight-reducing agents: from the well known to the unexpected. *British Journal of Clinical Pharmacology*, 71, 2011, pp. 250–253. DOI: 10.1111/j.1365-2125.2010.03822.x.
8. Diefenbach, I. C. F. – Friedrich, M. – Santos, M. R. – Bittencourt, C. F.: Development and validation of a column high-performance liquid chromatographic method for determination of sibutramine in capsules. *Journal of AOAC International*, 92, 2009, pp. 148–151. ISSN: 1060-3271.
9. Deconinck, E. – Verlinde, K. – Courselle, P. – de Beer, J. O.: A validated ultra high pressure liquid chromatographic method for the characterisation of confiscated illegal slimming products containing anorexics. *Journal of Pharmaceutical and Biomedical Analysis*, 59, 2012, pp. 38–43. DOI: 10.1016/j.jpba.2011.09.036.
10. Dunn, J. D. – Gryniewicz-Ruzicka, C. M. – Kauffman, J. F. – Westenberger, B. J. – Buhse, L. F.: Using a portable ion mobility spectrometer to screen dietary supplements for sibutramine. *Journal of Pharmaceutical and Biomedical Analysis*, 54, 2011, pp. 469–474. DOI: 10.1016/j.jpba.2010.09.017.
11. Moreira, A. P. – Martini, M. – de Carvalho L. M.: Capillary electrophoretic methods for the screening and determination of pharmacologic adulterants in herbal-based pharmaceutical formulations. *Electrophoresis*, 35, 2014, pp. 3212–3230. DOI:

- 10.1002/elps.201400059.
12. Stypułkowska, K. – Błazewicz, A. – Maurin, J. – Sarna, K. – Fijałek, Z.: X-ray powder diffractometry and liquid chromatography studies of sibutramine and its analogues content in herbal dietary supplements. *Journal of Pharmaceutical and Biomedical Analysis*, 56, 2011, pp. 969–975. DOI: 10.1016/j.jpba.2011.08.028.
 13. Ariburnu, E. – Uludag, M. F. – Yalcinkaya, H. – Yesilad, E.: Comparative determination of sibutramine as an adulterant in natural slimming products by HPLC and HPTLC densitometry. *Journal of Pharmaceutical and Biomedical Analysis*, 64–65, 2012, pp. 77–81. DOI: 10.1016/j.jpba.2012.02.004.
 14. Phattanawasin, P. – Sotanaphun, U. – Sukwattanasinit, T. – Akkarawarathorn, J. – Kitchaiya, S.: Quantitative determination of sibutramine in adulterated herbal slimming formulations by TLC-image analysis method. *Forensic Science International*, 219, 2012, pp. 96–100. DOI: 10.1016/j.forsci-int.2011.12.004.
 15. Reeuwijk, N. M. – Venhuis, B. J. – de Kaste, D. – Hoogenboom, R. L. A. P. – Rietjens, I. M. C. M. – Martena M. M.: Active pharmaceutical ingredients in herbal food supplements for weight loss sampled on the Dutch market. *Food Additives and Contaminants, Part A*, 31, 2014, pp. 1783–1793. DOI: 10.1080/19440049.2014.958574.
 16. Kim, H. J. – Lee, J. H. – Park, H. J. – Cho, S. H. – Cho, S. – Kim, W. S.: Monitoring of 29 weight loss compounds in food and dietary supplements by LC-MS/MS. *Food Additives and Contaminants, Part A*, 31, 2014, pp. 777–783. DOI: 10.1080/19440049.2014.888497.
 17. Fijałek, Z.: Market surveillance of suspected illegal products MSSIP001 : Slimming dietary supplements. Strasbourg : European Directorate for the Quality of Medicines & HealthCare, Council of Europe, sine dato. <https://www.edqm.eu/medias/fichiers/study_report_on_slimming_dietary_supplements.pdf>
 18. RASFF for safer food : The Rapid Alert System for Food and Feed : 2014 annual report. Brussels : European Commission – Health and Food Safety, 2015. ISBN: 978-92-79-46909-1. ISSN: 2363-0965. DOI: 10.2875/945461.
 19. Lachenmeier, D.W. – Löbell-Behrends, S. – Böse, W. – Marx, G.: Does European Union food policy privilege the internet market? Suggestions for a specialized regulatory framework, *Food Control*, 30, 2013, pp. 705–713. DOI: 10.1016/j.foodcont.2012.07.034.
 20. Mathon, C. – Ankli, A. – Reich, E. – Bieri, S. – Christen P.: Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC-UV densitometry. *Food Additives and Contaminants, Part A*, 31, 2014, pp. 15–20. DOI: 10.1080/19440049.2013.861934.
 21. Council Directive 2002/46/EC on the approximation of the laws of the Member States relating to food supplements. *Official Journal of the European Union, L 183*, 2002, pp. 51–57. <<http://data.europa.eu/eli/dir/2002/46/oj>>
 22. Pravilnik o dodacima prehrani. Zagreb : Narodne novine Službeni list Republike Hrvatske, 46, 22.4.2011. ISSN: 1333-9273. In Croatian.
 23. Bae, S. K. – Cao, S. – Seo, K. A. – Kim, H. – Kim, M. J. – Shon, J. H. – Liu, K. H. – Zhou, H. H. – Shin, J. G.: Cytochrome P450 2B6 catalyzes the formation of pharmacologically active sibutramine (*N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N,N*-dimethylamine) metabolites in human liver microsomes. *Drug Metabolism and Disposition*, 36, 2008, pp. 1679–1688. DOI: 10.1124/dmd.108.020727.
 24. Tachjian, A. – Maria, V. – Jahangir, A.: Use of herbal products and potential interactions in patients with cardiovascular diseases. *Journal of the American College of Cardiology*, 55, 2010, pp. 515–525. DOI: 10.1016/j.jacc.2009.07.074.
 25. Jordan, M. A.: Interactions with drugs and dietary supplements used for weight loss. In: El-Shemy, H. A. (Ed.): *Drug discovery*. Rijeka : InTech, 2013, pp. 107–155. ISBN: 978-953-51-0906-8, DOI: 10.5772/51145.
 26. Baxter, K. (Ed.): *Stockley's drug interactions - A source book of interactions, their mechanisms, clinical importance and management*. 8th ed. London : Pharmaceutical Press, 2008. ISBN: 978 0 85369 754 1.
 27. Anderson, I. M.: Selective serotonin reuptake inhibitors versus tricyclic antidepressants: a meta-analysis of efficacy and tolerability. *Journal of Affective Disorders*, 58, 2000, pp. 19–36. DOI: 10.1016/S0165-0327(99)00092-0.

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