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DEVELOPMENT AND VALIDATION OF THE METHODOLOGY FOR DETERMINATION OF SIBUTRAMINE IN MEDICINES

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This study is devoted to the development and validation of a method for determination of sibutramine in medicines for their quality control in order to further develop regulatory documentation in the Russian Federation. The extraction solvent and chromatographic separation conditions were selected for the optimal determination of sibutramine in medicines by high performance liquid chromatography (HPLC) using an ultraviolet detector (UV). The specificity, linearity, correctness of the method have been proved, the relative error of a single determination does not exceed 1.5% (0.70%), the calculated value of the Student's test tcalc (2.14) is less than the tabular ttable (2.36), the coefficient of variation (RSD) does not exceed 1% (0.93%), the intermediate precision was assessed, the relative error of which is 0.16%. The detection limit was determined at the level of 0.004 mg/ml; the limit of quantitative determination is 0.1 mg/ml. The methodology is correct, there is no systematic error, therefore, it is suitable for further use and inclusion into the regulatory documents.

Keywords: sibutramine, medicines, HPLC-UV, validation, quality control, regulatory documentation

The development and validation of methods for determining active pharmaceutical ingredient (API) as a part of medicines is essential and necessary for the development and maintenance of the pharmaceutical quality system.

Currently, obesity is a fairly common disease among the population.

The number of obese patients in Russia in 2019 compared to 2018 increased by 15.8% (registered cases in 2018–446,663, in 2019–517,357), which indicates an increase in the incidence rate every year [1].

Sibutramine is a highly effective medicine for the treatment of obesity. In the Russian Federation, studies have been conducted to prove the safety and pharmacological effectiveness of this medicine. According to the results of the six-month program "Vesna" (Spring), 44% of patients got rid of the diagnosis "obesity", also a decrease in glucose, low-density lipoprotein, cholesterol and an increase in high-density lipoprotein levels were found and decrease in blood pressure was noted [2,3]. The PrimaVera study confirmed the positive effect of sibutramine on the dynamics of changes in the body weight of patients (51% of the participants got rid of the diagnosis of "obesity") and the absence of serious side effects and risks associated with taking the medicine [4].

Sibutramine is a norepinephrine and serotonin reuptake inhibitor. The pharmacological effect is due to the formation of metabolites (M1-desmethylsibutramine and M2-didesmethylsibutramine), the half – life of which is 14 h and 16 h, respectively, for sibutramine the half – life is 1.1 h [5,6].

According to IUPAC (International Union for Pure and Applied Chemistry), sibutramine hydrochloride monohydrate-cyclobutane methanamine, 1-(4-chlorophenyl)-N, N-dimethyl-α- (2-methylpropyl)-, hydrochloride, monohydrate, (±)–; (±)– 1-(p-chlorophenyl)-α-isobutyl-N, N-dimethylcyclobutanmethylamine hydrochloride monohydrate (Fig. 1) is a slightly water-soluble powder of white to cream color [7].

To this date, the pharmaceutical market of the Russian Federation presents the following medicines containing sibutramine: Reduxin (capsules: 10; 15 mg of sibutramine hydrochloride + + 158.5 mg; 153.5 mg of microcrystalline cellulose), Reduxin Met (capsules: 10; 15 mg of sibutramine hydrochloride + 158.5 mg; 153.5 mg of microcrystalline cellulose; separately attached tablets of metformin 850 mg), Reduxin Forte (tablets: 10; 15 mg of sibutramine hydrochloride + 850 mg

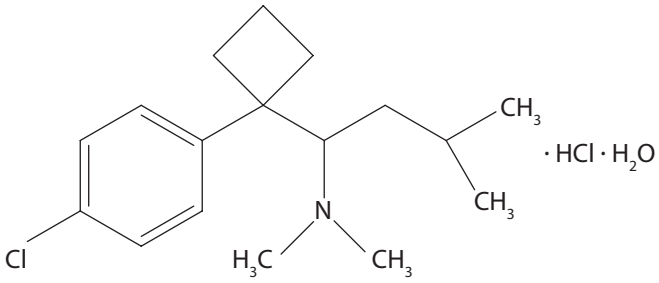

FIG. 1. *Structural formula of sibutramine*

metformin) (Promomed Rus LLC, Russia); Goldline (capsules: 10; 15 mg sibutramine hydrochloride), Goldline Plus (capsules: 10; 15 mg of sibutramine hydrochloride + 158.5 mg; 153.5 mg of microcrystalline cellulose) (Izvarino Pharma, LLC, Russia). These medicines are released strictly according to a doctor's prescription, since sibutramine hydrochloride is included in the List of potent and toxic substances and is subject to strict record keeping and storage [8].

Therefore, it is necessary to develop regulatory documentation for quality control of sibutramine hydrochloride API against the background of medicine components.

The purpose of this study is to develop and validate a method for determining sibutramine in medicinal products by HPLC-UV.

MATERIALS AND METHODS

The objects of the study were a standard sample of sibutramine hydrochloride (Tocris, UK), microcrystalline cellulose (Sigma Aldrich, USA), metformin hydrochloride (Supelco, USA).

RESULTS AND DISCUSSION

The optimal solvent was selected experimentally [9, 10]. Methanol of various concentrations was used as an extraction solvent, and the dependence of the sibutramine peak area on the solvent concentration was studied, as a result of which we

selected anhydrous methanol (J.T. Baker, Poland) as the solvent.

The chromatographic determination of sibutramine was performed using a high-performance liquid chromatograph Agilent 1100 (Agilent Technologies, USA) with a UV detector. A chromatographic column made of stainless steel C18 NUCLEOSIL (Macherry-Nagel, Germany) with dimensions of 4.6 mm × 150 mm and sorbent particles of 5 μ was used as a stationary phase; the temperature of the column thermostat was 40°C; the flow rate is 1 ml/min.

Various mobile phases were tested to obtain the best separations in the shortest possible time in order to optimize the analytical procedure. Mobile phases of acetonitrile buffer (pH 3–7) were tested at different ratios of organic and aqueous components (20–80%). The results that meet the requirements of the regulatory documentation were achieved using the mobile phase components in mixture of 0.05 M formate buffer pH=4.0 (ammonium formate-Honeywell, Germany, formic acid-Sigma-Aldrich, USA) and acetonitrile (PanReac AppliChem, Germany) at the ratio of 40:60 (by volume).

The analytical wavelength was chosen based on the obtained spectrum of methanol (Fig. 2) – 225 nm.

The completeness of sibutramine extraction was determined from the dosage form on model mixtures of the sibutramine substance and microcrystalline cellulose at the ratio corresponding to medicine (10 mg (15 mg) + 158.5 mg).

For the preparation of 9 model mixtures, about 0.010 g of the sibutramine hydrochloride substance (exact sample weight) and 0.159 g of microcrystalline cellulose were placed in measuring flasks with a volume of 10 ml, then, 8 ml of methanol was added, put into an ultrasonic bath for 10 minutes, the volume was brought to the mark, mixed, filtered through a membrane filter with a size of 0.2 microns (nylon). Then 1 ml of the resulting solution was placed into a 10 ml flask, 10 ml of methanol was added, and mixed.

The solution of the Reference Standard (RS) of sibutramine hydrochloride was prepared in a similar way for the model mixtures (the concentration of sibutramine hydrochloride was 0.10 mg/ml).

The method of quantitative determination of sibutramine using the HPLC-UV method was performed according to GPM 1.1.0012.15 SP XIV "Validation of analytical methods".

The specificity was determined based on the resulted chromatograms of the solvent, Reference Standard, placebo (microcrystalline cellulose, metformin), and model mixtures (Fig. 3–7).

The retention time of sibutramine is 5 minutes. Excipients do not interfere with the determination of API, therefore, the method meets the value of the specificity of the analytical method.

The linearity was determined on 9 model mixtures (the content of sibutramine hydrochloride from 80 to 120%) (Fig. 8).

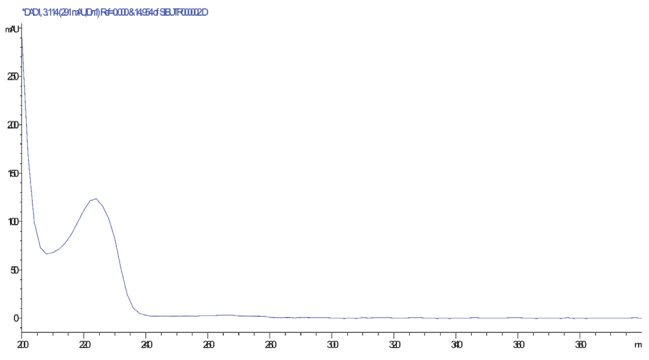

FIG. 2. *Sibutramine spectrum*

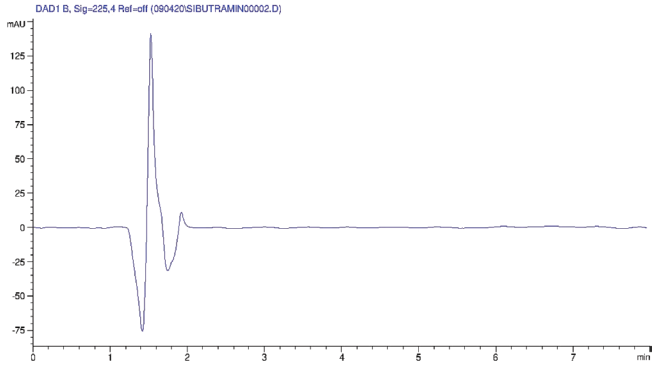

FIG. 3. *Solvent chromatogram*

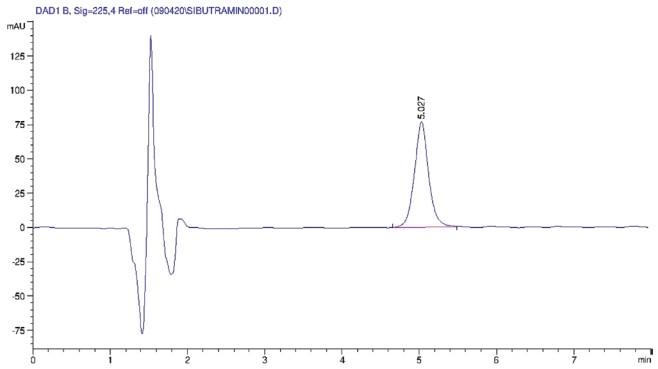

FIG. 4. *Chromatogram of the Reference Standard of sibutramine hydrochloride*

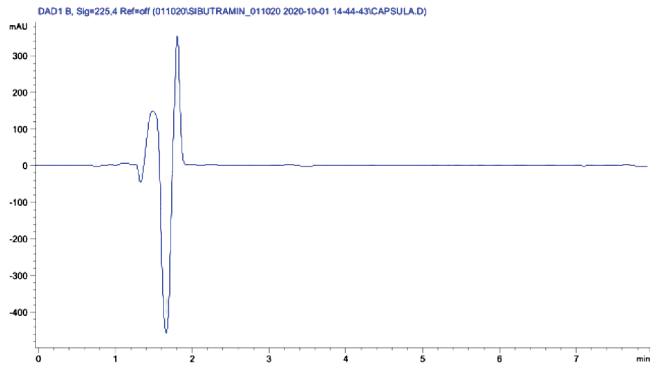

FIG. 5. *Chromatogram of microcrystalline cellulose*

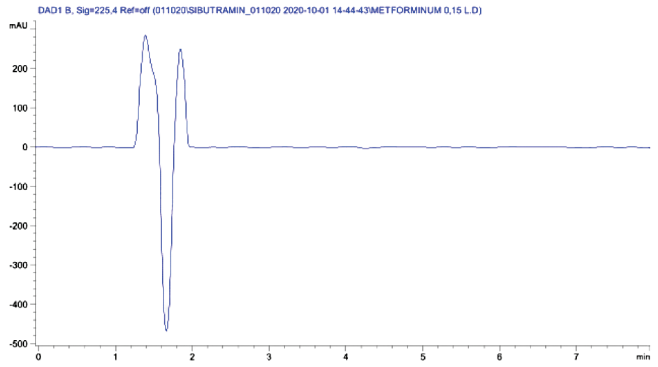

FIG. 6. *Chromatogram of metformin hydrochloride*

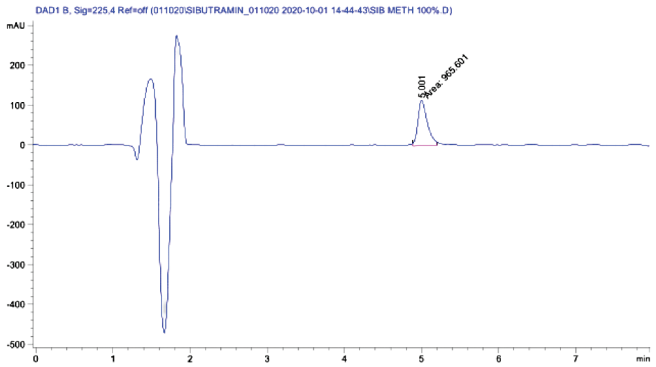

FIG. 7. *Chromatogram of the model mixture*

The value of the correlation coefficient R2 is 0.9992 (more than 0.9950) at y = 9672.9 x – – 18.066 (x – concentration (mg/ml), y – peak area).

The correctness of the method was proved by determining the degree of extraction of sibutramine introduced into the model mixtures.

Table 1 shows that the extraction of sibutramine from the model mixtures under experimental conditions is complete, the relative error of a single determination does not exceed 1.5% (0.70%). The confidence interval $\bar{X} \pm \Delta\bar{X}$ (100.22% ± 0.70%) includes 100%, the calculated value of the Student's test t_{calc}(2.14) is less than the tabular t_{tabl}(2.36), therefore, the method is correct and there is no systematic error.

To assess the *precision (convergence)* of the results, a coefficient of variation (RSD) not exceeding 1% (0.93%) was calculated.

In the study of two solutions prepared similarly to the model mixtures (the mass of microcrystalline cellulose is 0.159 g) by different analytical chemists, the *intermediate precision* was determined. The first chemist had the sample weight of the Reference Standard (RS) of sibutramine equal to 10.0 mg, the second chemist had 10.1 mg. Each

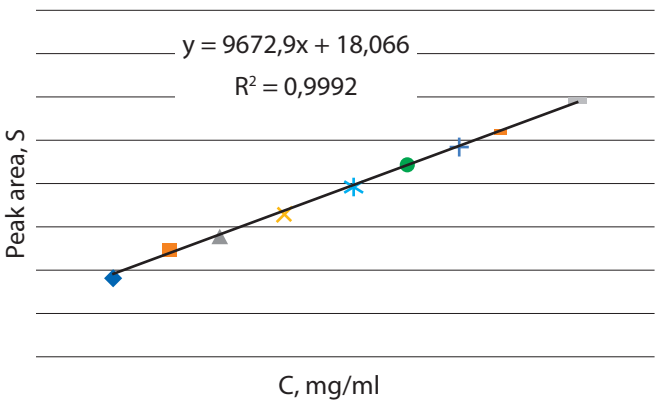

FIG. 8. *Graph of the linear dependence of the peak area (S) on the concentration (C) of the sibutramine solution*

Table 1

RESULTS OF QUANTITATIVE DETERMINATION OF SIBUTRAMINE IN MODEL MIXTURES

No.	Taken, mg/ml (C_1)	Determined, mg/ml (C_2)	Absolute error, mg/ml ($d = C_2 - C_1$)	Relative error, % ($Y = d \times 100/C_1$)	Determined, %	Metrological characteristics (P = 95%, n = 9)
1	0.0800	0.0796	-0.0004	-0.50	99.50	n = 9 $\bar{X} = 100.22$ S = 0.93 $S\bar{x} = 0.31$ $\Delta\bar{X} = 0.70$ $\bar{\varepsilon} = 0.70\%$ $t_{calc} = 2.14$ $t_{tabl} = 2.36$ RSD = 0.93%
2	0.0847	0.0851	0.0004	0.47	100.47	
3	0.0900	0.0893	-0.0007	-0.78	99.22	
4	0.0949	0.0947	-0.0002	-0.21	99.79	
5	0.0999	0.1010	0.0011	1.10	101.10	
6	0.1052	0.1061	0.0009	0.86	100.86	
7	0.1101	0.1105	0.0004	0.36	100.36	
8	0.1151	0.1139	-0.0012	-1.04	98.96	
9	0.1200	0.1221	0.0021	1.75	101.75	

solution was chromatographed 5 times in three repetitions.

The average sibutramine content for 10 measurements is 100.14%, the standard deviation is 0.16%, and the relative standard deviation of RSD is 0.16%. The results obtained meet the acceptance criteria for the *intermediate precision* value (the RSD value should be no more than 3%) for 10 parallel measurements.

The limit of detection of sibutramine using this validated technique is 0.004 mg /ml, the limit of quantification is 0.01 mg/ml.

CONCLUSION

The developed method meets the requirements of GPM 1.1.0012.15 SP XIV "Validation of analytical methods" according to the following parameters: specificity, linearity, correctness, convergence and intermediate precision.

The method is suitable for the determination of sibutramine in medicinal products and can be included in the regulatory documentation for dosage forms (capsules, tablets) containing sibutramine, for quality control of medicines on the pharmaceutical market.

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