Short Communication

VALIDATION OF ASSAY PROCEDURES OF AFOBAZOLEIN MICROCAPSULES

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ABSTRACT

Objective: The aim of this work was focused on a simple, rapid, accurate and sensitive method for quantitative analysis of afobazole in microcapsules (afb-m) using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

Methods: The analytical procedure was based on the fractionation characteristics of the afobazole in the chromatography column. The chromatography parameters «Zorbax extend-C18» column (150×2.1 mm i.d. 5 µm particle size), at 40 °C. The isocratic mobile phase was 5.0 M ammonium phosphate buffer (pH 7.0): acetonitrile (30:70; v/v) at a flow rate of at 1.0 ml. min^{-1}. The determinations were performed using UV-Vis detector set at 220 nm.

Results: An assay procedure for afb-m has been validated from indices, such as specificity, correctness, precision, and linearity. The registered retention time of the afobazole stock solution and the sample solution was 1.5 min. The determined accuracy of the method was in the range of 99.67%-100.67%. The analytical curve was linear (r^2-0.9996) over a wide concentration range (50%-150%). The method showed sufficient precision, with a relative standard deviation (RSD) smaller than 2%.

Conclusion: The HPLC method developed in this study showed specificity and selectivity with linearity in the working range and good precision and accuracy, making it very suitable for quantification of afb-m. The developed assay procedure of afb-m was meeting all the requirements of ICH validation criteria and can be applied for standardisation drug form afb-m.

Keywords: Afobazole, HPLC, Microcapsules, Validation

The present days, in the family of particular anxiolytics drugs, appeared-a selective anxiolytic afobazole®. Afobazole has a complex of the anxiolytic effect manifested by reducing mental and somatic (vegetative) anxiety symptoms with a light psychoactive effect [1]. There are no muscle relaxant properties, a negative impact on memory and attention, also using afobazole was not forming an addiction and does not develop withdrawal symptoms. The tableted dosage formulation of afobazole was frequently prescribed by medical consultants, as a result of popularity and effectiveness and lack of the variety of afobazole dosage formulations requires novel dosage forms [2-4].

The physicochemical, biopharmaceutical and technological research was carried out by the department of pharmaceutical chemistry and technology in Voronezh state university was proposed the enhance dosage formulation: a microencapsulated formulation of afobazole, obtained by dispersing in the "liquid-liquid" system [5-9].

The validation of an analytical method must demonstrate that it fulfils all the requirements of the analytical applications, ensuring the reliability of the results. For this reason, the tests must show that its specificity, linearity, precision, sensitivity, accuracy are adequate for the analysis. The quantitative determination method of afobazole in microcapsules must be validated following the requirements criteria: specificity, linearity, accuracy, and precision according to the recommendations of the international council for harmonization of technical requirements for pharmaceuticals for human use (ICH) [10, 11]. The main purpose of this work was conducting validation procedure of afb-m.

In this experiment was used: liquid chromatograph Agilent 1100 (Agilent technologies, Japan), UV diode array detector (Agilent technologies, Japan), «Zorbax extend-C18» column (Agilent technologies, Japan), acetonitrile (uf210, Russia), ammonium phosphate (manufactured by Sigma in Germany, and Fluka in Switzerland). Afobazole (fabomotizole) standard samples were synthesized by the Italian firm Erregiere and chemical-technological

laboratory research and technology department of the research institute of pharmacology in Russia, was meeting the requirements of the regulatory document "afobazole 42-13869-05.

The object of this research was the microencapsulated afobazole (afb-m). The afb-m used in the studies was obtained by the method of dispersion in the "liquid-liquid" system. The method was performed on the liquid chromatograph agilent 1100 and determination conducted by the UV-vis diode array detector at the wavelength 220 nm. The following conditions were applied: reversed phase 2,1x150 mm size «Zorbax extend-C18» column filled with the 5 µm size particles, 1.0 ml. min^{-1}mobile phase (MP) flow rate, column temperature 40 °C, multi-wavelength detection mode and the column thermos tasting temperature mode. For the preparation of MP was used acetonitrile and the buffer system (pH-7.0) which was consisting of 5.0 M ammonium phosphate solution with a volume ratio of 30:70 (v/v). For the microencapsulation process used a standard sample of afobazole with 98.5-101% purity without further purification.

The standard solution of afobazole was prepared using afobazole standard samples. Accurately weighed 0.030g was transferring into 100 ml volumetric flask and was dissolved five minutes in the ultrasonic bath by adding 80 ml of MP. After the full dissolution of afobazole, the solution volume was adjusted with the same solvent to 100 ml. The solution was accurately stirred for the solution homogeneity and filtered through a membrane filter with a pore size of 0.2-0.45 mm.

Accurately weighed 0.100 g porphyrized microcapsules powder was used to prepare the test solution and followed the same procedure, which was used to preparing the standard solution with discarding the first portion of the filtrate. The only fresh solutions were used in the experiment work.

The chromatogram of the afobazole standard solution was obtained by applying above described chromatographic conditions. The

specificity and selectivity describe the capacity of the analytical method to measure the drug in the presence of impurities, excipients, degradation products or matrix components. These parameters were determined by comparing the chromatograms of the afobazole standard solution and test solution. The chromatogram of the afobazole standard solution presented a peak in the time retention of 1.5

min (fig. 1). The chromatogram of afb-m solution (test solution) (fig. 2) showed a peak and retention time similar to afobazole standard solution. The components of the microcapsules do not interfere with the analysis; therefore, no peak is observed in the region of the main peak of afobazole (fig. 2). The chromatogram peaks are well resolved, indicating the high specificity of the method.
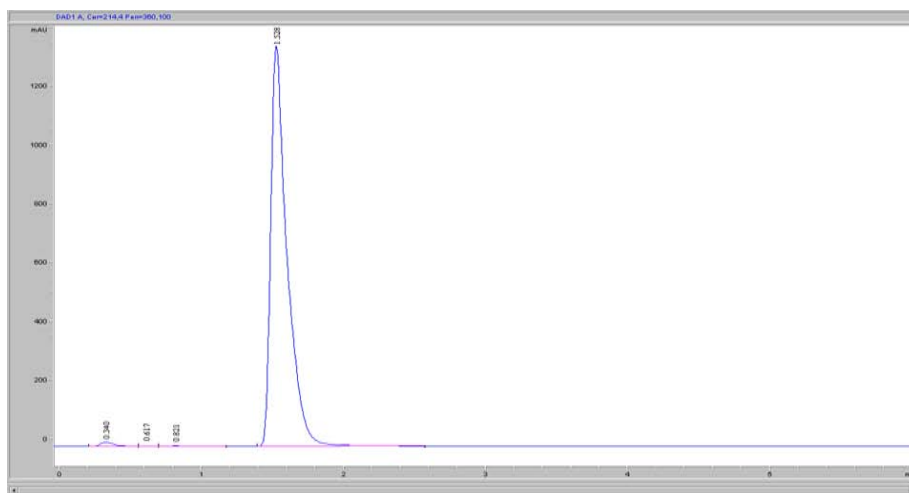

Fig. 1: HPLC chromatogram of the afobazole standard sample solution
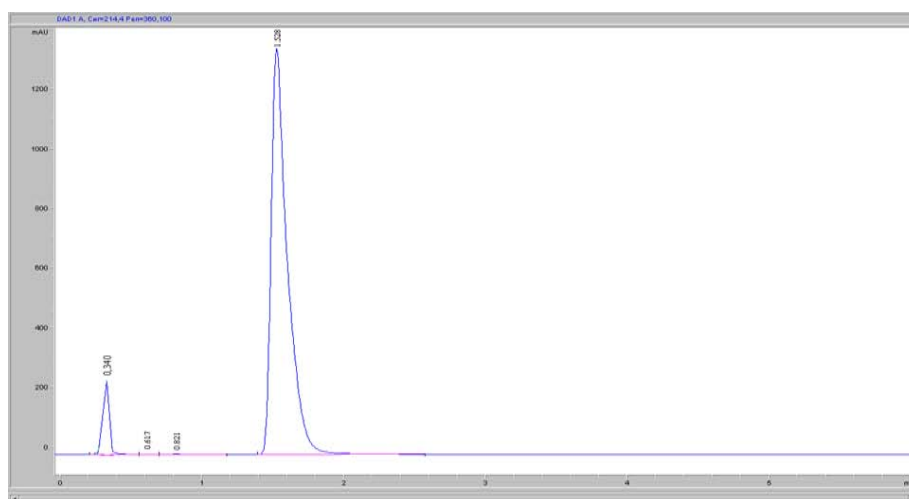

Fig. 2: HPLC chromatogram of the afobazole extraction from the microcapsules

Accuracy is one of the most important parameters of an analytical methodology, and it can be expressed as the percent recovery of known amounts of drug added to a sample.

For studies of the determination accuracy of the analytical method was carried out using afobazole model mixtures solution with a concentration range 50%-150% of standard solution concentration. The openability value (R) of the model mixtures and afobazole standard solution were measured. The percentage concentration of afobazole (X %) in the samples and model mixtures was calculated using the formula (1).

$$X = \frac{S_i * m_0 * 100 * 100}{S_{0i} * m * 100} = \frac{S_i * m_0 * 100}{S_{0i} * m}, (1)$$

Where,

m_0–a weighed portion of the afobazole standard sample used to prepare the reference solution, (g);

m–a weighed portion of the microcapsule powder used to prepare the test solution sample, (g);

S_i-the mean value of the peak areas, computed from the chromatogram of the afobazole test solution;

S_{0i}-the mean value of the peak areas, calculated from the chromatogram of the afobazole reference solution.

The precision was calculated from an average of six determinations of a homogeneous sample. The precision assays were expressed as relative standard deviation (RSD) 0.47, indicating that the method presents a good precision. The detailed precision data are shown in table 1.

The linearity of the method was established by analysing of afobazole model mixtures series consists in the range 50-150% of standard solution concentration. The analytical curve was constructed by plotting the area under the curve (AUC) of the main peak versus drug concentration, with a calculating correlation coefficient r (fig. 3).
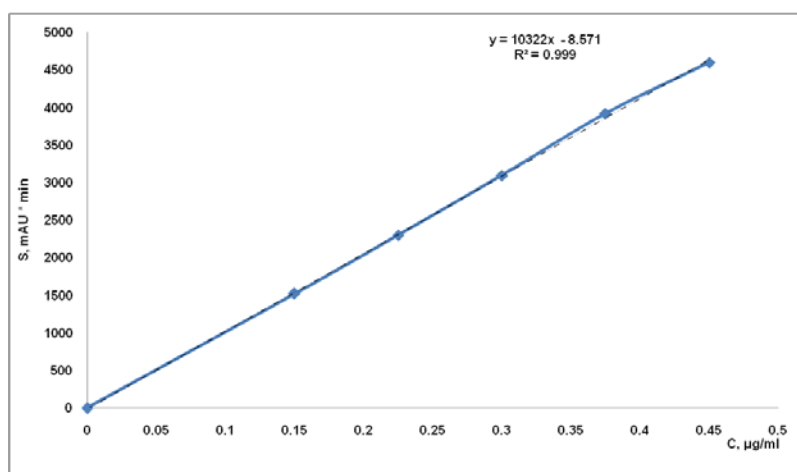
Fig. 3: The variation of the area under the chromatographic peak against to the afobazole concentration in the solution

The characteristics of the validation method were presented in table 1.

Table 1: Validation characteristics of the methodology for determining the afobazole in the microcapsules

The characteristics of the validation	Admissibility criteria	Results			Compliance with the criterion of admissibility
Specificity		Overlapping the retention time peaks in the chromatograms of the afobazole sample solution and standard solution.			Complied
Accuracy	R+ΔR (openability) must belong to the interval 98-102%	% 50 75 100 125 150	R-ΔR 99.67 98.30 99.33 98.33 99.67	R+ΔR 101.67 99.30 100.33 99.33 100.67	Complied
Precision-repeatability	RSD ≤ 2 % (n=6)	Sample number 1 2 3 4 5 6 RSD, %	R, % 100.43 98.77 99.56 101.34 101.56 99.15 0.47		Complied
Linearity	1. A linear dependency of peak area against to the afobazole concentration in the sample; 2. The correlation coefficient $R^2 \geq$ 0,99	1. Observing linear dependence of the peak area against to the concentration afobazole (fig. 2); 2. The correlation coefficient R^2 = 0,9996			Complied

As the shown in fig. 3, the analytical curve for the afobazole standard sample was constructed by plotting the area under the curve (AUC) of the main peak versus drug concentration. It was found to be linear over a wide concentration range (50%-150%) with a correlation coefficient of 0.9996. The straight line (trend line) equation obtained from the experimental results was found to be: y= 10322x-8,5714. The linearity parameters were founded in the experiment corresponds to the all the requirements criteria, the correlation coefficient was more than 0.99, which corresponds to the requirements of the state standard Russia ISO 5725 and ICH Q2 (R1).

The results were given in table 1. was demonstrating, that the analytical method has sufficient accuracy over the selected concentration range (from 50% to 150%). The relative standard deviation (RSD, %) did not exceed 2%. Thus, the afobazole determination method in the microcapsules was valid to concerning "correctness" and besides that, afobazole analysis technique has sufficient accuracy and precision and correctness. There were no any registered significant systematic errors in this method.

CONCLUSION

The results show that the HPLC method presented here can be considered suitable for the analytical determination of afobazole in microcapsules. The described method was me*et al.* l the validation requirements criteria and can make the conclusion that the validation procedure was accomplished. As a result of the high selectivity and acceptable level of sensitivity of the developed quantification method for afb-m can be recommended to apply for quality control purposes of afobazole in dosage formulations.

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CONFLICTS OF INTERESTS

Declared none

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