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DEVELOPMENT OF AN HPLC METHOD FOR SIBUTRAMINE IDENTIFICATION. SEARCH FOR UNDECLARED SIBUTRAMINE IN BIOLOGICALLY ACTIVE ADDITIVES APPETITE SUPPRESSANTS

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In this study an available reversed-phase HPLC method for the identification of sibutramine with UV detection and isocratic elution mode was developed, the evaluation of the method applicability and determination of validation indicators were carried out. It has been shown that the developed method meets the current international requirements. To search for sibutramine, the developed method has been used in some biologically active additives ("Lipocarnit", "Pineapple extract", "Turboslim night" and "Turboslim day").

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***Keywords*:** sibutramine, HPLC analysis, concentration, validation, BAA.

Introduction. Biologically active additives (BAA) have a special place in the world markets of food and healthcare products. It can be concluded from the fact that the volume of the global market for BAA in 2019 reached 123.28 billion US dollars with an annual growth trend of 8.2% [1]. Actively circulating advertisement and the influence of a complete nutrition campaign lead the general public at large to increasingly focus on the use of BAA in their diet. However, to create a seeming efficiency, some BAA manufacturers striving for super profit use prohibited compounds during production, the presence of which in BAA is not mentioned either on the label or in the instructions for use. The detection of undeclared compounds and the prevention of consumption of potentially hazardous BAA is a priority for food and drug circulation regulating organizations and quality control laboratories.

Taking into account the above mentioned, as well as advanced experience of the US FDA and a number of foreign researchers in the detection of undeclared compounds in BAA, it becomes obvious that the study of the composition of BAA on the market, the development of innovative detection methods is not only relevant, but also a necessity from the viewpoint of maintaining the health of the population [2, 3]. Sibutramine is one of these undeclared compounds. People with obesity usually try a complex of remedies that do not often give the expected results. In this case sibutramine is the only choice. However, they do not even realize the dangerous

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consequences of using sibutramine. Sibutramine is prohibited in some European countries, but in Russia it can still be purchased in pharmacies. Sibutramine is the main active agent in some BAA [1–3].

The drug targets those parts of the brain that are responsible for hunger reducing their activity. Such an effect leads to a decrease in appetite and feeling of satiety. The desire to eat decreases.

Sibutramine is an anorexigenic drug that enhances the feeling of satiety. It belongs to the group of psychotropic drugs for the treatment of obesity and is used in the complex therapy of overweight patients. It is used only as a restrictive measure in a hospital under strict medical supervision [2, 4].

Sibutramine is a synthetic drug developed about 25 years ago as an antidepressant by the American company "Abbott Laboratories". Clinical trials have not confirmed its effectiveness, but have shown that it has a strong anorexic (appetite suppressant) effect. In 1997, sibutramine was officially approved by the FDA of the US Department of Health, after which it began to be widely used around the world as a weight-loss drug; sibutramine tablets were prescribed to people of any age and with any ailments [1].

However, the highly effective new drug was soon found to have a number of extremely dangerous side effects. Sibutramine causes depression, such as drug addiction. It also increases the risk of developing cardiovascular disease, as evidenced by the increased mortality rate of heart attack and stroke among patients taking this drug for losing weight [5].

In-depth research by independent medical experts confirmed the danger of this drug and it was confiscated from the market by the FDA after 2010 due to the "risk of side effects" [6].

Sibutramine is prohibited in most European countries. The reason for prohibition was the decision of the European Medicines Agency (EMEA) of 21.01.2010 to suspend the licensing and sale of sibutramine-containing drugs in the EU, as the risks assessed during the use of these drugs outweigh the results. Many countries, including the Ukraine, imposed sanctions after the EU countries [2, 6, 7].

Since 24.01.2008, sibutramine has been included in the list of highly active drugs approved by the Government of the Russian Federation. This means that the sale of sibutramine-containing drugs in pharmacies is allowed only under a special prescription and is subject to special registration [4].

Sibutramine is also included in the World Anti-Doping Agency's (2010) list of prohibited compounds [2].

Based on the above mentioned, the aim of this work was to develop an HPLC method for quantitative and qualitative identification of sibutramine, for which the following tasks have been planned to be implemented:

- development of chromatographic conditions for the HPLC method and optimization for sibutramine model solution;
- implementation of the method expertise according to some indicators (selectivity, stability, linearity, accuracy, precision, determination of quantitative and qualitative identification limits);
- implementation of qualitative identification of undeclared sibutramine in some BAA sold in pharmacies by the HPLC method.

Experimental Part.

Equipment. The chromatographic method for sibutramine model solution was developed using reversed-phase high performance liquid chromatography (HPLC) Waters Separations module 2695 (USA). Altima C18, 5u, 250×4.6 mm^2 was used as a chromatographic column. Prior to the research, eluents and samples were prepared in accordance with the requirements of the HPLC method.

Preparation of the Eluent. The eluent used for the development of reversed-phase HPLC method was methanol and 0.1% aqueous solution of trifluoroacetic acid, in a ratio of 80:20, pH 3.5. For the preparation of the buffer, 1 *mL* of trifluoroacetic acid was added to 1 *L* of deionized water, pH was adjusted to 3.5, stirred well, then filtered through a vacuum filter system with 0.45 μm perforated membrane filters. The filtrate was then transferred to the capacity in an HPLC device intended for eluents.

On the analytical scale (Sartorius cp2p, Italy) standard samples of sibutramine were weighed ranging from 0.4 to 5.0 *mg*.

The weight stuff was transferred to the appropriate HPLC bottles and dissolved in 1 *mL* of the mobile phase; the bottles were kept in ultrasound for 10 *min* and then stirred on a VORTEX core stirrer for 10 *min*. The solution was filtered through PTFE 0.2 μm perforated filters and placed on a platform for samples of the HPLC automatic injection system, which had a certain numbering.

After operating the device and making the appropriate adjustments, completing the necessary data and the parameters in the relevant subsections of Waters Separations module 2695 chromatograph software (Empower), the study was carried out. The chromatographic conditions are presented in Tab. 1.

Table 1

Chromatographic conditions of sibutramine

Chromatographic column	Nautilus-E C18, 5u, 250×4.6 mm^2
Detector wavelength	225 *nm*
Flow rate	1 *mL*/*min*
Injection volume	10 μL
Column temperature	30℃
Pump operating mode	isocratic
Mobile phase	80:20 v/v methanol, 0.1% aqueous solution of trifluoroacetic acid, pH 3.5

To verify the applicability of the developed method, any newly developed method should be subject to expertise and validation in accordance with the requirements of the international guidelines [6–8].

Results and Discussion. In particular, it is necessary to study the following indicators of the method: selectivity, precision, linearity, lower limit of detection, lower limit of quantitation, accuracy.

***Selectivity*.** The selectivity of the analytical method characterizes the ability to accurately identify the target compound in the presence of degradation products and by-products in the sample. One of the permissible indicators of selectivity is the absence of peak superposition or slight superposition, in which the ratio of the surface of the substance to the surface of the target compound is less than 0.2. To evaluate the indicator, a mobile phase injection was performed followed by three injections of the test samples.

Retention time deviation should not exceed 1% and surfaces – 2%. Results of selectivity evaluation are presented in Tab. 2.

Table 2

Results of selectivity evaluation

Sibutramine, *mg*/*mL*	Retention time, *min*	Surface (AU)
Sample 1	3.636	5492999
Sample 2	3.622	5493956
Sample 3	3.606	5509321
Sample 4	3.614	5491987
Sample 5	3.588	5512435
Sample 6	3.592	5489299
Average value	3.609667	5498333
SD	0.018217	9890.37
RSD, %	0.504678	0.179879

The chromatogram of a sibutramine model solution is presented in Fig. 1.

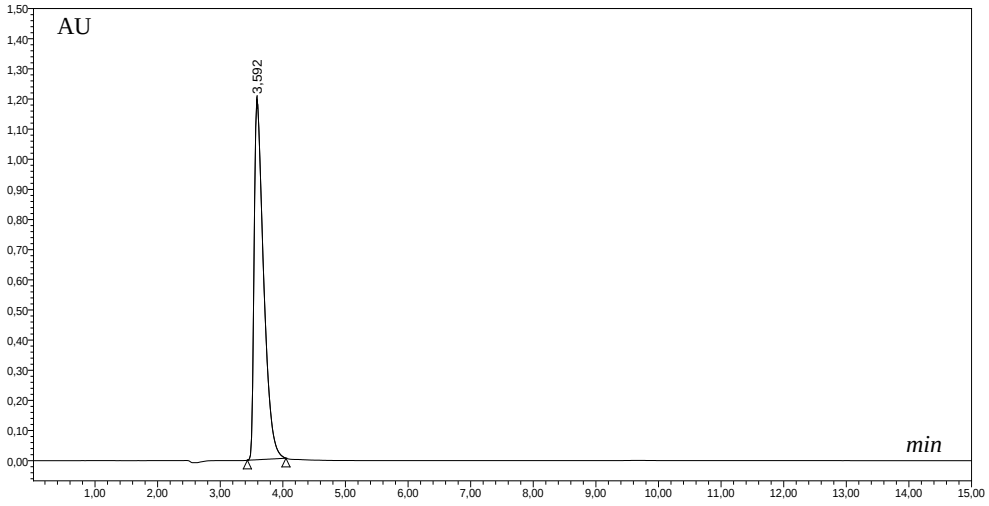

Fig. 1. Chromatogram of a sibutramine model solution.

Precision. In determining the accuracy and precision of the method, the influence of various factors on the stability of the validating method is evaluated. Precision also includes in-laboratory precision, which is characterized by research data obtained on different days and via different devices performed by various specialists. In our case, the study was conducted on two different days.

In the case of in-laboratory precision the relative standard deviation value may not exceed 5%. Results of in-laboratory precision are presented in Tab. 3.

Linearity. The linearity of the analytical method provides a direct relative dependence on the concentration of the target compound and the surface. To test the linearity of the method, solutions with 6 different concentrations were prepared with two injections each. Results of linearity determination are presented in Tab. 4.

Table 3

Results of in-laboratory precision

Sample	Sibutramine	
	day 1	day 2
Sample 1	5492999	5498999
Sample 2	5493956	5410625
Sample 3	5509321	5515489
Sample 4	5491987	5478912
Sample 5	5512435	5564661
Sample 6	5489299	5469899
Average value	5498333	5506431
RSD, %	0.179879	0.6098
Average RSD, %	0.394998	

Table 4

Results of linearity determination

Sample	Sibutramine	
	concentration, *mg*/*mL*	surface (AU)
Calibration 1	0.425	1802653
Calibration 2	1.205	3499818
Calibration 3	2.075	5492999
Calibration 4	3.102	8226256
Calibration 5	4.21	10782788
Calibration 6	5.13	12694406
Correlation coefficient *r*	0.995	

The results of two injections were averaged and based on the obtained results the correlation coefficient was calculated through the Excel Software and a linearity curve was constructed. As it can be seen from the result obtained, the correlation coefficient meets the international requirements. The linearity curve is presented below.

The linearity range is ±20% of the average value of the entire linearity range. The latter does not require additional expertise and the data of the linearity curve can be taken into consideration. The linearity range is considered to be a working range of quantitation. In the case of our method, 2.66 *mg*/*mL* to 2.13–3.2 *mg*/*mL* is considered to be the linearity range.

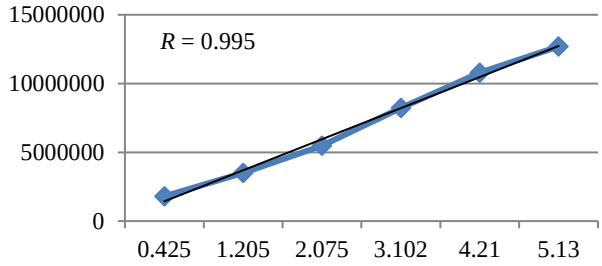

Fig. 2. The linearity curve of sibutramine.

Lower Limit of Detection. Lower Limit of Quantitation. Depending on the sensitivity of the device and method, as well as the properties of the substance, the minimum concentrations for the detection and quantitation of different substances

may vary. The lower limit of detection is the minimum concentration of a substance in the test solution, of which at lower concentrations the substance cannot be detected by this method. The lower limit of quantitation is the minimum concentration of a substance, in the test solution of which at lower concentrations the substance cannot be quantified by this method.

The calculations were performed by the Excel software taking into account the indicators of the linearity curve. The calculations were done by the following equations:

$$LOD = 3.3SD/S, \quad LOQ = 10SD/S,$$

where LOD and LOQ are the lower limits of detection and quantitation, respectively; SD is a standard deviation; S is a component that characterizes the slope of the linearity curve.

The smaller the limits, the greater the sensitivity of the method. Lower limits of detection and quantitation are SD=292751.6; S=2258312; LOD=0.427789 *mg*/*mL*, LOQ=1.296329 *mg*/*mL*.

Accuracy. The accuracy of the method is the difference between the concentration determined from the linearity curve and the previously known concentration in percent. The results are presented in the Tab. 5.

To check the accuracy, solutions of known concentrations in the linearity range were prepared and injected twice from each. The results obtained were placed in the linearity curve and concentrations were calculated by extrapolation according to the method of the linearity curve. The similarity coefficient of the obtained and previously known concentrations was calculated which should not exceed ± 2.5% .

Table 5

Results of accuracy assessment

Standard concentration, *mg*/*mL*	Peak surface	Calculated concentration, *mg*/*mL*	Calculated concentration, %
0.425	1802653	0.434711	102.285
1.205	3499818	1.186231	98.44237
2.075	5492999	2.068828	99.70256
3.102	8226256	3.279138	105.7104
4.21	10782788	4.411192	104.7789
5.13	12694406	5.257673	102.4887
Mean (*n*=6)			102.2347
SD			2.807643

As the results of the method expertise meet the international requirements, we have studied Reduxin capsules by HPLC according to the presented method. The results are presented in the Fig. 3.

The developed method allows the identification of sibutramine in Reduxin. The peak identified on the chromatogram corresponds to the storage time of the sibutramine standard.

Taking into account the risks associated with the use of sibutramine, the presented method was used to assess the presence of sibutramine in BAA sold in pharmacies in Armenian. In particular, “Lipocarnit”, “Pineapple extract”, “Turboslim night” and “Turboslim day” have been studied.

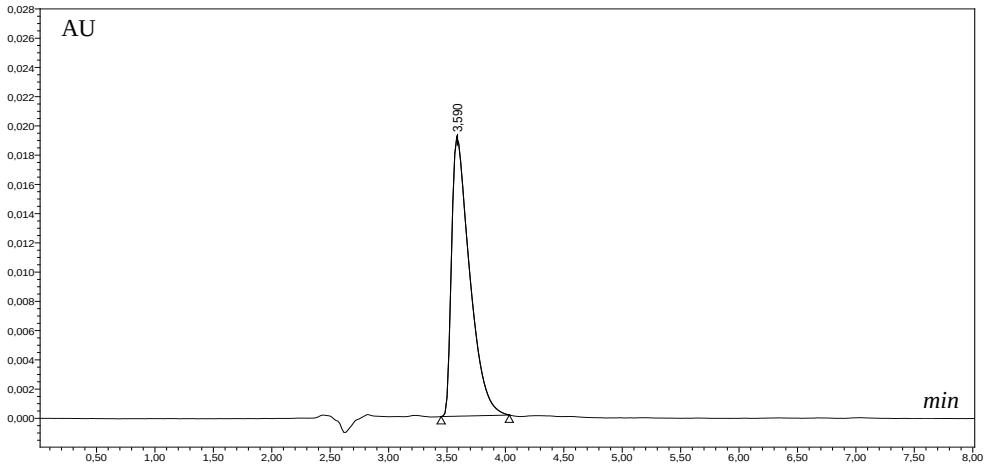

Fig. 3. Chromatogram of “Reduxin” by HPLC.

All products presented by manufacturers are registered as biologically active additives and are used as appetite suppressants and weight-loss drugs.

The HPLC results of the latter are presented below in Fig. 4–7.

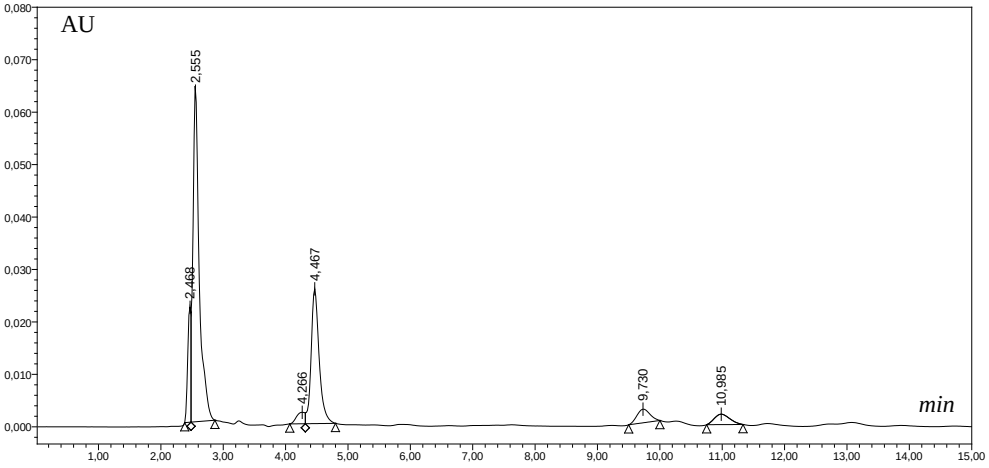

Fig. 4. Chromatogram of “Lipocarnit” sample by HPLC.

Chromatograms for identification of some biologically active substances available in the pharmaceutical market of the Republic of Armenia are shown in Figs 4–7. Further research and analysis of the results obtained in accordance with the developed method confirmed that sibutramine was not found in the studied BAA available in the RA pharmaceutical market.

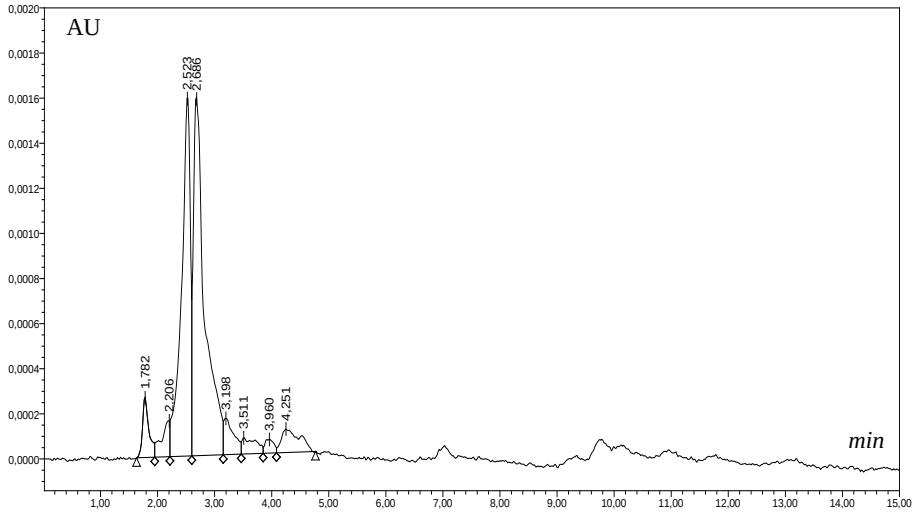

Fig. 5. Chromatogram of “Pineapple extract” sample by HPLC.

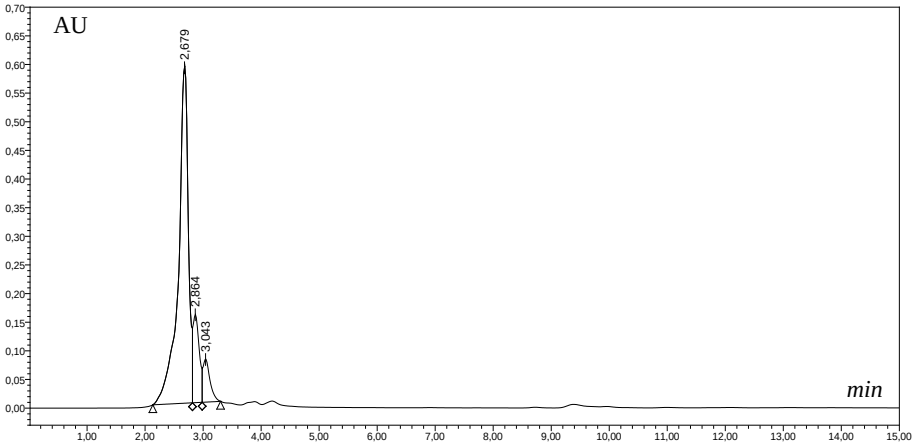

Fig. 6. Chromatogram of “Turboslim night” sample by HPLC.

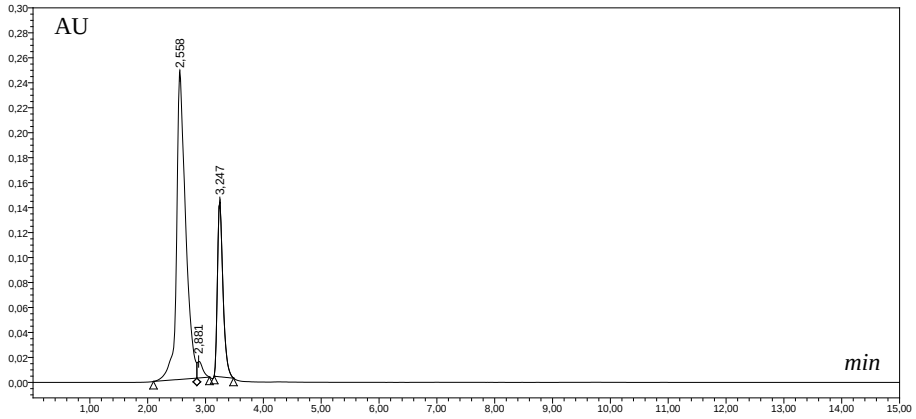

Fig. 7. Chromatogram of “Turboslim day” sample by HPLC.

Conclusion. The applicability of the developed method has been confirmed as a result of proper validation of the requirements of international guidelines. According to the expertise the results meet the international requirements. Further research and analysis of the results obtained in accordance with the developed method confirmed that sibutramine was not found in the studied BAA available in the RA pharmaceutical market.

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Ռ. Ե. ԻՍՐԱՅԵԼՅԱՆ, Տ. Հ. ՍԱՐԳՍՅԱՆ

ՍԻԲՈՒՏՐԱՄԻՆԻ ՆՈՒՅՆԱԿԱՆԱՑՄԱՆ ԲԱՀՔ ՄԵԹՈԴԻ ՄՇԱԿՈՒՄ: ՉՀԱՅՏԱՐԱՐԱԳՐՎԱԾ ՍԻԲՈՒՏՐԱՄԻՆԻ ՈՐՈՆՈՒՄ ԱԽՈՐԺԱԿԸ ԸՆԿՃՈՂ ԿԵՆՍԱԲԱՆՈՐԵՆ ԱԿՏԻՎ ՀԱՎԵԼՈՒՄՆԵՐՈՒՄ

Աշխատանքում մշակվել է սիբուտրամինի մատչելի ՀՖ ԲԱՀՔ նույնականացման մեթոդ ՈւՄ դետեկցմամբ և իզոկրատիկ էլուցման ռեժիմով, իրականացվել է մեթոդի կիրառելիության գնահատում, վալիդացիոն ցուցանիշների որոշում: Ցույց է տրվել, որ մշակված մեթոդը համապատասխանում

է գործող միջազգային պահանջներին, մշակված մեթոդը կիրառվել է որոշ ԿԱՀ-երում (“Липокарнит”, “Экстракт ананас”, “Турбослим ночь” և “Турбослим день”) սիբուտրամինի նույնականացման համար:

Р. Е. ИСРАЕЛЯН, Т. О. САРГСЯН

РАЗРАБОТКА МЕТОДА ВЭЖХ ДЛЯ ИДЕНТИФИКАЦИИ СИБУТРАМИНА. ПОИСК НЕОБЪЯВЛЕННОГО СИБУТРАМИНА В СОСТАВЕ БИОЛОГИЧЕСКИ АКТИВНЫХ ДОБАВОК ДЛЯ ПОДАВЛЕНИЯ АППЕТИТА

В этом исследовании разработан доступный метод обращенно-фазовой высокоэффективной жидкостной хроматографии для идентификации сибутрамина с УФ-детектированием и изократическим режимом элюирования, проведена оценка применимости метода и параметров валидации. Показано, что метод удовлетворяет современным требованиям международных стандартов. Методика использовалась для поиска сибутрамина в некоторых БАД (“Липокарнит”, “Экстракт ананаса”, “Турбослим ночь” и “Турбослим день”).