ANALYSIS AND STANDARDIZATION OF THE NEW ANXIOLYTIC DRUG AFOBAZOLE

S. E. Milkina,[1] O. B. Stepanenko,[2] L. N. Grushevskaya,[2] N. I. Avdyunina,[2] B. M. Pyatin,[2] V. L. Bagirova,[1] and E. B. Nechaeva[1]

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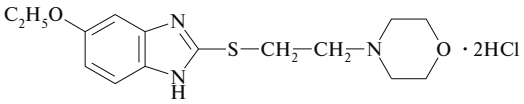
The physicochemical properties of the new anxiolytic drug afobazole, which belongs to the group of 2-mercaptobenzimidazole derivatives, have been studied with a view to its pharmacopoeial standardization. It is suggested to check afobazole for the presence of impurities by means of TLC and HPLC. Methods for the standardization of afobazole are developed and a project for the pharmacopoeial article of manufacturer for this drug is formulated.

As is known, the class of 2-mercaptobenzimidazole derivatives contains substances possessing gastroprotector, adaptogen, cardiotropic, and anxiolytic properties [1]. The new original domestic drug afobazole also belonging to this class and exhibits pronounced anxiolytic action that is not accompanied by side effects typical of tranquilizers of the benzodiazepine series [2]. Afobazole does not produce sedative (calming), hypnotic, and myorelaxant effects. At present, this drug is in the stage of clinical testing as a specific anxiolytic agent.

The chemical structure of afobazole corresponds to 5-ethoxy-2-[2-(morpholino)ethylthio]benzimidazole dihydrochloride.



The parent substance is synthesized via the alkylation of 5-ethoxybenzimidazole-2-thione with N-(2-chloroethyl)morpholine hydrochloride. The reaction proceeds on boiling in an alkaline aqueous alcohol medium. The reaction product is isolated by extraction with chloroform. The technical product is obtained by adding a hydrogen chloride solution in anhydrous ethanol to the base solution in an organic solvent until obtaining pH 1 – 2. Finally, pharmacopoeial afobazole is obtained by recrystallization from ethanol.

The present investigation was aimed at determining the physicochemical properties of afobazole, developing analytical procedures for the identification and determination of afobazole, and establishing maximum storage duration and justified parameters of quality of the parent substance I.

EXPERIMENTAL PART

Afobazole appears as a crystalline powder of white color, sometimes with a creamy tint, readily soluble in water (1 g per 5 – 8 ml), sparingly soluble in ethyl alcohol (1 g per 13 – 14 ml), slightly soluble in chloroform (1 g per 275 – 280 ml), and practically insoluble in ether (1 g is not dissolved in 100,000 ml).

The parent compound was characterized with respect to the drug solution transparency, color, and pH at a concentration of 1%. This solution is virtually colorless (coloration does not exceed 7b grade standard). With respect to transparency, the optical density of 1% afobazole solution does not exceed turbidity standard I. The 1% aqueous afobazole solution has pH within 2.0 – 3.0.

The afobazole composition and structure are confirmed by elemental analyses and spectroscopic measurements. The IR spectra in the 4000 – 400 cm^{-1} wavenumber range were recorded on a Perkin-Elmer Model 580 spectrophotometer (Sweden) using samples prepared as KBr pellets or nujol mulls. Since it was found that the characteristic absorption bands of afobazole were more clearly pronounced in the spectra of samples palletized with KBr, this method of sample preparation is recommended for spectroscopic identification of the parent drug. The IR spectrum of afobazole exhibits a broad absorption band with a maximum at 3240 cm^{-1}, which is typical of NH groups, and displays characteristic

[1] Institute for Drug Standardization, State Scientific Center for Drug Expertise and Control, Ministry of Public Health of the Russian Federation, Moscow, Russia.
[2] Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia.

bands at 1635 cm^{-1} (NH bending vibrations in ammonium ion), 1615, 1505, and 1465 cm^{-1} (C=N, C=C), 1450 and 1315 (CH bending), and 1180 and 1050 cm^{-1} (Ar–O–Alk chains).

The ^1H NMR spectra were measured on a Bruker AC-250 spectrometer (Germany) using a standard Bruker control program package. The chemical shifts were determined using TMS as the internal standard. The ^1H NMR spectrum in DMSO displayed signals from protons of all moieties of afobazole molecule (δ, ppm): 1.37 (t, 3H, CH_3), 3.37 (m, 7H, CH_2NCH_2), 3.58 (t, 2H, CH_2S), 3.91 (m, 4H, CH_2OCH_2), 3.93 (t, 2H, CH_2N), 4.08 (q, 2H, CH_2CH_3), 7.04 (dd, 1H, H-6), 7.15, (d, 1H, H-4), 7.59 (d, 1H, H-7).

The UV spectrum of afobazole dissolved in 0.01 M aqueous hydrochloric acid solution was measured in a wavelength interval from 210 to 350 nm. The spectrum exhibits a characteristic shape with maximum absorption at 302 ± 2 nm.

Based on these results, we propose to identify afobazole using IR and UV spectroscopy and a qualitative reaction for chlorides. The parent substance of afobazole melts at 190 – 196°C with decomposition and the formation of a yellow melt.

The content of foreign impurities in the parent substance of afobazole was evaluated using TLC. According to the scheme of synthesis, the possible impurities in the parent substance (compound I) can be the initial, intermediate, and side products of drug synthesis, in particular, 5-ethoxy-2-mercaptobenzimidazole (compound II, $R_f = 0.54$) and chloroethylmorpholine (compound III, $R_f = 0.62$), and the products of afobazole oxidation. The samples were dissolved in a chloroform – dietnylamine (9 : 1) mixture.

The spots of afobazole on the TLC trace were observed at $R_f = 0.23 – 0.32$. The adsorption bands of components were revealed by exposure to UV radiation with a wavelength of 254 nm.

The mobile phase for TLC was chosen taking into account selectivity, eluent strength, and chemical stability of the components. Preliminarily, we have tried various systems and established that the best separation of components is achieved in solvent mixtures possessing a basic character. The optimum separation of afobazole, initial compounds, and nonidentified impurities was obtained in a mixture of acetone, hexane, and concentrated aqueous ammonia solution with a component ratio of 20 : 20 : 0.5. This system ensures clear separation of afobazole (I) from the intermediate products (II, III) formed in the last stage of drug synthesis.

TLC analyses of the samples taken from several commercial batches of afobazole showed the absence of impurity III. However, there were two other nonidentified impurities with $R_f = 0.40$ and 0.18, which were revealed under UV irradiation. The content of each impurity did not exceed 0.15% and their total content was within 0.3%. Taking into account these results, we suggest to establish the possible content of each individual impurity as not exceeding 0.2% and the maximum total impurity content as 0.5%.

The best chromatographic separation of afobazole from impurities was achieved with a 150 × 4.0 mm column filled with Diaspher-110-C_{18} (7 μm). The optimum mobile phase was selected after preliminary experiments with methanol – water (50 : 50), acetonitrile – water (50 : 50), and methanol – water (pH 7.6, 50 : 50) systems. The optimum separation of afobazole from impurities was obtained with the latter system at an eluent flow rate of 1 ml/min. The UV detector was tuned to 302 nm. The reference samples were 5-ethoxy-2-mercaptobenzimidazole (II) and 4-(2-chloroethyl)morpholine hydrochloride (III). Chromatographic analysis of the samples taken from several commercial batches of afobazole confirmed the absence of impurity III. However, some of the samples contained impurity II (at an amount not exceeding 0.2%) and some nonidentified impurities probably representing the products of afobazole oxidation (their content did not exceed 0.6%). The total impurity content did not exceed 1%.

The weight loss of freshly prepared afobazole samples was determined at 130°C. For the samples taken from various batches, the weight loss varied from 0.7 to 1.07%. After a one-month storage under natural conditions in glass bottles with plastic screw caps, the weight loss increased to 1.93 – 4.02%.

The samples of afobazole were also studied with respect to hygroscopicity. The water content in saturated samples was determined either by measuring the weight loss at 130°C (this method showed a water content of 11.2%) or by titration according to Fischer (this technique showed about 12%).

The quantitative analysis for afobazole was performed by the method of nonaqueous acid-base titration in a mixture of formic acid and acetic anhydride. The titration end point was determined potentiometrically and by the change in the color of Crystal Violet indicator. The content of the parent substance in the product of synthesis fell within the interval from 98.5 to 100.5% (as calculated with respect to dry substance).

The stability of afobazole on storage was studied under natural conditions and using the accelerated aging test at 60°C. Upon expiration of the storage time corresponding to two years of storage under the natural conditions, the samples were subjected to all tests used for the drug quality evaluation. Based on these data, the maximum storage time of afobazole was established at 2 years.

The methods of analysis and the quality characteristics developed in this work were used as a basis in formulating the Pharmacopoeial Clause for the parent substance of afobazole.

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