IDENTIFICATION AND QUANTITATIVE DETERMINATION OF SPECIFIC IMPURITY IN MEXIDOL AND EMOXYPINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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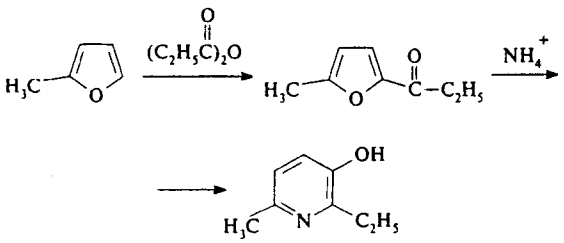
The drugs mexidol (I) and emoxypine (II), highly efficient antioxidants with a broad spectrum of action [1], are synthesized from 6-methyl-2-ethylpyridine-3-ol (III). Mexidol is an adduct of compound III with succinic acid, and emoxypine is hydrochloride of compound III.

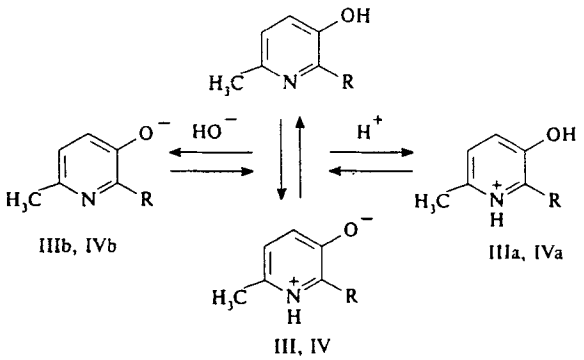
The major component of these drugs, compound III was synthesized by acylation of 2-methylfuran with propionic acid anhydride followed by rearrangement of 2-methyl-5-propylfuran with ring opening in the presence of ammonia and ammonium salts [2].



III

In the course of synthesis of compound III small amounts (less that 1%) of impurity 2,6-dimethylpyridine-3-ol (IV) may form, because propionic acid anhydride may contain the impurities of acetic anhydride and acetic-propionic anhydrides. Because of the higher toxicity of compound IV, its content in drugs I and II should not exceed 0.5%. The quality control of mexidol and emoxypine necessitates the development of a reliable technique for quantitative determination of dimethyloxypyridine impurity in these drugs.

The presence of the basic pyridine nitrogen atom and the acid phenol hydroxy group in molecules of compounds III and IV suggests that these compounds exist in solutions both in molecular and zwitterionic forms. The presence of acids and bases in aqueous solutions of compounds III and IV shifts the chemical equilibrium toward the predominance of cationic (IIIa and IVa) or anionic (IIIb and IVb) forms, respectively.



IIIb, IVb IIIa, IVa

III, IV

III: R = C_2H_5, IV: R = CH_3

Taking into account the amphoteric nature of compounds III and IV and their high solubility in water and polar organic solvents we used the HPLC technique (reversed phase mode) with the reagents being capable of maintaining the pH value.

When developing the HPLC procedure for separation of compounds III and IV, we studied the effect of the following factors on chromatographic behavior of the compounds: the type of sorbent (Octadecyl Si 100, Separon CSG C18), the nature and concentration of the organic modifier of the mobile phase (methanol, acetonitrile, ethanol, and acetonitrile), the concentration of the ion-pair reagent (sodium octylsulfonate), and the temperature of chromatographic analysis. Due to the low content of impurity IV (about 0.1%) in the experimental lots of drugs I and II, special attention was paid to the effectiveness of separation of compounds III and IV, to the symmetry of chromatographic peaks, and duration of the assay. Thus the proposed procedure can be used for rapid quality control of substances I and II.

The procedure for chromatographic separation and quantitative determination of the compounds was developed on

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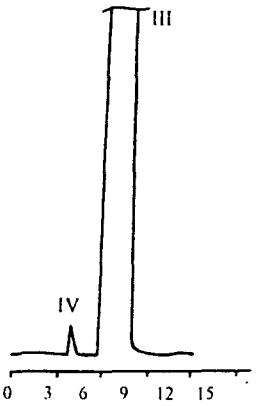

Fig. 1. HPLC of the sample of commercial mexidol I containing 0.25 mol. % dimethylhydroxypyridine IV.

samples of substances I and II that meet the requirements of the pharmacopoeial articles; samples of the experimental lots of I and II, obtained and purified using different techniques; samples of artificial mixtures of compounds III and IV containing 0.1 to 2.5 mol. % compound IV were also analyzed. Identification of compounds III and IV in the studied samples was performed by the retention time of the individual compound and by the method of internal standard.

Two pronounced absorption maxima at $\lambda = 226$ nm (ε 4850) and $\lambda = 296$ nm (ε 9786) are observed in the UV spectra of solutions of compounds III and IV possessing almost the same chromophores in the mobile phase of optimum composition (water – acetonitrile, 9 : 1, pH 3.0, concentration of ion-pair reagent 50 mg / liter). Thus the detection of the major substance III and impurity IV was carried in HPLC conditions at the wavelengths indicated above. We failed to adapt the HPLC procedure used to study the pharmacokinetics of mexindol [3] to the problem in question, because of significant asymmetry of the peak of the major substance observed at concentrations of artificial mixtures of III and IV ranging from 1 to 10 mg / ml with relative content of IV 0.1 to 0.5 mol. % which plagues the quantitative processing of the chromatograms.

The most effective separation of related compounds III and IV with high symmetry of the intensive peak of III both for artificial mixtures and for commercial samples of I and II was obtained on Octadecyl Si-100 sorbent when using a water–acetonitrile (9 : 1) mobile phase at pH 3.0 (sodium dihydrophosphate–phosphoric acid) with addition of 50 mg / liter of the ion-pair reagent sodium octylsulfonate. The optimum temperature of chromatographic analysis was 60°C. The relative molar content of admixture IV in the analyzed samples was calculated by the method of area normalization.

TABLE 1. Metrological Characteristics of Statistical Data Processing of the Mexidol Samples for Relative Content of Dimethylhydroxypyridine impurity IV*.

Compound	$\bar{X}$	S	$\Delta\bar{X}$	ε, % ($p = 95\%$)
Ia	0.5014	0.0107	0.0099	5.21
Ib	0.2471	0.0076	0.0070	7.49

A typical chromatogram of mexidol substance containing 0.25 mol. % compound IV is shown in Fig. 1. The main chromatographic characteristics of compounds III and IV under selected separation conditions for the artificial mixture with relative content of impurity IV 2.5 mol. % are: the retention time 6.30 (III) and 4.13 (IV) min, respectively; resolution R_s 1.5 (IV and III); selectivity α (III, IV) 1.86; and the capacity coefficients 1.04 (III) and 0.56 (IV).

It follows from the chromatogram profile (Fig. 1) and chromatographic characteristics of the analyzed compounds that selected chromatographic conditions ensure definite separation of methylethylhydroxypyridine (III) and impurity of dimethylhydroxypyridine (IV) and quantitative determination of the impurity content in substances I and II.

Note that solutions of drugs I and II in the mobile chromatographic phase are unstable in air. At concentrations of the analyzed samples I and II of 1 mg / ml, the peaks attributed to additional impurities were observed in chromatograms 2 h after the solutions were prepared. For this reason freshly prepared solutions of drugs I and II were used for quantitative determination of compound IV.

Table 1 summarizes metrological characteristics of statistical data processing for seven measurements of two samples of commercial mexidol I. The presented characteristics indicate that the proposed procedure possess high reproducibility. The time required for chromatographic analysis of the sample did not exceed 14 min.

The use of chromatographic column with Separon SGX C 18 sorbent (250 × 4 mm, 5 μm, ELSIKO), ammonia–phosphate buffer (aqua ammonia – phosphoric acid, pH 7.0), phosphate buffer (sodium dihydrophosphate–phosphoric acid, pH 3.0), methanol, ethanol, and acetonitrile as organic modifiers of mobile phase (from 10 to 50%), sodium octylsulfonate as the ion-pair reagent (at concentration less than 50 mg / ml) and chromatographic temperature lower than 60°C did not ensure satisfactory separation of compounds III and IV. Under these conditions chromatographic resolution R_s (III and IV) did not exceed 1.0. The asymmetry of the intensive peak of the major substance determined a decreased reproducibility of quantitative measurements.

The proposed procedure provides for identification and quantitative determination of impurity IV in mexidol and emoxypine at a level of 0.05 mol. %.

EXPERIMENTAL PART

The chromatograms were recorded on a LC-6A chromatograph fitted with a SPD-M6A spectrophotometric detector with a diode matrix and a CTO 6A (Shimadzu) thermostat. The detector sensitivity was 0.05 units of optical density per scale, the detection wavelength H = 296 nm. A Rheodyne injector (loop volume 20 μl) was used for the sample introduction. Separation of the analyzed compounds was carried out

on a column (250 × 4 mm) packed with an Octadecyl Si-100 sorbent (particle size 5 μm). The chromatograms were processed using SPDM6A software.

In this study the following reagents were used: acetonitrile for HPLC, water bidistillate, sodium dihydrophosphate dihydrate of analytical grade, reagent-grade phosphoric acid, and sodium octylsulfonate (for HPLC). The mobile phase consisted of 900 ml of 0.01 M aqueous solution of sodium dihydrophosphate (pH 3 was achieved by addition of phosphoric acid), 100 ml of acetonitrile and 50 mg of sodium octylsulfonate. The mobile phase was filtered through a membrane filter (pore diameter 0.45 μm). To remove the dissolved gases helium was bubbled through the eluent. The analyzed samples were dissolved in the mobile phase (concentration 1 mg/ml). Chromatographic analysis was carried out at 60°C; the mobile phase rate was 1.2 ml/min.

The UV absorption spectra of the solutions of compounds III and IV (at concentrations 0.10 mmole/liter in the mobile phase) were recorded on a Specord M-40 (Germany).

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