PHARMACEUTICAL ANALYSIS AND STANDARDIZATION OF AFOBAZOLE IN SOLID DOSAGE FORM (TABLETS)

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The investigation was aimed at developing procedures for the pharmaceutical analysis and standardization of the new domestic anxiolytic afobazole in solid dosage form (tablets). The main pharmacopoeial tests have been carried out and methods for the determination (TLC, HPLC), identification (TLC, UV spectrophotometry), and quantitative assay (UV spectrophotometry, HPLC) have been worked out. The test for uniformity of composition and the dissolution test were carried out with the aid of UV spectrophotometry.

Key words: afobazole, tablets, pharmaceutical analysis.

Afobazole (**I**), 5-ethoxy-2-[2-(morpholino)ethylthio]-benzimidazole dihydrochloride, is a new original selective anxiolytic that was invented at Zakusov State Institute of Pharmacology (ZSIP), RAMS. Pharmacological studies of the new drug showed that the anxiolytic activity of **I** was not associated with the side effects typical of other selective anxiolytics [1].

A solid dosage form of **I** (tablets, 5 mg) was developed in ZSIP [2]. The goal of the present work was to develop a procedure for pharmaceutical analysis, to establish the quality standard, and to study the stability on storage of the solid dosage form of **I**.

EXPERIMENTAL PART

Serial batches of tablets of **I** (5 mg) were studied using TLC, HPLC, and UV spectrophotometry under conditions developed previously for the substance and a solution for injection of **I** [3, 4].

TLC analysis was performed on Kieselgel 60 F254 plates (Merck) using acetone:hexane:NH_4OH (conc.) (20:20:0.5) with detection of spots in UV light at 254 nm.

The HPLC method was developed in a Beckman liquid chromatograph (USA) with a fixed volume loop (20 μL) and a variable-wavelength spectrophotometric detector. Studies were carried out on a steel column (250 mm × 4.6 mm) packed with C_{18} sorbent [5 μm, Luna C18(2)]. The mobile phase was a mixture of CH_3CN, MeOH, and phosphate buffer (0.02M, pH 7.3) (100:100:240) (v/v/v). The elution was isocratic at flow rate 1 mL/min and analytical wavelength 302 nm.

Absorption of drug samples in the UV spectral region was measured on a UV-1700 spectrophotometer (Shimadzu, Japan).

RESULTS AND DISCUSSION

The tablets were white or white with a barely discernable cream tint and were cylindrical with a beveled edge.

The average tablet mass was 0.150 g. The deviation from the average mass was less than 7.5% and was actually ± 5.5%. The disintegration time was from 2 to 5 min.

The purity of tablets of **I** was estimated by TLC using conditions selected previously for determining the purity of **I** drug substance. The active ingredient was extracted from a ground tablet by $CHCl_3$:Et_2NH (9:1). The $CHCl_3$ extract was placed at the origin in an amount equivalent to 100 μg of **I** [3]. Adsorption bands were detected using UV light and iodine vapor. The impurity content was estimated visually by comparing the sizes and intensities of impurity adsorption

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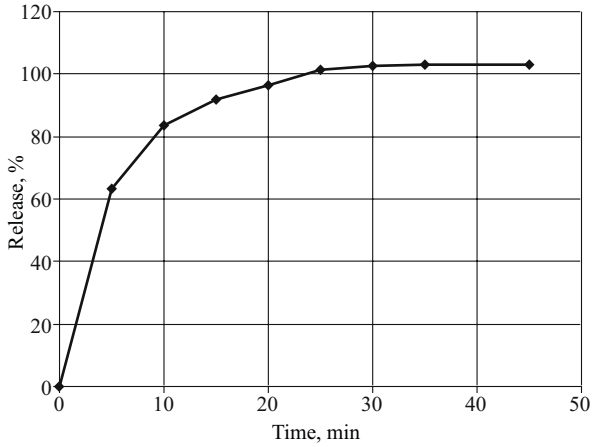

Fig. 1. Dissolution kinetics of tablets of **I** (5 mg) in HCl solution (0.01M).

bands and solutions of a working standard of **I** placed on the chromatographic plate in an amount equivalent to 0.2 μg (0.2%) and 0.1 μg (0.1%) of **I**.

The operability of the chromatographic system was checked using the R_s values of **I** ($R_f \sim 0.27$) and 5-ethoxy-2-mercaptobenzimidazole ($R_f \sim 0.54$), which fell in the range 2.0 ± 0.3 and using the color intensity of the adsorption band of standard **I** placed in an amount equivalent to 0.1 μg (spot should be clearly visible).

Chromatographic analysis of tablets found in certain samples, like in the drug substance, two unidentified impurities, the content of each of which was less than 0.2% and the total content of which was less than 0.4%. Like in the drug substance, a synthetic intermediate, chloroethylmorpholine, that is detected in iodine vapor was not observed in the tablets. However, the sample of **I** contained a different synthetic intermediate, 5-ethoxy-2-mercaptobenzimidazole, which is detected in UV light, at a content less than 0.2%.

We decided not to include determination of the chloroethylmorpholine impurity in the procedure because it was not observed in either the drug substance or the tablets.

The following procedure was developed based on the results. Tablets ground to a powder (0.6 g) were placed into a tube with a ground-glass stopper, shaken for 5 min with $CHCl_3$:Et_2NH (9:1, 2 mL), and filtered through paper. A portion (0.01 mL, 100 μg) of the resulting solution was placed at the origin of Silicagel plates with a fluorescent indicator (254 nm).

References of **I** were placed alongside (solution B, 0.02 mg/mL, 0.01 mL; solution C, 0.01 mg/mL, 0.01 mL).

Plates with deposited samples were dried in air for 10 min, placed into a chamber with the solvent mixture acetone:hexane:NH_4OH (conc.) (20:20:0.5), and chromatographed in ascending mode. When the solvent front had traveled 10 cm from the origin, the plates were removed from the chamber, dried in air for 10 min, and viewed in UV light at 254 nm.

The chromatogram of the tested solution showed the principal spot and a spot at the origin. Additional spots, each of which had a size and intensity less than that of solution B of **I** (less than 0.2% of a single impurity), were allowed. The total impurities should not exceed 0.4%.

The analytical results were considered reliable if the requirements of the test for verification of suitability of the chromatography system were satisfied.

An HPLC procedure was developed as an alternate method for determining the impurity content in tablets of **I**.

Solutions of **I** with concentration 1 mg/mL were prepared for the studies. For this, ground tablet powder (0.075 g) was placed into a 25-mL volumetric flask, shaken with mobile phase (15 mL) for 10 min, adjusted to the mark with mobile phase, stirred, and filtered through a 0.45 μm Millipore filter. The resulting solutions were chromatographed, producing at least five chromatograms for each solution.

The content of a single impurity in the drug was estimated by internal normalization of peak areas averaged over five chromatograms of the tested solution.

An extract of a placebo had practically no absorption at 302 nm and did not interfere with the determination of the impurities.

The suitability of the chromatography system was checked by preparing a solution of **I** (0.005 g) and 5-ethoxy-2-mercaptobenzimidazole (0.005 g) in mobile phase (100 mL). The suitability of the chromatography system was evaluated using the efficiency of the chromatography column (at least 4,000 theoretical plates), the asymmetry coefficient of the peak for **I** (less than 1.5), the separation coefficient of the peaks for **I** and 5-ethoxy-2-mercaptobenzimidazole (at least 6), and the relative standard deviation calculated for the peak areas of **I** in five parallel measurements (less than 2%).

Serial batches of tablets of **I** were analyzed by the developed procedure. Up to three unidentified impurities were observed in serial batches and in **I** drug substance. In addition, one sample had an impurity of 5-ethoxy-2-mercaptobenzimidazole. The content of each of these impurities was less than 0.3%. The total impurity content was less than 0.6%.

The content of **I** in the tablets was assayed quantitatively using UV spectrophotometry and HPLC methods.

The content of **I** in the tablets was quantitatively assayed by spectrophotometry in HCl solution (0.01M). The absorption maximum of **I** in this solution fell in the range 302 ± 2 nm. The optical density at 302 nm was a linear function of concentration in the range 0.001 – 0.03 mg/mL (correlation coefficient +0.998). The optical density at a concentration of 0.015 mg/mL was about 0.620. This concentration was selected as the working value.

The procedure was developed using model mixtures of **I** drug substance and a placebo in a ratio analogous to the tablet composition. The weight of **I** was about 0.025 g; of the placebo, about 0.725 g (masses of **I** and the placebo were in-

creased five times in order to lower the effect of weighing errors).

The active ingredient was extracted from the model mixtures by HCl solution (0.01M) by shaking for 10 min and filtering suspended matter through a 0.45 μm Millipore filter. The resulting solutions were adjusted to a concentration of 0.015 mg/mL. The placebo solution was prepared analogously.

The studies showed that the placebo solution had practically no absorption at the analytical wavelength (302 nm). The error in the quantitative determination of **I** in the model mixtures was less than 1.5% (Table 1).

The following procedure was developed for quantitative determination of **I** in tablets. About 0.15 g (accurate weight) of ground tablet powder is placed into a 100-mL volumetric flask, shaken in HCl solution (70 mL, 0.01M) for 10 min, adjusted to the mark with the same solvent, and filtered through a 0.45 μm Millipore filter, discarding the first portions of the filtrate. A 50-mL volumetric flask is charged with the resulting solution (15 mL), adjusted to the mark with HCl solution (0.01M), and stirred (tested solution). A solution of a working standard sample (WSS) of **I** at a concentration of 0.015 mg/mL in HCl (0.01M) is prepared in parallel. The optical density of the tested solution and the WSS solution are measured at 302 nm in a 10-mm cuvette.

The quantitative content of **I** in tablets was calculated as from 4.75 to 5.29 mg per tablet using the aforementioned procedure (Table 2).

A spectrophotometric method was used for quantitative assay of **I** in "Uniformity of composition" and "Dissolution" tests.

The Uniformity of composition test was performed under the same conditions as the Quantitative assay test. The deviations from the average of the content of **I** were less than 15% and actually (6.5%.

The following method was developed in order to check the Dissolution test. The test was conducted according to requirements of OFS 42-0003-04 on a rotating basket apparatus. The dissolution medium was HCl solution (0.01M, 500 mL); solvent temperature, 37 ± 1°C; basket rotation rate, 100 rpm; test time, 20 min.

An aliquot of the solution (20 mL) was taken from each vessel after 20 min and filtered through "blue-ribbon" filter paper or a 0.45 μm Millipore filter, discarding the first portions of the filtrate.

A solution of the WSS at a concentration of 0.01 mg/mL was prepared in parallel.

The optical density of the resulting solution and the WSS solution was measured at 302 nm in a 10-mm cuvette.

The dissolution kinetics of **I** from tablets was studied using the aforementioned procedure (Fig. 1). The dissolution norms of **I** were found. At least 80% of the active ingredient should be dissolved after 45 min.

All samples of tablets of **I** agreed with the established norms.

An HPLC procedure was developed as an alternate method for quantitative determination of **I** in tablets.

Model mixtures showed that the absorbance at 302 nm was linear with concentration in the range 0.0001 – 0.1 mg/mL (correlation coefficient 0.999). The working concentration was selected as 0.008 mg/mL. The active ingredient was extracted from the model mixtures and the ground tablet powder using mobile phase. The extract from the placebo did not absorb at 302 nm and did not interfere with the determination.

The following procedure was developed for quantitative determination of **I** in tablets using HPLC. About 0.3 g (accurate weight) of ground tablet powder is placed into a 50-mL volumetric flask, shaken with mobile phase (30 mL) for 10 min, adjusted to the mark with the same solvent, stirred, and filtered through a 0.45 μm Millipore filter or blue-ribbon filter paper.

The resulting solution (1 mL) is placed into a 25-mL volumetric flask, adjusted to the mark with mobile phase, and stirred. A solution of the WSS at a concentration of 0.008 mg/mL is prepared in parallel.

TABLE 1. Quantitative Determination of **I** in Model Mixtures

Afobazole added, g (m_1)	Afobazole found, g (m_2)	Absolute error, g ($d = m_2 - m_1$)	Relative error, % $X = (d \cdot 100/m_1)$	Afobazole found in % of m_1	Metrological characteristics (P = 95%)
0.0258	0.0260	+ 0.0002	0.78	100.78	$n = 9$
0.0245	0.0242	– 0.0003	1.22	99.78	$X = 99.92$
0.0251	0.0254	+ 0.0003	1.19	101.20	$S = 1.24$
0.0239	0.0235	– 0.0004	1.67	97.91	$S_x = 0.41$
0.0255	0.0254	– 0.0001	0.39	99.61	$\Delta\bar{X} = 1.00$
0.0252	0.0249	– 0.0003	1.19	98.81	$\varepsilon = 1.00$ %
0.0261	0.0265	+ 0.0004	1.53	101.53	
0.0243	0.0245	+ 0.0002	0.82	100.82	
0.0257	0.0254	– 0.0003	1.17	98.83	
0.0264	0.0263	– 0.0001	0.38	99.62	$n = 5$
	0.0264	0	0	100.00	$X = 99.85$
	0.0262	– 0.0002	0.76	99.24	$S = 0.58$
	0.0266	+ 0.0002	0.76	100.76	$S_{\bar{x}} = 0.26$
	0.0263	– 0.0001	0.38	99.62	$\Delta\bar{X} = 0.72$
					$\bar{\varepsilon} = 0.72$ %

TABLE 2. Quantitative Assay of Tablets of **I** (5 mg) by UV Spectrophotometry

Series No.	Quantative assay, mg/mL	Metrological characteristics (P = 95%, n = 5)		
		S	$\Delta\bar{X}$	$\bar{\varepsilon}$, %
1	5.29	0.034	0.042	0.80
2	4.75	0.043	0.053	1.13
3	5.04	0.042	0.052	1.03
4	5.00	0.019	0.024	0.47
5	5.12	0.058	0.072	1.41

TABLE 3. Quantitative Assay of Tablets of **I** (5 mg) by HPLC

Series No.	Quantative assay, mg/mL	Metrological characteristics (P = 95%, n = 5)		
		S	$\Delta\overline{X}$	$\overline{\varepsilon}$, %
1	5.32	0.052	0.065	1.22
2	4.81	0.031	0.039	0.80
3	5.11	0.067	0.083	1.63
4	4.98	0.059	0.073	1.47
5	5.09	0.046	0.057	1.12

The resulting solutions are chromatographed producing at least five chromatograms for each solution.

The Suitability of the chromatography system test is carried out the same as the determination of the index of "Impurities".

Analysis of tablets of **I** showed that the quantitative content of **I** in them was from 4.81 to 5.32 mg per tablet (Table 3).

Development of procedures for analyzing tablets of **I** showed that the impurity content can be determined by TLC (principal method) and HPLC (alternate method); the quantitative content, by UV spectrophotometry and HPLC. However, the Dissolution and Uniformity of composition tests are more convenient and economical to perform using the UV spectrophotometry method because of the large amount of sample. Therefore, the UV spectrophotometry procedure was included in the draft FSP for the quantitative tests. The HPLC procedure was proposed as an alternate for quantitative assay of **I** in tablets.

The agreement of R_f values or retention times of the tested sample with those of the WSS solution and the agreement of the maximum in the UV spectrum of the tested solution with that in the spectrum of the WSS were proposed for determining the identity of **I** in tablets.

The stability of tablets of **I** on storage was determined by the accelerated aging method [5] and under natural conditions. The quality of the studied samples was practically unchanged after a period equivalent to two years of storage had passed.

The completed studies resulted in the development of a pharmaceutical analytical procedure, a study of the stability on storage, and the development of quality norms for tablets of **I** (5 mg). These were included in the draft FSP for the drug.

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